3M Medical Department Study: T-6316.1 Analytical Report: FACT TOX-003
LRN-U2104

Study Title

104-Week Dietary Carcinogenicity Study with Narrow Range N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats

Analytical Laboratory Report Title

Determination of the Presence and Concentration of PFOS, PFOSA, PFOSAA, EtFOSE-OH, M556, and PFOSEA in Serum and Liver Samples of Crl:CD®(SD) IGS BR Rats Exposed to N-Ethyl Perfluorooctanesulfonamido Ethanol

Data Requirement

Not Applicable

Author

3M Environmental Laboratory

Study Completion Date

June 06, 2001

Performing Laboratories

Liver and Serum Analyses

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

Project Identification

3M Medical Department Study: T-6316.1
Covance In-Life Study: 6329-228
Analytical Report: FACT TOX-003
3M Laboratory Request No. U2104

Total Number of Pages

211

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This page has been reserved for specific country requirements.

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GLP Compliance Statement

Analytical Laboratory Report Title: Determination of the Presence and Concentration of PFOS, PFOSA, PFOSAA, EtFOSE-OH, M556, and PFOSEA in Serum and Liver Samples of Crl:CD®(SD) IGS BR Rats Exposed to N-Ethyl Perfluorooctanesulfonamido Ethanol

Study Identification Numbers: T-6316.1, FACT TOX-003, LRN-U2104

This study was conducted in compliance with United States Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations 21 CFR Part 58, with the exceptions in the bulleted list below. The analytical phase completed at the 3M Environmental Laboratory was performed in accordance with 3M Environmental Laboratory 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.
- Sample storage stability will not be determined.
- Characterization of the analytical standards is underway, but has not yet been completed (21 CFR 58.105 (a)). Lot No. 59905 of the surrogate, THPFOS, will not be characterized for purity since quantities of this standard are exhausted. Lot 936 of EtFOSE-OH, and Lot L2353 and TN-A-1886 of PFOSA will not be characterized since quantities of these standards are exhausted.
- The electronic data systems in use have not been validated and there is not an electronic audit trail of corrections currently available (21 CFR 58.130 (e)). Authenticated hard copies of chromatograms and associated documents will be considered as the original raw data.
- There were instances in the raw data where corrections were not made according to 21CFR58.130(e) GLP requirements. Data was crossed out with no reason given, or original entries were obscured or contained "write-overs" with no explanation, initial or date provided.
- Some reagents (00-022-2, 0.5M TBA solution and 00-003-28, 0.5M TBA solution) were not labeled with storage requirements.
- Persons responsible for equipment were not identified (021188-55 IEC Cetra GP*R and N-Evap.)

(See next page for signatures)

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GLP Compliance Statement continued

John L. Butenhoff, Ph. D., *Study Director* — Date: June 6, 2001

Marvin T. Case, D.V.M., Ph.D., *Sponsor Representative* — Date: 6 June 2001

Kristen J. Hansen, Ph.D., *Principal Analytical Investigator* — Date: 05/31/01

William K. Reagen, Ph.D., Laboratory Manager — Date: 05/31/01

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GLP Study—Quality Assurance Statement

Analytical Laboratory Report Title: Determination of the Presence and Concentration of PFOS, PFOSA, PFOSAA, EtFOSE-OH, M556, and PFOSEA in Serum and Liver Samples of Crl:CD®(SD) IGS BR Rats Exposed to N-Ethyl Perfluorooctanesulfonamido Ethanol

Study Identification Numbers: T-6316.1, FACT TOX-003, LRN-U2104

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

Inspection Dates	Phase	Date Reported to	
		Management	Study Director
4/20/00	Sample prep	8/8/00	8/8/00
6/30/00, 7/5/00	Sample prep	7/21/00	7/21/00
4/23/01—4/30/01 4/30/01—5/1/01	Data	5/2/01	5/2/01
5/22/01	Report	5/23/01	5/23/01

Casey Howell — *QAU Representative*

Jun 11, 2001 — Date

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Study Personnel and Contributors

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Liver and Serum Analyses
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Kathleen M. Stock*
Anh-Dao Vo
Bob W. Wynne*
Richard Youngblom*

*Contract lab professional service employees

Location of Archives

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

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Introduction and Purpose

The purpose of the study is to determine the presence and concentration of PFOS, PFOSA, PFOSAA, EtFOSE-OH, M556, and PFOSEA in serum and liver specimens collected from Covance Study No.: 6329-228 titled: 104-Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats. The Covance in-life study was initiated on April 17, 1998. The analytical phase of the study was initiated on May 22, 1998.

Test System

A total of 140 males and 140 female rats were used as the test system. Table 1 outlines the rat population demographics and dosage levels for study 6329-228.

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

Table 1. Test System Population Demographics for Study 6329-228

Study Group	Number of Animals		Approximate Dietary Levels of EtFOSE-OH (ppm)
	Male	Female	
Group 8 Control	70	70	0
Group 9 Treated	70	70	1

Specimen Collection and Analysis

Specimens were collected by Covance (study 6329-228) and sent to the 3M Environmental Laboratory for analysis. On week 4, 20 serum specimens and 20 liver specimens were collected from Groups 8 and 9 males and females. On week 14, 20 serum specimens and 20 liver specimens were collected from Groups 8 and 9 males and females. On week 53, 20 serum specimens and 20 liver specimens were collected from Groups 8 and 9 males and females. On week 105, 40 liver specimens were collected from Groups 8 and 9 males and females. On week 105, 40 serum specimens were collected from Groups 8 and 9 males and females. The number and type of specimens collected for analyses in the analytical phase of this study are presented.

Specimens Collected from Study Groups 8 and 9
Serum Specimens—100 specimens
Liver Specimens—100 specimens

Liver and sera specimens were shipped to the 3M Environmental Laboratory frozen and on dry ice.

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Sera and liver samples were extracted beginning on May 27, 1998 using an ion pairing reagent and either ethyl acetate or 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-ESMSMS) in the multiple reaction monitoring mode. PFOS, PFOSA, PFOSAA, EtFOSE-OH, M556, and PFOSEA levels were evaluated by external calibration using extracted curves. Analytical details are included in this report.

Specimen Receipt and Maintenance

The 3M Environmental Laboratory received liver and serum specimens collected at predetermined time points during and at the end of the *in-life* phase of Covance Study 6329-228 from Covance May 1998 through June 2000. All specimens were received frozen on dry ice and were immediately transferred to storage at -20°C ±10°C.

Control matrices used in liver and sera analyses performed during TOX-003 were obtained from commercial sources and are presented in Appendix A. Samples analyzed at the 3M Environmental Laboratory will be maintained for a period of 10 years and will be stored at the laboratory at -20°C ±10°C.

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Chemical Characterization of the Reference Standards

Chemical characterization information on the analytical reference materials used in this study is presented in tabular form below.

Table 2. Characterization of the Analytical Reference Standards in Study FACT TOX-003

Reference Standard / Formula	Acronym	Source	Expiration Date	Storage Conditions	Chemical Lot Number	Physical Description	Purity
Potassium Perfluorooctanesulfonate $C_8F_{17}SO_3$-K+	KPFOS[a]	3M	08/31/01	Ambient temperature	171	Light colored powder	86.4%
		3M	2010	Ambient temperature	193	White crystals	88.0%
		3M	2010	Ambient temperature	215	White powder	TBD[c]
N-Ethyl Perfluorooctanesulfonamido ethyl alcohol $C_8F_{17}SO_2N(C_2H_5)CH_2CH_2OH$	EtFOSE-OH	3M	11/26/01	Ambient temperature	936	Amber waxy solid	NA*
		3M	2010	Ambient temperature	Unknown (SD013)	Amber waxy solid	88.9%
Sodium Perfluorooctanesulfonylamido(ethyl) acetate $C_8F_{17}SO_2N(CH_2CH_3)CH_2COO$-Na	PFOSAA[b]	3M	01/01/2010	Ambient temperature	617	Yellow to amber liquid	TBD[d]
Perfluorooctanesulfonylamido(ethyl)acid $C_8F_{17}SO_2N(CH_2CH_3)CH_2COO$- H		3M	01/01/2010	Ambient temperature	Unknown (98-0207-0303-3)	Light yellow powder	TBD[c]
Perfluorooctanesulfonylamide $C_8F_{17}SO_2NH_2$	PFOSA	3M	01/01/2010	Ambient temperature	L2353	Amber to brown waxy solid	NA*
		3M	01/01/2010	Ambient temperature	L15709	Light yellow waxy solid	TBD[c]
Perfluorooctanesulfonylethylamide $C_8F_{17}SO_2NHCH_2CH_3$	PFOSEA	3M	01/01/2010	Ambient temperature	529	Amber waxy solid	TBD[c]
		3M	01/01/2010	Ambient temperature	Unknown (TN-A-1885)	Amber waxy solid	NA*
M556 $C_8F_{17}SO_2N(H)CH_2COOH$	M556	3M	01/01/2010	Ambient temperature	NB113047-80	White powder	TBD[c]
1H, 1H, 2H, 2H-Tetrahydroperfluorooctanesulfonic acid $C_8H_4F_{13}SO_3H$	THPFOS	ICN	01/01/2010	Ambient temperature	59909	Brown powder	NA*

*This lot is exhausted and cannot be characterized.
NR—Not recorded
NA—Not applicable
TBD—To be determined

[a]Target analyte is PFOS, $C_8F_{17}SO_3^-$
[b]Target analyte is $C_8F_{17}SO_2\{(CH_2CH_3)(CH_2COO^-)\}$
[c]Unless otherwise indicated, at the time of quantitation, the purity for all analytes was assumed to be 100%.
[d]For reference lot 617, a purity of 53.82% was used for PFOSAA result quantitation.

Dose Confirmation Analyses

Dose preparation methods and analysis were performed by Covance, using a validated analytical method provided by Covance (MP-M312-MA), and are reported separately (Reference Covance 6329-228).

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Method Summaries

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

Data collected prior to November 1999 was reworked in 2000 to accommodate improvements in data reduction methods. Both the original and "reworked" data are archived; reworked data is presented in the final results. The improved methods are documented in the form of method modifications.

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

3M Environmental Laboratory

PREPARATORY METHODS

- **FACT-M-1.0**. "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry". This method was used for week 4 samples extracted on 6/9/98 and week 14 samples extracted on 7/17/98.
- **FACT-M-3.0**, "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactants from Serum for Analysis using HPLC-Electrospray/Mass Spectrometry". This method was used for week 4 samples extracted on 5/27/98 and week 14 samples extracted on 7/15/98.
- **ETS-8-4.1**, "Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds from Serum for Analysis using HPLC-Electrospray/Mass Spectrometry". This method was used for week 53 samples extracted on 4/20/00, and week 105 samples extracted on 6/29/00.
- **ETS-8-6.0**, "Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry". This method was used for week 53 and week 105 samples extracted on 4/14/00.

  An ion-pairing reagent was added to the sample and the analyte ion pair was partitioned into ethyl acetate (FACT-M-1.0 and FACT-M-3.0) or MtBE (ETS-8-4.1 and ETS-8-6.0). The extract was transferred to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract was reconstituted in 1.0 mL of methanol, and then filtered through a 3 cc plastic syringe attached to a 0.2 µm nylon filter into glass autovials.

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ANALYTICAL METHODS

- **FACT-M-2.0**, "Analysis of Liver Extracts for Fluorochemicals Using HPLC-Electrospray/Mass Spectrometry".
- **FACT-M-4.0**, "Analysis of Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry".
- **ETS-8-5.1**, "Analysis of Potassium Perfluorooctanesulfonate or other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry".
- **ETS-8-7.0**, "Analysis of Potassium Perfluorooctanesulfonate or other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry".

The analyses were performed by monitoring one or more product ions selected from a single primary ion characteristic of 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 further to produce ion 99 (FSO_3^-). The characteristic product ion 99 was monitored for quantitative analysis.

ANALYTICAL EQUIPMENT

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

Liquid Chromatograph: Hewlett-Packard® Series 1100 Liquid Chromatograph system
Analytical column: Keystone® Betasil™ C_{18} 2x50 mm (5 µm)
Column temperature: Ambient
Mobile phase components:
Component A: 2mM ammonium acetate
Component B: methanol
Flow rate: 300 µL/min
Injection volume: 10 µL
Solvent Gradient: 9.0 minutes

Time (minutes)	*%B*
0.0	40%
1.0	40%
4.5	95%
6.5	95%
7.0	40%
9.0	40%

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Mass Spectrometer: Micromass® API/Mass Spectrometer Quattro II™ Triple Quadrupole system
Software: Mass Lynx™ 3.1, 3.2, 3.3, 3.4
Cone Voltage: 30–60 V
Collision Gas Energy: 25–45 eV
Mode: Electrospray Negative
Source Block Temperature: 150°C ±10°C
Electrode: Z-spray
Analysis Type: Multiple Reaction Monitoring (MRM)

Table 3. Target Ions Monitored in 3M Laboratory Analyses

Target Analyte	Primary Ion (AMU)	Product Ion (AMU)
PFOS	499	80, 99, 130
PFOSA	498	78
PFOSAA	584	83, 169
EtFOSE-OH	630	59
PFOSEA	526	65
M556	556	65, 78, 83, 169
THPFOS	427	80

Data Quality Objectives and Data Integrity

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

- **Linearity:** The coefficient of determination (r^2) equal to or greater than 0.980.
- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest acceptable standard in the calibration curve.
- **Acceptable Precision:** Precision is better than 30% for the method.
- **Acceptable Spike Recoveries:** 70–130%.

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Data Summary, Analyses, and Results

Data quality objectives for the analytical phase of this study outlined in the 3M Environmental Laboratory protocol for FACT TOX-003 (see Appendix B) were met with the exceptions noted in this report.

Summary of Quality Control Analyses Results

- **Linearity:** The coefficient of determination (r^2) of the standard curve was ≥0.980.
- **Calibration Standards:** Quantitation of the target analytes was based on linear regression analysis weighted 1/x of a single, opening or two bracketing extracted matrix curves for each group of samples. Rat and rabbit sera were used for matrix curves in sera analyses, while rabbit liver was used for matrix curves in liver analyses. High or low points on the curve may have been deactivated to provide a better linear fit over the curve range most appropriate to the data. Low curve points with peak areas less than two times that of the extraction blanks were deactivated to disqualify a data range that may have been significantly affected by background levels of the analyte. Occasionally, a single mid-range curve point that was an obvious outlier may have been deactivated. Quantitation of each analyte was based on the response of one or more specific product ion(s) using the multiple reaction monitoring mode of the instrument (see Appendix C, Analytical Methods). Calibration standards were prepared to run, undiluted, approximately within the linear range of the instrument (approximately 5–1000 ng/g).
- **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 surrogate matrix blanks. (See Appendix D).
- **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 rat sera and liver, rabbit sera and liver were selected as suitable surrogate matrices for method blanks.
- **Precision:** Instrumental precision was determined by replicate injections of a single serum extract from a related study (TOX-001). Instrumental precision was determined for PFOS, PFOSAA, and M556; variation was less than 8.5% for all targeted analytes.
- **Matrix Spikes:** Sera—Matrix spike samples were prepared from control rat sera along with each batch of sera samples. Samples were spiked between 75–250 ng/mL, levels that approximate the levels detected in Groups 8 and 9 samples, depending upon the analyte. All spikes were prepared to run, undiluted, within a ten-fold limit of the linear range of the calibration curve. For some analytes, samples for some high dose animals may be much higher than the range of these prepared spikes. Sera matrix spike recoveries are presented in tabular form below.

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Table 4. Matrix Spikes—Sera

	# of Spikes Prepared	Average Spike Recovery	Range	Standard Deviation
PFOS	8	111%	73–144%	27%
PFOSA	8	107%	79–126%	16%
PFOSAA	8	106%	74–140%	26%
M556*	4	139%	108–174%	35%
EtFOSE-OH	4	39%	16–61%	25%
PFOSEA	4	66%	36–90%	28%

*M556 was identified as a metabolite of EtFOSE-OH after initiation of the analytical work. Standard material was unavailable and had to be synthesized. No M556 data is provided for the Week 4 through Week 14.

Liver—Matrix spike samples were prepared from a control group rat liver specimen along with each batch of liver samples. Samples were spiked between 100–500 ng/g, levels that approximate the levels detected in Groups 8 and 9 samples, depending on the analyte. All spikes were prepared to run, undiluted, within a ten-fold limit of the linear range of the calibration curve. For some analytes, samples for some high dose animals may be much higher than the range of these prepared spikes. Liver matrix spike recoveries are presented in tabular form below.

Table 5. Matrix Spikes—Liver

	# of Spikes Prepared	Average Spike Recovery	Range	Standard Deviation
PFOS	12	94%	65–137%	23%
PFOSA	12	87%	58–132%	23%
PFOSAA	12	96%	53–200%	39%
M556*	6	103%	80–122%	18%
EtFOSE-OH	4	118%	77–154%	39%
PFOSEA	6	108%	69–144%	27%

*M556 was identified as a metabolite of EtFOSE-OH after initiation of the analytical work. Standard material was unavailable and had to be synthesized. No M556 data or QC is provided for the Week 4 through Week 14 samples.

- **Surrogates:** The surrogate (THPFOS) was added to all samples and standards, with the exception of Week 4 liver and sera samples. THPFOS was not used for quantitation, but was used to monitor for gross (±50%) instrument failure.

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Statement of Data Quality

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the sera and liver data can be considered to be accurate to within one standard deviation of the average fortified samples recovery. Refer to Tables 4 and 5 for matrix spikes from each analyte and matrix.

The results of quality control analyses (curve fit, CCVs, and MS/MSDs) for EtFOSE-OH and PFOSEA were inconsistent and indicate that data presented for this analyte should be considered to be qualitative only. Values for these analytes are presented in data tables in the spirit of full disclosure, but should not be used in any quantitative assessment of the data.

Summary of Sample Results

PFOS results (those obtained using lot # 171 and #193) and EtFOSE-OH results (those obtained with lot # SD013) have been corrected for purity of the analytical reference material. Uncorrected results are noted in the data tables.

- **Samples from Control Animals:** The target analytes were often detected in the sera and liver samples from the control animals. These levels were usually lower than those found in the low dose test animals.
- **Samples from Dosed Animals:** In general, target analytes were present in the sera and liver samples in the dose group. Detailed sample data tables are presented in Appendices D and E.

Statistical Methods and Calculations

Statistical methods were limited to the calculation of means and standard deviations. See Appendix F for example calculations used to generate the liver and serum sample data in FACT TOX-003.

Statement of Conclusion

Under the conditions of the present studies, the concentrations of PFOS, PFOSA, PFOSAA, EtFOSE-OH, M556, and PFOSEA were determined in the sera and liver of rats dosed with N-Ethyl Perfluoroctanesulfonamido Ethanol during the in-life phase of the study.

References

Covance Study No.: 6329-228 titled: 104-Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats

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Appendix A: Chemical Characterization and Control Matrices

Table 6. Characterization of the Control Matrices Used for Sera Analyses in Study FACT TOX-003

Control Matrix	Rat Serum	Rat Serum	Rabbit Serum	Rabbit Serum
Source	Sigma	Sigma	Sigma	Sigma
Expiration Date	2010	01/01/2010	01/01/2010	2010
Storage Conditions	-20°C ±10°C	-20°C ±10°C	-20°C ±10°C	-20°C ±10°C
Chemical Lot #	17H9306	19H89291	47H4641	118H8418
Physical Description	Rat Serum	Rat Serum	Rabbit Serum	Rabbit Serum

Table 7. Characterization of the Control Matrices Used for Liver Analyses in Study FACT TOX-003

Control Matrix	Rabbit Liver	Rabbit Liver	Rabbit Liver
Source	Covance Laboratories, Inc.	Covance Laboratories, Inc.	Covance Laboratories, Inc.
Expiration Date	2010	2010	2010
Storage Conditions	-20°C ±10°C	-20°C ±10°C	-20°C ±10°C
Chemical Lot #	F00014	F00007	F00016
Physical Description	Rabbit Liver	Rabbit Liver	Rabbit Liver

Table 8. Characterization of Test Article in Study FACT TOX-003

	Test Article
Chemical Name	**EtFOSE-OH** N-Ethyl Perfluorooctanesulfonamido ethanol T-6316
Source	3M
Expiration Date	11/26/01
Storage Conditions	Ambient temperature
Chemical Lot #	(30035, 30037, 30039)
Physical Description	Light yellow, waxy solid/flakes
Purity	97.4%

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Appendix B: Protocol, Amendments and Deviations

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COVANCE
THE DEVELOPMENT SERVICES COMPANY

Sponsor:

3M
St. Paul, Minnesota

PROTOCOL

Study Title:

T-6316.1

104-Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats

Date:

April 1, 1998

Performing Laboratory:

Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704-2595

Laboratory Study Identification:

Proposal No. 23124A

Covance 6329-228
3M T-6316.1

Study
104-Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats

Purpose
To assess the carcinogenicity of the test material when administered in the diet to rats for at least 104 weeks

Sponsor
3M
Toxicology Services
Building 220-2E-02, 3M Center
St. Paul, Minnesota 55144-1000

Study Monitor
Andrew M. Seacat, PhD
3M
Telephone No.: 612.575.3161
Facsimile No.: 612.733.1773

Study Location
Covance Laboratories Inc.
3301 Kinsman Boulevard
Madison, Wisconsin 53704-2595

Mailing Address: PO Box 7545
Madison, Wisconsin 53707-7545

Study Director
Peter J. Thomford, PhD
Covance Laboratories Inc.
Telephone No.: 608.241.7207
Facsimile No.: 608.242.2736

Toxicologist
Thomas E. Ryan, BS
Covance Laboratories Inc.

Proposed Study Timetable
In-Life Start Date: April 6, 1998
In-Life End Date: April 5, 2000

Regulatory Compliance
This study will be conducted in compliance with the Food and Drug Administration Good Laboratory Practice Regulations as set forth in Title 21 of the US Code of Federal Regulations, Part 58, issued December 22, 1978 (effective June 20, 1979), and with any applicable amendments.

Animal Care and Use Statement
All procedures in this protocol are in compliance with the Animal Welfare Act Regulations, 9 CFR 1-4. In the opinion of the Sponsor and study director, the study does not unnecessarily duplicate any previous work.

Quality Assurance
The protocol, study conduct, and final report will be audited by the Covance Quality Assurance Unit (QAU). The proliferation cell nuclear antigen evaluation, data, and report will be audited by the QAU of Pathology Associates International.

Test Material

Identification
T-6316 (Narrow Range N-Ethyl Perfluorooctanesulfonamido Ethanol, NEtFOSE)

Lot Numbers
The lot numbers will be maintained in the raw data.

Purity
98.1% NEtFOSE (w/w)

Stability
Responsibility of the Sponsor

Storage Conditions
At room temperature

Characteristics
Information on synthesis methods, composition, or other characteristics that define the test material is on file with the Sponsor.

Reserve (Archive) Samples
A reserve sample (approximately 5 g) will be taken and stored at room temperature. This sample will be transferred to the Sponsor after completion of the in-life phase to be retained in accordance with 21 CFR 58.195.

Disposition of Test Material
After authorization from the Sponsor, any remaining test material will be returned to:

Andrew M. Seacat, PhD
3M
Toxicology Services
Building 220-2E-02, 3M Center
St. Paul, Minnesota 55144-1000
Telephone No.: 612.575.3161
Facsimile No.: 612.733.1733

Animals

Species
Rat

Strain
Crl:CD®(SD)IGS BR

Source
Charles River Laboratories, Inc., Raleigh, North Carolina

Age at Initiation of Treatment
Preferably 6 weeks of age, but not more than 8 weeks of age

Weight at Initiation of Treatment
100 to 300 g

Number and Sex
140 males and 140 females

Identification
Implantable microchip identification device

Husbandry

Housing
Individual (may be group-housed during acclimation)

Diet
Certified Rodent Diet #5002, meal, (PMI Nutrition International) *ad libitum,* unless otherwise specified. The diet is routinely analyzed by the manufacturer for nutritional components and environmental contaminants. Results of specified nutrient and contaminant analyses are on file at Covance-Madison.

Water
Ad libitum. Samples of the water are routinely analyzed for specified microorganisms and environmental contaminants. The results are on file at Covance-Madison.

Contaminants
There are no known contaminants in the diet or water at levels that might interfere with this study.

Environment
Environmental controls for the animal room will be set to maintain 18 to 26°C, a relative humidity of 30 to 70%, a 12-hour light/12-hour dark cycle, and a minimum of 10 room air changes/hour. The light/dark cycle may be interrupted to accommodate study-related activities.

Acclimation

At least 1 week

Randomization

Selection of animals for the study will be based on body weights, clinical observations, and other data as appropriate. Animals will be assigned to treatment groups using a computerized blocking procedure designed to achieve body weight balance with respect to treatment groups. At the time of randomization, the weight variation of the animals of each sex used will not exceed ±2 standard deviations of the mean weight, and the mean body weight for each group of each sex will not be statistically different at the 5.0% probability level.

Justification

Rats historically have been used in safety evaluation studies and are recommended by appropriate regulatory agencies.

Group Designations and Dietary Levels

Group[a]	Number of Animals Male	Female	Dietary Levels (ppm NEtFOSE)[b]
8 (Control)[c,d,e]	70	70	0
9 (Treated)[d,e]	70	70	1

a Groups will be designated as Groups 8 and 9. This study will be located in the same animal room as Covance 6329-212.
b T-6316 is 98.1% n-ethyl perfluorooctanesulfonamido ethanol (NEtFOSE); dose levels are expressed as ppm of NEtFOSE.
c The control animals will receive the basal diet only.
d Five animals/sex/group will be sacrificed during Weeks 4 and 14 for hepatocellular proliferation rate measurements and biochemical analyses (palmitoyl-CoA oxidation).
e Ten animals/sex/group will be designated as interim sacrifice animals and will be sacrificed after at least 78 weeks of treatment.

Dosing Procedures

Method of Administration

Dietary. Animals will receive test diet for at least 104 weeks.

Reason for Dosing Route

The potential human exposure is by the oral route.

Dose Preparation

Dose preparations will be mixed at least once every 4 weeks according to the study-specific mixing procedure developed by Covance. Dose concentrations will be based on the NEtFOSE content as supplied; the Sponsor has stated the T-6316 is 98.1% NEtFOSE (w/w) as supplied. All dose preparations will be stored at room temperature.

Retention Samples

Samples (approximately 100 g) will be taken from each dose preparation and stored at room temperature. Unless used for analyses, these samples will be discarded at least 1 month after completion of the in-life phase.

Dose Analyses

By Covance using a method supplied by the Sponsor and validated by Covance

Homogeneity

Homogeneity will be determined for the 1-ppm diet preparation mixed for Week 1. One sample (approximately 100 g) each from the top, middle, and bottom of the diet preparation will be collected, divided into three subsamples for extraction and analysis, and analyzed for test material content. All samples will be stored at room temperature until analyzed within 7 days of mixing. Homogeneity analysis will be repeated if the batch size changes by more than 30%.

Stability

The middle homogeneity sample will be analyzed on the day of mixing and serve as stability time 0. Two additional samples (approximately 100 g each) will be taken from the 1-ppm diet preparation mixed for Week 1 to establish stability. One sample will be stored at room temperature for at least 19 days, then analyzed. The other sample will be stored at room temperature after at least 32 days, then analyzed.

Dose Confirmation

Samples (approximately 100 g each) will be collected from all dose preparations and analyzed in duplicate. Homogeneity samples collected from the middle of the dose preparation for Week 1 will be used for dose confirmation results. All samples will be stored at room temperature until analyzed.

Observation of Animals

Clinical Observations

Each animal will be observed twice daily (a.m. and p.m.) for mortality and moribundity, findings will be recorded as they are observed.

Once prior to treatment and weekly thereafter, each animal will be removed from its cage and examined; abnormal findings or an indication of normal will be recorded. The following information on each grossly visible or palpable mass will be recorded.

time of onset
location
size (small or large)
appearance
progression

Body Weights

Prior to treatment (at randomization), weekly for Weeks 1 through 17, once every 4 weeks thereafter, and at Week 105

Food Consumption

Weekly for Weeks 1 through 16 and once every 4 weeks thereafter

Clinical Pathology

Frequency and Number of Animals

Unscheduled Collection

When possible, a blood film will be made and held for possible future examination from animals sacrificed at unscheduled intervals.

Scheduled Collections

Hematology, clinical chemistry, urinalysis, and urine chemistry will be done on 10 animals/sex/group during Weeks 14, 27, and 53.

A blood film will be made and held for possible future examination from animals at scheduled sacrifices after at least 78 and 104 weeks of treatment.

Method of Collection

Hematology, Clinical Chemistry, Urinalyses, and Urine Chemistry

Animals will be fasted overnight; blood will be collected from a jugular vein. The anticoagulant will be potassium EDTA for hematology tests. Samples for clinical chemistry will be collected without anticoagulant. Urine will be collected chilled overnight (approximately 16 hours).

Blood Films

Blood films will be taken as part of the necropsy procedure.

Tests

Hematology

red blood cell (erythrocyte) count	platelet count
hemoglobin	white blood cell (leukocyte) count
hematocrit	differential blood cell count
mean corpuscular volume	blood cell morphology
mean corpuscular hemoglobin	reticulocyte smear (made, but not examined)
mean corpuscular hemoglobin concentration	

Clinical Chemistry

glucose	alanine aminotransferase
urea nitrogen	gamma glutamyltransferase
creatinine	aspartate aminotransferase
total protein	calcium
albumin	inorganic phosphorus
globulin	sodium
cholesterol	potassium
total bilirubin	chloride

Urinalysis

appearance	glucose
volume	ketones
specific gravity	bilirubin
pH	blood
protein	microscopic examination of sediment
urobilinogen	

Urine Chemistry

sodium	16 hour excretion of:
potassium	sodium
	potassium

Serum Analyses

Frequency and Number of Animals

Five animals/sex/group during Weeks 4 and 14 (animals selected for hepatocellular proliferation and biochemical analyses), five animals/sex/group (from animals selected for interim sacrifice) after at least 78 weeks of treatment, and five animals/sex/group at the terminal sacrifice

Method of Collection

Animals will be fasted overnight; blood (approximately 2 mL) will be collected from a jugular vein. Samples will be collected without anticoagulant.

Sample Handling

Blood samples will be allowed to clot at room temperature and centrifuged. Serum samples will be harvested and stored in a freezer set to maintain -60 to -80°C. Samples will be packed on dry ice and shipped to:

Kris J. Hansen, PhD
3M Environmental Technology and Safety Services
935 Bush Avenue
Building 2-3E-09
St. Paul, Minnesota 55133-3331
Telephone No.: 612.778.6018
Facsimile No.: 612.778.6176

Samples will be retained by the Sponsor for possible future analysis.

Termination

Unscheduled Sacrifices and Deaths

Necropsies will be done. Animals to be sacrificed will be anesthetized with sodium pentobarbital, weighed, and exsanguinated. A blood film will be taken as part of the necropsy procedure for sacrificed animals.

Scheduled Sacrifices

Interim Sacrifices

During Week 4, five animals/sex/group will be fasted overnight, bled for serum samples, anesthetized with sodium pentobarbital, weighed, and exsanguinated. The abdominal cavity of each animal will be opened, the liver will be removed and weighed, and liver samples will be collected. Animals will be discarded after liver collection.

During Week 14, five animals/sex/group will be fasted overnight, bled for serum samples, anesthetized with sodium pentobarbital, weighed, and exsanguinated. The abdominal cavity of each animal will be opened, the liver will be removed, weighed, and liver samples collected. Animals will be discarded after liver collection.

After at least 78 weeks of treatment, 10 animals/sex/group will be fasted overnight, bled for serum samples (five animals/sex/group), anesthetized with sodium pentobarbital, weighed, exsanguinated, and necropsied. A blood film will be taken as part of the necropsy procedure.

Terminal Sacrifice

After at least 104 weeks of treatment, the remaining animals will be fasted overnight, bled for serum samples (five animals/sex/group), anesthetized with sodium pentobarbital, weighed, exsanguinated, and necropsied. A blood film will be taken as part of the necropsy procedure.

Postmortem Procedures

Necropsy

The necropsy will include an examination of the external features of the carcass; all external body orifices; the abdominal, thoracic, and cranial cavities; organs; and tissues.

Cell Proliferation Tissue Collection and Immunohistochemical Evaluation

At the Week 4 and 14 interim sacrifices, representative samples of the left lateral lobe of the liver and any macroscopic lesions of the liver will be collected and preserved in zinc formalin.

After fixation, each sample of liver will be embedded in paraffin, and the paraffin blocks will be shipped to:

Sandra R. Eldridge, PhD
Pathology Associates International
15 Worman's Mill Court, Suite I
Frederick, Maryland 21701
Telephone No.: 301.663.1644, ext. 2201
Facsimile No: 301.663.8994

Proliferation cell nuclear antigen (PCNA) evaluation will be done on the samples. In addition, liver sections will be stained with hematoxylin and eosin and examined microscopically. Results will be provided for inclusion in the final report.

Palmitoyl-CoA Oxidase Tissue Collection and Analyses

At the Week 4 and 14 interim sacrifices, a sample (approximately 500 mg) of the right lateral lobe of the liver will also be collected from each animal and flash-frozen in liquid nitrogen. The liver tissue will be stored in a freezer set to maintain -60 to -80°C until analyzed by Covance for palmitoyl-CoA oxidase activity.

Organ Weights

At the Week 79 and terminal sacrifices, the following organs (when present) will be weighed; paired organs will be weighed separately:

adrenal (2)
brain
kidney (2)
liver
lung
ovary (2)
spleen
testis (2)
thyroid (2) with parathyroid
uterus with cervix

Organ-to-body weight percentages and organ-to-brain weight ratios will be calculated.

Bone Marrow Smear

From the femur of each animal at the Week 79 and terminal sacrifices only; made but not examined

Additional Liver Sample Collection

A portion of the liver will be collected from five animals/sex/group at the interim and terminal sacrifices and stored in a freezer set to maintain -60 to -80°C. Samples will be packed on dry ice and shipped to Kris J. Hansen, PhD, 3M Environmental Technology and Safety Services. Samples will be retained by the Sponsor for possible future analysis.

Tissue Preservation

The following tissues (when present) from each animal will be preserved in 10% neutral-buffered formalin:

adrenal (2)
brain
cecum
cervix
colon
duodenum
epididymis (2)
esophagus
eye (2)
femur with bone marrow (articular surface of the distal end)
Harderian gland
heart
ileum
jejunum
kidney (2)
lesions
liver
lung with mainstem bronchi
lymph node (mesenteric)
mammary gland (females only)
ovary (2)
pancreas
pituitary
prostate
rectum
salivary gland [mandibular (2)]
sciatic nerve
seminal vesicle (2)
skeletal muscle (thigh)

skin
spinal cord (cervical, thoracic, and lumbar)
spleen
sternum with bone marrow
stomach
testis (2)
thymus
thyroid (2) with parathyroid
trachea
urinary bladder
uterus
vagina

Histopathology
Tissues will be held for possible future examination.

Reports
One copy of each draft report will be sent to the Sponsor. The report for this study will be included as an appendix to the report for Covance 6329-212. The report will include the following information:

Experimental Design and Methods

Results
dose analyses
mortality
clinical observations
body weights
body weight changes
food consumption
test material consumption
clinical pathology results
palmitoyl CoA oxidase activities
macroscopic observations
cell proliferation assessments (provided by the Sponsor's designee)

Statistical Evaluation
body weights
body weight changes
food consumption
survival rates
clinical pathology values
palmitoyl CoA oxidase activities

Statistical methods will be those presented in Attachments Nos. 1 and 2. For each sex, Group 9 will be compared to Group 8 (Control).

At the end of 1 year after issuance of the audited draft report, if no requested revisions or instructions to finalize have been communicated by the Sponsor, then the audited draft report will be considered 'final' and issued as the final report, signed by the study director, and submitted to the Sponsor.

Any modifications or changes to the audited draft report requested 1 year after issuance will be performed at additional cost to the Sponsor.

Record Retention

All raw data, documentation, records, protocol, specimens, and final report generated as a result of this study, including those items listed below, will be archived in the storage facilities of Covance-Madison for a period of 1 year following submission of the final report to the Sponsor. All raw data stored on magnetic media, the protocol and protocol amendments, study correspondence, and the original report will be retained by Covance. One year after submission of the final report, all of the aforementioned materials will be sent to the Sponsor, and a return fee will be charged. The Sponsor may elect to have the materials retained in the Covance archives for an additional period of time, and Covance will charge a storage fee. If the Sponsor chooses to have Covance dispose of the materials, a disposal fee will be charged.

protocol and protocol amendments
dose preparation records
in-life records
  animal receipt
  acclimation
  animal room maintenance
  randomizations
  dose administration
  clinical observations
  body weights
  food consumption
  sample collection
clinical pathology records
anatomical pathology records
statistical analyses
study correspondence
tissue specimens (wet)
blood and bone marrow slides
final report (original signed copy)

The following supporting records will be retained at Covance-Madison but will not be archived with the study data.

feed analysis records
water analysis records
animal room environment records
refrigerator and freezer temperature records
room temperature records for test material storage
instrument calibration and maintenance records

PCNA evaluation data and paraffin blocks and tissue slides will be retained by Pathology Associates International.

Serum and liver samples sent to the Sponsor will be retained by the Sponsor

PROTOCOL APPROVAL

Andrew M. Seacat

4/17/98

Andrew M. Seacat, PhD
Study Monitor
3M

Date

Peter J. Thomford

4/1/98

Peter J. Thomford, PhD
Study Director
Covance Laboratories Inc.

Date

Attachment No. 1

Statistical Analyses

Levene's test will be done to test for variance homogeneity. In the case of heterogeneity of variance at $p \leq 0.05$, transformations will be used to stabilize the variance. Comparison tests will take variance heterogeneity into consideration.

One-way analysis of variance (ANOVA) will be used (if applicable) to analyze body weights, body weight changes, food consumption, continuous clinical pathology values, and organ weight data. If the ANOVA is significant, Student's t-test will be used for control versus treated group comparisons.

If the ANOVA shows significance for body weights at Week 1, one-way analysis of covariance (ANCOVA) will be used to analyze body weights, with initial body weights as the covariate. If the ANCOVA is significant, covariate-adjusted means will be used for control versus treated group comparisons.

Group comparisons (Group 9 versus Group 8) will be evaluated at the 5.0% two-tailed probability level. Only data collected on or after the first day of treatment will be analyzed statistically.

Attachment No. 2

Adjusted survival data are analyzed by the National Cancer Institute (NCI) lifetable package. The tests include: Graphical (Kaplan-Meier product-limit estimation curves), Cox-Tarone binary regression methods for trend and heterogeneity, and Gehan-Breslow nonparametric methods for trend and heterogeneity.

COVANCE
THE DEVELOPMENT SERVICES COMPANY

PROTOCOL AMENDMENT NO. 1

Covance 6329-228

104-Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats

Sponsor:	3M, St. Paul, Minnesota
Study Monitor:	Andrew M. Seacat, PhD
Testing Facility:	Covance Laboratories Inc., Madison, Wisconsin
Study Director:	Peter J. Thomford, PhD

This amendment modifies the following portions of the protocol:

Effective April 23, 1998

1. **Page 11, Termination.** To reflect the decision to anesthetize animals to be sacrificed with carbon dioxide, delete the text in this section and replace with the following:

   Unscheduled Sacrifices and Deaths
   Necropsies will be done. Animals to be sacrificed will be anesthetized with carbon dioxide, weighed, and exsanguinated. A blood film will be taken as part of the necropsy procedure for sacrificed animals.

Scheduled Sacrifices

Interim Sacrifices

During Week 4, five animals/sex/group will be fasted overnight, bled for serum samples, anesthetized with carbon dioxide, weighed, and exsanguinated. The abdominal cavity of each animal will be opened, the liver will be removed and weighed, and liver samples will be collected. Animals will be discarded after liver collection.

During Week 14, five animals/sex/group will be fasted overnight, bled for serum samples, anesthetized with carbon dioxide, weighed, and exsanguinated. The abdominal cavity of each animal will be opened, the liver will be removed and weighed, and liver samples will be collected. Animals will be discarded after liver collection.

After at least 78 weeks of treatment, 10 animals/sex/group will be fasted overnight, bled for serum samples (five animals/sex/group), anesthetized with carbon dioxide, weighed, exsanguinated, and necropsied. A blood film will be taken as part of the necropsy procedure.

Terminal Sacrifice

After at least 104 weeks of treatment, the remaining animals will be fasted overnight, bled for serum samples (five animals/sex/group), anesthetized with carbon dioxide, weighed, exsanguinated, and necropsied. A blood film will be taken as part of the necropsy procedure.

2 **Page 12, Postmortem Procedures, Cell Proliferation Tissue Collection and Immunohistochemical Evaluation, Paragraph 1.** To reflect the decision to collect liver samples for proliferation cell nuclear antigen evaluation at Week 14 only, delete the text in this paragraph and replace with the following:

At the Week 14 interim sacrifice, representative samples of the left lateral lobe of the liver and any macroscopic lesions of the liver will be collected and preserved in zinc formalin.

Effective April 28, 1998

3. **Page 2, Alternate Study Monitor.** To include the alternate study monitor in the protocol, add the following after "Study Monitor:"

Alternate Study Monitor
Marvin T. Case, DVM, PhD
3M Toxicology Services
Telephone No.: 612.733.5180
Facsimile No.: 612.733.1773

4. **Page 6, Group Designations and Dietary Levels, Footnote e.** To reflect the decision to change the Week 79 interim sacrifice to Week 53, delete the text in this footnote and replace with the following:

Ten animals/sex/group will be designated as interim sacrifice animals and will be sacrificed after at least 52 weeks of treatment.

5. **Page 9, Clinical Pathology, Frequency and Number of Animals, Scheduled Collections, Paragraph 2.** The Week 79 interim sacrifice will be moved to Week 53, therefore blood films will be prepared from animals sacrificed after 52 weeks of treatment. To reflect this decision, delete the text in this paragraph and replace with the following:

A blood film will be made and held for possible future examination from animals at scheduled sacrifices after at least 52 and 104 weeks of treatment.

6. **Page 10, Serum Analyses, Frequency and Number of Animals.** The Week 79 interim sacrifice will be moved to Week 53, therefore serum samples will be collected from animals sacrificed after 52 weeks of treatment. To reflect this decision, delete the text in this section and replace with the following:

Five animals/sex/group during Weeks 4 and 14 (animals selected for hepatocellular proliferation and biochemical analyses), five animals/sex/group (from animals selected for interim sacrifice) after at least 52 weeks of treatment, and five animals/sex/group at the terminal sacrifice

7. **Page 11, Termination, Scheduled Sacrifices, Interim Sacrifices, Paragraph 3.** To reflect the decision to change the Week 79 interim sacrifice to Week 53, delete the text in this paragraph and replace with the following:

After at least 52 weeks of treatment, 10 animals/sex/group will be fasted overnight, bled for serum samples (five animals/sex/group), anesthetized with carbon dioxide, weighed, exsanguinated, and necropsied. A blood film will be taken as part of the necropsy procedure.

8. **Page 12, Postmortem Procedures, Organ Weights.** To reflect the decision to record organ weights at the Week 53 interim sacrifice only, delete the text in this section and replace with the following:

At the Week 53 interim sacrifice, the following organs (when present) will be weighed; paired organs will be weighed separately:

adrenal (2)	ovary (2)
brain	spleen
kidney (2)	testis (2)
liver	thyroid (2) with parathyroid
lung	uterus with cervix

Organ-to-body weight percentages and organ-to-brain weight ratios will be calculated.

9. **Page 13, Postmortem Procedures, Bone Marrow Smear.** The Week 79 interim sacrifice will be moved to Week 53, therefore bone marrow smears will be collected from animals sacrificed after 52 weeks of treatment. To reflect this decision, delete the text in this section and replace with the following:

From the femur of each animal at the Week 53 and terminal sacrifices only; made but not examined

10. **Page 14, Reports, Paragraph 1.** To reflect the decision to provide unaudited summary reports after the Week 14 and 53 interim sacrifices, delete the text in this paragraph and replace with the following:

After the Week 14 and 53 interim sacrifices, unaudited summary reports will be sent to the Sponsor in conjunction with the summary reports for Covance 6329-212. The summary reports will include a brief description of methods and results and summary tables of in-life data, clinical pathology data, and anatomical pathology data. After completion of the study, one copy of each draft report will be sent to the Sponsor. The report for this study will be included as an appendix to the report for Covance 6329-212. The report will include the following information:

Effective May 15, 1998

11. **Page 10, Serum Analyses, Method of Collection.** To reflect the decision to increase the volume collected for serum analyses at the Week 53 and 105 sacrifices, delete the text in this section and replace with following:

Animals will be fasted overnight; blood (approximately 2 mL at the Week 4 and 14 sacrifices, approximately 3 mL at the Week 53 and 105 sacrifices) will be collected from a jugular vein. Samples will be collected without anticoagulant.

AMENDMENT APPROVAL

Andrew M. Seacat, PhD
Study Monitor
3M

Date: 6/8/98

Peter J. Thornford, PhD
Study Director
Covance Laboratories Inc.

Date: 6/2/98

COVANCE
THE DEVELOPMENT SERVICES COMPANY

PROTOCOL AMENDMENT NO. 2

Covance 6329-228

104-Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats

Sponsor:	3M, St. Paul, Minnesota
Study Monitor:	Andrew M. Seacat, PhD
Testing Facility:	Covance Laboratories Inc., Madison, Wisconsin
Study Director:	Peter J. Thomford, PhD

This amendment modifies the following portions of the protocol:

Effective April 1, 1998

1. **Page 4.** To include the vehicle used to dissolve the test material before mixing with the diet, add the following section.

Vehicle

Identification
Acetone

Lot Numbers
The lot numbers will be maintained in the raw data.

Purity
On file with the manufacturer

Stability
On file with the manufacturer

Storage Conditions
At room temperature

Characteristics
Information on synthesis methods, composition, or other characteristics that define the vehicle is on file with the manufacturer.

Effective June 30, 1998

2. **Page 7, Dosing Procedure, Retention Samples.** To reflect the decision to send retention samples of control diet to the Sponsor, delete the text in this section and replace with the following:

Samples (approximately 100 g) will be taken from each dose preparation during the in-life phase and stored at room temperature. Unless used for analyses, retention samples from the treated groups will be discarded at least 1 month after completion of the in-life phase. Samples from control diets prepared for Weeks 14 and before will be shipped to:

Kris J. Hansen, PhD
3M Environmental Technology and Safety Services
935 Bush Avenue
Building 2-3E-09
St. Paul, Minnesota 55133-3331
Telephone No.: 651.778.6018
Facsimile No.: 651.778.6176

Effective July 20, 1998

3. **Page 2, Study Monitor and Alternate Study Monitor.** To indicate the change in the area code, delete the text in these sections and replace with the following:

Study Monitor
Andrew M. Seacat, PhD
3M
Telephone No.: 651.575.3161
Facsimile No.: 651.733.1773

Alternate Study Monitor
Marvin T. Case, DVM, PhD
3M Toxicology Services
Telephone No.: 651.733.5180
Facsimile No.: 651.733.1773

4. **Page 4, Disposition of Test Material.** To indicate the change in the area code, delete the text in these section and replace with the following:

After authorization from the Sponsor, any remaining test material will be returned to:

Andrew M. Seacat, PhD
3M
Toxicology Services
Building 220-2E-02, 3M Center
St. Paul, Minnesota 55144-1000
Telephone No.: 651.575.3161
Facsimile No.: 651.733.1733

5. **Page 10, Serum Analyses, Sample Handling.** To indicate the change in the area code for the Sponsor, delete the text in these section and replace with the following:

Blood samples will be allowed to clot at room temperature and centrifuged. Serum samples will be harvested and stored in a freezer set to maintain -60 to -80 C. Samples will be packed on dry ice and shipped to:

Kris J. Hansen, PhD
3M Environmental Technology and Safety Services
935 Bush Avenue
Building 2-3E-09
St. Paul, Minnesota 55133-3331
Telephone No.: 651.778.6018
Facsimile No.: 651.778.6176

Samples will be retained by the Sponsor for possible future analysis.

Effective April 9, 1999

6. **Page 9, Clinical Pathology, Frequency and Number of Animals, Scheduled Collections, Paragraph 2.** Hematology tests will be done for animals sacrificed at Week 53 and blood films will not be necessary. Therefore, delete this paragraph and replace with the following.

   A blood film will be made and held for possible future examination from animals at scheduled sacrifice after at least 104 weeks of treatment.

7. **Page 11, Termination, Scheduled Sacrifices, Interim Sacrifices, Paragraph 3.** To reflect the decision to collect clinical pathology samples from animals at the Week 53 interim sacrifice, delete the text in this paragraph and replace with the following:

   After at least 52 weeks of treatment, 10 animals/sex/group will be fasted overnight, bled for clinical pathology tests (all animals) and serum samples (five animals/sex/group), anesthetized with carbon dioxide, weighed, exsanguinated, and necropsied.

AMENDMENT APPROVAL

Andrew M. Seacat, PhD
Study Monitor
3M

6/14/99
Date

Peter J. Thornford, PhD
Study Director
Covance Laboratories Inc.

02 June 1999
Date

COVANCE
THE DEVELOPMENT SERVICES COMPANY

PROTOCOL AMENDMENT NO. 3

Covance 6329-228

104-Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats

Sponsor:	3M, St. Paul, Minnesota
Study Monitor:	Andrew M. Seacat, PhD
Testing Facility:	Covance Laboratories Inc., Madison, Wisconsin
Study Director:	Peter J. Thomford, PhD

This amendment modifies the following portion of the protocol.

Effective January 26, 2000

1. **Page 14, Postmortem Procedures, Histopathology.** The Sponsor has requested that all tissues from animals in Group 8 and selected tissues from animals in Group 9 be examined microscopically. To reflect this decision, delete the text in this section and replace with the following.

Tissues (as appropriate) from each animal in Group 8 will be embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically.

Lesions, liver, lungs, kidneys, pancreas, thyroid, testes, and mammary glands (females) from each animal in Group 9 will be embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically. Parathyroids will be processed with the thyroids, but will not be examined.

Effective February 18, 2000

2. **Page 10, Serum Analyses, Frequency and Number of Animals.** To reflect the decision to collect serum samples from 10 animals/sex/group at termination, delete the text in this section and replace with the following:

   Five animals/sex/group during Weeks 4 and 14 (animals selected for hepatocellular proliferation and biochemical analyses), five animals/sex/group (from animals selected for interim sacrifice) after at least 52 weeks of treatment, and 10 animals/sex/group at the terminal sacrifice

3. **Page 11, Termination, Scheduled Sacrifices, Terminal Sacrifice.** To reflect the decision to collect serum samples from 10 animals/sex/group at termination, delete the text in this section and replace with the following:

   After at least 104 weeks of treatment, the remaining animals will be fasted overnight, bled for serum samples (10 animals/sex/group), anesthetized with sodium pentobarbital, weighed, exsanguinated, and necropsied. A blood film will be taken as part of the necropsy procedure.

4. **Page 13, Postmortem Procedures, Additional Liver Sample Collection.** decision to collect liver samples from 10 animals/sex/group at termination, delete the text in this section and replace with the following:

   A portion of the liver will be collected from five animals/sex/group at the interim sacrifice and 10 animals/sex/group at the terminal sacrifice and stored in a freezer set to maintain -60 to -80°C. Samples will be packed on dry ice and shipped to Kris J. Hansen, PhD, 3M Environmental Technology and Safety Services. Samples will be retained by the Sponsor for possible future analysis.

AMENDMENT APPROVAL

Andrew M. Seacat, PhD
Study Monitor
3M

2/25/2000
Date

Peter J. Thomford, PhD
Study Director
Covance Laboratories Inc.

23 FEB 2000
Date

3M Environmental Laboratory

Protocol - Analytical Study Phase

104-Week Dietary Carcinogenicity Study with Narrow Range N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats

In-Vivo Study Reference Number: Covance # 6329-228
(RE) LAC 7/27/99
Study Number: FACT-~~051498.1~~ 050598.2
Test Material: T-6316

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

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

Proposed Experimental Start Date: May 25, 1998
Proposed Experimental Termination Date: December 30, 2001

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

Author: Lisa Clemen

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

D T Bacon 7/15/99
Dale Bacon Date
Study Director Management

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

1.0 PURPOSE

1.1 According to this analytical protocol, the 3M Environmental Laboratory will analyze the tissue and fluid samples from the Covance study number 6329-228, "104-Week Dietary Carcinogenicity with Narrow Range N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats." The collected data will be provided to the sponsor for use in the assessment of toxicological effects of the test material when administered in the diets of rats for at least 104 weeks.

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

2.0 REGULATORY COMPLIANCE

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

2.1.1 The analytical phase is being conducted as a separate study and therefore has a separate Study Director, protocol, and final report, from those listed in the Covance protocol 6329-228.

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

2.1.3 Sample storage stability will not be determined.

3.0 TEST MATERIALS

3.1 Control, and reference Materials and Matrices

3.1.1 Analytical Reference Material: T-6316, from 3M ICP/PCP Division

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

3.1.3 Analytical Control Material: None.

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

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

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

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

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

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

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

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

4.0 EXPERIMENTAL - Overview

4.1 The tissues from animals dosed as described (Covance# 6329-228), will be available for analysis for fluorine-containing compounds. At the discretion of the Study Director, a series of analytical tests can be performed. All high dose and control sera and livers will be analyzed initially using HPLC-electrospray mass spectrometry to identify fluorine-containing compounds of interest present in the sera and liver (if any). The screening for organic fluoride in liver via combustion may be performed to present definitive data for fluorine in the liver. Based on the findings from these analyses, additional samples, tissues, or fluids may be analyzed at the discretion of the Study Director to determine the presence of fluorochemicals in these matrices.

5.0 EXPERIMENTAL - Methods

5.1 Methods (attached):

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

6.0 DATA ANALYSIS

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

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

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

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

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

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

6.4.3 Certified copies or originals of the written validated methods.

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

6.4.5 Certified copies or originals of study specific raw data.

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

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

7.0 MAINTENANCE OF RAW DATA AND RECORDS

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

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

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

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

8.0 REFERENCES

8.1 Approved AMDT standard operating procedures.

8.2 Approved ETS standard operating procedures.

9.0 ATTACHMENTS

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

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

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

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

Study Title

104-Week Dietary Carcinogenicity Study with Narrow Range N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats

PROTOCOL AMENDMENT NO. 1

Amendment Date:

20 January 2000

Performing Laboratory

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

Laboratory Project Identification

ET&SS LRN-U2104
FACT TOX-003
Covance Study: 6329-228
3M Medical Department Study: T-6316.1

3M Environmental Laboratory

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

1. ***PROTOCOL READS:***
   The following methods will be used:
   FACT-M-1.0, Extraction of Perfluorooctanesulfonate from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry
   FACT-M-2.0, Analysis of Liver Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry
   FACT-M-3.0, Extraction of Perfluorooctanesulfonate from Sera for Analysis using HPLC-Electrospray/Mass Spectrometry
   FACT-M-4.0, Analysis of Sera Extracts for Fluorochemicals using HPLC-Electrospray/Mass Spectrometry
   AMEND TO READ:
   The following methods will be used:
   ETS-8-4.1, Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry
   ETS-8-5.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry
   ETS-8-6.0, Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry
   ETS-8-7.0, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry
   REASON:
   The methods originally listed were superseded during the course of the study.

2. ***PROTOCOL READS:***
   There is no independent section of the protocol that addresses sample retention.
   AMEND TO READ:
   Specimens will be maintained in the 3M Environmental Laboratory specimen archives. Any specimens sent to sub-contract laboratories will be returned to the 3M Environmental Laboratory upon completion of analysis and submission of the sub-contract laboratory(s) final report. Specimens analyzed at sub-contract laboratories will be returned with the following documentation: the signed original chain of custody and records of storage conditions while at the sub-contract facility.
   REASON:
   To define in detail the appropriate disposition of specimens analyzed at subcontract laboratories.

3. ***PROTOCOL READS:***
Section 7 states that the following raw data and records will be retained in the study folder in the archives according to AMDT-S-8: Approved protocol and amendments; approved methods; data summaries; study correspondence; shipping records; raw data; and electronic copies of data. Additionally, Section 7 states that supporting records to be retained separately from the study folder in the archives according to AMDT-S-8 will include at least the following: Approved validation reports; training records; calibration records; instrument maintenance logs; Standard Operating Procedures, Equipment Procedures, and Methods; and appropriate specimens.
AMEND TO READ:
Section 7 states: "The original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including: approved protocol and amendments, study correspondence, shipping records, raw data, approved final report, and electronic copies of data will be retained in the archives of the 3M Environmental Laboratory. All corresponding training records, calibration records, instrument maintenance logs, standard operating procedures, equipment procedures, and methods will be retained in the archives of the facility performing each analysis."
REASON:
To direct subcontract laboratories in the disposition of the items listed above.

4. ***PROTOCOL READS:***
The study director for the present study was identified in the protocol as Kristen J. Hansen, Ph.D.
AMEND TO READ:
The role of study director for the present study was reassigned to John L. Butenhoff, Ph.D., as of 20 January 2000. The previous study director, Kristen Hansen, has been reassigned to the role of Principle Analytical Investigator.
REASON:
The role of study director was reassigned in an effort to ensure compliance with Good Laboratory Practice Standards that outline study personnel requirements (refer to 21 CFR Part 58).

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

Amendment Approval

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

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

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

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

Study Title

104-Week Dietary Carcinogenicity Study with Narrow Range N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats

PROTOCOL AMENDMENT NO. 3

Amendment Date:

May 4, 2001

Performing Laboratory

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

Project Identification

3M Medical Department Study: T-6316.1
Covance In-Life Study: 6329-228
Analytical Report: FACT TOX-003
3M Laboratory Request No. U2104

Exact Copy of Original
LAC 04/04/01
Initial Date

3M Environmental Laboratory

Protocol FACT TOX-003
Amendment No. 3

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

1. ***PROTOCOL READS:***
PURPOSE: The FACT TOX-003 protocol does not clearly identify the intended analytes for the study.

AMEND TO READ:
PURPOSE: Rat sera and liver samples will be analyzed for PFOS, PFOSA, PFOSAA, PFOSEA, EtFOSE-OH and M556.

REASON:
The target analytes will be clearly specified.

3M Environmental Laboratory

Protocol FACT TOX-003
Amendment No. 3

Amendment Approval

Marvin T Case — 25 May 2001

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

John 2. Butenhoff — June 5, 2001

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

Study Title

104-Week Dietary Carcinogenicity Study with Narrow Range N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats

PROTOCOL AMENDMENT NO. 2

Amendment Date:

January 8, 2001

Performing Laboratory

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

Laboratory Project Identification

ET&SS LRN-U2104
FACT TOX-003
Covance 6329-228
3M Medical Department Study: T-6316.1

3M Environmental Laboratory

Protocol FACT TOX-003
Amendment No. 2

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

1. PROTOCOL READS:

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

AMEND TO READ:

1.2 If matrix spike studies provide accurate representation of recovery of endogenous levels of PFOS, PFOSA, PFOSAA, PFOSEA, M556, and EtFOSE-OH, the 3M Environmental Laboratory will provide semi-quantitative data for sera and liver samples collected from test animals.

REASON:

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

3M Environmental Laboratory

Protocol FACT TOX-003
Amendment No. 2

Amendment Approval

Marvin T Case — 10 Jan 2001

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

John L. Butenhoff — 10 Jan 2001

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

Record of Deviation

I. Identification

Study / Project No.
TOX03

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

Document Number ETS 8-7.0

Date(s) of occurrence 6-8-98

II. Description:

Required Procedure/process:
A curve is to be run prior to and following the sample extracts. The average of the two curves will be plotted by linear regression.

Actual Procedure/process:
The final curve was excluded since it was incomplete. The data is bracketed by the first curve and CCVs that met acceptance criteria.

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

A method deviation was written.

Recorded By Marlene Nyi Date 5-8-01

IV. Impact on Study / Project

No adverse impact on the outcome of this study. LAC 05/11/01 lgh 05/22/01

Authorized By *(Study Director / Project Lead)* John T. Butterhoff May 15, 2001 Date

Study Director: John Butterhoff Sponsor Representative: Marv Case Marvin Case 14 May 2001
3M Environmental Laboratory
Form ETS-4-8.0
Deviation No. 1 (assigned by Study Director or Project Lead at the end of study or project)
REVIEWED LAC 06/6.

Record of Deviation

I. Identification

Study / Project No. TOX003 Covance 6329-228

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

Document Number	Date(s) of occurrence
ETS-8-5.1	7-13-98

II. Description:

Required Procedure/process:

The CCVs must be within ±30% of the theoretical value.

Actual Procedure/process:

Data was entered even though the CCVS did not meet the acceptance criteria. Samples had been reanalized on RB300 and the criteria was still not met.

III. Actions Taken:

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

Data was entered from the analysis from 7-13-98 and was flagged as not meeting criteria for CCV.

Recorded By Marlene Hug Date 1-5-01

IV. Impact on Study / Project

The flagged data will not meet the accuracy parameters defined for the study.

KJH 01/05/01

Authorized By *(Study Director / Project Lead)* Marvin T Case 10 Jan 2001 John Butenhoff 10 JAN 2001

Sponsor Representative: Marvin Case Study Director: John Butenhoff

3M Environmental Laboratory

Form ETS-4-8.0

Deviation No. 2

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

Record of Deviation

I. Identification

Study / Project No. FACT-TOX-003 Covance 6329-228

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

Document Number(s): FACT-M-4.0, ETS-8-5.1

Date(s) of occurrence: 07/13/98, 04/21/00, 04/24/00, 08/15/00, 08/28/00, 08/30/00

II. Description:

Required Procedure/process:
Section 10.1.1 and 10.1.2 states: "Analyze a method blank and a matrix blank prior to each calibration curve."

Actual Procedure/process:
On several occasions, the blanks were either a) analyzed after the calibration curve or b) not analyzed at all for a particular MS analysis (08/30/00, Wk4, Serum).

III. Actions Taken:

(such as amendment issued, SOP revision, etc.)
This deviation was written.

Recorded By Lisa A. Clemen Date 05/03/01

IV. Impact on Study / Project

All blanks were analyzed at least once during the course of the study. When blanks were analyzed after the calibration curve, all control-of-bias practices were followed. In one analysis, blanks were not analyzed since these blanks had been run previously and did not require reanalysis. In this particular analysis (08/30/00, Wk 4, Serum) instrument blanks were used to determine limits of quantitation. This deviation has no adverse affect on these study data.

kjh 05/04/01

Authorized By *(Study Director / Project Lead)* John T. Butenhoff Date May 15, 2001

Study Director: John Butenhoff
Sponsor Representative: Marv Case Marvin T Case 8 May 2001

3M Environmental Laboratory
Form ETS-4-8.0

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

Record of Deviation

I. Identification

Study / Project No.
FACT-TOX-3 Covance 6329-228

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

Document Number	Date(s) of occurrence
FACT-M-3.0 & ETS-8-4.1	7/15/98

II. Description:

Required Procedure/process:
Method requires 1.0ml of serum to be used in sample preparation.

Actual Procedure/process:
Initial sample submitted for analysis was less than 1.0ml. All of sample was used in sample prep - volumes ranged from 0.5 to 1.0ml (1 out of 20 samples at 1.0ml)

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

Submitted deviation

Recorded By	Date
R. Wynne	8/9/00

IV. Impact on Study / Project

Recent work indicates that sample volumes ≥0.5 ml do not impact results.

Authorized By *(Study Director / Project Lead)*	Date
Glenn T. Butterhoff 9/6/00	KH 08/28/00 Marvin Case 15 Sept 2000

Study Director: John Butterhoff Sponsor: Rep. Marv Case

3M Environmental Laboratory
Form ETS-4-8.0

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

Record of Deviation

I. Identification

Study / Project No.tox003

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

Document Number:FACT-M-2.0 Date(s) of occurrence: 08/07/1998

II. Description:

Required Procedure/process:
Section **10.2.1** Analyze a matrix spike and matrix spike duplicate with each analysis. With a minimum of 2 spikes per batch.

Actual Procedure/process: The matrix spike and duplicates were analyzed in an earlier run and were not re-run with this analysis.

III. Actions Taken:

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

This deviation from the stated method is noted.

Recorded By: Harold Johnson 5.4.01 Date May 4, 2001

IV. Impact on Study / Project

This will have no impact on the study.

hh 05/04/01

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

John T. Butenhoff May 15, 2001

Study Director: John Butenhoff
Sponsor Representative: Mary Case Maureen T Case 8 May 2001
3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 5
(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification

Study / Project No.
FACT-TOX-003 Covance 6329-228

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

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

II. Description:

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

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

III. Actions Taken:

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

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

Recorded By	Date
Lisa A. Clemen	07/27/99

IV. Impact on Study / Project

This deviation will not adversely affect the outcome of this study

Authorized By (Study Director / Project Lead)	Date
John J. Butenhoff May 15, 2001 Kiter Hon	7/28/99

Study Director John Butenhoff Sponsor Representative. Mary Case Mary T Case 8 May 2001

3M Environmental Laboratory
Form ETS-4-8.0

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

Record of Deviation

I. Identification

Study / Project No. FACT-TOX-003 Covance 6329-228

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

Document Number(s): ETS-8-7.0 Date(s) of occurrence: 04/14/00, 04/17/00

II. Description:

Required Procedure/process:
Section 14.3.1 states, "The r^2 value for the calibration curve must be 0.980 or better."
Section 14.3.6 states, "A valid curve must contain at least five active points."

Actual Procedure/process:
The EtFOSE-OH calibration curve for the Week 104 liver generated on 04/14/00 had an r^2 value of 0.9687.
A four point curve was used to plot the EtFOSE-OH calibration curve for the Week 53 liver, generated on 04/17/00.

III. Actions Taken:

(such as amendment issued, SOP revision, etc.)
These data were flagged in the raw data and this deviation was written.

Recorded By: Lisa A. Clemen Date: 05/03/01

IV. Impact on Study / Project

EtFOSE-OH data is flagged as qualitative, so these data will not be adversely affected.
kjr 05/04/01

Authorized By *(Study Director / Project Lead)*: John T. Butenhoff May 15, 2001 Date:

Study Director: John Butenhoff
Sponsor Representative: Marv Case Marvin T Case 8 May 2001

3M Environmental Laboratory
Form ETS-4-8.0

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

Record of Deviation

I. Identification

Study / Project No.
Fact - TOX-003 / Covance 6329-228

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

Document Number	Date(s) of occurrence
ETS-9-27.0, Section 13.1	June 30, 2000 & July 5, 2000

II. Description:

Required Procedure/process:

When a Bottle-top Dispenser is used on a GLP project, it must be calibrated before use daily at the volume the Bottle-top Dispenser will be used on the project, and by the operator who will be using the Bottle-top Dispenser on the project.

Actual Procedure/process:

The Calibration Check logbook indicated that March 1, 2000 was the last time the Bottle-top Dispenser was calibrated.

III. Actions Taken:

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

The Bottle-top Dispenser will be calibrated before use daily when GLP projects are conducted at the the volume used on the project and by the operator who will be using the Bottle-top Dispenser on the project. This deviation was written.

Recorded By	Date
Kelly Kuehlwein	8/8/00

IV. Impact on Study / Project

Standards & samples were prepared identically, therefore no impact on the study.

kh 08/29/00

Authorized By *(Study Director / Project Lead)*	Date
John T. Butenhoff 9/13/00	Marvin T. Case 13 Sept 2000

Study Director: John Butenhoff Sponsor Rep: Marv Case

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 8
(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification

Study / Project No. FACT-TOX-003 Covance 6329-228

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

Document Number(s): ETS-8-5.1 Date(s) of occurrence: 08/30/00

II. Description:

Required Procedure/process:
A five point curve or better is used for plotting the calibration curve.

Actual Procedure/process:
A four point curve was used for plotting the PFOSAA calibration curve for the Week 4 serum curve, generated on 08/30/00.

III. Actions Taken:

(such as amendment issued, SOP revision, etc.)
These data were flagged in the raw data and this deviation was written.

Recorded By: Lisa A. Clemen Date: 05/03/01

IV. Impact on Study / Project

These data will not be adversely affected.
all other QC parameters were met. bjh 05/04/01

Authorized By *(Study Director / Project Lead)*: John T. Butenhoff Date: May 15, 2001

Study Director: John Butenhoff
Sponsor Representative: Mary Case Mary T Case 8 May 2001

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 9
(assigned by Study Director or Project Lead at the end of study or project)

3M Medical Department Study: T-6316.1 Analytical Report: FACT TOX-003
LRN-U2104

Appendix C: Extraction and Analytical Methods

This appendix includes the following methods:

Preparatory Methods

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

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

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

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

Analytical Methods

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

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

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

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

3M ENVIRONMENTAL LABORATORY

METHOD

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

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

Revision Date: N/A

Author: Lisa Clemen

Approved By:

[signature] 5/26/98
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Technical Reviewer Date

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

1.0 SCOPE AND APPLICATION

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

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

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

2.0 Summary of Method

2.1 This method describes how to extract potassium perfluorooctanesulfonate (PFOS) or other fluorochemical surfactants from liver using ion pairing reagent and 5.0 mLs of ethyl acetate. An ion pairing reagent is added to each sample and partitioned into ethyl acetate. Four mLs of extract is removed to a centrifuge tube and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL methanol then filtered through a 3 cc plastic syringe attached to a 0.2 µm filter into glass autovials.

3.0 Definitions

3.1 None.

4.0 Warnings and Cautions

4.1 **Health and Safety Warnings:**

4.1.1 Use universal precautions when handling animal livers, they may contain pathogens.

5.0 Interferences

5.1 There are no known interferences at this time.

6.0 Equipment

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

6.1.1 Ultra-Turrax T25 Grinder for grinding liver samples
6.1.2 Vortex mixer, VWR, Vortex Genie 2
6.1.3 Centrifuge, Mistral 1000 or IEC
6.1.4 Shaker, Eberbach or VWR
6.1.5 Nitrogen Evaporator, Organomation
6.1.6 Balance

7.0 Supplies and Materials

7.1 Gloves
7.2 Dissecting scalpels
7.3 Eppendorf or disposable pipettes
7.4 Nalgene bottles, capable of holding 250 mL and 1 L
7.5 Glass, type A, volumetric flasks
7.6 40 mL glass I-CHEM vials
7.7 Plastic sampule vials, Wheaton, 6 mL
7.8 Polypropylene centrifuge tubes, 15 mL
7.9 Labels

7.10 Syringes, capable of measuring 10 µL to 50 µL

7.11 Glass, type A, volumetric pipettes

7.12 Graduated pipettes

7.13 Electronic pipettor, Eppendorf or equivalent

7.14 Timer

7.15 Disposable plastic 3 cc syringes

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

7.17 Crimp cap autovials

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

8.0 Reagents and Standards

8.1 Reagents

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

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

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

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

8.1.4 Sodium carbonate/Sodium Bicarbonate Buffer (J.T. Baker or equivalent), (Na_2CO_3/$NaHCO_3$) 0.25M: Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and dilute to volume with Milli-Q™ water. Store in a 1 L nalgene bottle.

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

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

8.1.7 Methanol, Omnisolv, glass distilled or HPLC grade.

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

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

8.2 Standards

8.2.1 Prepare PFOS standards for the standard curve.

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

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

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

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

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

9.0 SAMPLE HANDLING

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

10.0 QUALITY CONTROL

10.1 Matrix Spikes

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

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

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

10.2 Continuing Calibration Checks

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

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

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

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

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare Liver Homogenate to Use for Standards

11.1.1 Weigh approximately 40 g of liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q™ water. Grind to a homogeneous solution.

11.1.2 If 40 g is not available, use appropriate amounts of liver and water in keeping with a 1:5 ratio.

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

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

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

Table 1
Approximate Spiking Amounts for Calibration Standards

Working Standard (Approx. Conc.)	µL	Approx. final conc. of PFOS in liver
-	-	Blank
0.50 ppm	4	0.010 ppm
0.50 ppm	20	0.050 ppm
0.50 ppm	40	0.100 ppm
5.0 ppm	10	0.250 ppm
5.0 ppm	20	0.500 ppm
5.0 ppm	30	0.750 ppm
50 ppm	4	1.000 ppm

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

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

12.0 PROCEDURES

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

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

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

12.4 Record the liver weight in the study notebook.

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

12.6 Add 2.5 mLs of water to sampule vial.

12.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, or until the sample is homogeneous.

12.8 Rinse the probe into the sample with 2.5 mLs water using a pipette.

12.9 Take the grinder apart and clean it with methanol after each sample. Follow AMDT-EP-22.

12.10 Cap the sample and vortex for 15 seconds.

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

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

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

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

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

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

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

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

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

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

12.21 Vortex mix for 30 seconds.

12.22 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial.

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

12.24 Cap and hold for electrospray mass spectrometry analysis.

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

13.0 Data Analysis and Calculations

13.1 Calculations:

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

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

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

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

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

14.0 Method Performance

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

15.0 Pollution Prevention and Waste Management

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

16.0 Records

16.1 Complete the extraction worksheet and tape into the study notebook.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

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

18.0 References

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

19.0 Affected Documents

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

20.0 Revisions

Revision Number.	Reason For Revision	Revision Date

Extraction Worksheet for FACT-M-1

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

Blank Liver Homogenate: Std.# Liver amount = g

Liver Extraction Method : **Date & Initials**

Vortex 15 sec.

Pipette 1 mL of Liver Solution

Pipette 1 mL of †0.5 M TBA, pH 10. Std. #_____

Pipette 2 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ Buffer Std. #_____

Pipette 5 mL of Ethyl Acetate TN-A-_____

Shake 20 min.

Centrifuge 20-25 min. Centrifuge Speed

Remove a 4 mL aliquot of organic layer

Put on Nitrogen Evaporator to dryness Evaporator Temperature

Add 1.0 mL of Methanol TN-A-_____

Vortex 30 sec.

Filter using a 3cc B-D syringe with a 0.2µm SRI filter into a 1.5 mL autosample vial

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

FACT-M-1.0
Extraction of PFOS from Liver

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3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: FACT-M-3.0 **Adoption Date:** 4/22/98

Revision Date: N/A

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Approved By:

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[signature] 4/22/98
Group Leader Date

[signature] 4/22/98
Technical Reviewer Date

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

1.0 SCOPE AND APPLICATION

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

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

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

2.0 Summary of Method

2.1 This method describes how to extract potassium perfluorooctanesulfonate (PFOS) or other anionic fluorochemical surfactants from serum using an ion pairing reagent and 5.0 mL of ethyl acetate. An ion pairing reagent is added to the sample and the analyte ion pair is partitioned into ethyl acetate. Four mL of extract are removed and put onto a nitrogen evaporator until dry. Each extract is reconstituted in 1.0 mL of methanol, then filtered through a 3 cc plastic syringe attached to a 0.2 µm nylon filter into glass autovials.

3.0 Definitions

3.1 None.

4.0 Warnings and Cautions

4.1 Health and Safety Warnings:

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

5.0 Interferences

5.1 There are no known interferences at this time.

6.0 Equipment

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

6.1.1 Vortex mixer, VWR, Vortex Genie 2
6.1.2 Centrifuge, Mistral 1000 or IEC
6.1.3 Shaker, Eberbach or VWR
6.1.4 Nitrogen evaporator, Organomation
6.1.5 Balance, (± 0.100 gm)

7.0 Supplies and Materials

7.1 Gloves
7.2 Eppendorf or disposable pipettes
7.3 Nalgene bottles, capable of holding 250 mL and 1 L
7.4 Glass, type A, volumetric flasks
7.5 40 mL glass I-CHEM vials
7.6 Polypropylene centrifuge tubes, 15 mL
7.7 Labels
7.8 Syringes, capable of measuring 10 µL to 50 µL
7.9 Glass, type A, volumetric pipettes
7.10 Graduated pipettes

7.11 Electronic pipettor, Eppendorf or equivalent

7.12 Timer

7.13 Disposable plastic 3 cc syringes

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

7.15 Crimp cap autovials

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

8.0 Reagents and Standards

8.1 Reagents

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

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

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

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

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

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

8.1.6 Other fluorochemicals, as appropriate.

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

8.1.8 Methanol, Omnisolv, glass distilled or HPLC grade.

8.1.9 Serum, frozen liquid from Sigma.

8.1.10 Control serum received with each sample set.

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

8.2 Standards

8.2.1 Prepare PFOS standards for the standard curve.

8.2.2 Prepare other fluorochemical standards, as appropriate.

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

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

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

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

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

9.0 Sample Handling

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

10.0 Quality Control

10.1 Matrix Blanks and Method Blanks

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

10.1.2 Two 1.0 mL aliquots of Milli-Q™ water are extracted following this procedure and used as method blanks.

10.2 Matrix Spikes

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

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

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

10.3 Continuing Calibration Checks

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

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

10.3.3 Prepare each continuing calibration check from the same serum used to prep the initial curve.

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

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare Serum Standards

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

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

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

11.1.4 Two 1 mL or appropriate aliquots serve as matrix blanks. Typically use the standard concentrations and spiking amounts listed in table 1 to spike, in duplicate, two standard curves for a total of fourteen samples.

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

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

Working Standard (Approx. Conc.)	µL	Approx. final conc. of PFOS in serum
-	-	Blank
0.500 ppm	20	0.010 ppm
5.00 ppm	5	0.025 ppm
5.00 ppm	10	0.050 ppm
5.00 ppm	20	0.100 ppm
50.0 ppm	5	0.250 ppm
50.0 ppm	10	0.500 ppm
50.0 ppm	15	0.750 ppm

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

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

12.0 PROCEDURES

12.1 Obtain frozen serum samples and allow to thaw.

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

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

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

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

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

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

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

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

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

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

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

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

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

12.15 Vortex mix for 30 seconds.

12.16 Attach a 0.2 µm nylon mesh filter to a 3 cc syringe and transfer the sample to this syringe. Filter into a 1.5 mL glass autovial.

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

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

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

13.0 Data Analysis and Calculations

13.1 Calculations:

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

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

14.0 Method Performance

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

15.0 Pollution Prevention and Waste Management

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

16.0 Records

16.1 Complete the extraction worksheet attached to this method, and tape into the study notebook.

17.0 Tables, Diagrams, Flowcharts, and Validation Data

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

18.0 References

18.1 None

19.0 Affected Documents

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

20.0 Revisions

Revision Number.	Reason For Revision	Revision Date

Extraction Worksheet for FACT-M-3

Study #	Sample Number 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. or Comments
-	H_2O Blank	-	-	-	
-	Serum Blank	-	-	-	
-					
-					
-					
-					
-					
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[1]		-	-	-	
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		-	-	-	

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

Blank Serum Std # Serum amount = g

Serum Extraction Method : **Date & Initials**

Vortex 15 sec.

Pipette Serum Volume mL

Pipette 1 mL of 0.5 M TBA, pH 10. Std. #

Pipette 2 mL of 0.25 Na_2CO_3/0.25M $NaHCO_3$ buffer Std. #

Pipette 5 mL of ethyl acetate TN-A-

Shake 20 min.

Centrifuge 20-25 min. Centrifuge speed:

Remove a 4 mL aliquot of organic layer

Put on Nitrogen Evaporator to dryness Evaporator #: Temperature:

Add methanol Volume mL TN-A-

Vortex 30 sec.

Filter using a 3cc B-D syringe with a 0.2µm SRI filter into a 1.5 mL autosample vial

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

FACT-M-3.0
Extraction of PFOS from Serum

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3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: ETS-8-4.1 **Adoption Date:** 03/01/99

Revision Date: 4/27/99

Author: Lisa Clemen, Glenn Langenburg

Approved By:

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

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

1.0 SCOPE AND APPLICATION

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

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

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

2.0 SUMMARY OF METHOD

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

2.2 These sample extracts are analyzed following method ETS-8-5.1 or other appropriate methods.

3.0 DEFINITIONS

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

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

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

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

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

3.6 M556: $C_8F_{17}SO_2N(H)(CH_2COOH)$

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

4.0 WARNINGS AND CAUTIONS

4.1 **Health and safety warnings**

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

5.0 INTERFERENCES

5.1 There are no interferences known at this time.

6.0 EQUIPMENT

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

6.1.1 Vortex mixer, VWR, Vortex Genie 2

6.1.2 Centrifuge, Mistral 1000 or IEC

6.1.3 Shaker, Eberbach or VWR

6.1.4 Nitrogen evaporator, Organomation

6.1.5 Balance (± 0.100 g)

7.0 SUPPLIES AND MATERIALS

7.1 Gloves

7.2 Eppendorf or disposable pipettes

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

7.4 Volumetric flasks, glass, type A

7.5 I-CHEM vials, glass, 40 mL glass

7.6 Centrifuge tubes, polypropylene, 15 mL

7.7 Labels

7.8 Oxford Dispenser – 3.0 to 10.0 mL

7.9 Syringes, capable of measuring 5 µL to 50 µL

7.10 Graduated pipettes

7.11 Syringes, disposable plastic, 3 cc

7.12 Syringe filters, nylon, 0.2 µm, 25 mm

7.13 Timer

7.14 Crimp cap autovials and caps

7.15 Crimpers

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

8.0 REAGENTS AND STANDARDS

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

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

8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent

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

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

8.6 Methyl-T-Butyl Ether, Omnisolv, glass distilled or HPLC grade

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

8.8 Serum or blood, frozen from supplier

8.9 **Fluorochemical standards**

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

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

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

8.9.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 570

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

8.9.6 M556 (3M Specialty Chemical Division), molecular weight = 557

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

8.9.8 Other fluorochemicals, as appropriate

8.10 Reagent preparation

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

8.10.1 10 N sodium hydroxide (NaOH): Weigh approximately 200 g NaOH. Pour into a 1000 mL beaker containing 500 mL Milli-Q™ water, mix until all solids are dissolved. Store in a 1 L Nalgene bottle.

8.10.2 1 N sodium hydroxide (NaOH): Dilute 10 N NaOH 1:10. Measure 10 mL of 10 N NaOH solution into a 100 mL volumetric flask and dilute to volume using Milli-Q™ water. Store in a 125 mL Nalgene bottle.

8.10.3 0.5 M tetrabutylammonium hydrogen sulfate (TBA): Weigh approximately 169 g of TBA into a 1 L volumetric containing 500 mL Milli-Q™ water. Adjust to pH 10 using approximately 44 to 54 mL of 10 N NaOH (While adding the last mL of NaOH, add slowly because the pH changes abruptly). Dilute to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

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

8.10.4 0.25 M sodium carbonate/sodium bicarbonate buffer (Na_2CO_3/$NaHCO_3$): Weigh approximately 26.5 g of sodium carbonate (Na_2CO_3) and 21.0 g of sodium bicarbonate ($NaHCO_3$) into a 1 L volumetric flask and bring to volume with Milli-Q™ water. Store in a 1 L Nalgene bottle.

8.11 Standards preparation

8.11.1 Prepare PFOS standards for the standard curve.

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

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

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

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

8.11.6 Dilute working standard 1 with methanol for a working standard 2 solution of approx. 5.0 ppm.

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

8.12 Surrogate stock standard preparation

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

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

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

9.0 SAMPLE HANDLING

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

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

10.0 QUALITY CONTROL

10.1 Solvent Blanks, Method blanks and matrix blanks

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

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

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

10.2 Matrix spikes

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

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

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

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

10.3 Continuing calibration checks

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

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

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

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

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

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

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

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

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

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

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

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

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

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

12.0 PROCEDURE

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

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

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

12.4 Record the initial volume on the extraction worksheet.

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

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

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

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

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

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

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

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

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

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

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

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

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

12.18 Vortex mix for 30 seconds.

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

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

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

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

13.0 Data Analysis and Calculations

13.1 Calculations

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

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

Final Concentration (µg/mL) of PFOS in matrix

14.0 Method Performance

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

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

14.2.1 Method blanks and matrix blanks.

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

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

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

15.0 Pollution Prevention and Waste Management

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

16.0 RECORDS

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

17.0 ATTACHMENTS

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values and summary

17.3 Attachment C, Calibration standard concentration worksheet

18.0 REFERENCES

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

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

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

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

Extraction Worksheet ETS-8-4.1

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

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

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

MDL/LOQ values for rabbit serum

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

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

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

Compound: PFOS

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

Compound: PFOSA

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

Compound: PFOSAA

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

Compound: EtFOSE-OH

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

Compound: PFOSEA

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

Compound: M556

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

Ion Pair Standard Curves – Fluids

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

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

Actual concentrations of standards in the FC mix

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

Calculated concentrations of standards in the sample matrix

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

Validated ranges – approximate concentrations

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

3M ENVIRONMENTAL LABORATORY

METHOD

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

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

Revision Date: NA

Author: Lisa Clemen, Robert Wynne

Approved By:

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

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

1.0 SCOPE AND APPLICATION

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

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

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

2.0 Summary of Method

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

2.2 These sample extracts are analyzed following method ETS-8-7.0 or other appropriate methods.

3.0 Definitions

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

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

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

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

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

3.6 M556: $C_8F_{17}SO_2N(H)(CH_2COOH)$

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

4.0 Warnings and Cautions

4.1 **Health and Safety Warnings:**

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

5.0 Interferences

5.1 There are no interferences known at this time.

6.0 Equipment

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

6.1.1 Ultra-Turrax T25 Grinder for grinding liver samples

6.1.2 Vortex mixer, VWR, Vortex Genie 2

6.1.3 Centrifuge, Mistral 1000 or IEC

6.1.4 Shaker, Eberbach or VWR

6.1.5 Nitrogen Evaporator, Organomation

6.1.6 Balance (sensitivity to 0.100 g)

7.0 SUPPLIES AND MATERIALS

7.1 Gloves

7.2 Dissecting scalpels

7.3 Eppendorf or disposable pipettes

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

7.5 Volumetric flasks, glass, type A

7.6 I-CHEM vials, 40 mL glass

7.7 Plastic sampule vials, Wheaton, 6 mL (or appropriate size)

7.8 Centrifuge tubes, polypropylene, 15 mL

7.9 Labels

7.10 Oxford Dispensor – 3.0 to 10.0 ml

7.11 Syringes, capable of measuring 5 µL to 50 µL

7.12 Graduated pipettes

7.13 Syringes, disposable plastic, 3 cc

7.14 Syringe filters, nylon, 0.2 µm, 25 mm

7.15 Timer

7.16 Crimp cap autovials and caps

7.17 Crimpers

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

8.0 REAGENTS AND STANDARDS

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

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

8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent

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

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

8.6 Methyl-*tert*-butyl ether, Omnisolv, glass distilled or HPLC grade

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

8.8 Liver, frozen from supplier

8.9 Dry ice from supplier

8.10 **Fluorochemical standards**

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

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

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

8.10.4 EtFOSE-OH (3M Specialty Chemical Division), molecular weight = 570

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

8.10.6 M556 (3M Specialty Chemical Division), molecular weight = 557

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

8.10.8 Other fluorochemicals, as appropriate

8.11 Reagent preparation

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

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

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

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

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

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

8.12 Standards preparation

8.12.1 Prepare PFOS standards for the standard curve.

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

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

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

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

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

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

8.13 Surrogate stock standard preparation

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

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

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

9.0 SAMPLE HANDLING

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

10.0 QUALITY CONTROL

10.1 Matrix blanks and method blanks

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

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

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

10.2 Matrix spikes

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

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

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

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

10.3 Continuing calibration verifications

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

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

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

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

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

11.1.1 Weigh approximately 40 g of liver into a 250 mL Nalgene bottle containing 200 mLs Milli-Q™ water. Grind to a homogeneous solution.

11.1.2 If 40 g is not available, use appropriate amounts of liver and water to ensure a 1:5 ratio.

11.1.3 Refer to **13.0** to calculate the actual density of liver homogenate and the concentration of solid liver tissue dispersed in 1.0 mL of homogenate solution.

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

11.1.6 Two 1 mL aliquots, or other appropriate volume, serve as matrix blanks.

11.1.7 Typically use the standard concentrations and spiking amounts listed in Table 1, at the end of this section, to spike, in duplicate, two standard curves, for a total of eighteen samples, two matrix blanks, and two method blanks.

11.1.8 Refer to validation reports **ETS-8-6.0 and ETS-8-7.0-V-1** or **Attachment B,** which lists the working ranges and the Linear Calibration Range (LCR) for calibration curves.

11.1.9 Use **Attachment C** as an aid in calculating the concentrations of the working standards. Refer to **13.0** to calculate actual concentrations of PFOS in calibration standards.

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

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

Table 1
Approximate Spiking Amounts for Calibration Standards

Working Standard (Approx. Conc.)	µl	Approx. final conc. of PFOS in liver
-	-	Blank
0.50 ppm	2	0.005 ppm
0.50 ppm	4	0.010 ppm
0.50 ppm	10	0.025 ppm
0.50 ppm	20	0.050 ppm
0.50 ppm	40	0.100 ppm
5.0 ppm	10	0.250 ppm
5.0 ppm	20	0.500 ppm
5.0 ppm	30	0.750 ppm
50 ppm	4	1.00 ppm

12.0 PROCEDURE

12.1 Obtain frozen liver samples.

12.2 Cut approximately 1 g of liver using a dissecting scalpel. This part of the procedure is best performed quickly, not allowing the liver to thaw.

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

12.4 Record the liver weight in the study notebook.

12.5 Return unused liver portions to freezer.

12.6 Add 2.5 mLs of water to sampule vial.

12.7 Grind the sample. Put the grinder probe in the sample and grind for about 2 minutes, or until the sample is homogeneous.

12.8 Rinse the probe into the sample with 2.5 mLs water using a pipette.

12.9 Take the grinder apart and clean it with methanol after each sample. Refer to AMDT-EP-22.

12.10 Cap the sample and vortex for 15 seconds. Label the sampule vial with the study number, weight, liver ID, date and analyst initials.

12.11 Pipette 1.0 mL, or other appropriate volume, of homogenate into a 15 mL polypropylene centrifuge tube. Label the centrifuge tube with the identical information as the sampule vial. Refer to attached worksheet for documenting the remaining steps.

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

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

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

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

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

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

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

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

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

12.21 Label a fresh 15 mL centrifuge tube with the same information as in **12.10.**

12.22 Remove 4.0 mL of the organic layer to the fresh 15 mL centrifuge tube.

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

12.24 Add 1.0 mL to each centrifuge tube using a graduated pipette.

12.25 Vortex mix for 30 seconds.

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

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

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

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

13.0 Data Analysis and Calculations

13.1 Calculations:

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

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

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

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

* 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:

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

*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					
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	
		-	-	-	

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

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

MDL/LOQ values for rabbit liver

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

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

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

Compound: PFOS

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

Compound: PFOSA

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

Compound: PFOSAA

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

Compound: EtFOSE-OH

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

Compound: PFOSEA

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

Compound: M556

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

Ion Pair Standard Curves – Tissue

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

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

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

Actual concentrations of standards in the FC mix

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

Calculated concentrations of standards in the sample matrix

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

Validated ranges – approximate concentrations

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

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: FACT-M-2.0 **Adoption Date:** 5/26/98

Revision Date: N/A

Author: Lisa Clemen

Approved By:

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

Kristen Hoz 5/26/98
Group Leader Date

Lisa A Clemen 5/27/98
Technical Reviewer Date

Initial RSD Date 6/5/01

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

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

Method Number: FACT-M-4.0 **Adoption Date:** 4/22/98

Revision Date: N/A

Author: Lisa Clemen

Approved By:

[signature] 4/22/98
Laboratory Manager Date

[signature] 4/22/98
Group Leader Date

Lisa Clemen 4/14/98
Technical Reviewer Date

E... Initial RSD 4/15/98 Date

1.0 SCOPE AND APPLICATION

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

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

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

2.0 SUMMARY OF METHOD

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

3.0 DEFINITIONS

3.1 None.

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

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

4.2 Cautions:

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

4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

5.1 Teflon should not be used for sample storage or any part of instrumentation that comes in contact with the sample or extract.

6.0 EQUIPMENT

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

6.1.1 Micromass Electrospray Mass Spectrometer

6.1.2 HP1100 low pulse solvent pumping system and autosampler.

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

7.1.1 Nitrogen gas, refrigerated liquid, regulated to approximately 100 psi.

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

7.1.3 Capped autovials or capped 15 mL centrifuge tubes.

8.0 REAGENTS AND STANDARDS

8.1 Reagents

8.1.1 Methanol, HPLC grade or equivalent.

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

8.1.3 Ammonium acetate, HPLC grade or equivalent.

8.2 Standards

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

9.0 Sample Handling

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

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

10.0 Quality Control

10.1 Matrix Blanks and Method Blanks

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

10.2 Matrix Spikes

10.2.1 Analyze a matrix spike and matrix spike duplicate with each analysis.

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

10.2.3 See section 13 to calculate percent recovery.

10.3 Continuing Calibration Checks

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

10.3.2 See section 13 to calculate percent difference.

10.4 System Suitability

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

11.0 Calibration and Standardization

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

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

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

12.0 PROCEDURES

12.1 Acquisition Set up

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

12.1.2 To create a method click on scan button in the Acquisition control panel and select SIR (Single Ion Recording). Set Ionization Mode as appropriate and mass to 499 or other appropriate masses. A scan is usually collected along with the SIRs. Save method.

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

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

12.2 Using the Autosampler

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

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

12.2.2.1 Sample size = 10 µL injection with a sample wash

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 15 minutes

12.2.2.4 Solvent ramp =

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

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

12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

12.3.1 Refer to AMDT-EP-31 for more details.

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

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

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

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

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

12.3.6.1 Drying gas 250-400 liters/hour

12.3.6.2 ESI nebulizing gas 10-15 liters/hour

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

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

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

12.3.8 Record tune parameters in the instrument log.

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

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

13.0 Data Analysis and Calculations

13.1 Calculations:

13.1.4 Calculate matrix spike percent recoveries using the following equation:

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

13.1.5 Calculate percent difference using the following equation:

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

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

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

14.0 Method Performance

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

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

15.0 Pollution Prevention and Waste Management

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

16.0 Records

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

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

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

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

16.5 Copy instrument runlog pages, including instrument parameters and sample results, and tape into appropriate study notebook.

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

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

17.0 Tables, Diagrams, Flowcharts, and Validation Data

17.1 Attachment A: FACT-M-4 Data reporting spreadsheet

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

18.0 References

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

19.0 Affected Documents

19.1 FACT-M-3.0, "Extraction of Fluorochemical Anions from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"

20.0 REVISIONS

Revision Number.	Reason For Revision	Revision Date

Laboratory Study

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

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

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

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: ETS-8-5.1 **Adoption Date:** 03/01/99

Revision Date: 4/26/99

Author: Lisa Clemen, Robert Wynne

Approved By:

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

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

1.0 SCOPE AND APPLICATION

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

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

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

2.0 Summary of Method

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

3.0 Definitions

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

3.2 **Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These charged droplets are produced by the application of a strong electrical field.

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

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

3.5 **Mass Lynx Software:** System software designed for the specific operation of these Quattro II triple quadrupole systems. Currently MassLynx has Windows 95 and WindowsNT 4.0 versions. All versions are similar. For more details see the manual specific to the instrument (Micromass Quattro II triple quadrupole MassLynx or MassLynx NT User's Guide).

4.0 Warnings and Cautions

4.1 **Health and Safety Warnings:**

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

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

4.2 Cautions:

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

4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

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

6.0 EQUIPMENT

6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.

6.1.1 Micromass Quattro II triple quadrupole Mass Spectrometer equipped with an electrospray ionization source

6.1.2 HP1100 low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

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

7.1.2 HPLC analytical column, specifics to be determined by the analyst and documented in the raw data.

7.1.3 Capped autovials or capped 15 mL centrifuge tubes

8.0 REAGENTS AND STANDARDS

8.1 Reagents

8.1.1 Methanol, HPLC grade or equivalent

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

8.1.3 Ammonium acetate, reagent grade or equivalent

8.2 Standards

8.2.1 Typically two method blanks, two matrix blanks, and eighteen matrix standards are prepared during the extraction procedure. See **ETS-8-4.1**.

9.0 SAMPLE HANDLING

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

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

10.0 QUALITY CONTROL

10.1 Solvent Blanks, Method Blanks and Matrix Blanks

10.1.1 Solvent blanks, method blanks and matrix blanks are prepared and analyzed with each batch to determine contamination or carryover.

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

10.2 Matrix Spikes

10.2.1 Matrix spikes are prepared and analyzed to determine the matrix effect on the recovery efficiency.

10.2.2 Matrix spike duplicates are prepared and analyzed to measure the precision and the recovery for each analyte.

10.2.3 Analyze a matrix spike and matrix spike duplicate per forty samples, with a minimum of 2 spikes per batch.

10.2.4 Matrix spike and matrix spike duplicate concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.3 Continuing Calibration Verifications

10.3.1 Continuing calibration verifications are analyzed to verify the continued accuracy of the calibration curve.

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

11.0 CALIBRATION AND STANDARDIZATION

11.1 Analyze the extracted matrix standards prior to and following each set of extracts. The average of two standard curves will be plotted by linear regression ($y = my + b$), weighted 1/x, not forced through zero, using MassLynx or other suitable software.

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

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

12.0 PROCEDURES

12.1 Acquisition Set up

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

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

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

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

12.2 Using the Autosampler

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

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

12.2.2.1 Sample size = 10 µL injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 13.5 minutes

12.2.2.4 Solvent ramp =

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

12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

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

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

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

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

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

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

12.3.6.1 Drying gas 250-400 liters/hour

12.3.6.2 ESI nebulizing gas 10-15 liters/hour

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

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

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

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

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

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

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

13.1.4 Calculate matrix spike percent recoveries using the following equation:

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

13.1.5 Calculate percent difference using the following equation:

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

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

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

14.0 METHOD PERFORMANCE

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

14.2 Solvent Blanks, Method Blanks, and Matrix Blanks

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

14.3 Calibration Curves

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

14.4 Matrix Spikes

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

14.5 Continuing Calibration Verifications

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

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

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

15.0 POLLUTION PREVENTION AND WASTE MANAGEMENT

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

16.0 RECORDS

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

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

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

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

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

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

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

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

18.0 REFERENCES

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

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

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

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

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

Laboratory Study

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

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

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

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: ETS-8-7.0 **Adoption Date:** 07/22/99

Revision Date: NA

Author: Lisa Clemen, Glenn Langenburg

Approved By:

[signature] 7/22/99
Laboratory Manager Date

[signature] 7/14/99
Group Leader Date

[signature] 07/14/99
Technical Reviewer Date

Initial RSD Date 6/5/01

Original

1.0 SCOPE AND APPLICATION

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

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

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

2.0 Summary of Method

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

3.0 Definitions

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

3.2 **Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These charged droplets are produced by the application of a strong electrical field.

3.3 **Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API Quattro II triple quadrupole mass spectrometer is equipped with two quadrupole mass selective detectors and a collision cell. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected. A single MS may be employed for ion detection or an ion may be selected in the first quadrupole, fragmented in the collision cell, and these fragments may be analyzed in the second quadrupole.

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

3.5 **Mass Lynx Software:** System software designed for the specific operation of these Quattro II triple quadrupole systems. Currently MassLynx has Windows 95 and WindowsNT 4.0 versions. All versions are similar. For more details refer to the manual specific to the instrument (Micromass Quattro II triple quadrupole MassLynx or MassLynx NT User's Guide).

4.0 Warnings and Cautions

4.1 **Health and Safety Warnings:**

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

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

4.2 Cautions:

4.2.1 Operate the solvent pumps below a back pressure of 400 bar (5800 psi). If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.

4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

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

6.0 EQUIPMENT

6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.

6.1.1 Micromass Quattro II triple quadrupole Mass Spectrometer equipped with an electrospray ionization source.

6.1.2 HP1100 low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

7.1.1 High purity grade air regulated to approximately 100 psi (house air system)

7.1.2 HPLC analytical column, specifics to be determined by the analyst and documented in the raw data

7.1.3 Capped autovials or capped 15 ml centrifuge tubes

8.0 REAGENTS AND STANDARDS

8.1 Reagents

8.1.1 Methanol, HPLC grade or equivalent

8.1.2 Milli-Q™ water (ASTM type I), all water used in this method should be ATSM type I, or equivalent, and be provided by a Milli-Q TOC Plus system or other vendor

8.1.3 Ammonium acetate, reagent grade or equivalent

8.1.3.1 When preparing different amounts than those listed, adjust accordingly.

8.1.3.2 2.0 mM ammonium acetate solution: Weigh approximately 0.300 g ammonium acetate. Pour into a 2000 mL volumetric container containing 2000 mL Milli-Q™ water, mix until all solids are dissolved. Store at room temperature.

8.2 Standards

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

9.0 Sample Handling

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

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

10.0 Quality Control

10.1 Method Blanks and Matrix Blanks

10.1.1 Solvent blanks, method blanks, and matrix blanks are prepared and analyzed with each batch to determine contamination or carryover.

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

10.2 Matrix Spikes

10.2.1 Matrix spikes are prepared and analyzed to determine the matrix effect on the recovery efficiency.

10.2.2 Matrix spike duplicates are prepared and analyzed to measure the precision and the recovery for each analyte.

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

10.2.4 Matrix spike and matrix spike duplicate concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.3 Continuing Calibration Checks

10.3.1 Continuing calibration verifications are analyzed to verify the continued accuracy of the calibration curve.

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

11.0 Calibration and Standardization

11.1 Analyze the extracted matrix standards prior to and following each set of sample extracts. The average of two standard curves will be plotted by linear regression ($y = mx + b$), weighted 1/x, not forced through the origin, using MassLynx or other suitable software.

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

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

12.0 PROCEDURES

12.1 Acquisition Set up

12.1.1 Set up the sample list.

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

12.1.1.2 Assign a method (MS file) for acquiring

12.1.1.3 Assign an HPLC program (Inlet file)

12.1.1.4 Type in sample descriptions and vial position numbers

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

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

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

12.2 Using the Autosampler

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

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

12.2.2.1 Sample size = 10 µL injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 9 minutes

12.2.2.4 Solvent ramp conditions

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

12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

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

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

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

12.3.4 Turn on the nitrogen.

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

12.3.6 Open the Inlet Editor.

12.3.6.1 Set HPLC pump to "On"

12.3.6.2 Set the flow to 10 - 500 uL/min or as appropriate

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

12.3.6.4 Allow to equilibrate for approximately 10 minutes.

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

12.3.7.1 Drying gas 250-400 liters/hour

12.3.7.2 ESI nebulizing gas 10-15 liters/hour

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

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

12.3.7.5 Source block temperature 150°

12.3.7.6 Desolvation temperature 250°

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

12.3.9 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, refer to appropriate MassLynx User's Guide). Ensure start and end sample number includes all samples to be analyzed.

13.0 Data Analysis and Calculations

13.1 Calculations:

13.1.4 Calculate matrix spike percent recoveries using the following equation:

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

13.1.5 Calculate percent difference using the following equation:

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

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

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

14.0 Method Performance

14.1 Method Detection Limit (MDL) and Limit of Quantitation (LOQ) are method, analyte, and matrix specific. 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 Medical Department Study: T-6316.1 Analytical Report: FACT TOX-003
LRN-U2104

Appendix D: Data Summary Tables

Table 9. FACT TOX-003 Data Summary for PFOS, PFOSA, PFOSAA, EtFOSE, M556 and PFOSEA—Rat Serum (µg/mL)

Timept	Group		PFOS Avg ± SD	PFOSA Avg ± SD	PFOSAA Avg ± SD	EtFOSE** Avg ± SD	M556 Avg ± SD	PFOSEA** Avg ± SD
Week 4	Group 8 0.0 ppm	M	<LOQ[a] (n=5)	<LOQ[b] (n=5)	0.00460 ± 0.00158* (n=5)	NA	NA	NA
		F	<LOQ[a] (n=5)	<LOQ[b] (n=5)	0.0103 ± 0.00375* (n=5)	NA	NA	NA
	Group 9 1.0 ppm	M	0.174 ± 0.0510 (n=5)	0.00500 ± 0.000125 (n=5)	0.247 ± 0.195* (n=5)	NA	NA	NA
		F	0.270 ± 0.0687 (n=5)	0.00778 ± 0.00254 (n=5)	0.294 ± 0.111* (n=5)	NA	NA	NA
Week 14	Group 8 0.0 ppm	M	0.0393 ± 0.0109 (n=5)	<LOQ[c] (n=5)	<LOQ[d] (n=5)	NA	NA	NA
		F	0.126 ± 0.0159 (n=5)	<LOQ[c] (n=5)	<LOQ[d] (n=5)	NA	NA	NA
	Group 9 1.0 ppm	M	2.19 ± 0.733 (n=5)	0.0110 ± 0.000716 (n=5)	0.497 ± 0.618 (n=5)	NA	NA	NA
		F	3.26 ± 0.629 (n=6)	0.0164 ± 0.00354 (n=6)	0.578 ± 0.425 (n=6)	NA	NA	NA
Week 53	Group 8 0.0 ppm	M	0.0490 ± 0.0352 (n=5)	<LOQ[e] (n=5)	<LOQ[f] (n=5)	<LOQ[g] (n=5)	<LOQ[h] (n=5)	<LOQ[i] (n=5)
		F	0.0727 ± 0.0565 (n=5)	<LOQ[e] (n=5)	<LOQ[f] (n=5)	<LOQ[g] (n=5)	<LOQ[h] (n=5)	<LOQ[i] (n=5)
	Group 9 1.0 ppm	M	1.35 ± 0.452 (n=5)	0.00672 ± 0.00251 (n=5)	0.297 ± 0.194 (n=5)	<LOQ[g] (n=5)	0.120 ± 0.0289 (n=5)	<LOQ[i] (n=5)
		F	3.02 ± 1.54 (n=5)	0.0102 ± 0.00252 (n=5)	0.273 ± 0.110 (n=5)	<LOQ[g] (n=5)	0.202 ± 0.0723 (n=5)	<LOQ[i] (n=5)

NA—Not applicable
*Qualitative data on 4 points in calibration curve
**EtFOSE and PFOSEA results are qualitative only.
[a]LOQ—Limit of Quantitation = 0.0230 µg/mL
[b]LOQ—Limit of Quantitation = 0.0494 µg/mL
[c]LOQ—Limit of Quantitation = 0.00989 µg/mL
[d]LOQ—Limit of Quantitation = 0.0282 µg/mL
[e]LOQ—Limit of Quantitation = 0.00516 µg/mL
[f]LOQ—Limit of Quantitation = 0.0253 µg/mL
[g]LOQ—Limit of Quantitation = 0.0225 µg/mL
[h]LOQ—Limit of Quantitation = 0.00494 µg/mL
[i]LOQ—Limit of Quantitation = 0.00978 µg/mL
NOTE: Results are expressed as group/gender averages ± the standard deviation associated with that group/gender.
NOTE: All PFOSA, PFOSAA, PFOSEA, M556, and PFOS Week 4 results are not corrected for purity of the reference standard material.
It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that, with the exception of PFOSEA and EtFOSE-OH, the sera and liver data can be considered to be accurate to within one standard deviation of the average fortified samples recovery.

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Table 9. (Continued) FACT TOX-003 Data Summary for PFOS, PFOSA, PFOSAA, EtFOSE, M556 and PFOSEA—Rat Serum (µg/mL)

Timept	Group		PFOS Avg ± SD	PFOSA Avg ± SD	PFOSAA Avg ± SD	EtFOSE** Avg ± SD	M556 Avg ± SD	PFOSEA** Avg ± SD
Week 105	Group 8 0.0 ppm	M	0.0137 ± 0.0181 (n=10)	<LOQ[e] (n=10)	<LOQ[j] (n=10)	<LOQ[k] (n=10)	<LOQ[l] (n=10)	<LOQ[m] (n=10)
		F	0.0256 ± 0.0145 (n=10)	<LOQ[e] (n=10)	<LOQ[j] (n=10)	<LOQ[k] (n=10)	<LOQ[l] (n=10)	<LOQ[m] (n=10)
	Group 9 1.0 ppm	M	1.12 ± 1.10 (n=10)	0.00720 ± 0.00330 (n=10)	0.252 ± 0.160 (n=10)	<LOQ[k] (n=10)	0.0645 ± 0.0445 (n=10)	<LOQ[m] (n=10)
		F	1.61 ± 0.631 (n=10)	0.00797 ± 0.00198 (n=10)	0.259 ± 0.156 (n=10)	<LOQ[k] (n=10)	0.0868 ± 0.0294 (n=10)	<LOQ[m] (n=10)

**EtFOSE and PFOSEA results are qualitative only.
[a]LOQ—Limit of Quantitation = 0.0230 µg/mL
[b]LOQ—Limit of Quantitation = 0.0494 µg/mL
[c]LOQ—Limit of Quantitation = 0.00989 µg/mL
[d]LOQ—Limit of Quantitation = 0.0282 µg/mL
[e]LOQ—Limit of Quantitation = 0.00516 µg/mL
[f]LOQ—Limit of Quantitation = 0.0253 µg/mL
[g]LOQ—Limit of Quantitation = 0.0225 µg/mL
[h]LOQ—Limit of Quantitation = 0.00494 µg/mL
[i]LOQ—Limit of Quantitation = 0.00978 µg/mL
[j]LOQ—Limit of Quantitation = 0.00503 µg/mL
[k]LOQ—Limit of Quantitation = 0.00448 µg/mL
[l]LOQ—Limit of Quantitation = 0.0248 µg/mL
[m]LOQ—Limit of Quantitation = 0.00493 µg/mL

NOTE: Results are expressed as group/gender averages ± the standard deviation associated with that group/gender.

NOTE: All PFOSA, PFOSAA, PFOSEA, M556, and PFOS Week 4 results are not corrected for purity of the reference standard material.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that, with the exception of PFOSEA and EtFOSE-OH, the sera and liver data can be considered to be accurate to within one standard deviation of the average fortified samples recovery.

3M Medical Department Study: T-6316.1 Analytical Report: FACT TOX-003
LRN-U2104

Table 10. FACT TOX-003 Data Summary for PFOS, PFOSA, PFOSAA, EtFOSE, M556 and PFOSEA—Rat Liver (µg/g)

Timept	Group		PFOS Avg ± SD	PFOSA Avg ± SD	PFOSAA Avg ± SD	EtFOSE* Avg ± SD	M556 Avg ± SD	PFOSEA* Avg ± SD
Week 4	Group 8 0.0 ppm	M	0.198 ± 0.0822 (n=5)	<LOQ[a] (n=5)	<LOQ[b] (n=5)	NR	NA	NA
		F	0.0891 ± 0.0315 (n=5)	<LOQ[a] (n=5)	<LOQ[b] (n=5)	NR	NA	NA
	Group 9 1.0 ppm	M	6.53 ± 0.628 (n=5)	0.187 ± 0.0401 (n=5)	0.683 ± 0.613 (n=5)	NR	NA	NA
		F	2.97 ± 0.937 (n=5)	0.222 ± 0.104 (n=5)	0.582 ± 0.193 (n=5)	NR	NA	NA
Week 14	Group 8 0.0 ppm	M	2.96 ± 4.83 (n=5)	0.0822 ± 0.156 (n=5)	0.330 ± 0.656 (n=5)	NR	NA	NA
		F	0.411 ± 0.0625 (n=5)	0.0130 ± 0.000872 (n=5)	0.0411 ± 0.0121 (n=5)	NR	NA	NA
	Group 9 1.0 ppm	M	12.4 ± 6.99 (n=5)	0.366 ± 0.221 (n=5)	2.89 ± 1.88 (n=5)	NR	NA	NA
		F	9.19 ± 1.53 (n=5)	0.580 ± 0.239 (n=5)	2.61 ± 2.49 (n=5)	NR	NA	NA
Week 53	Group 8 0.0 ppm	M	1.44 ± 1.18 (n=5)	<LOQ[c] (n=5)	<LOQ[d] (n=5)	<LOQ[e] (n=5)	<LOQ[f] (n=5)	<LOQ[g] (n=5)
		F	0.178 ± 0.115 (n=5)	0.00647 ± 0.0000786 (n=5)	<LOQ[d] (n=5)	<LOQ[e] (n=5)	<LOQ[f] (n=5)	<LOQ[g] (n=5)
	Group 9 1.0 ppm	M	37.1 ± 10.9 (n=5)	0.204 ± 0.0769 (n=5)	0.906 ± 0.605 (n=5)	<LOQ[e] (n=5)	0.336 ± 0.0682 (n=5)	<LOQ[g] (n=5)
		F	25.2 ± 13.9 (n=5)	0.242 ± 0.0999 (n=5)	0.527 ± 0.131 (n=5)	<LOQ[e] (n=5)	0.267 ± 0.0660 (n=5)	<LOQ[g] (n=5)

*EtFOSE and PFOSEA results are qualitative only.
NR—No result
NA—Not applicable

[a]LOQ—Limit of Quantitation = 0.0121 µg/g
[b]LOQ—Limit of Quantitation = 0.0341 µg/g
[c]LOQ—Limit of Quantitation = 0.00644 µg/g
[d]LOQ—Limit of Quantitation = 0.0125 µg/g
[e]LOQ—Limit of Quantitation = 0.0559 µg/g
[f]LOQ—Limit of Quantitation = 0.0307 µg/g
[g]LOQ—Limit of Quantitation = 0.0308 µg/g
[h]LOQ—Limit of Quantitation = 0.0322 µg/g
[i]LOQ—Limit of Quantitation = 0.0314 µg/g

NOTE: Results are expressed as group/gender averages ± the standard deviation associated with that group/gender.

NOTE: All PFOSA, PFOSAA, PFOSEA, M556 and EtFOSE-OH Week 4 and Week 14 results are not corrected for purity of the reference standard material.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the sera and liver data can be considered to be accurate to within one standard deviation of the average fortified samples recovery.

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Table 10. (Continued) FACT TOX-003 Data Summary for PFOS, PFOSA, PFOSAA, EtFOSE, M556 and PFOSEA—Rat Liver (µg/g)

Timept	Group		PFOS Avg ± SD	PFOSA Avg ± SD	PFOSAA Avg ± SD	EtFOSE* Avg ± SD	M556 Avg ± SD	PFOSEA* Avg ± SD
Week 105	Group 8 0.0 ppm	M	0.0653 ± 0.0923 (n=10)	<LOQ[h] (n=10)	<LOQ[i] (n=10)	<LOQ[e] (n=10)	<LOQ[f] (n=10)	<LOQ[g] (n=10)
		F	0.0872 ± 0.0522 (n=10)	<LOQ[h] (n=10)	<LOQ[i] (n=10)	<LOQ[e] (n=10)	<LOQ[f] (n=10)	<LOQ[g] (n=10)
	Group 9 1.0 ppm	M	6.21 ± 3.80 (n=10)	0.371 ± 0.187 (n=10)	0.736 ± 0.551 (n=10)	<LOQ[e] (n=10)	0.250 ± 0.0865 (n=10)	<LOQ[g] (n=10)
		F	9.63 ± 4.35 (n=10)	0.550 ± 0.112 (n=10)	0.771 ± 0.411 (n=10)	<LOQ[e] (n=10)	0.336 ± 0.0748 (n=10)	<LOQ[g] (n=10)

*EtFOSE and PFOSEA results are qualitative only.
NR—No result
NA—Not applicable

[a]LOQ—Limit of Quantitation = 0.0121 µg/g
[b]LOQ—Limit of Quantitation = 0.0341 µg/g
[c]LOQ—Limit of Quantitation = 0.00644 µg/g
[d]LOQ—Limit of Quantitation = 0.0125 µg/g
[e]LOQ—Limit of Quantitation = 0.0559 µg/g
[f]LOQ—Limit of Quantitation = 0.0307 µg/g
[g]LOQ—Limit of Quantitation = 0.0308 µg/g
[h]LOQ—Limit of Quantitation = 0.0322 µg/g
[i]LOQ—Limit of Quantitation = 0.0314 µg/g

NOTE: Results are expressed as group/gender averages ± the standard deviation associated with that group/gender.

NOTE: All PFOSA, PFOSAA, PFOSEA, M556 and EtFOSE-OH Week 4 and Week 14 results are not corrected for purity of the reference standard material.

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the sera and liver data can be considered to be accurate to within one standard deviation of the average fortified samples recovery.

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Appendix E: Data Spreadsheets

Covance# 6329-228

Study:	TOX003 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats
Product Number/Test Substance:	T-6316 (EtFOSE OH)
Matrix:	Rat Serum
Method/Revision:	FACT-M-3.0 & FACT-M-4.0, ETS-8-5.1
Analytical Equipment System Number:	Machine 041098
Instrument Software/Version:	MassLynx 3.1
Filename:	See Last to Right
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Date of Extraction/Analyst:	5/27/98 OK
Date of Analysis/Analyst:	07/13/98, 08/30/00 MEE/MMH
Date of Data Reduction/Analyst:	2/14/00, 8/31/00 mmh

Sample Data

WEEK 4 RAT SERA

			lot 215								lot L-2353						lot 617								
Group Dose	Sample #	Extraction Vol. Ratio	PFOS Correction Factor	PFOS Purity Correction Factor	PFOS Dilution Factor	PFOS Conc. ug/mL	Filename	Concentration of PFOS ug/mL or % Rec		Average PFOS ug/mL	RSD Std. Dev.	PFOSA Purity Correction Factor	PFOSA Dilution Factor	PFOSA Conc. ug/mL	Filename	Concentration of PFOSA ug/mL or % Rec	Average PFOSA ug/mL	RSD Std. Dev.	PFOSAA Old Purity Correction Factor	PFOSAA Purity Correction Factor	PFOSAA Dilution Factor	PFOSAA Conc. ug/mL	Filename	Concentration of PFOSAA ug/mL or % Rec		Average PFOSAA ug/mL	RSD Std. Dev.
Method Blk	H2O Blk-1	1	0.9275	Unknown	1	0.00	M71398011	<LOQ (0.0230 ug/mL)				Unknown	1	1.99	M71398011	<LOQ (0.0494 ug/mL)			0.5382	Unknown	1	NR	NR	NR			
Method Blk	H2O Blk-2	1	0.9275	Unknown	NR	NR	NR	NR		<LOQ	NA	Unknown	1	NR	NR	NR	<LOQ	NA	0.5382	Unknown	1	NR	NR	NR		NR	NR
Matrix Blk	Rat Serum Blk-1	1	0.9275	Unknown	NR	NR	NR	NR				Unknown	1	NR	NR	NR			0.5382	Unknown	1	NR	NR	NR			
Matrix Blk	Rat Serum Blk-2	1	0.9275	Unknown	1	0.00	M71398002	<LOQ (0.0230 ug/mL)		<LOQ	NA	Unknown	1	0.00	M71398002	<LOQ (0.0494 ug/mL)	<LOQ	NA	0.5382	Unknown	1	NR	NR	NR		NR	NR
QC-75 ppb	RTS-052698-MS	1	NA	NA	1	106	M71398041	144%				NA	1	82.6	A083000016	112%			NA	NA	1	86.9	A083000016	115%	A		
	RTS-052698-MSD	1	NA	NA	1	70.8	M71398042	96%		120%	40%	NA	1	81.7	A083000017	111%	111%	1%	NA	NA	1	90.9	A083000017	120%	A	117%	5%
	RTS-052698-MS	1	NA	NA	1	166	A083000016	226%	2nd			NA	NA	NA	NA	NA			NA	NA	NA	NA	NA	NA			
	RTS-052698-MSD	1	NA	NA	1	142	A083000017	193%	2nd	210%	16%	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA		NA	NA
Group 8	C92332F	1	0.9275	Unknown	1	21.5	M71398013	<LOQ (0.0230 ug/mL)	1			Unknown	1	0.84	A083000021	<LOQ (0.0494 ug/mL)			0.5382	Unknown	1	18.7	A083000021	0.0101	A		
Control	C92352F	1	0.9275	Unknown	1	11.0	M71398014	<LOQ (0.0230 ug/mL)	1			Unknown	1	0.84	A083000022	<LOQ (0.0494 ug/mL)			0.5382	Unknown	1	19.1	A083000022	0.0103	A		
0.0 ppm	C92355F	1	0.9275	Unknown	1	5.22	M71398015	<LOQ (0.0230 ug/mL)	1			Unknown	1	0.70	A083000023	<LOQ (0.0494 ug/mL)			0.5382	Unknown	1	11.7	A083000023	0.00629	A		
	C92366F	1	0.9275	Unknown	1	5.61	M71398016	<LOQ (0.0230 ug/mL)	1		NA	Unknown	1	0.78	A083000024	<LOQ (0.0494 ug/mL)		NA	0.5382	Unknown	1	16.1	A083000024	0.00864	A		36.3
	C92374F	1	0.9275	Unknown	1	3.08	M71398017	<LOQ (0.0230 ug/mL)	1	<LOQ	NA	Unknown	1	0.83	A083000025	<LOQ (0.0494 ug/mL)	<LOQ	NA	0.5382	Unknown	1	30.5	A083000025	0.0164	A	0.0103	0.00375
	C92192M	1	0.9275	Unknown	1	0.00	M71398020	<LOQ (0.0230 ug/mL)	1			Unknown	1	0.63	A083000030	<LOQ (0.0494 ug/mL)			0.5382	Unknown	1	5.68	A083000030	0.00306	A		
	C92196M	1	0.9275	Unknown	1	0.00	M71398021	<LOQ (0.0230 ug/mL)	1			Unknown	1	0.71	A083000031	<LOQ (0.0494 ug/mL)			0.5382	Unknown	1	11.9	A083000031	0.00640	A		
	C92207M	1	0.9275	Unknown	1	0.00	M71398022	<LOQ (0.0230 ug/mL)	1			Unknown	1	0.62	A083000032	<LOQ (0.0494 ug/mL)			0.5382	Unknown	1	5.45	A083000032	0.00293	A		
	C92210dM	1	0.9275	Unknown	1	0.00	M71398023	<LOQ (0.0230 ug/mL)	1		NA	Unknown	1	0.57	A083000033	<LOQ (0.0494 ug/mL)		NA	0.5382	Unknown	1	8.82	A083000033	0.00475	A		34.3
	C92259dM	1	0.9275	Unknown	1	0.00	M71398024	<LOQ (0.0230 ug/mL)	1	<LOQ	NA	Unknown	1	0.66	A083000034	<LOQ (0.0494 ug/mL)	<LOQ	NA	0.5382	Unknown	1	10.9	A083000034	0.00584	A	0.00460	0.00158
Group 9	C92435F	1	0.9275	Unknown	10	28.3	M71398027	0.262	3			Unknown	1	7.25	A083000038	0.00725			0.5382	Unknown	1	383	A083000038	0.206	A		
Low Dose	C92402F	1	0.9275	Unknown	10	36.0	M71398028	0.334	3			Unknown	1	8.85	A083000039	0.00885			0.5382	Unknown	1	509	A083000039	0.274	A		
1.0 ppm	C92403F	1	0.9275	Unknown	10	19.8	M71398029	0.183	3			Unknown	1	5.24	A083000040	0.00524			0.5382	Unknown	1	336	A083000040	0.181	A		
	C92467F	1	0.9275	Unknown	10	37.1	M71398030	0.344	3		25.4	Unknown	1	11.6	A083000041	0.0116		32.7	0.5382	Unknown	1	664	A083000041	0.358	A		37.9
	C92470F	1	0.9275	Unknown	10	24.6	M71398031	0.229	3	0.270	0.0687	Unknown	1	5.95	A083000042	0.00595	0.00778	0.00254	0.5382	Unknown	1	837	A083000042	0.451	4A	0.294	0.111
	C92264M	1	0.9275	Unknown	5	34.7	M71398034	0.161	2			Unknown	1	4.37	A083000047	< LOQ (0.0494 ug/mL)			0.5382	Unknown	1	330	A083000047	0.177	A		
	C92280M	1	0.9275	Unknown	5	41.7	M71398035	0.193	2			Unknown	1	3.49	A083000048	< LOQ (0.0494 ug/mL)			0.5382	Unknown	1	249	A083000048	0.134	A		
	C92284M	1	0.9275	Unknown	5	29.4	M71398036	0.136	2			Unknown	1	5.22	A083000049	0.00522			0.5382	Unknown	1	369	A083000049	0.198	A		
	C92300M	1	0.9275	Unknown	5	27.2	M71398037	0.126	2		29.4	Unknown	1	4.18	A083000050	< LOQ (0.0494 ug/mL)		2.51	0.5382	Unknown	1	248	A083000050	0.134	A		78.9
	C92329dM	1	0.9275	Unknown	5	54.5	M71398038	0.253	2	0.174	0.0510	Unknown	1	4.89	A083000051	< LOQ (0.0494 ug/mL)	0.00500	0.000125	0.5382	Unknown	1	1100	A083000051	0.592	4A	0.247	0.195

NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate

Data Entered/By: 12/19/00, 1/5/01 mmh
Data Verified/ By: 03/20/01 kjh, 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC/

1 = CCVs failed. Data is estimated and is biased high
2 = CCVs failed. Data is estimated and is biased low.
3 = CCVs failed. Data is estimated.
4 = Extract above calibration range. Data is estimated.
A = Qualitative only, 4 calibration points were used to plot the calibration curve. LAC 05/03/01

		PFOS	PFOSA	PFOSAA
07/13/98	Initial LOQ ug/mL	24.8	4.94	10.5
	Corrected LOQ ug/mL	0.0230	0.00494	0.0108
08/30/00	Initial LOQ ug/mL	9.76	4.94	5.20
	Corrected LOQ ug/mL	0.00905	0.00494	0.00533

Covance# 6329-228

Study:	TOX003, 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats
Product Number(Test Substance)	T-6316 (EtFOSE-OH)
Matrix:	Rat Serum
Method/Revision:	FACT-M-3.0 & FACT-M-4.0; ETS-B-5.1
Analytical Equipment System Number	Machine 0410#8
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:	7/13/98 LAS/SAH
Date of Analysis/Analyst:	7/17/98, 8/7/98 MEE/HOJ
Date of Data Reduction/Analyst	2/10/00 mmh

Sample Data

WEEK 14 RAT SERA

					lot 193							Lot L-2353						lot 617						
Group Dose	Sample #	Extraction Vol. Ratio	PFOS Correction Factor	PFOS Purity Correction Factor	PFOS Dilution Factor	PFOS Conc. ug/mL	Filename	Concentration of PFOS ug/mL or % Rec	Average PFOS ug/mL	RSD Std. Dev.	PFOSA Purity Correction Factor	PFOSA Dilution Factor	PFOSA Conc. ug/mL	Filename	Concentration of PFOSA ug/mL or % Rec	Average PFOSA ug/mL	RSD Std. Dev.	PFOSAA Old Purity Correction Factor	PFOSAA Purity Correction Factor	PFOSAA Dilution Factor	PFOSAA Conc. ug/mL	Filename	Concentration of PFOSAA ug/mL or % Rec	Average PFOSAA ug/mL	RSD Std. Dev.
Method Blk	H20 Blk-1	1	0.9275	Unknown	1	1.21	M71798002	<LOQ (0.0230 ug/mL)			Unknown	1	1.50	M71798002	<LOQ (0.00989 ug/mL)			0.5382	Unknown	1	0.00	M71798002	<LOQ (0.0282 ug/mL)		
	H20 Blk-2	1	0.9275	Unknown	1	0.00	M71798043	<LOQ (0.0230 ug/mL)	<LOQ	NA	Unknown	1	0.00	M71798043	<LOQ (0.00989 ug/mL)	<LOQ	NA	0.5382	Unknown	1	0.00	M71798043	<LOQ (0.0282 ug/mL)	<LOQ	NA
Matrix Blk	Rat Serum Blk-1	1	0.9275	Unknown	1	4.68	M71798003	<LOQ (0.0230 ug/mL)			Unknown	1	1.59	M71798003	<LOQ (0.00989 ug/mL)			0.5382	Unknown	1	0.00	M71798003	<LOQ (0.0282 ug/mL)		
	Rat Serum Blk-2	1	0.9275	Unknown	1	2.04	M71798005	<LOQ (0.0230 ug/mL)	<LOQ	NA	Unknown	1	0.00	M71798044	<LOQ (0.00989 ug/mL)	<LOQ	NA	0.5382	Unknown	1	0.00	M71798003	<LOQ (0.0282 ug/mL)	<LOQ	NA
QC-100 ppb	RTS07158-MS	1	NA	NA	1	107	M71798040	109%			NA	1	102	M71798040	103%			NA	NA	1	76.6	M71798040	74%		
	RTS07158-MSD	1	NA	NA	1	114	M71798041	117%	113%	7%	NA	1	109	M71798041	110%	107%	7%	NA	NA	1	85.1	M71798041	82%	78%	11%
Group 8	C92255M	1	0.9275	Unknown	1	58.9	M71798012	0.0546			Unknown	1	1.95	M71798012	<LOQ (0.00989 ug/mL)			0.5382	Unknown	1	0.10	M71798012	<LOQ (0.0282 ug/mL)		
Control	C92256M	1	0.9275	Unknown	1	31.0	M71798013	0.0288			Unknown	1	1.69	M71798013	<LOQ (0.00989 ug/mL)			0.5382	Unknown	1	0.00	M71798013	<LOQ (0.0282 ug/mL)		
0.0 ppm	C92257M	1	0.9275	Unknown	1	40.5	M71798014	0.0376			Unknown	1	1.59	M71798014	<LOQ (0.00989 ug/mL)			0.5382	Unknown	1	0.00	M71798014	<LOQ (0.0282 ug/mL)		
	C92258M	1	0.9275	Unknown	1	39.0	M71798015	0.0362		27.8	Unknown	1	1.63	M71798015	<LOQ (0.00989 ug/mL)		NA	0.5382	Unknown	1	0.00	M71798015	<LOQ (0.0282 ug/mL)		NA
	C92260M	1	0.9275	Unknown	1	**	NA	**	0.0393	0.0109	Unknown	1	**	NA	**	<LOQ	NA	0.5382	Unknown	1	**	NA	**	<LOQ	NA
	C92396F	1	0.9275	Unknown	1	152	M71798018	0.141			Unknown	1	1.69	M71798018	<LOQ (0.00989 ug/mL)			0.5382	Unknown	1	3.35	M71798018	<LOQ (0.0282 ug/mL)		
	C92397F	1	0.9275	Unknown	1	130	M71798019	0.120			Unknown	1	1.64	M71798019	<LOQ (0.00989 ug/mL)			0.5382	Unknown	1	0.00	M71798019	<LOQ (0.0282 ug/mL)		
	C92398F	1	0.9275	Unknown	1	120	M71798020	0.111			Unknown	1	1.35	M71798020	<LOQ (0.00989 ug/mL)			0.5382	Unknown	1	0.00	M71798020	<LOQ (0.0282 ug/mL)		
	C92399F	1	0.9275	Unknown	1	156	M71798021	0.144		12.6	Unknown	1	1.54	M71798021	<LOQ (0.00989 ug/mL)		NA	0.5382	Unknown	1	0.00	M71798021	<LOQ (0.0282 ug/mL)		NA
	C92400F	1	0.9275	Unknown	1	121	M71798022	0.112	0.126	0.0159	Unknown	1	1.61	M71798022	<LOQ (0.00989 ug/mL)	<LOQ	NA	0.5382	Unknown	1	0.00	M71798022	<LOQ (0.0282 ug/mL)	<LOQ	NA
Group 9	C92325M	1	0.9275	Unknown	1	1534	M71798025	1.42	1		Unknown	1	11.2	M71798025	0.0112			0.5382	Unknown	1	437	M71798025	0.235		
Low Dose	C92326M	1	0.9275	Unknown	25	142	A80798014	3.30			Unknown	1	11.6	M71798026	0.0116			0.5382	Unknown	25	118	A80798015	1.59		
1.0 ppm	C92327M	1	0.9275	Unknown	25	77.4	A80798015	1.80			Unknown	1	11.6	M71798027	0.0116			0.5382	Unknown	1	553	M71798027	0.297		
	C92328M	1	0.9275	Unknown	25	83.0	A80798016	1.92		33.5	Unknown	1	7.98	M71798028	<LOQ (0.00989 ug/mL)	6.48		0.5382	Unknown	1	194	M71798028	0.104		124
	C92330M	1	0.9275	Unknown	25	108	A80798017	2.51	2.19	0.733	Unknown	1	10.8	M71798029	0.0108	0.0110	0.000716	0.5382	Unknown	1	470	M71798029	0.253	0.497	0.618
	C92464F 1/2	1	0.9275	Unknown	50	79.3	A80798020	3.68			Unknown	1	17.5	M71798032	0.0175			0.5382	Unknown	50	37.8	A80798020	1.02		
	C92464F 2/2	1	0.9275	Unknown	1	3384	M71798033	3.14	1		Unknown	1	17.9	M71798033	0.0179			0.5382	Unknown	1	1275	M71798033	0.686	1*		
	C92465F	1	0.9275	Unknown	50	59.9	A80798021	2.78			Unknown	1	14.7	M71798034	0.0147			0.5382	Unknown	1	587	M71798034	0.316		
	C92466F	1	0.9275	Unknown	50	79.6	A80798022	3.69			Unknown	1	12.9	M71798035	0.0129			0.5382	Unknown	1	300	M71798035	0.162		
	C92468F	1	0.9275	Unknown	50	50.1	A80798023	2.32		19.3	Unknown	1	13.1	M71798036	0.0131		21.6	0.5382	Unknown	1	317	M71798036	0.171		73.5
	C92469F	1	0.9275	Unknown	50	85.4	A80798024	3.96	3.26	0.629	Unknown	1	22.2	M71798037	0.0222	0.0164	0.00354	0.5382	Unknown	50	41.5	A80798024	1.12	0.578	0.425

* Sample went to dryness after initial injection and prior to dilutions.
1 Exceed above calibration range. Data is estimated.
** Sample spilled during extraction, no sample remaining.

NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate

Data Entered/By: 12/22/00 & 1/8/01 mmh
Data Verified/ By: 03/20/01 hjh, 5/22/01 mmh
Purity Entered/Verified 03/29/01 LAC/

		PFOS	PFOSA	PFOSAA
07/13/98	Initial LOQ ug/mL	24.8	4.94	10.5
	Corrected LOQ ug/mL	0.0230	0.00494	0.00565
07/17/98	Initial LOQ ug/mL	24.8	9.89	52.4
	Corrected LOQ ug/mL	0.0230	0.00989	0.0282
08/07/98	Initial LOQ ug/mL	24.8	9.89	52.4
	Corrected LOQ ug/mL	0.0230	0.00989	0.0282

Covance# 6329-228

Study	TOX(03), 104 Week Dietary Carcinogenicity Study with Narrow Range (98 1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats
Product Number/Test Substance	T-6316 (EtFOSE-OH)
Matrix	Rat Serum
Method/Revision	ETS-8-4.1 & ETS-8-5.1
Analytical Equipment System Number	Ruby 100699
Instrument Software/Version	Analyst 3.4
Filename	See listing to the right
R-Squared Value	See Attachments
Slope	See Attachment
Y-Intercept	See Attachments
Dates of Extraction/Analyst	04/20/00 SAL/KJK
Dates of Analysis/Analyst	04/21/00, 04/24/00, 08/15/00, 08/28/00 LAS/MMH/AHO/SAH
Date of Data Reduction/Analyst	05/05/00, 04/25/00, 08/16/00, 08/30/00 MMH/LAS

Sample Data

WEEK 53 RAT SERA

Group Dose	Sample #	Extraction Vol. mL	Surrogate Verified	PFOS Old Purity Correction Factor	PFOS Purity Correction Factor	PFOS Dilution Factor	PFOS Conc. ng/mL	Filename (lot 171)	Concentration of PFOS ng/mL or % Rec	21mm PFOS ng/mL	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	PFOSA Old Purity Correction Factor	PFOSA Purity Correction Factor	PFOSA Dilution Factor	PFOSA Conc. ng/mL	Filename (lot L-15709)	Concentration of PFOSA ng/mL or % Rec	Mean PFOSA ng/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	04200-H2O Blk-3	1	NA	0.9949	0.8640	1	0.00	R042100016	< LOQ (0.00428 ug/mL)			NA	0.9510	Unknown	1	0.00	R042100016	< LOQ (0.00516 ug/mL)		
	04200-H2O BB-4	1	NA	0.9949	0.8640	1	0.00	R042100017	< LOQ (0.00428 ug/mL)	<LOQ	NA	NA	0.9510	Unknown	1	0.20	R042100017	< LOQ (0.00516 ug/mL)	<LOQ	NA
Matrix Blk	RBS04200 Sera Blk-3	1	NA	0.9949	0.8640	1	0.00	R042100018	< LOQ (0.00428 ug/mL)			NA	0.9510	Unknown	1	0.00	R042100018	< LOQ (0.00516 ug/mL)		
	RBS04200 Sera Blk-4	1	NA	0.9949	0.8640	1	0.00	R042100019	< LOQ (0.00428 ug/mL)	<LOQ	NA	NA	0.9510	Unknown	1	0.00	R042100019	< LOQ (0.00516 ug/mL)	<LOQ	NA
	RTS04200 Sera Blk-3	1	NA	0.9949	0.8640	1	0.00	R042100020	< LOQ (0.00428 ug/mL)			NA	0.9510	Unknown	1	0.00	R042100020	< LOQ (0.00516 ug/mL)		
	RTS04200 Sera Blk-4	1	NA	0.9949	0.8640	1	0.00	R042100024	< LOQ (0.00428 ug/mL)	<LOQ	NA	NA	0.9510	Unknown	1	0.00	R042100034	< LOQ (0.00516 ug/mL)	<LOQ	NA
QC	RTS04200-MS-250-3	1	NA	NA	NA	1	182	R042100025	73%			NA	NA	NA	1	196	R042100025	79%		
	RTS04200-MSD-250-3	1	NA	NA	NA	1	197	R042100026	79%	76%	8%	NA	NA	NA	1	222	R042100026	90%	84%	12%
	RTS04200-MS-250-3	1	NA	NA	NA	1	NA	NA	NA			NA	NA	NA	1	NA	NA	NA		
	RTS04200-MSD-250-3	1	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	1	NA	NA	NA	NA	NA
Group 8 Control 0.0 ppm	CV2195M	1	NA	0.9949	0.8640	1	117	R042100027	0.101			NA	0.9510	Unknown	1	0.00	R042100027	< LOQ (0.00516 ug/mL)		
	CV2199M	1	NA	0.9949	0.8640	1	35.9	R042100028	0.0312			NA	0.9510	Unknown	1	0.00	R042100028	< LOQ (0.00516 ug/mL)		
	CV2205M	1	NA	0.9949	0.8640	1	32.0	R042100032	0.0278			NA	0.9510	Unknown	1	0.00	R042100032	< LOQ (0.00516 ug/mL)		
	CV2234M	1	NA	0.9949	0.8640	1	18.9	R042100033	0.0164		71.7	NA	0.9510	Unknown	1	0.00	R042100033	< LOQ (0.00516 ug/mL)		NA
	CV2246M	1	NA	0.9949	0.8640	1	79.1	R042100034	0.0687	0.0490	0.0352	NA	0.9510	Unknown	1	0.00	R042100034	< LOQ (0.00516 ug/mL)	<LOQ	NA
	CV2341F	1	NA	0.9949	0.8640	1	2.47	R042100035	< LOQ (0.00428 ug/mL)			NA	0.9510	Unknown	1	0.00	R042100035	< LOQ (0.00516 ug/mL)		
	CV2356F	1	NA	0.9949	0.8640	1	25.3	R042100036	0.0219			NA	0.9510	Unknown	1	0.00	R042100036	< LOQ (0.00516 ug/mL)		
	CV2372F	1	NA	0.9949	0.8640	1	104	R042100040	0.0905			NA	0.9510	Unknown	1	0.00	R042100040	< LOQ (0.00516 ug/mL)		
	CV2376F	1	NA	0.9949	0.8640	1	148	R042100041	0.129		77.7	NA	0.9510	Unknown	1	0.00	R042100041	< LOQ (0.00516 ug/mL)		NA
	CV2396F	1	NA	0.9949	0.8640	1	136	R042100042	0.118	0.0727	0.0565	NA	0.9510	Unknown	1	0.00	R042100042	< LOQ (0.00516 ug/mL)	<LOQ	NA
Group 9 Low Dose 1.0 ppm	CV2267M	1	NA	0.9949	0.8640	10	210	D081500025	1.82			NA	0.9510	Unknown	1	3.55	R042100043	< LOQ (0.00516 ug/mL)		
	CV2274M	1	NA	0.9949	0.8640	10	140	D081500026	1.21			NA	0.9510	Unknown	1	4.54	R042100044	< LOQ (0.00516 ug/mL)		
	CV2287M	1	NA	0.9949	0.8640	10	191	D081500027	1.65			NA	0.9510	Unknown	1	6.85	R042100048	0.00720		
	CV2284M	1	NA	0.9949	0.8640	10	164	D081500028	1.43		33.4	NA	0.9510	Unknown	1	10.4	R042100049	0.0109		37.3
	CV2320M	1	NA	0.9949	0.8640	1	759	D081500029_A	0.659	1.35	0.452	NA	0.9510	Unknown	1	4.09	R042100050	< LOQ (0.00516 ug/mL)	0.00672	0.00251
	CV2406F	1	NA	0.9949	0.8640	10	417	D081500032	3.62			NA	0.9510	Unknown	1	8.52	R042100051	0.00896		
	CV2411F	1	NA	0.9949	0.8640	10	450	D081500033	3.90			NA	0.9510	Unknown	1	8.41	R042100052	0.00884		
	CV2418F	1	NA	0.9949	0.8640	10	452	D081500034	3.92			NA	0.9510	Unknown	1	9.49	R042100056	0.00998		
	CV2422F	1	NA	0.9949	0.8640	10	386	D081500035	3.35		51.1	NA	0.9510	Unknown	1	13.9	R042100057	0.0146		24.7
	CV2438F	1	NA	0.9949	0.8640	1	338	R042100058	0.294	3.02	1.54	NA	0.9510	Unknown	1	8.21	R042100058	0.00863	0.0102	0.00252

* *Recoveries confirmed.* LAC 09/03/00

A = Estimated concentration, above the calibration range LAC 05/10/01

NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
M556 = $C_8F_{17}SO_2N((H)CH_2COO)$
PFOSEA = Perfluorooctane sulfonyl ethylamide

Date Entered/By: 05/12/00, 06/01/00, 08/22/00, 08/23/00, 09/03/00 LAC/MMH 05/09/01 mmh
Date Verified/ By: 03/30/01 tjh, 05/10/01 LAC, 5/23/01 mmh
Purity Entered/Verified: 03/29/01 LAC/

		PFOS	PFOSA	PFOSAA	EtFOSE	M556	PFOSEA
04/21/00	Initial LOQ ng/mL	4.93	4.91	49.1	24.7	49.3	9.71
	Corrected LOQ ng/mL	0.00428	0.00516	0.0503	0.0225	0.0494	0.00978
04/24/00	Initial LOQ ng/mL	NA	NA	24.7	NA	97.6	NA
	Corrected LOQ ng/mL	NA	NA	0.0253	NA	0.0977	NA
08/15/00	Initial LOQ ng/mL	4.93	NA	NA	4.92	24.8	24.6
	Corrected LOQ ng/mL	0.00428	NA	NA	0.00448	0.0248	0.0248
08/28/00	Initial LOQ ng/mL	4.93	4.91	NA	NA	4.93	NA
	Corrected LOQ ng/mL	0.00428	0.00516	NA	NA	0.00494	NA

See Week 53

Covance# 6329-228

Study:	TOX003: 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats
Product Number/Test Substance:	T-6316 (EtFOSE-OH)
Matrix:	Rat Serum
Method/Revision:	ETS-8-4.1 & ETS-8-5.1
Analytical Equipment System Number:	Ruby 100699
Instrument Software/Version:	Masslynx 3.4
Filename:	See listing to the right
R Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Dates of Extraction/Analyst:	04/20/00 SAL/KJK
Dates of Analysis/Analyst:	04/21/00, 04/24/00, 08/15/00, 08/28/00 IAS/MMH/HOJ/SAH
Date of Data Reduction/Analyst:	05/05/00, 04/25/00, 08/16/00, 08/30/00 MMH/IAS

Sample Data

WEEK 53 RAT SERA

			lot 98-0207-030-3									lot SD013							
Group Dose	Sample #	Surrogate Verified	PFOSAA Old Purity Correction Factor	PFOSAA Purity Correction Factor	PFOSAA Dilution Factor	PFOSAA Conc. ng/mL	Filename	Concentration of PFOSAA ng/mL or % Rec	Mean PFOSAA ng/mL	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	EtFOSE Old Purity Correction Factor	EtFOSE Purity Correction Factor	EtFOSE Dilution Factor	EtFOSE Conc. ng/mL	Filename	Concentration of EtFOSE ng/mL or % Rec	Mean EtFOSE ng/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	04200-H2O Blk-3	NA	0.9760	Unknown	1	0.00	R042400025	< LOQ (0.0253 ng/mL)			NA	0.9770	0.8890	1	0.00	R042100016	< LOQ (0.0225 ng/mL)		
	04200-H2O Blk-4	NA	0.9760	Unknown	1	0.00	R042400026	< LOQ (0.0253 ng/mL)	<LOQ	NA	NA	0.9770	0.8890	1	0.00	R042100017	< LOQ (0.0225 ng/mL)	<LOQ	NA
Matrix Blk	RBS04200-Sera Blk-3	NA	0.9760	Unknown	1	0.00	R042400027	< LOQ (0.0253 ng/mL)			NA	0.9770	0.8890	1	0.00	R042100018	< LOQ (0.0225 ng/mL)		
	RBS04200-Sera Blk-4	NA	0.9760	Unknown	1	0.00	R042400028	< LOQ (0.0253 ng/mL)	<LOQ	NA	NA	0.9770	0.8890	1	0.00	R042100019	< LOQ (0.0225 ng/mL)	<LOQ	NA
	RTS04200-Sera Blk-3	NA	0.9760	Unknown	1	0.00	R042400029	< LOQ (0.0253 ng/mL)			NA	0.9770	0.8890	1	0.00	R042100020	< LOQ (0.0225 ng/mL)		
	RTS04200-Sera Blk-4	NA	0.9760	Unknown	1	0.00	R042400033	< LOQ (0.0253 ng/mL)	<LOQ	NA	NA	0.9770	0.8890	1	0.00	R042100024	< LOQ (0.0225 ng/mL)	<LOQ	NA
QC	RTS04200-MS-250-3	NA	NA	NA	1	207	R042100025	83%			NA	NA	NA	1	40.1	R042100025	16% *		
	RTS04200-MSD-250-3	NA	NA	NA	1	244	R042100026	99%	91%	17%	NA	NA	NA	1	45.9	R042100026	18% *	17%	13%
	RTS04200-MS-250-3	NA	NA	NA	1	NA	NA	NA			NA	NA	NA	1	78.8	D081500021	32% 2nd*		
	RTS04200-MSD-250-3	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	1	90.2	D081500022	36% 2nd*	34%	13%
Group 8 Control 0.0 ppm	C92195M	NA	0.9760	Unknown	1	0.00	R042400036	< LOQ (0.0253 ng/mL)			NA	0.9770	0.8890	1	0.00	R042100027	< LOQ (0.0225 ng/mL)		
	C92199M	NA	0.9760	Unknown	1	0.00	R042400037	< LOQ (0.0253 ng/mL)			NA	0.9770	0.8890	1	0.00	R042100028	< LOQ (0.0225 ng/mL)		
	C92205M	NA	0.9760	Unknown	1	0.00	R042400041	< LOQ (0.0253 ng/mL)			NA	0.9770	0.8890	1	0.00	R042100032	< LOQ (0.0225 ng/mL)		
	C92238/M	NA	0.9760	Unknown	1	0.00	R042400042	< LOQ (0.0253 ng/mL)		NA	NA	0.9770	0.8890	1	0.00	R042100033	< LOQ (0.0225 ng/mL)		NA
	C92246/M	NA	0.9760	Unknown	1	0.00	R042400043	< LOQ (0.0253 ng/mL)	<LOQ	NA	NA	0.9770	0.8890	1	0.00	R042100034	< LOQ (0.0225 ng/mL)	<LOQ	NA
	C92341F	NA	0.9760	Unknown	1	0.00	R042400044	< LOQ (0.0253 ng/mL)			NA	0.9770	0.8890	1	0.00	R042100035	< LOQ (0.0225 ng/mL)		
	C92356F	NA	0.9760	Unknown	1	0.00	R042400045	< LOQ (0.0253 ng/mL)			NA	0.9770	0.8890	1	0.00	R042100036	< LOQ (0.0225 ng/mL)		
	C92372F	NA	0.9760	Unknown	1	0.00	R042400049	< LOQ (0.0253 ng/mL)			NA	0.9770	0.8890	1	0.00	R042100040	< LOQ (0.0225 ng/mL)		
	C92376F	NA	0.9760	Unknown	1	0.00	R042400050	< LOQ (0.0253 ng/mL)		NA	NA	0.9770	0.8890	1	0.00	R042100041	< LOQ (0.0225 ng/mL)		NA
	C92390F	NA	0.9760	Unknown	1	0.00	R042400051	< LOQ (0.0253 ng/mL)	<LOQ	NA	NA	0.9770	0.8890	1	0.00	R042100042	< LOQ (0.0225 ng/mL)	<LOQ	NA
Group 9 Low Dose 1.0 ppm	C92267/M	NA	0.9760	Unknown	1	92.3	R042100043	0.0946			NA	0.9770	0.8890	1	0.00	R042100043	< LOQ (0.0225 ng/mL)		
	C92276/M	NA	0.9760	Unknown	1	273	R042100044	0.279			NA	0.9770	0.8890	1	0.00	R042100044	< LOQ (0.0225 ng/mL)		
	C92287/M	NA	0.9760	Unknown	1	596	R042100048	0.610			NA	0.9770	0.8890	1	0.00	R042100048	< LOQ (0.0225 ng/mL)		
	C92288/M	NA	0.9760	Unknown	1	304	R042100049	0.311		65.3	NA	0.9770	0.8890	1	0.00	R042100049	< LOQ (0.0225 ng/mL)		NA
	C92320/M	NA	0.9760	Unknown	1	186	R042100050	0.191	0.297	0.194	NA	0.9770	0.8890	1	0.00	R042100050	< LOQ (0.0225 ng/mL)	<LOQ	NA
	C92406F	NA	0.9760	Unknown	1	189	R042100051	0.194			NA	0.9770	0.8890	1	0.00	R042100051	< LOQ (0.0225 ng/mL)		
	C92413F	NA	0.9760	Unknown	1	201	R042100052	0.206			NA	0.9770	0.8890	1	0.00	R042100052	< LOQ (0.0225 ng/mL)		
	C92418F	NA	0.9760	Unknown	1	332	R042100056	0.340			NA	0.9770	0.8890	1	0.00	R042100056	< LOQ (0.0225 ng/mL)		
	C92423F	NA	0.9760	Unknown	1	426	R042100057	0.436		60.3	NA	0.9770	0.8890	1	0.00	R042100057	< LOQ (0.0225 ng/mL)		NA
	C92436F	NA	0.9760	Unknown	1	186	R042100058	0.191	0.273	0.110	NA	0.9770	0.8890	1	0.00	R042100058	< LOQ (0.0225 ng/mL)	<LOQ	NA

* Recoveries confirmed: LAC 09/03/00

A = Estimated concentration, above the calibration range 1

Date Entered/By:	05/12/00, 06/01/00, 08/22/00, 08/
Date Verified/ By:	03/20/01 kjh, 05/10/01 LAC, M
Purity Entered/Verified:	03/29/01 LAC/

04/21/00	Initial LOQ ng/mL
	Corrected LOQ ng/mL
04/24/00	Initial LOQ ng/mL
	Corrected LOQ ng/mL
08/15/00	Initial LOQ ng/mL
	Corrected LOQ ng/mL
08/28/00	Initial LOQ ng/mL
	Corrected LOQ ng/mL

Covance# 6329-228

Study	TOX003, 104 Week Dietary Carcinogenicity Study with Narrow Range (R.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats
Product Number/Test Substance	T-6316 (EtFOSE Oil)
Matrix	Rat Serum
Method/Revision	ETS 8-4.1 & ETS-8-5.1
Analytical Equipment System Number	Ruby 100699
Instrument Software/Version	Analyst 1.4
Principle	See listing to the right
R-Squared Value	See Attachments
Slope	See Attachments
Y-Intercept	See Attachments
Dates of Extraction/Analyst	04/20/00 SAL/KJK
Dates of Analysis/Analyst	04/21/00, 04/24/00, 05/15/00, 08/28/00 IAS/MMH/HJJ/SAH
Date of Data Reduction/Analyst	05/05/00, 04/25/00, 08/16/00, 08/30/00 MMH/IAS

Sample Data

WEEK 53 RAT SERA

lot NB113047-80

lot 529

Group Dose	Sample #	Surrogate Verified	M556 Old Purity Correction Factor	M556 Purity Correction Factor	M556 Dilution Factor	M556 Conc. ng/mL	Filename	Concentration of M556 ng/mL or % Rec	Mean M556 ng/mL	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	PFOSEA Old Purity Correction Factor	PFOSEA Purity Correction Factor	PFOSEA Dilution Factor	PFOSEA Conc. ng/mL	Filename	Concentration of PFOSEA ng/mL or % Rec	Mean PFOSEA ng/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	04200-H2O Blk-3	NA	0.9989	Unknown	1	0.67	R082800015	<LOQ (0.00494 ng/mL)			NA	0.9930	Unknown	1	0.00	R042100016	< LOQ (0.00978 ng/mL)		
	04200-H2O Blk-4	NA	0.9989	Unknown	1	0.34	R082800020	<LOQ (0.00494 ng/mL)	<LOQ	NA	NA	0.9930	Unknown	1	0.00	R042100017	< LOQ (0.00978 ng/mL)	<LOQ	NA
Matrix Blk	RBS04200-Sera Blk-3	NA	0.9989	Unknown	1	0.88	R082800016	<LOQ (0.00494 ng/mL)			NA	0.9930	Unknown	1	0.00	R042100018	< LOQ (0.00978 ng/mL)		
	RBS04200-Sera Blk-4	NA	0.9989	Unknown	1	0.00	R082800021	<LOQ (0.00494 ng/mL)	<LOQ	NA	NA	0.9930	Unknown	1	0.00	R042100019	< LOQ (0.00978 ng/mL)	<LOQ	NA
	RTS04200-Sera Blk-3	NA	0.9989	Unknown	1	0.00	R082800017	<LOQ (0.00494 ng/mL)			NA	0.9930	Unknown	1	0.00	R042100020	< LOQ (0.00978 ng/mL)		
	RTS04200-Sera Blk-4	NA	0.9989	Unknown	1	0.00	R082800022	<LOQ (0.00494 ng/mL)	<LOQ	NA	NA	0.9930	Unknown	1	0.00	R042100024	< LOQ (0.00978 ng/mL)	<LOQ	NA
QC	RTS04200-MS-250-3	NA	NA	NA	1	268	R042400034	108%			NA	NA	NA	1	89.2	R042100025	36% *		
	RTS04200-MSD-250-3	NA	NA	NA	1	275	R042400035	110%	109%	2%	NA	NA	NA	1	119	R042100026	48% *	42%	28%
	RTS04200-MS-250-3	NA	NA	NA	1	NA	NA	NA			NA	NA	NA	1	106	D081500021	43% 2nd*		
	RTS04200-MSD-250-3	NA	NA	NA	1	NA	NA	NA	NA	NA	NA	NA	NA	1	133	D081500022	54% 2nd*	48%	22%
Group 8 Control 0.0 ppm	C92195M	NA	0.9989	Unknown	1	3.43	R082800025	<LOQ (0.00494 ng/mL)			NA	0.9930	Unknown	1	0.00	R042100027	< LOQ (0.00978 ng/mL)		
	C92199M	NA	0.9989	Unknown	1	0.00	R082800026	<LOQ (0.00494 ng/mL)			NA	0.9930	Unknown	1	0.00	R042100028	< LOQ (0.00978 ng/mL)		
	C92205M	NA	0.9989	Unknown	1	0.00	R082800027	<LOQ (0.00494 ng/mL)			NA	0.9930	Unknown	1	0.00	R042100032	< LOQ (0.00978 ng/mL)		
	C92218M	NA	0.9989	Unknown	1	0.00	R082800028	<LOQ (0.00494 ng/mL)		NA	NA	0.9930	Unknown	1	0.00	R042100033	< LOQ (0.00978 ng/mL)		NA
	C92246M	NA	0.9989	Unknown	1	0.00	R082800029	<LOQ (0.00494 ng/mL)	<LOQ	NA	NA	0.9930	Unknown	1	0.00	R042100034	< LOQ (0.00978 ng/mL)	<LOQ	NA
	C92341F	NA	0.9989	Unknown	1	1.63	R082800032	<LOQ (0.00494 ng/mL)			NA	0.9930	Unknown	1	0.00	R042100035	< LOQ (0.00978 ng/mL)		
	C92350F	NA	0.9989	Unknown	1	0.00	R082800033	<LOQ (0.00494 ng/mL)			NA	0.9930	Unknown	1	0.00	R042100036	< LOQ (0.00978 ng/mL)		
	C92372F	NA	0.9989	Unknown	1	0.00	R082800034	<LOQ (0.00494 ng/mL)			NA	0.9930	Unknown	1	0.00	R042100040	< LOQ (0.00978 ng/mL)		
	C92376F	NA	0.9989	Unknown	1	0.00	R082800035	<LOQ (0.00494 ng/mL)		NA	NA	0.9930	Unknown	1	0.00	R042100041	< LOQ (0.00978 ng/mL)		NA
	C92390F	NA	0.9989	Unknown	1	0.00	R082800036	<LOQ (0.00494 ng/mL)	<LOQ	NA	NA	0.9930	Unknown	1	0.00	R042100042	< LOQ (0.00978 ng/mL)	<LOQ	NA
Group 9 Low Dose 1.0 ppm	C92267M	NA	0.9989	Unknown	1	94.6	R042400052	0.0947			NA	0.9930	Unknown	1	0.00	R042100043	< LOQ (0.00978 ng/mL)		
	C92274M	NA	0.9989	Unknown	1	136	R042400053	0.136			NA	0.9930	Unknown	1	0.00	R042100044	< LOQ (0.00978 ng/mL)		
	C92287M	NA	0.9989	Unknown	1	162	R042400057	0.162			NA	0.9930	Unknown	1	0.00	R042100048	< LOQ (0.00978 ng/mL)		
	C92288M	NA	0.9989	Unknown	1	106	R042400058	0.106		24.2	NA	0.9930	Unknown	1	0.00	R042100049	< LOQ (0.00978 ng/mL)		NA
	C92320M	NA	0.9989	Unknown	1	98.9	R042400059	0.0990	0.120	0.0289	NA	0.9930	Unknown	1	0.00	R042100050	< LOQ (0.00978 ng/mL)	<LOQ	NA
	C92406F	NA	0.9989	Unknown	1	207	R042400060	0.207			NA	0.9930	Unknown	1	0.00	R042100051	< LOQ (0.00978 ng/mL)		
	C92411F	NA	0.9989	Unknown	1	202	R042400061	0.202		1	NA	0.9930	Unknown	1	0.00	R042100052	< LOQ (0.00978 ng/mL)		
	C92418F	NA	0.9989	Unknown	1	183	R042400065	0.183			NA	0.9930	Unknown	1	0.00	R042100056	< LOQ (0.00978 ng/mL)		
	C92422F	NA	0.9989	Unknown	1	311	R042400066	0.311		35.8	NA	0.9930	Unknown	1	0.00	R042100057	< LOQ (0.00978 ng/mL)		NA
	C92438F	NA	0.9989	Unknown	1	109	R042400067	0.109	0.202	0.0723	NA	0.9930	Unknown	1	0.00	R042100058	< LOQ (0.00978 ng/mL)	<LOQ	NA

* Recoveries confirmed. LAC 09/03/00

A = Estimated concentration, above the calibration range. 1

Date Entered/By: 05/12/00, 06/01/00, 08/22/00, 08/
Date Verified/ By: 03/20/01 hjh, 05/10/01 LAC. 5/
Purity Entered/Verified: 03/29/01 LAC/

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
M556 = C8F17SO2N((H)C)H2COO)
PFOSEA = Perfluorooctane sulfonyl ethylamido

04/21/00	Initial LOQ ng/mL
	Corrected LOQ ng/mL
04/24/00	Initial LOQ ng/mL
	Corrected LOQ ng/mL
05/15/00	Initial LOQ ng/mL
	Corrected LOQ ng/mL
08/28/00	Initial LOQ ng/mL
	Corrected LOQ ng/mL

Covance# 6329-228

Study:	TOX003, 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats	TOX003, 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamidoethanol
Product Number/Test Substance:	T-6316 (EtFOSE-OH)	T-6316 (EtFOSE-OH)
Matrix:	Rat Serum	Rat Serum
Method/Revision:	ETS-8-4.1 & ETS-8-5.1	ETS-8-4.1 & ETS-8-5.1
Analytical Equipment System Number:	Davey 070799	Davey 070799
Instrument Software/Version:	MassLynx 3.4	MassLynx 3.4
Filename:	See Attachments	See Attachments
R-Squared Value:	See Attachments	See Attachments
Slope:	See Attachments	See Attachments
Y-Intercept:	See Attachments	See Attachments
Dates of Extraction/Analyst:	06/29/00 SAL/KJK	06/29/00 SAL/KJK
Dates of Analysis/Analyst:	07/06/00, 07/19/00, 07/24/00, 08/24/00 MMH/SAH	07/06/00, 07/19/00, 07/24/00, 08/24/00 MMH/SAH
Date of Data Reduction/Analyst:	07/07/00, 07/25/00, 07/27/00, 08/28/00 MMH/HOJ	07/07/00, 07/25/00, 07/27/00, 08/28/00 MMH/HOJ

Sample Data

WEEK 105 RAT SERA

lot 171 | lot L-15709 | lot 98-0207-036-3

Group Dose	Sample #	Extraction Vol. mL	Surrogate Verified	PFOS Old Purity Correction Factor	PFOS Purity Correction Factor	PFOS Dilution Factor	PFOS Conc. ng/mL	Filename	Concentration of PFOS ng/mL or % Rec	Mean PFOS ng/mL	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	PFOSA Old Purity Correction Factor	PFOSA Purity Correction Factor	PFOSA Dilution Factor	PFOSA Conc. ng/mL	Filename	Concentration of PFOSA ng/mL or % Rec	Mean PFOSA ng/mL	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	PFOSA Old Purity Correction Factor	PFOSA Purity Correction Factor	PFOSAA Dilution Factor	PFOSAA Conc. ng/mL	Filename
Method Blk	06290-H2O Blk-5	1	NA	0.9949	0.8640	1	0.00	R072400007	<LOQ (0.00428 ng/mL)			NA	0.9510	Unknown	1	0.00	D070600037	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.16	R072400007
	06290-H2O Blk-6	1	NA	0.9949	0.8640	1	0.33	R072400021	<LOQ (0.00428 ng/mL)	<LOQ	NA	NA	0.9510	Unknown	1	0.00	D070600021	<LOQ (0.00516 ng/mL)	<LOQ	NA	NA	0.9760	Unknown	1	1.95	R072400021
Matrix Blk	RBS06290-Sera Blk-5	1	NA	0.9949	0.8640	1	0.39	R072400008	<LOQ (0.00428 ng/mL)			NA	0.9510	Unknown	1	0.00	D070600008	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.04	R072400008
	RBS06290-Sera Blk-6	1	NA	0.9949	0.8640	1	0.47	R072400022	<LOQ (0.00428 ng/mL)	<LOQ	NA	NA	0.9510	Unknown	1	0.00	D070600022	<LOQ (0.00516 ng/mL)	<LOQ	NA	NA	0.9760	Unknown	1	1.05	R072400022
Matrix Blk	RTS06290-Sera Blk-5	1	NA	0.9949	0.8640	1	3.23	R072400009	<LOQ (0.00428 ng/mL)			NA	0.9510	Unknown	1	0.00	D070600009	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.07	R072400009
	RTS06290-Sera Blk-6	1	NA	0.9949	0.8640	1	3.95	R072400023	<LOQ (0.00428 ng/mL)	<LOQ	NA	NA	0.9510	Unknown	1	0.00	D070600023	<LOQ (0.00516 ng/mL)	<LOQ	NA	NA	0.9760	Unknown	1	1.40	R072400023
QC	RTS06290-MS-250 ppb-5	1	NA	NA	NA	1	340	D070600024	135% *			NA	NA	NA	1	307	D070600024	124%			NA	NA	NA	1	342	D070600024
	RTS06290-MSD-250 ppb-5	1	NA	NA	NA	1	345	D070600025	137% *	136%	1%	NA	NA	NA	1	310	D070600025	126%	125%	1%	NA	NA	NA	1	347	D070600025
	RTS06290-MS-250 ppb-5	1	NA	NA	NA	1	343	R072400034	137% 2nd*			NA	NA	NA	NA	NA		NA			NA	NA	NA	NA	NA	
	RTS06290-MSD-250 ppb-5	1	NA	NA	NA	1	365	R072400025	146% 2nd*	141%	6%	NA	NA	NA	NA	NA		NA	NA		NA	NA	NA	NA	NA	
Group 8 Control 0.0 ppm	C92197/M	1	NA	0.9949	0.8640	1	4.58	R072400029	<LOQ (0.00428 ng/mL)			NA	0.9510	Unknown	1	0.00	D070600028	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.93	R072400029
	C92205/M	1	NA	0.9949	0.8640	1	3.01	R072400030	<LOQ (0.00428 ng/mL)			NA	0.9510	Unknown	1	0.00	D070600029	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.60	R072400030
	C92206/M	1	NA	0.9949	0.8640	1	3.39	R072400031	<LOQ (0.00428 ng/mL)			NA	0.9510	Unknown	1	0.00	D070600030	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.51	R072400031
	C92209/M	1	NA	0.9949	0.8640	1	3.37	R072400032	<LOQ (0.00428 ng/mL)			NA	0.9510	Unknown	1	0.00	D070600031	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.35	R072400032
	C92214/M	1	NA	0.9949	0.8640	1	27.8	R072400033	0.0242			NA	0.9510	Unknown	1	0.00	D070600032	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.75	R072400033
	C92222/M	1	NA	0.9949	0.8640	1	7.75	R072400037	0.00673			NA	0.9510	Unknown	1	0.00	D070600035	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	2.31	R072400037
	C92231/M	1	NA	0.9949	0.8640	1	6.06	R072400038	<LOQ (0.00428 ng/mL)			NA	0.9510	Unknown	1	0.00	D070600036	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.47	R072400038
	C92233/M	1	NA	0.9949	0.8640	1	2.41	R072400039	<LOQ (0.00428 ng/mL)			NA	0.9510	Unknown	1	0.00	D070600037	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.95	R072400039
	C92239/M	1	NA	0.9949	0.8640	1	70.1	R072400040	0.0608		132	NA	0.9510	Unknown	1	0.00	D070600038	<LOQ (0.00516 ng/mL)		NA	NA	0.9760	Unknown	1	1.40	R072400040
	C92252/M	1	NA	0.9949	0.8640	1	23.0	R072400041	0.0200	0.0137	0.0181	NA	0.9510	Unknown	1	0.00	D070600039	<LOQ (0.00516 ng/mL)	<LOQ	NA	NA	0.9760	Unknown	1	1.23	R072400041
	C92331/F	1	NA	0.9949	0.8640	1	41.2	R072400045	0.0358			NA	0.9510	Unknown	1	0.00	D070600042	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.62	R072400045
	C92334/F	1	NA	0.9949	0.8640	1	50.6	R072400046	0.0439			NA	0.9510	Unknown	1	0.00	D070600043	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.35	R072400046
	C92348/F	1	NA	0.9949	0.8640	1	53.9	R072400047	0.0468			NA	0.9510	Unknown	1	0.00	D070600044	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.39	R072400047
	C92354/F	1	NA	0.9949	0.8640	1	15.9	R072400048	0.0138			NA	0.9510	Unknown	1	0.00	D070600045	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.55	R072400048
	C92358/F	1	NA	0.9949	0.8640	1	4.43	R072400049	<LOQ (0.00428 ng/mL)			NA	0.9510	Unknown	1	0.00	D070600046	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.27	R072400049
	C92360/F	1	NA	0.9949	0.8640	1	23.6	R072400053	0.0205			NA	0.9510	Unknown	1	0.00	D070600049	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.04	R072400053
	C92362/F	1	NA	0.9949	0.8640	1	8.99	R072400054	0.00781			NA	0.9510	Unknown	1	0.00	D070600050	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.04	R072400054
	C92369/F	1	NA	0.9949	0.8640	1	36.3	R072400055	0.0333			NA	0.9510	Unknown	1	0.00	D070600051	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	1.75	R072400055
	C92374/F	1	NA	0.9949	0.8640	1	30.1	R072400056	0.0262		56.5	NA	0.9510	Unknown	1	0.00	D070600052	<LOQ (0.00516 ng/mL)		NA	NA	0.9760	Unknown	1	1.17	R072400056
	C92381/F	1	NA	0.9949	0.8640	1	27.8	R072400057	0.0241	0.0256	0.0145	NA	0.9510	Unknown	1	0.00	D070600053	<LOQ (0.00516 ng/mL)	<LOQ	NA	NA	0.9760	Unknown	1	1.29	R072400057
Group 9 Low Dose 1.0 ppm	C92261/M	1	NA	0.9949	0.8640	1	684	D070600056	0.594			NA	0.9510	Unknown	1	5.58	D070600056	0.00587			NA	0.9760	Unknown	1	247	D070600056
	C92266/M	1	NA	0.9949	0.8640	10	110	R072400061	0.953			NA	0.9510	Unknown	1	3.82	D070600057	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	121	D070600057
	C92270/M	1	NA	0.9949	0.8640	20	183	R072400062	3.17			NA	0.9510	Unknown	1	14.4	D070600058	0.0152			NA	0.9760	Unknown	1	640	D070600058
	C92278/M	1	NA	0.9949	0.8640	10	100	R072400063	0.868			NA	0.9510	Unknown	1	9.79	D070600059	0.0103			NA	0.9760	Unknown	1	362	D070600059
	C92281/M	1	NA	0.9949	0.8640	1	495	D070600060	0.430			NA	0.9510	Unknown	1	3.59	D070600060	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	196	D070600060
	C92284/M	1	NA	0.9949	0.8640	20	174	R072400064	3.01			NA	0.9510	Unknown	1	6.06	D070600063	0.00637			NA	0.9760	Unknown	1	343	D070600063
	C92309/M	1	NA	0.9949	0.8640	1	99.0	D070600064	0.0860			NA	0.9510	Unknown	1	2.05	D070600064	<LOQ (0.00516 ng/mL)			NA	0.9760	Unknown	1	134	D070600064
	C92311/M	1	NA	0.9949	0.8640	1	503	D070600065	0.437			NA	0.9510	Unknown	1	8.05	D070600065	0.00846			NA	0.9760	Unknown	1	225	D070600065
	C92318/M	1	NA	0.9949	0.8640	10	154	R072400065	1.33		97.8	NA	0.9510	Unknown	1	3.52	D070600066	<LOQ (0.00516 ng/mL)		45.8	NA	0.9760	Unknown	1	164	D070600066
	C92321/M	1	NA	0.9949	0.8640	1	387	D070600067	0.336	1.12	1.10	NA	0.9510	Unknown	1	1.41	D070600067	<LOQ (0.00516 ng/mL)	0.00720	0.00330	NA	0.9760	Unknown	1	131	D070600067
	C92410/F	1	NA	0.9949	0.8640	20	131	R072400069	2.27			NA	0.9510	Unknown	1	8.73	D070600070	0.00918			NA	0.9760	Unknown	1	161	D070600070
	C92416/F	1	NA	0.9949	0.8640	20	137	R072400070	2.38			NA	0.9510	Unknown	1	11.3	D070600071	0.0119			NA	0.9760	Unknown	1	505	D070600071
	C92419/F	1	NA	0.9949	0.8640	1	623	D070600072	0.541			NA	0.9510	Unknown	1	8.39	D070600072	0.00882			NA	0.9760	Unknown	1	138	D070600072
	C92421/F	1	NA	0.9949	0.8640	10	101	R072400071	0.879			NA	0.9510	Unknown	1	5.59	D070600073	0.00588			NA	0.9760	Unknown	1	255	D070600073
	C92430/F	1	NA	0.9949	0.8640	10	199	R072400072	1.73			NA	0.9510	Unknown	1	7.13	D070600074	0.00750			NA	0.9760	Unknown	1	183	D070600074
	C92431/F	1	NA	0.9949	0.8640	10	124	R072400073	1.08			NA	0.9510	Unknown	1	5.49	D070600077	0.00577			NA	0.9760	Unknown	1	175	D070600077
	C92439/F	1	NA	0.9949	0.8640	20	108	R072400077	1.88			NA	0.9510	Unknown	1	7.26	D070600078	0.00763			NA	0.9760	Unknown	1	121	D070600078
	C92443/F	1	NA	0.9949	0.8640	10	148	R072400078	1.28			NA	0.9510	Unknown	1	7.29	D070600079	0.00767			NA	0.9760	Unknown	1	203	D070600079
	C92451/F	1	NA	0.9949	0.8640	20	113	R072400079	1.96		39.1	NA	0.9510	Unknown	1	9.23	D070600080	0.00971		24.9	NA	0.9760	Unknown	1	555	D070600080
	C92452/F	1	NA	0.9949	0.8640	20	124	R072400080	2.15	1.61	0.631	NA	0.9510	Unknown	1	5.37	D070600081	0.00565	0.00797	0.00198	NA	0.9760	Unknown	1	231	D070600081

* High recoveries confirmed with 07/24/00 and 08/24/00 analysis LAC 08/21/00 and 09/03/00

NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
M556 = $C_8F_{17}SO_2N(H)CH_2COO^-$
PFOSEA = Perfluorooctane sulfonyl ethylamide

Date Entered/By: 07/13/00, 08/15/00, 08/21/00, 09/03/00 LAC
Date Verified/ By: 03/20/01 kjk, 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC

		PFOS	PFOSA	PFOSAA	EtFOSE	M556	PFOSEA
07/06/00	Initial LOQ ng/mL	4.93	4.91	4.91	4.92	4.93	4.90
	Corrected LOQ ng/mL	0.0428	0.00516	0.0503	0.00448	0.00494	0.00493
07/19/00	Initial LOQ ng/mL	4.93	NA	4.91	NA	24.8	NA
	Corrected LOQ ng/mL	0.00428	NA	0.00503	NA	0.0248	NA
07/24/00	Initial LOQ ng/mL	4.93	NA	4.91	NA	24.8	NA
	Corrected LOQ ng/mL	0.00428	NA	0.00503	NA	0.0248	NA
08/24/00	Initial LOQ ng/mL	9.76	NA	NA	NA	24.8	NA
	Corrected LOQ ng/mL	0.00848	NA	NA	NA	0.0248	NA

FACT-M 4.0

Covance# 6329-228

Study: [illegible]nside Ethanol in Rats
Product Number(Test Substance):
Matrix:
Method/Revision:
Analytical Equipment System Number:
Instrument Software/Version:
Filename:
R-Squared Value:
Slope:
Y-Intercept:
Dates of Extraction/Analyst:
Dates of Analysis/Analyst:
Date of Data Reduction/Analyst:

Sample Data

WEEK 105 RAT SERA lot SD013

Group Dose	Sample #	Concentration of PFOSAA ug/mL or % Rec	Mean PFOSAA ug/mL	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	EtFOSE Old Purity Correction Factor	EtFOSE Purity Correction Factor	EtFOSE Dilution Factor	EtFOSE Conc. ug/mL	Filename	Concentration of EtFOSE ug/mL or % Rec	Mean EtFOSE ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	06290-H2O Blk-5	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.40	D070600007	<LOQ (0.00448 ug/mL)		
	06290-H2O Blk-6	<LOQ (0.00503 ug/mL)	<LOQ	NA	NA	0.9770	0.8890	1	1.42	D070600021	<LOQ (0.00448 ug/mL)	<LOQ	NA
Matrix Blk	RBS06290-Sera Blk-5	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.00	D070600008	<LOQ (0.00448 ug/mL)		
	RBS06290-Sera Blk-6	<LOQ (0.00503 ug/mL)	<LOQ	NA	NA	0.9770	0.8890	1	1.54	D070600022	<LOQ (0.00448 ug/mL)	<LOQ	NA
Matrix Blk	RTS06290-Sera Blk-5	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.37	D070600009	<LOQ (0.00448 ug/mL)		
	RTS06290-Sera Blk-6	<LOQ (0.00503 ug/mL)	<LOQ	NA	NA	0.9770	0.8890	1	0.00	D070600023	<LOQ (0.00448 ug/mL)	<LOQ	NA
QC	RTS06290-MS-250 ppb-5	138%			NA	NA	NA	1	148	D070600024	60%		
	RTS06290-MSD-250 ppb-5	140%	139%	2%	NA	NA	NA	1	151	D070600025	61%	60%	2%
	RTS06290-MS-250 ppb-5	NA			NA	NA	NA	NA	NA	NA	NA		
	RTS06290-MSD-250 ppb-5	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Group 8 Control 0.0 ppm	C92197dM	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	1.28	D070600028	<LOQ (0.00448 ug/mL)		
	C92203dM	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.00	D070600029	<LOQ (0.00448 ug/mL)		
	C92208dM	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.95	D070600030	<LOQ (0.00448 ug/mL)		
	C92209dM	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.56	D070600031	<LOQ (0.00448 ug/mL)		
	C92218dM	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.15	D070600032	<LOQ (0.00448 ug/mL)		
	C92222dM	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.17	D070600035	<LOQ (0.00448 ug/mL)		
	C92231dM	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.41	D070600036	<LOQ (0.00448 ug/mL)		
	C92233dM	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.38	D070600037	<LOQ (0.00448 ug/mL)		
	C92239dM	<LOQ (0.00503 ug/mL)		NA	NA	0.9770	0.8890	1	0.12	D070600038	<LOQ (0.00448 ug/mL)		NA
	C92253dM	<LOQ (0.00503 ug/mL)	<LOQ	NA	NA	0.9770	0.8890	1	0.97	D070600039	<LOQ (0.00448 ug/mL)	<LOQ	NA
	C9233dF	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.87	D070600042	<LOQ (0.00448 ug/mL)		
	C92334dF	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.77	D070600043	<LOQ (0.00448 ug/mL)		
	C92348dF	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.34	D070600044	<LOQ (0.00448 ug/mL)		
	C92354dF	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.36	D070600045	<LOQ (0.00448 ug/mL)		
	C92358dF	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.85	D070600046	<LOQ (0.00448 ug/mL)		
	C92360dF	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.57	D070600049	<LOQ (0.00448 ug/mL)		
	C92362dF	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.06	D070600050	<LOQ (0.00448 ug/mL)		
	C92369dF	<LOQ (0.00503 ug/mL)			NA	0.9770	0.8890	1	0.27	D070600051	<LOQ (0.00448 ug/mL)		
	C92374dF	<LOQ (0.00503 ug/mL)		NA	NA	0.9770	0.8890	1	0.25	D070600052	<LOQ (0.00448 ug/mL)		NA
	C9238dF	<LOQ (0.00503 ug/mL)	<LOQ	NA	NA	0.9770	0.8890	1	0.24	D070600053	<LOQ (0.00448 ug/mL)	<LOQ	NA
Group 9 Low Dose 1.0 ppm	C92261dM	0.253			NA	0.9770	0.8890	1	0.92	D070600056	<LOQ (0.00448 ug/mL)		
	C92266dM	0.124			NA	0.9770	0.8890	1	0.54	D070600057	<LOQ (0.00448 ug/mL)		
	C92270dM	0.656			NA	0.9770	0.8890	1	0.79	D070600058	<LOQ (0.00448 ug/mL)		
	C92278dM	0.371			NA	0.9770	0.8890	1	2.57	D070600059	<LOQ (0.00448 ug/mL)		
	C92281dM	0.201			NA	0.9770	0.8890	1	0.15	D070600060	<LOQ (0.00448 ug/mL)		
	C92288dM	0.249			NA	0.9770	0.8890	1	1.32	D070600063	<LOQ (0.00448 ug/mL)		
	C92309dM	0.138			NA	0.9770	0.8890	1	2.19	D070600064	<LOQ (0.00448 ug/mL)		
	C92311dM	0.231			NA	0.9770	0.8890	1	1.37	D070600065	<LOQ (0.00448 ug/mL)		
	C92316dM	0.168		63.4	NA	0.9770	0.8890	1	0.08	D070600066	<LOQ (0.00448 ug/mL)		NA
	C92321dM	0.135	0.252	0.160	NA	0.9770	0.8890	1	0.06	D070600067	<LOQ (0.00448 ug/mL)	<LOQ	NA
	C92410F	0.165			NA	0.9770	0.8890	1	0.72	D070600070	<LOQ (0.00448 ug/mL)		
	C92416F	0.517			NA	0.9770	0.8890	1	0.63	D070600071	<LOQ (0.00448 ug/mL)		
	C92419F	0.142			NA	0.9770	0.8890	1	0.61	D070600072	<LOQ (0.00448 ug/mL)		
	C92423F	0.261			NA	0.9770	0.8890	1	0.56	D070600073	<LOQ (0.00448 ug/mL)		
	C92430F	0.187			NA	0.9770	0.8890	1	0.96	D070600074	<LOQ (0.00448 ug/mL)		
	C92431F	0.179			NA	0.9770	0.8890	1	1.03	D070600077	<LOQ (0.00448 ug/mL)		
	C92439F	0.123			NA	0.9770	0.8890	1	1.00	D070600078	<LOQ (0.00448 ug/mL)		
	C92443F	0.208			NA	0.9770	0.8890	1	0.26	D070600079	<LOQ (0.00448 ug/mL)		
	C92451F	0.569		60.2	NA	0.9770	0.8890	1	0.00	D070600080	<LOQ (0.00448 ug/mL)		NA
	C92452F	0.236	0.259	0.156	NA	0.9770	0.8890	1	0.20	D070600081	<LOQ (0.00448 ug/mL)	<LOQ	NA

* High recoveries confirmed with 07/24/00 and 08/24/00 ana

Date Entered/By: 07/13/00, 08/15/00, 08/21/00, 09/
Date Verified/ By: 03/20/01 kjh, 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC/

07/06/00 Initial LOQ ng/mL
Corrected LOQ ng/mL
07/19/00 Initial LOQ ng/mL
Corrected LOQ ng/mL
07/24/00 Initial LOQ ng/mL
Corrected LOQ ng/mL
08/24/00 Initial LOQ ng/mL
Corrected LOQ ng/mL

Covance# 6329-228

Study:	TOX003, 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats
Product Number(Test Substance):	T-6316 (EtFOSE-OH)
Matrix:	Rat Serum
Method#/Revision:	ETS-8-4.1 & ETS-8-5.1
Analytical Equipment System Number:	Davey 070799
Instrument Software/Version:	MassLynx 3.4
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Dates of Extraction/Analyst:	06/29/00 SAL/KJK
Dates of Analysis/Analyst:	07/6&/00, 07/19/00, 07/24/00, 08/24/00 MMH/SAH
Date of Data Reduction/Analyst:	07/07A0, 07/25/00, 07/27/00, 08/28/00 MMH/HOJ

Sample Data

WEEK 105 RAT SERA

kt NB110347.B0 kt 529

Group Dose	Sample #	Surrogate Verified	M556 Old Purity Correction Factor	M556 Purity Correction Factor	M556 Dilution Factor	M556 Conc. ug/mL	Filename	Concentration of M556 ug/mL or % Rec	Mean M556 ug/mL	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	PFOSEA Old Purity Correction Factor	PFOSEA Purity Correction Factor	PFOSEA Dilution Factor	PFOSEA Conc. ng/mL	Filename	Concentration of PFOSEA ug/mL or % Rec	Mean PFOSEA ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	06290-H2O Blk-5	NA	0.9989	Unknown	1	0.00	A082400005	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	0.00	D070600007	<LOQ (0.00493 ug/mL)		
	06290-H2O Blk-6	NA	0.9989	Unknown	1	0.00	A082400019	<LOQ (0.0248 ug/mL)	<LOQ	NA	NA	0.9930	Unknown	1	2.19	D070600021	<LOQ (0.00493 ug/mL)	<LOQ	NA
Matrix Blk	RBS06290-Sera Blk-5	NA	0.9989	Unknown	1	0.00	A082400006	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	1.09	D070600008	<LOQ (0.00493 ug/mL)		
	RBS06290-Sera Blk-6	NA	0.9989	Unknown	1	0.00	A082400020	<LOQ (0.0248 ug/mL)	<LOQ	NA	NA	0.9930	Unknown	1	1.89	D070600022	<LOQ (0.00493 ug/mL)	<LOQ	NA
Matrix Blk	RTS06290-Sera Blk-5	NA	0.9989	Unknown	1	0.00	A082400007	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	0.85	D070600009	<LOQ (0.00493 ug/mL)		
	RTS06290-Sera Blk-6	NA	0.9989	Unknown	1	0.00	A082400021	<LOQ (0.0248 ug/mL)	<LOQ	NA	NA	0.9930	Unknown	1	1.03	D070600023	<LOQ (0.00493 ug/mL)	<LOQ	NA
QC	RTS06290-MS-250 ppb-5	NA	NA	NA	1	431	A071900024	134% *			NA	NA	NA	1	222	D070600024	90%		
	RTS06290-MSD-250 ppb-5	NA	NA	NA	1	412	A071900025	166% *	170%	5%	NA	NA	NA	1	223	D070600025	90%	90%	1%
	RTS06290-MS-250 ppb-5	NA	NA	NA	1	381	A082400022	154% 2nd*			NA	NA	NA	NA	NA	NA	NA		
	RTS06290-MSD-250 ppb-5	NA	NA	NA	1	378	A082400023	153% 2nd*	153%	1%	NA	NA	NA	NA	NA	NA	NA	NA	NA
Group 8 Control 0.0 ppm	C92197bM	NA	0.9989	Unknown	1	0.00	A082400026	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	0.00	D070600028	<LOQ (0.00493 ug/mL)		
	C92203bM	NA	0.9989	Unknown	1	0.00	A082400027	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	0.00	D070600029	<LOQ (0.00493 ug/mL)		
	C92208bM	NA	0.9989	Unknown	1	0.00	A082400028	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	0.82	D070600030	<LOQ (0.00493 ug/mL)		
	C92209bM	NA	0.9989	Unknown	1	0.00	A082400029	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	1.29	D070600031	<LOQ (0.00493 ug/mL)		
	C92218bM	NA	0.9989	Unknown	1	0.00	A082400030	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	0.98	D070600032	<LOQ (0.00493 ug/mL)		
	C92222bM	NA	0.9989	Unknown	1	0.00	A082400033	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	1.20	D070600035	<LOQ (0.00493 ug/mL)		
	C92231bM	NA	0.9989	Unknown	1	0.00	A082400034	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	1.07	D070600036	<LOQ (0.00493 ug/mL)		
	C92233bM	NA	0.9989	Unknown	1	0.00	A082400035	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	0.00	D070600037	<LOQ (0.00493 ug/mL)		
	C92239bM	NA	0.9989	Unknown	1	0.00	A082400036	<LOQ (0.0248 ug/mL)		NA	NA	0.9930	Unknown	1	0.00	D070600038	<LOQ (0.00493 ug/mL)		NA
	C92252bM	NA	0.9989	Unknown	1	0.00	A082400037	<LOQ (0.0248 ug/mL)	<LOQ	NA	NA	0.9930	Unknown	1	1.42	D070600039	<LOQ (0.00493 ug/mL)	<LOQ	NA
	C9233MF	NA	0.9989	Unknown	1	0.00	A082400040	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	0.97	D070600042	<LOQ (0.00493 ug/mL)		
	C9234bF	NA	0.9989	Unknown	1	0.00	A082400041	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	0.00	D070600043	<LOQ (0.00493 ug/mL)		
	C9234bF	NA	0.9989	Unknown	1	0.00	A082400042	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	0.84	D070600044	<LOQ (0.00493 ug/mL)		
	C9235aF	NA	0.9989	Unknown	1	0.00	A082400043	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	1.09	D070600045	<LOQ (0.00493 ug/mL)		
	C9235bF	NA	0.9989	Unknown	1	0.00	A082400044	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	0.00	D070600046	<LOQ (0.00493 ug/mL)		
	C9236aF	NA	0.9989	Unknown	1	0.00	A082400047	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	0.98	D070600049	<LOQ (0.00493 ug/mL)		
	C9236bF	NA	0.9989	Unknown	1	0.00	A082400048	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	0.82	D070600050	<LOQ (0.00493 ug/mL)		
	C9236wF	NA	0.9989	Unknown	1	0.00	A082400049	<LOQ (0.0248 ug/mL)			NA	0.9930	Unknown	1	0.00	D070600051	<LOQ (0.00493 ug/mL)		
	C9237aF	NA	0.9989	Unknown	1	0.00	A082400050	<LOQ (0.0248 ug/mL)		NA	NA	0.9930	Unknown	1	1.08	D070600052	<LOQ (0.00493 ug/mL)		NA
	C9238bF	NA	0.9989	Unknown	1	0.00	A082400051	<LOQ (0.0248 ug/mL)	<LOQ	NA	NA	0.9930	Unknown	1	1.14	D070600053	<LOQ (0.00493 ug/mL)	<LOQ	NA
Group 9 Low Dose 1.0 ppm	C92261bM	NA	0.9989	Unknown	1	44.2	A071900076	0.0447			NA	0.9930	Unknown	1	1.74	D070600056	<LOQ (0.00493 ug/mL)		
	C92266bM	NA	0.9989	Unknown	1	53.1	A071900077	0.0532			NA	0.9930	Unknown	1	0.83	D070600057	<LOQ (0.00493 ug/mL)		
	C92270bM	NA	0.9989	Unknown	1	140	A071900078	0.140			NA	0.9930	Unknown	1	1.22	D070600058	<LOQ (0.00493 ug/mL)		
	C92276bM	NA	0.9989	Unknown	1	96.4	A071900079	0.0965			NA	0.9930	Unknown	1	1.37	D070600059	<LOQ (0.00493 ug/mL)		
	C92281bM	NA	0.9989	Unknown	1	21.1	A082400054	0.0211			NA	0.9930	Unknown	1	1.06	D070600060	<LOQ (0.00493 ug/mL)		
	C9228bM	NA	0.9989	Unknown	1	129	A071900083	0.129			NA	0.9930	Unknown	1	1.39	D070600063	<LOQ (0.00493 ug/mL)		
	C9230bM	NA	0.9989	Unknown	1	15.6	A082400055	0.0156			NA	0.9930	Unknown	1	2.43	D070600064	<LOQ (0.00493 ug/mL)		
	C9231bM	NA	0.9989	Unknown	1	42.4	A071900085	0.0425			NA	0.9930	Unknown	1	3.13	D070600065	<LOQ (0.00493 ug/mL)		
	C9231bM	NA	0.9989	Unknown	1	77.6	A071900086	0.0777		68.9	NA	0.9930	Unknown	1	1.04	D070600066	<LOQ (0.00493 ug/mL)		NA
	C9232bM	NA	0.9989	Unknown	1	25.7	A071900087	0.0257	0.0645	0.0445	NA	0.9930	Unknown	1	1.17	D070600067	<LOQ (0.00493 ug/mL)	<LOQ	NA
	C9241bF	NA	0.9989	Unknown	1	124	A071900090	0.125			NA	0.9930	Unknown	1	0.00	D070600070	<LOQ (0.00493 ug/mL)		
	C9241bF	NA	0.9989	Unknown	1	97.4	A071900091	0.0975			NA	0.9930	Unknown	1	1.45	D070600071	<LOQ (0.00493 ug/mL)		
	C9241wF	NA	0.9989	Unknown	1	93.4	A071900092	0.0935			NA	0.9930	Unknown	1	0.98	D070600072	<LOQ (0.00493 ug/mL)		
	C9242bF	NA	0.9989	Unknown	1	106	A071900093	0.106			NA	0.9930	Unknown	1	0.96	D070600073	<LOQ (0.00493 ug/mL)		
	C9243bF	NA	0.9989	Unknown	1	88.5	A071900094	0.0886			NA	0.9930	Unknown	1	0.00	D070600074	<LOQ (0.00493 ug/mL)		
	C9243UF	NA	0.9989	Unknown	1	43.1	A071900097	0.0431			NA	0.9930	Unknown	1	1.20	D070600077	<LOQ (0.00493 ug/mL)		
	C9243wF	NA	0.9989	Unknown	1	59.2	A071900098	0.0592			NA	0.9930	Unknown	1	1.23	D070600078	<LOQ (0.00493 ug/mL)		
	C9244bF	NA	0.9989	Unknown	1	56.2	A071900099	0.0563			NA	0.9930	Unknown	1	1.17	D070600079	<LOQ (0.00493 ug/mL)		
	C9245UF	NA	0.9989	Unknown	1	130	A071900100	0.130		33.8	NA	0.9930	Unknown	1	1.15	D070600080	<LOQ (0.00493 ug/mL)		NA
	C9245ZF	NA	0.9989	Unknown	1	69.1	A071900101	0.0692	0.0868	0.0294	NA	0.9930	Unknown	1	1.00	D070600081	<LOQ (0.00493 ug/mL)	<LOQ	NA

* High recoveries confirmed with 07/24/00 and 08/24/00 analysis.

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
M556 = $C_8F_{17}SO_2N(H)CH_2COO^-$
PFOSEA = Perfluorooctane sulfonyl ethylamide

Date Entered/By: 07/13/00, 08/15/00, 08/21/00, 09/0
Date Verified/ By: 03/20/01 kjk, 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC/

07/0x/00 Initial LOQ ug/mL
Corrected LOQ ug/mL
07/19/00 Initial LOQ ug/mL
Corrected LOQ ug/mL
07/24/00 Initial LOQ ug/mL
Corrected LOQ ug/mL
08/24/00 Initial LOQ ug/mL
Corrected LOQ ug/mL

Study:	TOX003, 104 Week Dietary Carcinogenicity
Product Number(Test Substance):	T-6316 (EtFOSE-OH)
Matrix:	Rat Serum
Method#/Revision:	ETS-8-4.1 & ETS-8-5.1
Analytical Equipment System Number:	Davey 070799
Instrument Software/Version:	MassLynx 3.4
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Dates of Extraction/Analyst:	06/29/00 SAL/KJK
Dates of Analysis/Analyst:	07/6&/00, 07/19/00, 07/24/00, 08/24/00, MM
Date of Data Reduction/Analyst:	07/07/00, 07/25/00, 07/27/00, 08/28/00 MM

Sample Data

WEEK 105 RAT SERA

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ng/mL or % Rec
Method Blk	06290-H2O Blk-5	0.00	<LOQ (0.00428 ng/mL)
	06290-H2O Blk-6	0.53	<LOQ (0.00428 ng/mL)
Matrix Blk	RBS06290-Sera Blk-5	0.39	<LOQ (0.00428 ng/mL)
	RBS06290-Sera Blk-6	0.47	<LOQ (0.00428 ng/mL)
Matrix Blk	RTS06290-Sera Blk-5	3.23	<LOQ (0.00428 ng/mL)
	RTS06290-Sera Blk-6	3.95	<LOQ (0.00428 ng/mL)
QC	RTS06290-MS-250 ppb-5	340	135% *
	RTS06290-MSD-250 ppb-5	345	137% *
	RTS06290-MS-250 ppb-5	343	137% 2nd*
	RTS06290-MSD-250 ppb-5	365	146% 2nd*
Group 8 Control 0.0 ppm	C92197bM	4.58	<LOQ (0.00428 ng/mL)
	C92203bM	3.01	<LOQ (0.00428 ng/mL)
	C92208bM	3.39	<LOQ (0.00428 ng/mL)
	C92209bM	3.37	<LOQ (0.00428 ng/mL)
	C92218bM	27.8	0.0242
	C92222bM	7.75	0.00673
	C92231bM	4.06	<LOQ (0.00428 ng/mL)
	C92233bM	2.41	<LOQ (0.00428 ng/mL)
	C92239bM	70.1	0.0608
	C92252bM	23.0	0.0200
	C9233MF	41.2	0.0358
	C9234bF	50.6	0.04399
	C9234bF	53.9	0.0468
	C9235aF	15.9	0.0138
	C9235bF	4.43	<LOQ (0.00428 ng/mL)
	C9236bF	23.6	0.0205
	C9236bF	8.99	0.00781
	C9236wF	38.3	0.0333
	C9237aF	30.1	0.0262
	C9238bF	27.8	0.0241
Group 9 Low Dose 1.0 ppm	C92261bM	684	0.596
	C92266bM	110	0.953
	C92270bM	183	3.17
	C92276bM	100	0.87
	C92281bM	495	0.430
	C9228bM	174	3.01
	C9230bM	99.0	0.0860
	C9231bM	503	0.437
	C9231bM	154	1.33
	C9232bM	387	0.336
	C9241bF	131	2.27
	C9241bF	137	2.38
	C9241wF	623	0.541
	C9242bF	101	0.88
	C9243bF	199	1.73
	C9243UF	124	1.08
	C9243wF	108	1.88
	C9244bF	148	1.28
	C9245UF	113	1.96
	C9245ZF	124	2.15

* High recoveries confirmed with 07/24/00 and 08/24/00 analysis. LAC 08/21/00 and 09/03/00

Date Entered/By: 07/13/00, 08/15/00, 08/21/00, 09/03/00 LAC
Date Verified/ By: 03/20/01 kjk, 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC/

Covance# 6329-228

Study:	TOX003, 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats		
Product Number(Test Substance):	T-6316 (EtFOSE-OH)		
Matrix:	Rat Liver	Filename:	See Below
Method/Revision:	FACT-M-1.0 & FACT-M-2.0, ETS-8-7.0	R-Squared Value:	See Attachments
Analytical Equipment System Number:	Madeline 041098, Amelia 062498, Soup020199	Slope:	See Attachments
Instrument Software/Version:	MassLynx 3.1	Y-Intercept:	See Attachments
Date of Extraction/Analyst:	6/09/98 RWW		
Date of Analysis/Analyst:	6/26/98, 6/30/98, 10/13/99 MEE/JJ/MMH		
Date of Data Reduction/Analyst:	02/08/00, 02/09/00, 06/08/00, 05/04/01 MMH/IAS		

Sample Data

WEEK 4 RAT LIVER REWORK lot 193

Group Dose	Sample #	Initial Wt. g	Total Mass of Liver g	PFOS Std Correction Factor	PFOS Purity Correction Factor	PFOS Conc. ug/g	PFOS Dilution Factor	PFOS Calc. Conc. ug/g	Filename	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	RBL06098-H2O Blk-1	1.0000	NA	0.9275	Unknown	17.2	1	16.0	M062698002	< LOQ (0.0552 ug/g)		
	RBL06098-H2O Blk-2	1.0000	NA	0.9275	Unknown	0.73	1	0.68	A06309802	< LOQ (0.0552 ug/g)	<LOQ	NA
Matrix Blk	RBL06098-Lvr Blk-1	1.0080	NA	0.9275	Unknown	14.5	1	13.3	M062698003	< LOQ (0.0552 ug/g)		
	RBL06098-Lvr Blk-2	1.0080	NA	0.9275	Unknown	5.95	1	5.47	A06309803	< LOQ (0.0552 ug/g)	<LOQ	NA
QC - 100 ppb	C92196M-MS	1.0106	NA	NA	NA	334	1	79.9	M062698040	67%		
	C92196M-MSD	1.0106	NA	NA	NA	368	1	113	M062698041	95%	81%	35%
QC - 100 ppb	C92196M-MS	1.0106	NA	NA	NA	413	1	158	A06309840	132% 2nd		
	C92196M-MSD	1.0106	NA	NA	NA	456	1	200	A06309841	168% 2nd	150%	24%
QC - 100 ppb	C92196M-MS	1.0106	NA	NA	NA	NA	NA	NA	NA	NA 3rd		
	C92196M-MSD	1.0106	NA	NA	NA	NA	NA	NA	NA	NA 3rd	NA	NA
QC - 500 ppb	C92192M-MS	1.0052	NA	NA	NA	965	1	608	S101399046	102%		
	C92192M-MSD	1.0052	NA	NA	NA	1067	1	710	S101399047	119%	110%	15%
QC - 500 ppb	C92192M-MS	1.0052	NA	NA	NA	NA	NA	NA	NA	NA 2nd		
	C92192M-MSD	1.0052	NA	NA	NA	NA	NA	NA	NA	NA 2nd	NA	NA
Group 8	C92192M	1.0052	NA	0.9275	Unknown	353	1	326	M062698012	0.326		
Control	C92196M	1.0106	NA	0.9275	Unknown	254	1	233	M062698013	0.233		
0.0 ppm	C92207M	1.0133	NA	0.9275	Unknown	173	1	158	M062698014	0.158		
	C92216M	1.0029	NA	0.9275	Unknown	155	1	144	M062698015	0.144		41.6
	C92259M	1.0334	NA	0.9275	Unknown	142	1	127	M062698016	0.127	0.198	0.0822
	C92332F	1.0267	NA	0.9275	Unknown	85.8	1	77.5	M062698019	0.0775		
	C92352F	1.0512	NA	0.9275	Unknown	85.9	1	75.8	M062698020	0.0758		
	C92355F	1.0140	NA	0.9275	Unknown	151	1	138	M062698021	0.138		
	C92366F	1.0217	NA	0.9275	Unknown	53.0	1	48.1	M062698022	< LOQ (0.0552 ug/g)		35.4
	C92373F	1.0285	NA	0.9275	Unknown	109	1	98.4	M062698023	0.0984	0.0891	0.0315
Group 9	C92264M	1.0069	NA	0.9275	Unknown	147	50	6788	A06309812	6.79		
Low Dose	C92280M	1.0390	NA	0.9275	Unknown	160	50	7152	A06309813	7.15		
1.0 ppm	C92284M	1.0242	NA	0.9275	Unknown	154	50	6978	A06309814	6.98		
	C92300M	1.0230	NA	0.9275	Unknown	126	50	5706	A06309815	5.71		9.62
	C92329M	1.0315	NA	0.9275	Unknown	134	50	6034	A06309816	6.03	6.53	0.628
	C92402F	1.0435	NA	0.9275	Unknown	797	5	3541	A06309833	3.54		
	C92435F	1.0030	NA	0.9275	Unknown	760	5	3513	A06309834	3.51		
	C92463F	1.0376	NA	0.9275	Unknown	858	5	3835	A06309835	3.84		
	C92467F	1.0071	NA	0.9275	Unknown	360	5	1658	A06309836	1.66		31.5
	C92470F	1.0170	NA	0.9275	Unknown	509	5	2322	A06309837	2.32	2.97	0.937

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

Date Entered/By: 04/17/00, 06/20/00 MMH/LAC, 05/08/01 mmh
Date Verified/ By: 03/22/01 bsj, 04/03/01 kjh, 05/10/01 LAC 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC / 04/03/01 KJH

AMDT# 050598.2
Covance# 6329-228

Study: TOX003. 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats
Product Number(Test Substance): T-6316 (EtFOSE-OH)
Matrix: Rat Liver
Method/Revision: FACT-M-1.0 & FACT-M-2.0, ETS-8-7.0
Analytical Equipment System Number: Madeline 041098, Amelia 062498, Soup020199
Instrument Software/Version: MassLynx 3.1
Date of Extraction/Analyst: 6/09/98 RWW
Date of Analysis/Analyst: 6/26/98, 6/30/98, 10/13/99 MEE/JJ/MMH
Date of Data Reduction/Analyst: 02/08/00, 02/09/00, 06/08/00, 05/04/01 MMH/IAS

Filename: See Below
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments

Sample Data

WEEK 4 RAT LIVER REWORK lot L-2353 lot 617

Group Dose	Sample #	PFOSA Purity Correction Factor	PFOSA Conc. ng/g	PFOSA Dilution Factor	PFOSA Calc. Conc. ng/g	Filename	Concentration of PFOSA ug/g or % Rec.	Mean PFOSA ug/g	RSD Std. Dev. MS/MSD RPD	PFOSAA Old Purity Correction Factor	PFOSAA Purity Correction Factor	PFOSAA Conc. ng/g	PFOSAA Dilution Factor	PFOSAA Calc. Conc. ng/g	Filename	Concentration of PFOSAA ug/g or % Rec.	Mean PFOSAA ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	RBL06098-H2O Blk-1	Unknown	0.00	1	0.00	M62698002	< LOQ (0.0121 ug/g)			0.5382	Unknown	0.00	1	0.00	M062698002	< LOQ (0.0341 ug/g)		
	RBL06098-H2O Blk-2	Unknown	0.00	1	0.00	A06309802	< LOQ (0.0121 ug/g)	<LOQ	NA	0.5382	Unknown	0.00	1	0.00	A06309802	< LOQ (0.0341 ug/g)	<LOQ	NA
Matrix Blk	RBL06098-Lvr Blk-1	Unknown	0.00	1	0.00	M62698003	< LOQ (0.0121 ug/g)			0.5382	Unknown	0.00	1	0.00	M062698003	< LOQ (0.0341 ug/g)		
	RBL06098-Lvr Blk-2	Unknown	1.27	1	1.26	A06309803	< LOQ (0.0121 ug/g)	<LOQ	NA	0.5382	Unknown	0.00	1	0.00	A06309803	< LOQ (0.0341 ug/g)	<LOQ	NA
QC - 100 ppb	C92196M-MS	NA	104	1	103	M62698040	85%			NA	NA	68.1	1	67.4	A06309840	53%		
	C92196M-MSD	NA	101	1	100	M62698041	83%	84%	3%	NA	NA	158	1	156	A06309841	123%	88%	79%
QC - 100 ppb	C92196M-MS	NA	107	1	106	A06309840	88% 2nd			NA	NA	227	1	225	S101399054	177% 2nd		
	C92196M-MSD	NA	124	1	122	A06309841	101% 2nd	94%	14%	NA	NA	254	1	251	S101399055	198% 2nd	188%	11%
QC - 100 ppb	C92196M-MS	NA	84.4	1	84.4	S101399054	70% 3rd			NA	NA	NA	NA	NA	NA	NA 3rd		
	C92196M-MSD	NA	68.7	1	68.0	S101399055	56% 3rd	63%	21%	NA	NA	NA	NA	NA	NA	NA 3rd	NA	NA
QC - 500 ppb	C92192M-MS	NA	382	1	380	S101399046	63%			NA	NA	647	1	644	S101399046	101%		
	C92192M-MSD	NA	352	1	350	S101399047	58%	60%	8%	NA	NA	763	1	759	S101399047	119%	110%	16%
QC - 500 ppb	C92192M-MS	NA	NA	NA	NA	NA	NA 2nd			NA	NA	NA	NA	NA	NA	NA 2nd		
	C92192M-MSD	NA	NA	NA	NA	NA	NA 2nd	NA	NA	NA	NA	NA	NA	NA	NA	NA 2nd	NA	NA
Group 8	C92192M	Unknown	0.00	1	0.00	M62698012	< LOQ (0.0121 ug/g)			0.5382	Unknown	0.00	1	0.00	M62698012	< LOQ (0.0341 ug/g)		
Control	C92196M	Unknown	0.00	1	0.00	M62698013	< LOQ (0.0121 ug/g)			0.5382	Unknown	0.00	1	0.00	M62698013	< LOQ (0.0341 ug/g)		
0.0 ppm	C92207M	Unknown	0.00	1	0.00	M62698014	< LOQ (0.0121 ug/g)			0.5382	Unknown	0.00	1	0.00	M62698014	< LOQ (0.0341 ug/g)		
	C92216M	Unknown	0.00	1	0.00	M62698015	< LOQ (0.0121 ug/g)		NA	0.5382	Unknown	0.00	1	0.00	M62698015	< LOQ (0.0341 ug/g)		NA
	C92259M	Unknown	0.00	1	0.00	M62698016	< LOQ (0.0121 ug/g)	<LOQ	NA	0.5382	Unknown	0.00	1	0.00	M62698016	< LOQ (0.0341 ug/g)	<LOQ	NA
	C92332F	Unknown	0.00	1	0.00	M62698019	< LOQ (0.0121 ug/g)			0.5382	Unknown	0.00	1	0.00	M62698019	< LOQ (0.0341 ug/g)		
	C92352F	Unknown	5.06	1	4.81	M62698020	< LOQ (0.0121 ug/g)			0.5382	Unknown	0.00	1	0.00	M62698020	< LOQ (0.0341 ug/g)		
	C92355F	Unknown	5.24	1	5.17	M62698021	< LOQ (0.0121 ug/g)			0.5382	Unknown	0.00	1	0.00	M62698021	< LOQ (0.0341 ug/g)		
	C92366F	Unknown	0.00	1	0.00	M62698022	< LOQ (0.0121 ug/g)		NA	0.5382	Unknown	0.00	1	0.00	M62698022	< LOQ (0.0341 ug/g)		NA
	C92373F	Unknown	0.00	1	0.00	M62698023	< LOQ (0.0121 ug/g)	<LOQ	NA	0.5382	Unknown	0.00	1	0.00	M62698023	< LOQ (0.0341 ug/g)	<LOQ	NA
Group 9	C92264M	Unknown	194	1	192	M62698026	0.192			0.5382	Unknown	160	5	428	M06309826	0.428		
Low Dose	C92280M	Unknown	244	1	234	M62698027	0.234			0.5382	Unknown	132	5	342	M06309827	0.342		
1.0 ppm	C92284M	Unknown	161	1	158	M62698028	0.158			0.5382	Unknown	225	5	590	M06309828	0.590		
	C92300M	Unknown	139	1	136	M62698029	0.136		21.5	0.5382	Unknown	111	5	293	M06309829	0.293		89.8
	C92329M	Unknown	220	1	214	M62698030	0.214	0.187	0.0401	0.5382	Unknown	675	5	1760	M06309830	1.76	0.683	0.613
	C92402F	Unknown	227	1	217	M62698033	0.217			0.5382	Unknown	248	5	641	M06309833	0.641		
	C92435F	Unknown	392	1	391	M62698034	0.391			0.5382	Unknown	229	5	613	M06309834	0.613		
	C92463F	Unknown	200	1	193	M62698035	0.193			0.5382	Unknown	172	5	446	M06309835	0.446		
	C92467F	Unknown	203	1	201	M62698036	0.201		47.2	0.5382	Unknown	133	5	355	M06309836	0.355		33.2
	C92470F	Unknown	107	1	105	M62698037	0.105	0.222	0.104	0.5382	Unknown	324	5	856	M06309837	0.856	0.582	0.193

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

Date Entered/By: 04/17/00, 06/20/00 MMH/LAC, 05/08/01 mmh
Date Verified/ By: 03/22/01 hoj, 04/03/01 kjh, 05/10/01 LAC 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC / 04/03/01 KJH

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

Date Entered/By: 04/17/00, 06/20/00 MMH/LAC, 05/08/01 mmh
Date Verified/ By: 03/22/01 hoj, 04/03/01 kjh, 05/10/01 LAC 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC / 04/03/01 KJH

Covance# 6329-228

Study:	TOX003, 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats		
Product Number(Test Substance):	T-6316 (EtFOSE-OH)		
Matrix:	Rat Liver	Filename:	See Below
Method/Revision:	FACT-M-1.0 & FACT-M-2.0, ETS-8-7.0	R-Squared Value:	See Attachments
Analytical Equipment System Number:	Madeline 041098, Amelia 062498, Soup020199	Slope:	See Attachments
Instrument Software/Version:	MassLynx 3.1	Y-Intercept:	See Attachments
Date of Extraction/Analyst:	6/09/98 RWW		
Date of Analysis/Analyst:	6/26/98, 6/30/98, 10/13/99 MEE/JJ/MMH		
Date of Data Reduction/Analyst:	02/08/00, 02/09/00, 06/08/00, 05/04/01 MMH/IAS		

Sample Data

WEEK 4 RAT LIVER REWORK lot 936

Group Dose	Sample #	EtFOSE Purity Correction Factor	EtFOSE Conc. ug/g	EtFOSE Dilution Factor	EtFOSE Calc. Conc. ug/g	Filename	Concentration of EtFOSE ug/g or % Rec.	Mean EtFOSE ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	RBL06098-H2O Blk-1	Unknown	NR	1	NR	NA	NR		
	RBL06098-H2O Blk-2	Unknown	NR	1	NR	NA	NR	NR	NR
Matrix Blk	RBL06098-Lvr Blk-1	Unknown	NR	1	NR	NA	NR		
	RBL06098-Lvr Blk-2	Unknown	NR	1	NR	NA	NR	NR	NR
QC - 100 ppb	C92196M-MS	NA	NR	1	NR	NA	NR		
	C92196M-MSD	NA	NR	1	NR	NA	NR	NR	NR
QC - 100 ppb	C92196M-MS	NA	NR	1	NR	NA	NR		
	C92196M-MSD	NA	NR	1	NR	NA	NR	NR	NR
QC - 100 ppb	C92196M-MS	NA	NR	1	NR	NA	NR		
	C92196M-MSD	NA	NR	1	NR	NA	NR	NR	NR
QC - 500 ppb	C92192M-MS	NA	NR	1	NR	NA	NR		
	C92192M-MSD	NA	NR	1	NR	NA	NR	NR	NR
QC - 500 ppb	C92192M-MS	NA	NR	1	NR	NA	NR		
	C92192M-MSD	NA	NR	1	NR	NA	NR	NR	NR
Group 8	C92192M	Unknown	NR	1	NR	NA	NR		
Control	C92196M	Unknown	NR	1	NR	NA	NR		
0.0 ppm	C92207M	Unknown	NR	1	NR	NA	NR		
	C92216M	Unknown	NR	1	NR	NA	NR		NR
	C92259M	Unknown	NR	1	NR	NA	NR	NR	NR
	C92332F	Unknown	NR	1	NR	NA	NR		
	C92352F	Unknown	NR	1	NR	NA	NR		
	C92355F	Unknown	NR	1	NR	NA	NR		
	C92366F	Unknown	NR	1	NR	NA	NR		NR
	C92373F	Unknown	NR	1	NR	NA	NR	NR	NR
Group 9	C92264M	Unknown	NR	1	NR	NA	NR		
Low Dose	C92280M	Unknown	NR	1	NR	NA	NR		
1.0 ppm	C92284M	Unknown	NR	1	NR	NA	NR		
	C92300M	Unknown	NR	1	NR	NA	NR		NR
	C92329M	Unknown	NR	1	NR	NA	NR	NR	NR
	C92402F	Unknown	NR	1	NR	NA	NR		
	C92435F	Unknown	NR	1	NR	NA	NR		
	C92463F	Unknown	NR	1	NR	NA	NR		
	C92467F	Unknown	NR	1	NR	NA	NR		NR
	C92470F	Unknown	NR	1	NR	NA	NR	NR	NR

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol

NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

Date Entered/By: 04/17/00, 06/20/00 MMH/LAC, 05/08/01 mmh
Date Verified/ By: 03/22/01 hoj, 04/03/01 kjh, 05/10/01 LAC 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC / 04/03/01 KJH

AMDT# 030598.2
Covance# 6329-228

Study: TOX003, 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats
Product Number(Test Substance): T-6316 (EtFOSE-OH)
Matrix: Rat Liver
Method/Revision: FACT-M-1.0 & FACT-M-2.0, ETS-8-7.0
Analytical Equipment System Number: Madeline 041098, Amelia 062498, Soup 020199
Instrument Software/Version: MassLynx 3.1, 3.2, 3.3
Date of Extraction/Analyst: 07/17/98 LAS/SAH
Date of Analysis/Analyst: 07/17/98, 7/29/99, 08/07/98, 10/12/99 HOJ/SAH/MEE/MMH
Date of Data Reduction/Analyst: 10/13/99, 10/26/99, 02/09/00 MMH

Filename: See Below
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments

Sample Data

WEEK 14 RAT LIVER REWORK

Group Dose	Sample #	Initial Wt. g	Total Mass of Liver g	PFOS Std Correction Factor	PFOS Purity Correction Factor	PFOS Conc. ug/g	PFOS Dilution Factor	PFOS Calc. Conc. ug/g	Filename	Concentration of PFOS ug/g or % Rec.	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD	PFOSA Purity Correction Factor	PFOSA Conc. ug/g	PFOSA Dilution Factor	PFOSA Calc. Conc. ug/g	Filename	Concentration of PFOSA ug/g or % Rec.	Mean PFOSA ug/g	RSD Std. Dev. MS/MSD RPD
		lot 193											lot L-2353							
Method Blk	H2O Blk-1	1.0000	NA	0.9275	Unknown	0.00	1	0.00	A71798002	< LOQ (0.0114 ug/g)			Unknown	4.20	1	4.20	A71798002	< LOQ (0.0124 ug/g)		
	H2O Blk-2	1.0000	NA	0.9275	Unknown	0.00	1	0.00	A71798044	< LOQ (0.0114 ug/g)	<LOQ	NA	Unknown	3.82	1	3.82	A71798044	< LOQ (0.0124 ug/g)	<LOQ	NA
Matrix Blk	Rabbit Liver Blk-1	40.13	NA	0.9275	Unknown	0.00	1	0.00	A71798003	< LOQ (0.0114 ug/g)			Unknown	4.99	1	0.124	A71798003	< LOQ (0.0124 ug/g)		
	Rabbit Liver Blk-2	40.13	NA	0.9275	Unknown	0.00	1	0.00	A71798045	< LOQ (0.0114 ug/g)	<LOQ	NA	Unknown	3.90	1	0.0972	A71798045	< LOQ (0.0124 ug/g)	<LOQ	NA
QC - 100 ppb	C92255M-MS	1.0093	NA	NA	NA	1041	1	76.9	A71798040	65%			NA	167	1	156	A71798040	129%		
	C92255M-MSD	1.0093	NA	NA	NA	1100	1	136	A71798041	114%	89%	55%	NA	171	1	160	A71798041	132%	131%	2%
	C92255M-MS	1.0093	NA	NA	NA	NA	NA	NA	NA	NA 2nd			NA	159	1	148	S101299046	122% 2nd		
	C92255M-MSD	1.0093	NA	NA	NA	NA	NA	NA	NA	NA 2nd	NA	NA	NA	96.0	1	86	S101299047	71% 2nd	97%	53%
Group 8	C92255M	1.0093	NA	0.9275	Unknown	963	1	885	A71798012	0.885			Unknown	9.20	1	9.12	A71798012	< LOQ (0.0124 ug/g)		
Control	C92256M	1.0115	NA	0.9275	Unknown	253	50	11589	A80798080	11.6			Unknown	366	1	362	A71798013	0.362		
0.0 ppm	C92257M	0.9920	NA	0.9275	Unknown	1055	1	987	A71798014	0.987			Unknown	8.14	1	8.21	A71798014	< LOQ (0.0124 ug/g)		
	C92258M	1.0150	NA	0.9275	Unknown	674	1	616	A71798015	0.616		163	Unknown	7.23	1	7.12	A71798015	< LOQ (0.0124 ug/g)		190
	C92260M	0.9984	NA	0.9275	Unknown	772	1	717	A71798016	0.717	2.96	4.83	Unknown	8.64	1	8.65	A71798016	< LOQ (0.0124 ug/g)	0.0822	0.156
	C92396F	1.0116	NA	0.9275	Unknown	490	1	449	A71798019	0.449			Unknown	12.6	1	12.4	A71798019	0.0124		
	C92397F	0.9949	NA	0.9275	Unknown	498	1	464	A71798020	0.464			Unknown	12.3	1	12.3	A71798020	< LOQ (0.0124 ug/g)		
	C92398F	1.0015	NA	0.9275	Unknown	491	1	455	A71798021	0.455			Unknown	13.4	1	13.4	A71798021	0.0134		
	C92399F	1.0009	NA	0.9275	Unknown	362	1	335	A71798022	0.335		15.2	Unknown	9.45	1	9.44	A71798022	< LOQ (0.0124 ug/g)		6.71
	C92400F	0.9943	NA	0.9275	Unknown	375	1	350	A71798023	0.350	0.411	0.0625	Unknown	14.3	1	14.4	A71798023	0.0144	0.0130	0.000872
Group 9	C92325M	0.9911	NA	0.9275	Unknown	531	1	497	A71798026	0.497			Unknown	7.10	1	7.16	A71798026	< LOQ (0.0124 ug/g)		
Low Dose	C92326M	0.9983	NA	0.9275	Unknown	254	80	18845	A80798081	18.8			Unknown	591	1	592	A71798027	0.592		
1.0 ppm	C92327M	0.9977	NA	0.9275	Unknown	177	80	13190	A80798082	13.2			Unknown	454	1	455	A71798028	0.455		
	C92328M	1.0095	NA	0.9275	Unknown	204	80	15021	A80798083	15.0		56.2	Unknown	461	1	456	A71798029	0.456		60.3
	C92330M	1.0098	NA	0.9275	Unknown	199	80	14637	A80798084	14.6	12.4	6.99	Unknown	318	1	314	A71798030	0.314	0.366	0.221
	C92464F	1.0167	NA	0.9275	Unknown	485	25	11053	A80798087	11.1			Unknown	528	1	520	A71798033	0.520		
	C92465F	0.9997	NA	0.9275	Unknown	407	25	9443	A80798088	9.44			Unknown	497	1	497	A71798034	0.497		
	C92466F	1.0043	NA	0.9275	Unknown	308	25	7110	A80798089	7.11			Unknown	525	1	522	A71798035	0.522		
	C92468F	1.0114	NA	0.9275	Unknown	362	25	8299	A80798090	8.30		16.7	Unknown	374	1	370	A71798036	0.370		41.1
	C92469F	1.0089	NA	0.9275	Unknown	437	25	10052	A80798091	10.1	9.19	1.53	Unknown	1001	1	992	A71798037	0.992	0.580	0.239

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

Date Entered/By: 04/17/00 mmh
Date Verified/ By: 12/19/00 hoj, 03/22/01 hoj, 04/03/01 kjh, 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC/ 04/03/01 KJH

AMDA# 050598.2
Covance# 6329-228

Study: TOX003, 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats
Product Number(Test Substance): T-6316 (EtFOSE-OH)
Matrix: Rat Liver
Method/Revision: FACT-M-1.0 & FACT-M-2.0, ETS-8-7.0
Analytical Equipment System Number: Madeline 041098, Amelia 062498, Soup 020199
Instrument Software/Version: MassLynx 3.1, 3.2, 3.3
Date of Extraction/Analyst: 07/17/98 IAS/SAH
Date of Analysis/Analyst: 07/17/98, 7/29/99, 08/07/98, 10/12/99 HOJ/SAH/MEE/MMH
Date of Data Reduction/Analyst: 10/13/99, 10/26/99, 02/09/00 MMH

Filename: See Below
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments

Sample Data

WEEK 14 RAT LIVER REWORK

Group Dose	Sample #	lot 617 PFOSAA Old Purity Correction Factor	PFOSAA Purity Correction Factor	PFOSAA Conc. ug/g	PFOSAA Dilution Factor	PFOSAA Calc. Conc. ug/g	Filename	Concentration of PFOSAA ug/g or % Rec.	Mean PFOSAA ug/g	RSD Std. Dev. MS/MSD RPD	lot 936 EtFOSE Purity Correction Factor	EtFOSE Conc. ug/g	EtFOSE Dilution Factor	EtFOSE Calc. Conc. ug/g	Filename	Concentration of EtFOSE ug/g or % Rec.	Mean EtFOSE ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	H2O Blk-1	0.5382	Unknown	NR	NR	NR	NR	NR			Unknown	NR	1	NR	NA	NR		
	H2O Blk-2	0.5382	Unknown	0.00	1	0.00	S101299004	<LOQ (0.0351 ug/g)	<LOQ	NA	Unknown	NR	1	NR	NA	NR	NR	NR
Matrix Blk	Rabbit Liver Blk-1	0.5382	Unknown	NR	NR	NR	NR	NR			Unknown	NR	1	NR	NA	NR		
	Rabbit Liver Blk-2	0.5382	Unknown	0.00	1	0.00	S101299005	<LOQ (0.0351 ug/g)	<LOQ	NA	Unknown	NR	1	NR	NA	NR	NR	NR
QC - 100 ppb	C92255M-MS	NA	NA	136	1	83.7	S101299046	66%			NA	NR	1	NR	NA	NR		
	C92255M-MSD	NA	NA	307	1	254	S101299047	200%	133%	101%	NA	NR	1	NR	NA	NR	NR	NR
	C92255M-MS	NA	NA	NA	NA	NA	NA	NA			NA	NR	1	NR	NA	NR		
	C92255M-MSD	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NR	1	NR	NA	NR	NR	NR
Group 8	C92255M	0.5382	Unknown	51.1	1	27.3	S101299014	<LOQ (0.0351 ug/g)			Unknown	NR	1	NR	NA	NR		
Control	C92256M	0.5382	Unknown	283	10	1504	S101299030	1.50			Unknown	NR	1	NR	NA	NR		
0.0 ppm	C92257M	0.5382	Unknown	21.7	1	11.8	S101299015	<LOQ (0.0351 ug/g)			Unknown	NR	1	NR	NA	NR		
	C92258M	0.5382	Unknown	31.0	1	16.4	S101299016	<LOQ (0.0351 ug/g)		199	Unknown	NR	1	NR	NA	NR		NR
	C92260M	0.5382	Unknown	72.5	1	39.1	S101299017	0.0391	0.330	0.656	Unknown	NR	1	NR	NA	NR	NR	NR
	C92396F	0.5382	Unknown	118	1	62.6	S101299018	0.0626			Unknown	NR	1	NR	NA	NR		
	C92397F	0.5382	Unknown	10.1	1	5.45	S101299022	<LOQ (0.0351 ug/g)			Unknown	NR	1	NR	NA	NR		
	C92398F	0.5382	Unknown	40.6	1	21.8	S101299023	<LOQ (0.0351 ug/g)			Unknown	NR	1	NR	NA	NR		
	C92399F	0.5382	Unknown	53.8	1	28.9	S101299024	<LOQ (0.0351 ug/g)		29.4	Unknown	NR	1	NR	NA	NR		NR
	C92400F	0.5382	Unknown	69.0	1	37.4	S101299025	0.0374	0.0411	0.0121	Unknown	NR	1	NR	NA	NR	NR	NR
Group 9	C92325M	0.5382	Unknown	79.3	1	43.1	S101299026	0.0431			Unknown	NR	1	NR	NA	NR		
Low Dose	C92326M	0.5382	Unknown	469	20	5052	A102599062	5.05			Unknown	NR	1	NR	NA	NR		
1.0 ppm	C92327M	0.5382	Unknown	537	10	2899	S101299042	2.90			Unknown	NR	1	NR	NA	NR		
	C92328M	0.5382	Unknown	461	10	2458	S101299032	2.46		65.2	Unknown	NR	1	NR	NA	NR		NR
	C92330M	0.5382	Unknown	747	10	3979	S101299033	3.98	2.89	1.88	Unknown	NR	1	NR	NA	NR	NR	NR
	C92464F	0.5382	Unknown	1149	10	6083	S101299034	6.08			Unknown	NR	1	NR	NA	NR		
	C92465F	0.5382	Unknown	421	10	2267	S101299038	2.27			Unknown	NR	1	NR	NA	NR		
	C92466F	0.5382	Unknown	142	5	380	S101299040	0.380			Unknown	NR	1	NR	NA	NR		
	C92468F	0.5382	Unknown	103	5	275	S101299041	0.275		95.1	Unknown	NR	1	NR	NA	NR		NR
	C92469F	0.5382	Unknown	381	20	4063	A102599063	4.06	2.61	2.49	Unknown	NR	1	NR	NA	NR	NR	NR

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol

NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

Date Entered/By: 04/17/00 mmh
Date Verified/ By: 12/19/00 hoj, 03/22/01 hoj, 04/03/01 kjh, 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC/ 04/03/01 KJH

Covance# 6329-228

Study: TOX003, 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats
Product Number(Test Substance): T-6316 (EtFOSE-OH)
Matrix: Rat Liver — Filename: See Below
Method/Revision: ETS 8-6.0 & ETS-8-7.0 — R Squared Value: See Attachments
Analytical Equipment System Number: Amelia 062498, Davey 070799 — Slope: See Attachments
Instrument Software/Version: MassLynx 3.3 & 3.4 — Y-Intercept: See Attachments
Date of Extraction/Analyst: 4/14/00 SAL/RWW
Date of Analysis/Analyst: 04/17/00, 05/03/00, 05/15/00 IAS/MMH/IAS
Date of Data Reduction/Analyst: 04/18/00, 05/04/00, 05/16/00, 05/08/01 IAS/MMH

Sample Data

WEEK 53 RAT LIVER

Group Dose	Sample #	Surrogate Verified	Initial Wt. g	Total Mass of Liver g	PFOS Old Purity Correction Factor	PFOS Purity Correction Factor	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Filename	Concentration of PFOS ng/g or % Rec		Mean PFOS ng/g	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	PFOSA Old Purity Correction Factor	PFOSA Purity Correction Factor	PFOSA Conc. ng/g	PFOSA Dilution Factor	PFOSA Calc. Conc. ng/g	Filename
					lot 171																lot 1.-15709
Method Blk	RBL041400-H2OBlk-1	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	A041700004	< LOQ (0.0267 ug/g)			NA	NA	0.9510	Unknown	2.59	1	2.51	A041700004
	RBL041400-H2OBlk-2	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	D041400004	< LOQ (0.0107 ug/g)		<LOQ	NA	NA	0.9510	Unknown	0.00	1	0.00	D041400004
Matrix Blk	RBL041400-LiverBlk-1	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	A041700005	< LOQ (0.0267 ug/g)			NA	NA	0.9510	Unknown	2.09	1	2.20	A041700005
	RBL041400-LiverBlk-2	NA	1.0000	NA	0.9949	0.8640	0.00	1	4.77	D041400005	< LOQ (0.0107 ug/g)		<LOQ	NA	NA	0.9510	Unknown	0.00	1	0.00	D041400005
QC - 300 ppb	C92197/M/G8-MS-1	NA	1.0453	NA	0.9949	0.8640	289	1	226	D041400017	78%				NA	NA	NA	239	1	224	D041400017
	C92197/M/G8-MSD-1	NA	1.0453	NA	0.9949	0.8640	287	1	224	D041400018	78%		78%	1%	NA	NA	NA	262	1	246	D041400018
	C92195/M/G8-MS-1	High - Not Confirmed	1.0243	NA	0.9949	0.8640	1192	1	403	A041700017	137%	A			NA	NA	NA	291	1	280	A041700017
	C92195/M/G8-MSD-1	High - Not Confirmed	1.0243	NA	0.9949	0.8640	1112	1	324	A041700018	110%	A	124%	22%	NA	NA	NA	196	1	188	A041700018
	RBL041400-MS-1	NA	1.0000	NA	0.9949	0.8640	232	1	232	D041400076	76%				NA	NA	NA	283	1	282	D041400075
	RBL041400-MSD-1	NA	1.0000	NA	0.9949	0.8640	261	1	261	D041400077	85%		80%	12%	NA	NA	NA	260	1	259	D041400076
Group 8	C92195M		1.0243	NA	0.9949	0.8640	920	1	780	A041700019	0.780	A			NA	0.9510	Unknown	4.08	1	4.19	A041700019
0.0 ppm	C92199M	High - Not Confirmed	1.0416	NA	0.9949	0.8640	171	20	2855	A050300019	2.86				High - Not Confirmed	0.9510	Unknown	2.72	1	2.75	A041700020
	C92205M	NA	1.0515	NA	0.9949	0.8640	741	1	612	A041700021	0.612				NA	0.9510	Unknown	2.67	1	2.67	A041700021
	C92238M	NA	0.9947	NA	0.9949	0.8640	433	1	378	A041700024	0.378			81.7	NA	0.9510	Unknown	4.06	1	4.29	A041700024
	C92246M	High - Not Confirmed	1.0013	NA	0.9949	0.8640	298	10	2581	A050300020	2.58		1.44	1.18	NA	0.9510	Unknown	3.08	1	3.23	A041700025
	C92341F	NA	1.0282	NA	0.9949	0.8640	0.00	1	0.00	A041700026	< LOQ (0.0267 ug/g)				NA	0.9510	Unknown	3.07	1	3.14	A041700026
	C92356F	NA	0.9926	NA	0.9949	0.8640	114	1	100	A041700027	0.100				NA	0.9510	Unknown	3.66	1	3.88	A041700027
	C92372F	NA	1.0245	NA	0.9949	0.8640	223	1	189	A041700028	0.189				NA	0.9510	Unknown	1.91	1	1.96	A041700028
	C92376F	NA	1.0418	NA	0.9949	0.8640	349	1	291	A041700031	0.291			64.6	NA	0.9510	Unknown	6.55	1	6.61	A041700031
	C92390F	NA	1.0329	NA	0.9949	0.8640	334	1	281	A041700032	0.281		0.178	0.115	NA	0.9510	Unknown	3.06	1	3.12	A041700032
Group 9	C92267M	High - Not Confirmed	1.0137	NA	0.9949	0.8640	497	100	42575	A050300028	42.6				NA	0.9510	Unknown	132	1	137	A041700033
1.0 ppm	C92274M	High - Not Confirmed	1.0853	NA	0.9949	0.8640	478	100	38248	A050300031	38.2				NA	0.9510	Unknown	147	1	142	A041700034
	C92287M	High - Not Confirmed	0.9901	NA	0.9949	0.8640	572	100	50148	A050300032	50.1				NA	0.9510	Unknown	227	1	241	A041700035
	C92288M	High - Not Confirmed	1.0555	NA	0.9949	0.8640	410	100	33720	A050300033	33.7			29.4	NA	0.9510	Unknown	321	1	320	A041700038
	C92320M	High - Not Confirmed	0.9916	NA	0.9949	0.8640	239	100	20904	A050300034	20.9		37.1	10.9	NA	0.9510	Unknown	170	1	181	A041700039
	C92406F	High - Not Confirmed	1.0459	NA	0.9949	0.8640	362	100	30075	A050300021	30.1				NA	0.9510	Unknown	289	1	290	A041700040
	C92411F	High - Not Confirmed	1.0815	NA	0.9949	0.8640	460	100	36954	A050300024	37.0				NA	0.9510	Unknown	197	1	192	A041700041
	C92418F	High - Not Confirmed	1.0528	NA	0.9949	0.8640	372	100	30647	A050300025	30.6				NA	0.9510	Unknown	179	1	179	A041700042
	C92422F	High - Not Confirmed	1.0250	NA	0.9949	0.8640	323	100	27346	A050300026	27.3			55.0	NA	0.9510	Unknown	386	1	396	A041700045
	C92438F		1.0810	NA	0.9949	0.8640	1516	1	1218	A041700046	1.22	A	25.2	13.9	NA	0.9510	Unknown	160	1	156	A041700046

A = Above linear range of the initial curve, estimated values.
B = Qualitative only

NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
PFOSEA = Perfluorooctane sulfonyl ethylamide
M556 = C8F17SO2N((H)CH2COO)

Date Entered/Analyst: 04/26/00 mmh, 09/15/00 LAC, 05/09/01 mmh
Date Verified/Analyst: 03/22/01 hoj, 04/03/01 kjh, 05/10/01 LAC 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC/04/03/01 KJH

Covance# 6329-228

Study: TOX003, 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats
Product Number(Test Substance): T-6316 (EtFOSE-OH)
Matrix: Rat Liver
Method/Revision: ETS-8-6.0 & ETS-8-7.0
Analytical Equipment System Number: Amelia 062498, Davey 070799
Instrument Software/Version: MassLynx 3.3 & 3.4
Date of Extraction/Analyst: 4/14/00 SAL/RWW
Date of Analysis/Analyst: 04/17/00, 05/03/00, 05/15/00 IAS/MMH/IAS
Date of Data Reduction/Analyst: 04/18/00, 05/04/00, 05/16/00, 05/08/01 IAS/MMH

Filename: See Below
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments

Sample Data

WEEK 53 RAT LIVER

lot 98-0207-0303-3 | lot SD013

Concentration of PFOSA ug/g or % Rec	Mean PFOSA ug/g	RSD Std. Dev. MS/MSD RPD	Group Dose	Sample #	Surrogate Verified	PFOSAA Old Purity Correction Factor	PFOSAA Purity Correction Factor	PFOSAA Conc. ug/g	PFOSAA Dilution Factor	PFOSAA Calc. Conc. ug/g	Filename	Concentration of PFOSAA ug/g or % Rec	Mean PFOSAA ug/g	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	EtFOSE Old Purity Correction Factor	EtFOSE Purity Correction Factor	EtFOSE Conc. ug/g
< LOQ (0.00644 ug/g)		NA	Method Blk	RBL041400-H2OBlk-1	NA	0.9760	Unknown	0.00	1	0.00	A041700004	< LOQ (0.0125 ug/g)		NA	NA	0.9770	0.8890	0.00
< LOQ (0.0322 ug/g)	<LOQ	NA		RBL041400-H2OBlk-2	NA	0.9760	Unknown	0.00	1	0.00	D041400004	< LOQ (0.0314 ug/g)	<LOQ	NA	NA	0.9770	0.8890	0.00
< LOQ (0.00644 ug/g)		NA	Matrix Blk	RBL041400-LiverBlk-1	NA	0.9760	Unknown	0.00	1	0.00	A041700005	< LOQ (0.0125 ug/g)		NA	NA	0.9770	0.8890	0.00
< LOQ (0.0322 ug/g)	<LOQ	NA		RBL041400-LiverBlk-2	NA	0.9760	Unknown	0.00	1	0.00	D041400005	< LOQ (0.0314 ug/g)	<LOQ	NA	NA	0.9770	0.8890	0.00
78%			QC - 300 ppb	C92197/MGB-MS-1	NA	0.9760	Unknown	224	1	215	D041400017	74%			NA	NA	NA	277
86%	82%	9%		C92197/MGB-MSD-1	NA	0.9760	Unknown	260	1	249	D041400018	86%	80%	15%	NA	NA	NA	231
94%				C92195/MGB-MS-1	NA	0.9760	Unknown	251	1	240	A041700017	82%			NA	NA	NA	472
63%	79%	39%		C92195/MGB-MSD-1	NA	0.9760	Unknown	254	1	243	A041700018	83%	82%	2%	NA	NA	NA	459
92%				RBL041400-MS-1	NA	0.9760	Unknown	232	1	222	D041400075	73%			NA	NA	NA	NR
85%	88%	9%		RBL041400-MSD-1	NA	0.9760	Unknown	266	1	266	D041400076	87%	80%	18%	NA	NA	NA	NR
< LOQ (0.00644 ug/g)			Group 8	C92195M	NA	0.9760	Unknown	0.00	1	0.00	A041700019	< LOQ (0.0125 ug/g)			NA	0.9770	0.8890	0.00
< LOQ (0.00644 ug/g)			0.0 ppm	C92199M	High - Not Confirmed	0.9760	Unknown	0.00	1	0.00	A041700020	< LOQ (0.0125 ug/g)			High - Not Confirmed	0.9770	0.8890	0.00
< LOQ (0.00644 ug/g)				C92205M	NA	0.9760	Unknown	0.00	1	0.00	A041700021	< LOQ (0.0125 ug/g)			NA	0.9770	0.8890	0.00
< LOQ (0.00644 ug/g)		NA		C92238M	NA	0.9760	Unknown	0.00	1	0.00	A041700024	< LOQ (0.0125 ug/g)		NA	NA	0.9770	0.8890	0.00
< LOQ (0.00644 ug/g)	<LOQ	NA		C92246M	NA	0.9760	Unknown	0.00	1	0.00	A041700025	< LOQ (0.0125 ug/g)	<LOQ	NA	NA	0.9770	0.8890	0.00
< LOQ (0.00644 ug/g)				C92341F	NA	0.9760	Unknown	0.00	1	0.00	A041700026	< LOQ (0.0125 ug/g)			NA	0.9770	0.8890	0.00
< LOQ (0.00644 ug/g)				C92356F	NA	0.9760	Unknown	0.00	1	0.00	A041700027	< LOQ (0.0125 ug/g)			NA	0.9770	0.8890	0.00
< LOQ (0.00644 ug/g)				C92372F	NA	0.9760	Unknown	4.40	1	4.40	A041700028	< LOQ (0.0125 ug/g)			NA	0.9770	0.8890	0.00
0.00661		1.22		C92376F	NA	0.9760	Unknown	10.1	1	9.96	A041700031	< LOQ (0.0125 ug/g)		NA	NA	0.9770	0.8890	0.00
< LOQ (0.00644 ug/g)	0.00647	0.0000786		C92390F	NA	0.9760	Unknown	0.00	1	0.00	A041700032	< LOQ (0.0125 ug/g)	<LOQ	NA	NA	0.9770	0.8890	0.00
0.137			Group 9	C92267M	NA	0.9760	Unknown	344	1	348	A041700033	0.348			NA	0.9770	0.8890	0.00
0.142			1.0 ppm	C92274M	NA	0.9760	Unknown	885	1	836	A041700034	0.836			NA	0.9770	0.8890	0.00
0.241				C92287M	NA	0.9760	Unknown	1872	1	1937	A041700035	1.94 A			NA	0.9770	0.8890	0.00
0.320		37.7		C92288M	NA	0.9760	Unknown	678	1	658	A041700038	0.658		66.8	NA	0.9770	0.8890	0.00
0.181	0.204	0.0769		C92320M	NA	0.9760	Unknown	729	1	753	A041700039	0.753	0.906	0.605	NA	0.9770	0.8890	0.00
0.290				C92406F	NA	0.9760	Unknown	464	1	455	A041700040	0.455			NA	0.9770	0.8890	0.00
0.192				C92411F	NA	0.9760	Unknown	406	1	384	A041700041	0.384			NA	0.9770	0.8890	0.00
0.179				C92418F	NA	0.9760	Unknown	583	1	567	A041700042	0.567			NA	0.9770	0.8890	0.00
0.396		41.2		C92422F	NA	0.9760	Unknown	729	1	728	A041700045	0.728		24.8	NA	0.9770	0.8890	0.00
0.156	0.242	0.0999		C92438F	NA	0.9760	Unknown	530	1	502	A041700046	0.502	0.527	0.131	NA	0.9770	0.8890	0.00

A = Above linear range of the initial curve, estimated valuea.
B = Qualitauve only

NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Raage N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
PFOSEA = Perfluorooctane sulfonyl ethylamide
M556 = C8F17SO2N((H)CH2COO)

Date Entered/Analyst: 04/26/00 mmh, 09/15/00 LAC, 05/09/01 mmh
Date Verified/Analyst: 03/22/01 hoj, 04/03/01 kjh, 05/10/01 LAC 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC/ 04/03/01 KJH

Covance# 6329-228

Study: TOX003, 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats
Product Number(Test Substance): T-6316 (EtFOSE-OH)
Matrix: Rat Liver
Method/Revision: ETS-8-6.0 & ETS-8-7.0
Analytical Equipment System Number: Amelia 062498, Davey 070799
Instrument Software/Version: MassLynx 3.3 & 3.4
Date of Extraction/Analyst: 4/14/00 SAL/RWW
Date of Analysis/Analyst: 04/17/00, 05/03/00, 05/15/00 IAS/MMH/IAS
Date of Data Reduction/Analyst: 04/18/00, 05/04/00, 05/16/00, 05/08/01 IAS/MMH
Filename: See Below
R-Squared Val: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments

Sample Data

WEEK 53 RAT LIVER lot NB113047-80 lot 529

EtFOSE Dilution Factor	EtFOSE Calc. Conc. ng/g	Filename	Concentration of EtFOSE ng/g or % Rec		Mean EtFOSE ng/g	RSD Std. Dev. MS/MSD RPD	Group Dose	Sample #	Surrogate Verified	M556 Old Purity Correction Factor	M556 Purity Correction Factor	M556 Conc. ng/g	M556 Dilution Factor	M556 Calc. Conc. ng/g	Filename	Concentration of M556 ng/g or % Rec	Mean M556 ng/g	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	PFOSEA Old Purity Correction Factor
1	0.00	A041700004	< LOQ (0.0559 ug/g)	B		NA	Method Blk	RBL041400-H2OBlk-1	NA	0.9989	Unknown	0.00	1	0.00	A041700004	< LOQ (0.0307 ug/g)		NA	NA	0.9930
1	0.00	D041400004	< LOQ (0.0559 ug/g)	B	<LOQ	NA		RBL041400-H2OBlk-2	NA	0.9989	Unknown	0.00	1	0.00	D041400004	< LOQ (0.0307 ug/g)	<LOQ	NA	NA	0.9930
1	0.00	A041700005	< LOQ (0.0559 ug/g)	B		NA	Matrix Blk	RBL041400-LiverBlk-1	NA	0.9989	Unknown	0.00	1	0.00	A041700005	< LOQ (0.0307 ug/g)		NA	NA	0.9930
1	0.00	D041400005	< LOQ (0.0559 ug/g)	B	<LOQ	NA		RBL041400-LiverBlk-2	NA	0.9989	Unknown	0.00	1	0.00	D041400005	< LOQ (0.0307 ug/g)	<LOQ	NA	NA	0.9930
1	265	D041400017	92%	B			QC - 300 ppb	C92197/M/G8-MS-1	NA	NA	NA	361	1	345	D041400017	119%			NA	NA
1	221	D041400018	77%	B	84%	18%		C92197/M/G8-MSD-1	NA	NA	NA	370	1	354	D041400018	122%	121%	2%	NA	NA
1	452	A041700017	154%	B				C92195/M/G8-MS-1	NA	NA	NA	316	1	303	A041700017	103%			NA	NA
1	439	A041700018	149%	B	151%	3%		C92195/M/G8-MSD-1	NA	NA	NA	331	1	317	A041700018	108%	105%	5%	NA	NA
NR	NR	NR	NR	B				RBL041400-MS-1	NA	NA	NA	256	1	245	D041400075	80%			NA	NA
NR	NR	NR	NR	B	NR	NR		RBL041400-MSD-1	NA	NA	NA	257	1	257	D041400076	84%	82%	5%	NA	NA
1	0.00	A041700019	< LOQ (0.0559 ug/g)	B			Group 8	C92195M	NA	0.9989	Unknown	0.00	1	0.00	A041700019	< LOQ (0.0307 ug/g)			NA	0.9930
1	0.00	A041700020	< LOQ (0.0559 ug/g)	B			0.0 ppm	C92199M	High - Not Confirmed	0.9989	Unknown	0.00	1	0.00	A041700020	< LOQ (0.0307 ug/g)			High - Not Confirmed	0.9930
1	0.00	A041700021	< LOQ (0.0559 ug/g)	B				C92205M	NA	0.9989	Unknown	0.00	1	0.00	A041700021	< LOQ (0.0307 ug/g)			NA	0.9930
1	0.00	A041700024	< LOQ (0.0559 ug/g)	B		NA		C92238M	NA	0.9989	Unknown	0.00	1	0.00	A041700024	< LOQ (0.0307 ug/g)		NA	NA	0.9936
1	0.00	A041700025	< LOQ (0.0559 ug/g)	B	<LOQ	NA		C92246M	NA	0.9989	Unknown	0.00	1	0.00	A041700025	< LOQ (0.0307 ug/g)	<LOQ	NA	NA	0.9930
1	0.00	A041700026	< LOQ (0.0559 ug/g)	B				C92341F	NA	0.9989	Unknown	0.00	1	0.00	A041700026	< LOQ (0.0307 ug/g)			NA	0.9930
1	0.00	A041700027	< LOQ (0.0559 ug/g)	B				C92356F	NA	0.9989	Unknown	0.00	1	0.00	A041700027	< LOQ (0.0307 ug/g)			NA	0.9930
1	0.00	A041700028	< LOQ (0.0559 ug/g)	B				C92372F	NA	0.9989	Unknown	0.00	1	0.00	A041700028	< LOQ (0.0307 ug/g)			NA	0.9930
1	0.00	A041700031	< LOQ (0.0559 ug/g)	B		NA		C92376F	NA	0.9989	Unknown	0.00	1	0.00	A041700031	< LOQ (0.0307 ug/g)		NA	NA	0.9930
1	0.00	A041700032	< LOQ (0.0559 ug/g)	B	<LOQ	NA		C92390F	NA	0.9989	Unknown	0.00	1	0.00	A041700032	< LOQ (0.0307 ug/g)	<LOQ	NA	NA	0.9930
1	0.00	A041700033	< LOQ (0.0559 ug/g)	B			Group 9	C92267M	NA	0.9989	Unknown	269	1	266	A041700033	0.266			NA	0.9930
1	0.00	A041700034	< LOQ (0.0559 ug/g)	B			1.0 ppm	C92274M	NA	0.9989	Unknown	359	1	331	A041700034	0.331			NA	0.9930
1	0.00	A041700035	< LOQ (0.0559 ug/g)	B				C92287M	NA	0.9989	Unknown	376	1	380	A041700035	0.380			NA	0.9930
1	0.00	A041700038	< LOQ (0.0559 ug/g)	B		NA		C92388M	NA	0.9989	Unknown	450	1	426	A041700038	0.426		20.3	NA	0.9930
1	0.00	A041700039	< LOQ (0.0559 ug/g)	B	<LOQ	NA		C92320M	NA	0.9989	Unknown	274	1	277	A041700039	0.277	0.336	0.0682	NA	0.9930
1	0.00	A041700040	< LOQ (0.0559 ug/g)	B				C92406F	NA	0.9989	Unknown	236	1	226	A041700040	0.226			NA	0.9930
1	0.00	A041700041	< LOQ (0.0559 ug/g)	B				C92411F	NA	0.9989	Unknown	324	1	300	A041700041	0.300			NA	0.9930
1	0.00	A041700042	< LOQ (0.0559 ug/g)	B				C92418F	NA	0.9989	Unknown	288	1	274	A041700042	0.274			NA	0.9930
1	0.00	A041700045	< LOQ (0.0559 ug/g)	B		NA		C92422F	NA	0.9989	Unknown	361	1	353	A041700045	0.353		24.7	NA	0.9930
1	0.00	A041700046	< LOQ (0.0559 ug/g)	B	<LOQ	NA		C92438F	NA	0.9989	Unknown	196	1	182	A041700046	0.182	0.267	0.0660	NA	0.9930

A = Above linear range of the initial curve, estimated values.
B = Qualitative only

NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

Date Entered/Analyst: 04/26/00 mmh 09/15/00 LAC, 05/09/01 mmh
Date Verified/Analyst: 03/22/01 hoj 04/03/01 kjh, 05/10/01 LAC 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC/ 04/03/01 KJH

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
PFOSEA = Perfluorooctane sulfosyl ethylamide
M556 = C8F17SO2N((H)CH2COO)

ETS-8.7.0

Covance# 6329-228

Study:	TOX003, 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats
Product Number(Test Substance):	T-6316 (EtFOSE-OH)
Matrix:	Rat Liver
Method/Revision:	ETS-8-6.0 & ETS-8-7.0
Analytical Equipment System Number:	Amelia 062498, Davey 070799
Instrument Software/Version:	MassLynx 3.3 & 3.4
Date of Extraction/Analyst:	4/14/00 SAL/RWW
Date of Analysis/Analyst:	04/17/00, 05/03/00, 05/15/00 IAS/MMH/IAS
Date of Data Reduction/Analyst:	04/18/00, 05/04/00, 05/16/00, 05/08/01 IAS/MMH

Filename:	See Attachments		
R-Squared Value:	See Attachments		
Slope:	See Attachments		
Y-Intercept:	See Attachments		

Sample Data

PFOSEA Purity Correction Factor	PFOSEA Conc. ng/g	PFOSEA Dilution Factor	PFOSEA Calc. Conc. ng/g	Filename	Concentration of PFOSEA ug/g or % Rec	Mean PFOSEA ug/g	RSD Std. Dev. MS/MSD RPD
Unknown	0.00	1	0.00	A041700004	< LOQ (0.0308 ug/g)		NA
Unknown	0.00	1	0.00	D041400004	< LOQ (0.0308 ug/g)	<LOQ	NA
Unknown	0.00	1	0.00	A041700005	< LOQ (0.0308 ug/g)		NA
Unknown	0.00	1	0.00	D041400005	< LOQ (0.0308 ug/g)	<LOQ	NA
NA	208	1	199	D041400017	69%		
NA	254	1	243	D041400018	84%	77%	20%
NA	442	1	423	A041700017	144%		
NA	353	1	338	A041700018	115%	130%	22%
NA	358	1	343	D041400075	112%		
NA	393	1	376	D041400076	123%	117%	9%
Unknown	0.00	1	0.00	A041700019	< LOQ (0.0308 ug/g)		
Unknown	0.00	1	0.00	A041700020	< LOQ (0.0308 ug/g)		
Unknown	0.00	1	0.00	A041700021	< LOQ (0.0308 ug/g)		
Unknown	0.00	1	0.00	A041700024	< LOQ (0.0308 ug/g)		NA
Unknown	0.00	1	0.00	A041700025	< LOQ (0.0308 ug/g)	<LOQ	NA
Unknown	0.00	1	0.00	A041700026	< LOQ (0.0308 ug/g)		
Unknown	0.00	1	0.00	A041700027	< LOQ (0.0308 ug/g)		
Unknown	0.00	1	0.00	A041700028	< LOQ (0.0308 ug/g)		
Unknown	0.00	1	0.00	A041700031	< LOQ (0.0308 ug/g)		NA
Unknown	0.00	1	0.00	A041700032	< LOQ (0.0308 ug/g)	<LOQ	NA
Unknown	0.00	1	0.00	A041700033	< LOQ (0.0308 ug/g)		
Unknown	0.00	1	0.00	A041700034	< LOQ (0.0308 ug/g)		
Unknown	0.00	1	0.00	A041700035	< LOQ (0.0308 ug/g)		
Unknown	0.00	1	0.00	A041700038	< LOQ (0.0308 ug/g)		NA
Unknown	0.00	1	0.00	A041700039	< LOQ (0.0308 ug/g)	<LOQ	NA
Unknown	0.00	1	0.00	A041700040	< LOQ (0.0308 ug/g)		
Unknown	0.00	1	0.00	A041700041	< LOQ (0.0308 ug/g)		
Unknown	0.00	1	0.00	A041700042	< LOQ (0.0308 ug/g)		
Unknown	0.00	1	0.00	A041700045	< LOQ (0.0308 ug/g)		NA
Unknown	0.00	1	0.00	A041700046	< LOQ (0.0308 ug/g)	<LOQ	NA

WEEK 53 RAT LIVER

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	PFOSA Calc. Conc. ng/g	Concentration of PFOSA ug/g or % Rec	Mean PFOSA ug/g	RSD Std. Dev. MS/MSD RPD	PFOSAA Calc. Conc. ng/g	Concentration of PFOSAA ug/g or % Rec
Method Blk	RBL041400-H2OBlk-1	0.00	< LOQ (0.0267 ug/g)			2.51	< LOQ (0.00644 ug/g)			0.00	< LOQ (0.0125 ug/g)
	RBL041400-H2OBlk-2	0.00	< LOQ (0.0107 ug/g)	<LOQ	NA	0.00	< LOQ (0.0322 ug/g)	<LOQ	NA	0.00	< LOQ (0.0314 ug/g)
Matrix Blk	RBL041400-LiverBlk-1	0.00	< LOQ (0.0267 ug/g)			2.20	< LOQ (0.00644 ug/g)			0.00	< LOQ (0.0125 ug/g)
	RBL041400-LiverBlk-2	4.77	< LOQ (0.0107 ug/g)	<LOQ	NA	0.00	< LOQ (0.0322 ug/g)	<LOQ	NA	0.00	< LOQ (0.0314 ug/g)
QC - 300 ppb	C92197/M/GB-MS-1	226	78%			224	78%			215	74%
	C92197/M/GB-MSD-1	224	78%	78%	1%	246	86%	82%	9%	249	86%
	C92195/M/GB-MS-1	403	137%			280	94%			240	82%
	C92195/M/GB-MSD-1	324	110%	124%	22%	188	63%	79%	39%	243	83%
	RBL041400-MS-1	232	76%			282	92%			222	73%
	RBL041400-MSD-1	261	85%	80%	12%	259	85%	88%	9%	266	87%
Group 8 0.0 ppm	C92195M	780	0.780 A			4.19	< LOQ (0.00644 ug/g)			0.00	< LOQ (0.0125 ug/g)
	C92199M	2855	2.86			2.75	< LOQ (0.00644 ug/g)			0.00	< LOQ (0.0125 ug/g)
	C92205M	612	0.612			2.67	< LOQ (0.00644 ug/g)			0.00	< LOQ (0.0125 ug/g)
	C92238M	378	0.378		81.7	4.29	< LOQ (0.00644 ug/g)		NA	0.00	< LOQ (0.0125 ug/g)
	C92246M	2581	2.58	1.44	1.18	3.23	< LOQ (0.00644 ug/g)	<LOQ	NA	0.00	< LOQ (0.0125 ug/g)
	C92341F	0.00	< LOQ (0.0267 ug/g)			3.14	< LOQ (0.00644 ug/g)			0.00	< LOQ (0.0125 ug/g)
	C92356F	100	0.100			3.88	< LOQ (0.00644 ug/g)			0.00	< LOQ (0.0125 ug/g)
	C92372F	189	0.189			1.96	< LOQ (0.00644 ug/g)			4.40	< LOQ (0.0125 ug/g)
	C92376F	291	0.291		64.6	6.61	0.00661		1.22	9.96	< LOQ (0.0125 ug/g)
	C92390F	281	0.281	0.178	0.115	3.12	< LOQ (0.00644 ug/g)	0.00647	0.00	0.00	< LOQ (0.0125 ug/g)
Group 9 1.0 ppm	C92267M	42575	42.6			137	0.137			348	0.348
	C92274M	38248	38.2			142	0.142			836	0.836
	C92287M	50148	50.1			241	0.241			1937	1.94
	C92288M	33720	33.7		29.4	320	0.320		37.7	658	0.658
	C92320M	20904	20.9	37.1	10.9	181	0.181	0.204	0.0769	753	0.753
	C92406F	30075	30.1			290	0.290			455	0.455
	C92411F	36954	37.0			192	0.192			384	0.384
	C92418F	30647	30.6			179	0.179			567	0.567
	C92422F	27346	27.3		55.0	396	0.396		41.2	728	0.728
	C92438F	1218	1.22 A	25.2	13.9	156	0.156	0.242	0.0999	502	0.502

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NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
PFOSEA = Perfluorooctane sulfonyl ethylamide
M556 = C8F17SO2N(H)CH2COO)

Date Entered/Analyst:	04/26/00 mmh, 09/15/00 LAC, 05/09/01 mmh
Date Verified/Analyst:	03/22/01 hoj, 04/03/01 kjh, 05/10/01 LAC 5/22/01 mmh
Purity Entered/Verified:	03/29/01 LAC/ 04/03/01 KJH

Covance# 6329-228

Study: TOX003, 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats
Product Number(Test Substance): T-6316 (EtFOSE-OH)
Matrix: Rat Liver
Method/Revision: ETS-8-6.0 & ETS-8-7.0
Analytical Equipment System Number: Amelia 062498, Davey 070799
Instrument Software/Version: MassLynx 3.3 & 3.4
Date of Extraction/Analyst: 4/14/00 SAL/RWW
Date of Analysis/Analyst: 4/17/00, 5/15/00 IAS/MMH/IAS
Date of Data Reduction/Analyst: 4/18/00, 5/16/00, 05/08/01 IAS/MMH

Filename: See Below
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments

Sample Data

WEEK 105 RAT LIVER

					lot 171										lot L-15709					
Group Dose	Sample #	Surrogate Verified	Initial Wt. g	Total Mass of Liver g	PFOS Old Purity Correction Factor	PFOS Purity Correction Factor	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Filename	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	PFOSA Old Purity Correction Factor	PFOSA Purity Correction Factor	PFOSA Conc. ng/g	PFOSA Dilution Factor	PFOSA Calc. Conc. ng/g	Filename
Method Blk	RBL041400-H2OBlk-1	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	A041700004	< LOQ (0.0267 ug/g)		NA	NA	0.9510	Unknown	2.39	1	2.51	A041700004
	RBL041400-H2OBlk-2	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	D041400004	< LOQ (0.0107 ug/g)	<LOQ	NA	NA	0.9510	Unknown	0.00	1	0.00	D041400004
Matrix Blk	RBL041400-LiverBlk-1	NA	1.0000	NA	0.9949	0.8640	0.00	1	0.00	A041700005	< LOQ (0.0267 ug/g)		NA	NA	0.9510	Unknown	2.09	1	2.20	A041700005
	RBL041400-LiverBlk-2	NA	1.0000	NA	0.9949	0.8640	0.00	1	4.77	D041400005	< LOQ (0.0107 ug/g)	<LOQ	NA	NA	0.9510	Unknown	0.00	1	0.00	D041400005
QC - 300 ppb	C92197/MGB-MS-1	NA	1.0453	NA	0.9949	0.8640	289	1	226	D041400017	78%			NA	0.9510	Unknown	239	1	224	D041400017
	C92197/MGB-MSD-1	NA	1.0453	NA	0.9949	0.8640	287	1	224	D041400018	78%	78%	1%	NA	0.9510	Unknown	262	1	246	D041400018
	C92195/MGB-MS-1	High - Not Confirmed	1.0243	NA	0.9949	0.8640	1192	1	403	A041700017	137%			NA	0.9510	Unknown	291	1	280	A041700017
	C92195/MGB-MSD-1	High - Not Confirmed	1.0243	NA	0.9949	0.8640	1112	1	324	A041700018	110%	124%	22%	NA	0.9510	Unknown	196	1	188	A041700018
	RBL041400-MS-1	NA	1.0000	NA	0.9949	0.8640	232	1	232	D041400076	76%			NA	0.9510	Unknown	283	1	282	D041400075
	RBL041400-MSD-1	NA	1.0000	NA	0.9949	0.8640	261	1	261	D041400077	85%	80%	12%	NA	0.9510	Unknown	269	1	259	D041400076
Group 8	C92197M	NA	1.0453	NA	0.9949	0.8640	53.1	1	44.1	D041400019	0.0441			NA	0.9510	Unknown	0.000	1	0.00	D041400019
0.0 ppm	C92203M	NA	1.0172	NA	0.9949	0.8640	3.44	1	2.94	D041400020	< LOQ (0.0107 ug/g)			NA	0.9510	Unknown	0.000	1	0.00	D041400020
	C92208M	NA	1.0160	NA	0.9949	0.8640	9.43	1	8.06	D041400021	< LOQ (0.0107 ug/g)			NA	0.9510	Unknown	0.000	1	0.00	D041400021
	C92209M	NA	1.0160	NA	0.9949	0.8640	0.00	1	0.00	D041400024	< LOQ (0.0107 ug/g)			NA	0.9510	Unknown	0.23	1	0.24	D041400024
	C92218M	NA	0.9845	NA	0.9949	0.8640	83.0	1	73.2	D041400025	0.0732			NA	0.9510	Unknown	0.000	1	0.00	D041400025
	C92222M	NA	1.0021	NA	0.9949	0.8640	3.96	1	3.43	D041400026	< LOQ (0.0107 ug/g)			NA	0.9510	Unknown	0.000	1	0.00	D041400026
	C92231M	NA	1.0036	NA	0.9949	0.8640	2.78	1	2.41	D041400027	< LOQ (0.0107 ug/g)			NA	0.9510	Unknown	0.000	1	0.00	D041400027
	C92233M	NA	0.9986	NA	0.9949	0.8640	0.00	1	0.00	D041400028	< LOQ (0.0107 ug/g)			NA	0.9510	Unknown	0.000	1	0.00	D041400028
	C92239M	NA	1.0052	NA	0.9949	0.8640	261	1	225	D041400031	0.225		141	NA	0.9510	Unknown	0.000	1	0.00	D041400031
	C92252M	NA	0.9930	NA	0.9949	0.8640	281	1	246	D041400032	0.246	0.0653	0.0923	NA	0.9510	Unknown	0.000	1	0.00	D041400032
	C92333F	NA	0.9977	NA	0.9949	0.8640	125	1	109	D041400033	0.109			NA	0.9510	Unknown	0.000	1	0.00	D041400033
	C92334F	NA	1.0419	NA	0.9949	0.8640	112	1	93.0	D041400034	0.0930			NA	0.9510	Unknown	0.37	1	0.37	D041400034
	C92348F	NA	1.0545	NA	0.9949	0.8640	186	1	153	D041400035	0.153			NA	0.9510	Unknown	0.09	1	0.090	D041400035
	C92354F	NA	1.0607	NA	0.9949	0.8640	16.9	1	13.8	D041400038	0.0138			NA	0.9510	Unknown	0.000	1	0.00	D041400038
	C92358F	NA	0.9868	NA	0.9949	0.8640	7.29	1	6.42	D041400039	< LOQ (0.0107 ug/g)			NA	0.9510	Unknown	0.08	1	0.09	D041400039
	C92360F	NA	1.0691	NA	0.9949	0.8640	109	1	88.4	D041400040	0.0884			NA	0.9510	Unknown	0.26	1	0.26	D041400040
	C92362F	NA	1.0245	NA	0.9949	0.8640	35.3	1	29.9	D041400041	0.0299			NA	0.9510	Unknown	0.53	1	0.54	D041400041
	C92369F	NA	1.0813	NA	0.9949	0.8640	140	1	112	D041400042	0.112			NA	0.9510	Unknown	0.000	1	0.00	D041400042
	C92374F	NA	1.0801	NA	0.9949	0.8640	138	1	111	D041400045	0.111		59.9	NA	0.9510	Unknown	0.47	1	0.46	D041400045
	C92383F	NA	1.0817	NA	0.9949	0.8640	187	1	150	D041400046	0.150	0.0872	0.0522	NA	0.9510	Unknown	0.33	1	0.32	D041400046
Group 9	C92261M	NA	0.9963	NA	0.9949	0.8640	289	20	5037	D051500017	5.04			NA	0.9510	Unknown	400	1	422	D041400047
1.0 ppm	C92266M	NA	0.9909	NA	0.9949	0.8640	429	20	7515	D051500018	7.51			NA	0.9510	Unknown	264	1	280	D041400048
	C92270M	NA	1.0076	NA	0.9949	0.8640	737	20	12702	D051500019	12.7			NA	0.9510	Unknown	795	1	829	D041400049
	C92278M	NA	1.0037	NA	0.9949	0.8640	218	20	3781	D051500020	3.78			NA	0.9510	Unknown	482	1	505	D041400052
	C92281M	NA	1.0052	NA	0.9949	0.8640	270	20	4660	D051500021	4.66			NA	0.9510	Unknown	235	1	246	D041400053
	C92283M	NA	1.0182	NA	0.9949	0.8640	636	20	10841	D051500025	10.8			NA	0.9510	Unknown	341	1	352	D041400054
	C92309M	NA	0.9823	NA	0.9949	0.8640	643	1	568	D041400055	0.57			NA	0.9510	Unknown	169	1	180	D041400055
	C92311M	NA	0.9877	NA	0.9949	0.8640	118	20	2084	D051500027	2.08			NA	0.9510	Unknown	355	1	378	D041400056
	C92318M	NA	0.9963	NA	0.9949	0.8640	498	20	8678	D051500028	8.68		61.2	NA	0.9510	Unknown	251	1	265	D041400059
	C92321M	NA	0.9981	NA	0.9949	0.8640	359	20	6249	D051500029	6.25	6.21	3.80	NA	0.9510	Unknown	245	1	258	D041400060
	C92410F	NA	1.0894	NA	0.9949	0.8640	672	20	10717	D051500033	10.7			NA	0.9510	Unknown	566	1	546	D041400061
	C92416F	NA	0.9916	NA	0.9949	0.8640	623	20	10911	D051500034	10.9			NA	0.9510	Unknown	729	1	773	D041400062
	C92419F	NA	1.0143	NA	0.9949	0.8640	127	20	2177	D051500035	2.18			NA	0.9510	Unknown	494	1	512	D041400063
	C92423F	NA	0.9980	NA	0.9949	0.8640	294	20	5114	D051500036	5.11			NA	0.9510	Unknown	592	1	624	D041400066
	C92430F	NA	0.9956	NA	0.9949	0.8640	479	20	8351	D051500045	8.35			NA	0.9510	Unknown	380	1	402	D041400074
	C92431F	NA	1.0113	NA	0.9949	0.8640	360	20	6178	D051500037	6.18			NA	0.9510	Unknown	465	1	484	D041400067
	C92439F	NA	1.0007	NA	0.9949	0.8640	570	20	9900	D051500041	9.90			NA	0.9510	Unknown	524	1	551	D041400068
	C92443F	High - Not Confirmed	0.9890	NA	0.9949	0.8640	934	20	16395	D051500042	16.4			NA	0.9510	Unknown	631	1	671	D041400069
	C92451F	NA	0.9974	NA	0.9949	0.8640	667	20	11614	D051500043	11.6		45.2	NA	0.9510	Unknown	464	1	490	D041400070
	C92452F	NA	0.9927	NA	0.9949	0.8640	856	20	14968	D051500044	15.0	9.63	4.35	NA	0.9510	Unknown	421	1	446	D041400073

A = Qualitative only

NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
PFOSEA = Perfluorooctane sulfonyl ethylamide
M556 = C8F17SO2N((H)CH2COO)

Date Entered/Analyst: 05/01/00, 05/26/00 MMH, CSH
Date Verified/Analyst: 03/22/01 hoj, 04/03/01 kjh, 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC / 04/03/01 KJH

AMDP1# 050598.2
Covance# 6329-228

Study:	TOX003. 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats
Product Number(Test Substance):	T-6316 (EtFOSE-OH)
Matrix:	Rat Liver
Method/Revision:	ETS-8-6.0 & ETS-8-7.0
Analytical Equipment System Number:	Amelia 062498, Davey 070799
Instrument Software/Version:	MassLynx 3.3 & 3.4
Date of Extraction/Analyst:	4/14/00 SAL/RWW
Date of Analysis/Analyst:	4/17/00, 5/15/00 IAS/MMH/IAS
Date of Data Reduction/Analyst:	4/18/00, 5/16/00, 05/08/01 IAS/MMH
Filename:	See Below
R-Squared Valu	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments

Sample Data

WEEK 105 RAT LIVER

lot 98-0207-0303-3

lot SD013

Concentration of PFOSA ug/g or % Rec	Mean PFOSA ug/g	RSD Std. Dev. MS/MSD RPD	Group Dose	Sample #	Surrogate Verified	PFOSAA Old Purity Correction Factor	PFOSAA Purity Correction Factor	PFOSAA Conc. ug/g	PFOSAA Dilution Factor	PFOSAA Calc. Conc. ug/g	Filename	Concentration of PFOSAA ug/g or % Rec	Mean PFOSAA ug/g	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	EtFOSE Old Purity Correction Factor	EtFOSE Purity Correction Factor	EtFOSE Conc. ug/g	EtFOSE Dilution Factor	EtFOSE Calc. Conc. ug/g
< LOQ (0.00644 ug/g)		NA	Method Blk	RBL041400-H2OBlk-1	NA	0.9760	Unknown	0.00	1	0.00	A041700004	< LOQ (0.0125 ug/g)		NA	NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)	<LOQ	NA		RBL041400-H2OBlk-2	NA	0.9760	Unknown	0.00	1	0.00	D041400004	< LOQ (0.0314 ug/g)	<LOQ	NA	NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.00644 ug/g)		NA	Matrix Blk	RBL041400-LiverBlk-1	NA	0.9760	Unknown	0.00	1	0.00	A041700005	< LOQ (0.0125 ug/g)		NA	NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)	<LOQ	NA		RBL041400-LiverBlk-2	NA	0.9760	Unknown	0.00	1	0.00	D041400005	< LOQ (0.0314 ug/g)	<LOQ	NA	NA	0.9770	0.8890	0.00	1	0.00
74%			QC	C92197M/GB-MS-1	NA	0.9760	Unknown	224	1	215	D041400017	74%			NA	0.9770	0.8890	277	1	265
86%	82%	9%		C92197MGB-MSD-1	NA	0.9760	Unknown	260	1	249	D041400018	86%	80%	15%	NA	0.9770	0.8890	231	1	221
94%				C92195/MGB-MS-1	NA	0.9760	Unknown	251	1	240	A041700017	82%			NA	0.9770	0.8890	472	1	452
63%	79%	39%		C92195/MGB-MSD-1	NA	0.9760	Unknown	254	1	243	A041700018	83%	82%	2%	NA	0.9770	0.8890	459	1	439
92%				RBL041400-MS-1	NA	0.9760	Unknown	232	1	222	D041400075	73%			NA	0.9770	0.8890	NR	NR	NR
85%	88%	9%		RBL041400-MSD-1	NA	0.9760	Unknown	266	1	266	D041400076	87%	80%	18%	NA	0.9770	0.8890	NR	NR	NR
< LOQ (0.0322 ug/g)			Group 8	C92197M	NA	0.9760	Unknown	0.00	1	0.00	D041400019	< LOQ (0.0314 ug/g)			NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)			0.0 ppm	C92203M	NA	0.9760	Unknown	0.00	1	0.00	D041400020	< LOQ (0.0314 ug/g)			NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)				C92208M	NA	0.9760	Unknown	0.00	1	0.00	D041400021	< LOQ (0.0314 ug/g)			NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)				C92209M	NA	0.9760	Unknown	0.00	1	0.00	D041400024	< LOQ (0.0314 ug/g)			NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)				C92218M	NA	0.9760	Unknown	0.00	1	0.00	D041400025	< LOQ (0.0314 ug/g)			NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)				C92222M	NA	0.9760	Unknown	0.00	1	0.00	D041400026	< LOQ (0.0314 ug/g)			NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)				C92231M	NA	0.9760	Unknown	0.00	1	0.00	D041400027	< LOQ (0.0314 ug/g)			NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)				C92233M	NA	0.9760	Unknown	0.00	1	0.00	D041400028	< LOQ (0.0314 ug/g)			NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)		NA		C92239M	NA	0.9760	Unknown	0.00	1	0.00	D041400031	< LOQ (0.0314 ug/g)		NA	NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)	<LOQ	NA		C92252M	NA	0.9760	Unknown	0.00	1	0.00	D041400032	< LOQ (0.0314 ug/g)	<LOQ	NA	NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)				C92233F	NA	0.9760	Unknown	0.00	1	0.00	D041400033	< LOQ (0.0314 ug/g)			NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)				C92334F	NA	0.9760	Unknown	0.00	1	0.00	D041400034	< LOQ (0.0314 ug/g)			NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)				C92348F	NA	0.9760	Unknown	0.00	1	0.00	D041400035	< LOQ (0.0314 ug/g)			NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)				C92354F	NA	0.9760	Unknown	0.00	1	0.00	D041400038	< LOQ (0.0314 ug/g)			NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)				C92358F	NA	0.9760	Unknown	0.00	1	0.00	D041400039	< LOQ (0.0314 ug/g)			NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)				C92360F	NA	0.9760	Unknown	0.00	1	0.00	D041400040	< LOQ (0.0314 ug/g)			NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)				C92362F	NA	0.9760	Unknown	0.00	1	0.00	D041400041	< LOQ (0.0314 ug/g)			NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)				C92369F	NA	0.9760	Unknown	0.00	1	0.00	D041400042	< LOQ (0.0314 ug/g)			NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)		NA		C92374F	NA	0.9760	Unknown	0.00	1	0.00	D041400045	< LOQ (0.0314 ug/g)		NA	NA	0.9770	0.8890	0.00	1	0.00
< LOQ (0.0322 ug/g)	<LOQ	NA		C92383F	NA	0.9760	Unknown	0.00	1	0.00	D041400046	< LOQ (0.0314 ug/g)	<LOQ	NA	NA	0.9770	0.8890	0.00	1	0.00
0.422			Group 9	C92261M	NA	0.9760	Unknown	574	1	591	D041400047	0.591			NA	0.9770	0.8890	0.00	1	0.00
0.280			1.0 ppm	C92266M	NA	0.9760	Unknown	383	1	396	D041400048	0.396			NA	0.9770	0.8890	0.00	1	0.00
0.829				C92270M	NA	0.9760	Unknown	112	20	2279	D051500019	2.28			NA	0.9770	0.8890	0.00	1	0.00
0.505				C92278M	NA	0.9760	Unknown	762	1	778	D041400052	0.778			NA	0.9770	0.8890	0.00	1	0.00
0.246				C92281M	NA	0.9760	Unknown	600	1	612	D041400053	0.612			NA	0.9770	0.8890	0.00	1	0.00
0.352				C92283M	NA	0.9760	Unknown	527	1	531	D041400054	0.531			NA	0.9770	0.8890	0.00	1	0.00
0.180				C92309M	NA	0.9760	Unknown	497	1	518	D041400055	0.518			NA	0.9770	0.8890	0.00	1	0.00
0.378				C92311M	NA	0.9760	Unknown	545	1	565	D041400056	0.565			NA	0.9770	0.8890	0.00	1	0.00
0.265		50.4		C92318M	NA	0.9760	Unknown	482	1	496	D041400059	0.496		74.9	NA	0.9770	0.8890	0.00	1	0.00
0.258	0.371	0.187		C92321M	NA	0.9760	Unknown	578	1	593	D041400060	0.593	0.736	0.551	NA	0.9770	0.8890	0.00	1	0.00
0.546				C92410F	NA	0.9760	Unknown	580	1	545	D041400061	0.545			NA	0.9770	0.8890	0.00	1	0.00
0.773				C92416F	NA	0.9760	Unknown	88.6	20	1831	D051500034	1.83			NA	0.9770	0.8890	0.00	1	0.00
0.512				C92419F	NA	0.9760	Unknown	420	1	424	D041400063	0.424			NA	0.9770	0.8890	0.00	1	0.00
0.624				C92423F	NA	0.9760	Unknown	813	1	835	D041400066	0.835			NA	0.9770	0.8890	60.4	1	55.1
0.402				C92430F	NA	0.9760	Unknown	482	1	496	D041400074	0.496			NA	0.9770	0.8890	0.00	1	0.00
0.484				C92431F	NA	0.9760	Unknown	698	1	707	D041400067	0.707			NA	0.9770	0.8890	0.00	1	0.00
0.551				C92439F	NA	0.9760	Unknown	404	1	414	D041400068	0.414			NA	0.9770	0.8890	0.00	1	0.00
0.671				C92443F	NA	0.9760	Unknown	753	1	780	D041400069	0.780			NA	0.9770	0.8890	0.00	1	0.00
0.490		20.3		C92451F	NA	0.9760	Unknown	862	1	885	D041400070	0.885		53.2	NA	0.9770	0.8890	0.00	1	0.00
0.446	0.550	0.112		C92452F	NA	0.9760	Unknown	772	1	796	D041400073	0.796	0.771	0.411	NA	0.9770	0.8890	0.00	1	0.00

A = Qualitative only

NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
PFOSEA = Perfuoroctane sulfonyl ethylamide
M556 = C8F17SO2N((H)CH2COO)

Date Entered/Analyst: 05/01/00, 05/26/00 MMH, CSH
Date Verified/Analyst: 03/22/01 boj, 04/03/01 kjh, 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC / 04/03/01 KJH

Covance# 6329-228

Filename	Concentration of EtFOSE ug/g or % Rec		Mean EtFOSE ug/g	RSD Std. Dev. MS/MSD RPD
A041700004	< LOQ (0.0559 ug/g)	A		NA
D041400004	< LOQ (0.0559 ug/g)	A	<LOQ	NA
A041700005	< LOQ (0.0559 ug/g)	A		NA
D041400005	< LOQ (0.0559 ug/g)	A	<LOQ	NA
D041400017	92%	A		
D041400018	77%	A	84%	18%
A041700017	154%	A		
A041700018	149%	A	151%	3%
NR	NR	A		
NR	NR	A	NR	NR
D041400019	< LOQ (0.0559 ug/g)	A		
D041400020	< LOQ (0.0559 ug/g)	A		
D041400021	< LOQ (0.0559 ug/g)	A		
D041400024	< LOQ (0.0559 ug/g)	A		
D041400025	< LOQ (0.0559 ug/g)	A		
D041400026	< LOQ (0.0559 ug/g)	A		
D041400027	< LOQ (0.0559 ug/g)	A		
D041400028	< LOQ (0.0559 ug/g)	A		
D041400031	< LOQ (0.0559 ug/g)	A		NA
D041400032	< LOQ (0.0559 ug/g)	A	<LOQ	NA
D041400033	< LOQ (0.0559 ug/g)	A		
D041400034	< LOQ (0.0559 ug/g)	A		
D041400035	< LOQ (0.0559 ug/g)	A		
D041400038	< LOQ (0.0559 ug/g)	A		
D041400039	< LOQ (0.0559 ug/g)	A		
D041400040	< LOQ (0.0559 ug/g)	A		
D041400041	< LOQ (0.0559 ug/g)	A		
D041400042	< LOQ (0.0559 ug/g)	A		
D041400045	< LOQ (0.0559 ug/g)	A		NA
D041400046	< LOQ (0.0559 ug/g)	A	<LOQ	NA
D041400047	< LOQ (0.0559 ug/g)	A		
D041400048	< LOQ (0.0559 ug/g)	A		
D041400049	< LOQ (0.0559 ug/g)	A		
D041400052	< LOQ (0.0559 ug/g)	A		
D041400053	< LOQ (0.0559 ug/g)	A		
D041400054	< LOQ (0.0559 ug/g)	A		
D041400055	< LOQ (0.0559 ug/g)	A		
D041400056	< LOQ (0.0559 ug/g)	A		
D041400059	< LOQ (0.0559 ug/g)	A		NA
D041400060	< LOQ (0.0559 ug/g)	A	<LOQ	NA
D041400061	< LOQ (0.0559 ug/g)	A		
D041400062	< LOQ (0.0559 ug/g)	A		
D041400063	< LOQ (0.0559 ug/g)	A		
D041400066	< LOQ (0.0559 ug/g)	A		
D041400074	< LOQ (0.0559 ug/g)	A		
D041400067	< LOQ (0.0559 ug/g)	A		
D041400068	< LOQ (0.0559 ug/g)	A		
D041400069	< LOQ (0.0559 ug/g)	A		
D041400070	< LOQ (0.0559 ug/g)	A		NA
D041400073	< LOQ (0.0559 ug/g)	A	<LOQ	NA

Covance# 6329-228

Study: TOX003, 104 Week Dietary Carcinogenicity Study with Narrow Raage (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats
Product Number(Test Substance): T-6316 (EtFOSE-OH)
Matrix: Rat Liver
Method/Revision: ETS-8-6.0 & ETS-8-7.0
Analytical Equipment System Number: Ameba 062498, Davey 070799
Instrument Software/Version: MassLynx 3.3 & 3.4
Date of Extraction/Analyst: 4/14/00 SAL/RWW
Date of Analysis/Analyst: 4/17/00, 5/15/00 IAS/MMH/IAS
Date of Data Reduction/Analyst: 4/18/00, 5/16/00, 05/08/01 IAS/MMH

Filename: See Below
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments

Sample Data

Rat Liver week 104

lot NB113047-80

lot 529

Group Dose	Sample #	Surrogate Verified	M556 Old Purity Correction Factor	M556 Purity Correction Factor	M556 Conc. ug/g	M556 Dilution Factor	M556 Calc. Conc. ng/g	Filename	Concentration of M556 ug/g or % Rec	Mean M556 ug/g	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	PFOSEA Old Purity Correction Factor	PFOSEA Purity Correction Factor	PFOSEA Conc. ng/g	PFOSEA Dilution Factor	PFOSEA Calc. Conc. ng/g	Filename	Concentration of PFOSEA ug/g or % Rec
Method Blk	RBL041400-H2OBlk-1	NA	0.9989	Unknown	0.00	1	0.00	A041700004	< LOQ (0.0307 ug/g)		NA	NA	0.9930	Unknown	0.00	1	0.00	A041700004	< LOQ (0.0308 ug/g)
	RBL041400-H2OBlk-2	NA	0.9989	Unknown	0.00	1	0.00	D041400004	< LOQ (0.0307 ug/g)	<LOQ	NA	NA	0.9930	Unknown	0.00	1	0.00	D041400004	< LOQ (0.0308 ug/g)
Matrix Blk	RBL041400-LiverBlk-1	NA	0.9989	Unknown	0.00	1	0.00	A041700005	< LOQ (0.0307 ug/g)		NA	NA	0.9930	Unknown	0.00	1	0.00	A041700005	< LOQ (0.0308 ug/g)
	RBL041400-LiverBlk-2	NA	0.9989	Unknown	0.00	1	0.00	D041400005	< LOQ (0.0307 ug/g)	<LOQ	NA	NA	0.9930	Unknown	0.00	1	0.00	D041400005	< LOQ (0.0308 ug/g)
QC	C92197/MGB-MS-1	NA	0.9989	Unknown	361	1	345	D041400017	119%			NA	0.9930	Unknown	208	1	199	D041400017	69.2%
	C92197/MGB-MSD-1	NA	0.9989	Unknown	370	1	354	D041400018	122%	121%	2.42%	NA	0.9930	Unknown	254	1	243	D041400018	84.4%
	C92195/MGB-MS-1	NA	0.9989	Unknown	316	1	303	A041700017	103%			NA	0.9930	Unknown	442	1	423	A041700017	144%
	C92195/MGB-MSD-1	NA	0.9989	Unknown	331	1	317	A041700018	108%	105%	4.58%	NA	0.9930	Unknown	353	1	338	A041700018	115%
	RBL041400-MS-1	NA	0.9989	Unknown	256	1	245	D041400075	80%			NA	0.9930	Unknown	358	1	343	D041400075	112%
	RBL041400-MSD-1	NA	0.9989	Unknown	257	1	257	D041400076	84%	82%	5%	NA	0.9930	Unknown	393	1	376	D041400076	123%
Group 8 0.0 ppm	C92197M	NA	0.9989	Unknown	0.00	1	0.00	D041400019	< LOQ (0.0307 ug/g)			NA	0.9930	Unknown	0.00	1	0.00	D041400019	< LOQ (0.0308 ug/g)
	C92203M	NA	0.9989	Unknown	0.00	1	0.00	D041400020	< LOQ (0.0307 ug/g)			NA	0.9930	Unknown	0.00	1	0.00	D041400020	< LOQ (0.0308 ug/g)
	C92208M	NA	0.9989	Unknown	0.00	1	0.00	D041400021	< LOQ (0.0307 ug/g)			NA	0.9930	Unknown	0.00	1	0.00	D041400021	< LOQ (0.0308 ug/g)
	C92209M	NA	0.9989	Unknown	0.00	1	0.00	D041400024	< LOQ (0.0307 ug/g)			NA	0.9930	Unknown	0.00	1	0.00	D041400024	< LOQ (0.0308 ug/g)
	C92218M	NA	0.9989	Unknown	0.00	1	0.00	D041400025	< LOQ (0.0307 ug/g)			NA	0.9930	Unknown	0.00	1	0.00	D041400025	< LOQ (0.0308 ug/g)
	C92222M	NA	0.9989	Unknown	0.00	1	0.00	D041400026	< LOQ (0.0307 ug/g)			NA	0.9930	Unknown	0.00	1	0.00	D041400026	< LOQ (0.0308 ug/g)
	C92231M	NA	0.9989	Unknown	0.00	1	0.00	D041400027	< LOQ (0.0307 ug/g)			NA	0.9930	Unknown	0.00	1	0.00	D041400027	< LOQ (0.0308 ug/g)
	C92233M	NA	0.9989	Unknown	0.00	1	0.00	D041400028	< LOQ (0.0307 ug/g)			NA	0.9930	Unknown	0.00	1	0.00	D041400028	< LOQ (0.0308 ug/g)
	C92239M	NA	0.9989	Unknown	0.00	1	0.00	D041400031	< LOQ (0.0307 ug/g)		NA	NA	0.9930	Unknown	0.00	1	0.00	D041400031	< LOQ (0.0308 ug/g)
	C92252M	NA	0.9989	Unknown	0.00	1	0.00	D041400032	< LOQ (0.0307 ug/g)	<LOQ	NA	NA	0.9930	Unknown	0.00	1	0.00	D041400032	< LOQ (0.0308 ug/g)
	C92333F	NA	0.9989	Unknown	0.00	1	0.00	D041400033	< LOQ (0.0307 ug/g)			NA	0.9930	Unknown	0.00	1	0.00	D041400033	< LOQ (0.0308 ug/g)
	C92334F	NA	0.9989	Unknown	0.00	1	0.00	D041400034	< LOQ (0.0307 ug/g)			NA	0.9930	Unknown	0.00	1	0.00	D041400034	< LOQ (0.0308 ug/g)
	C92348F	NA	0.9989	Unknown	0.00	1	0.00	D041400035	< LOQ (0.0307 ug/g)			NA	0.9930	Unknown	0.00	1	0.00	D041400035	< LOQ (0.0308 ug/g)
	C92354F	NA	0.9989	Unknown	0.00	1	0.00	D041400038	< LOQ (0.0307 ug/g)			NA	0.9930	Unknown	0.00	1	0.00	D041400038	< LOQ (0.0308 ug/g)
	C92358F	NA	0.9989	Unknown	0.00	1	0.00	D041400039	< LOQ (0.0307 ug/g)			NA	0.9930	Unknown	0.00	1	0.00	D041400039	< LOQ (0.0308 ug/g)
	C92360F	NA	0.9989	Unknown	0.00	1	0.00	D041400040	< LOQ (0.0307 ug/g)			NA	0.9930	Unknown	0.00	1	0.00	D041400040	< LOQ (0.0308 ug/g)
	C92362F	NA	0.9989	Unknown	0.00	1	0.00	D041400041	< LOQ (0.0307 ug/g)			NA	0.9930	Unknown	0.00	1	0.00	D041400041	< LOQ (0.0308 ug/g)
	C92369F	NA	0.9989	Unknown	0.00	1	0.00	D041400042	< LOQ (0.0307 ug/g)			NA	0.9930	Unknown	0.00	1	0.00	D041400042	< LOQ (0.0308 ug/g)
	C92374F	NA	0.9989	Unknown	0.00	1	0.00	D041400045	< LOQ (0.0307 ug/g)		NA	NA	0.9930	Unknown	0.00	1	0.00	D041400045	< LOQ (0.0308 ug/g)
	C92383F	NA	0.9989	Unknown	0.00	1	0.00	D041400046	< LOQ (0.0307 ug/g)	<LOQ	NA	NA	0.9930	Unknown	0.00	1	0.00	D041400046	< LOQ (0.0308 ug/g)
Group 9 1.0 ppm	C92261M	NA	0.9989	Unknown	230	1	231	D041400047	0.231			NA	0.9930	Unknown	0.00	1	0.00	D041400047	< LOQ (0.0308 ug/g)
	C92266M	NA	0.9989	Unknown	314	1	317	D041400048	0.317			NA	0.9930	Unknown	0.00	1	0.00	D041400048	< LOQ (0.0308 ug/g)
	C92270M	NA	0.9989	Unknown	402	1	399	D041400049	0.399			NA	0.9930	Unknown	0.00	1	0.00	D041400049	< LOQ (0.0308 ug/g)
	C92278M	NA	0.9989	Unknown	313	1	312	D041400052	0.312			NA	0.9930	Unknown	0.00	1	0.00	D041400052	< LOQ (0.0308 ug/g)
	C92281M	NA	0.9989	Unknown	194	1	193	D041400053	0.193			NA	0.9930	Unknown	0.00	1	0.00	D041400053	< LOQ (0.0308 ug/g)
	C92283M	NA	0.9989	Unknown	306	1	300	D041400054	0.300			NA	0.9930	Unknown	0.00	1	0.00	D041400054	< LOQ (0.0308 ug/g)
	C92309M	NA	0.9989	Unknown	84	1	85.9	D041400055	0.0859			NA	0.9930	Unknown	0.00	1	0.00	D041400055	< LOQ (0.0308 ug/g)
	C92311M	NA	0.9989	Unknown	212	1	214	D041400056	0.214			NA	0.9930	Unknown	0.00	1	0.00	D041400056	< LOQ (0.0308 ug/g)
	C92318M	NA	0.9989	Unknown	240	1	241	D041400059	0.241		34.7	NA	0.9930	Unknown	0.00	1	0.00	D041400059	< LOQ (0.0308 ug/g)
	C92321M	NA	0.9989	Unknown	201	1	202	D041400060	0.202	0.250	0.0865	NA	0.9930	Unknown	0.00	1	0.00	D041400060	< LOQ (0.0308 ug/g)
	C92410F	NA	0.9989	Unknown	454	1	417	D041400061	0.417			NA	0.9930	Unknown	0.00	1	0.00	D041400061	< LOQ (0.0308 ug/g)
	C92416F	NA	0.9989	Unknown	486	1	491	D041400062	0.491			NA	0.9930	Unknown	0.00	1	0.00	D041400062	< LOQ (0.0308 ug/g)
	C92419F	NA	0.9989	Unknown	318	1	313	D041400063	0.313			NA	0.9930	Unknown	0.00	1	0.00	D041400063	< LOQ (0.0308 ug/g)
	C92423F	NA	0.9989	Unknown	385	1	386	D041400066	0.386			NA	0.9930	Unknown	0.00	1	0.00	D041400066	< LOQ (0.0308 ug/g)
	C92430F	NA	0.9989	Unknown	245	1	246	D041400074	0.246			NA	0.9930	Unknown	0.00	1	0.00	D041400074	< LOQ (0.0308 ug/g)
	C92431F	NA	0.9989	Unknown	267	1	265	D041400067	0.265			NA	0.9930	Unknown	0.00	1	0.00	D041400067	< LOQ (0.0308 ug/g)
	C92439F	NA	0.9989	Unknown	339	1	339	D041400068	0.339			NA	0.9930	Unknown	0.00	1	0.00	D041400068	< LOQ (0.0308 ug/g)
	C92443F	NA	0.9989	Unknown	302	1	306	D041400069	0.306			NA	0.9930	Unknown	0.00	1	0.00	D041400069	< LOQ (0.0308 ug/g)
	C92451F	NA	0.9989	Unknown	310	1	311	D041400070	0.311		22.2	NA	0.9930	Unknown	0.00	1	0.00	D041400070	< LOQ (0.0308 ug/g)
	C92452F	NA	0.9989	Unknown	288	1	291	D041400073	0.291	0.336	0.0748	NA	0.9930	Unknown	0.00	1	0.00	D041400073	< LOQ (0.0308 ug/g)

A = Qualitative only

NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Raage N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
PFOSEA = Perfuorooctane sulfosyl ethylamide
M556 = C8F17SO2N((H)CH2COO)

Date Entered/Analyst: 05/01/00, 05/26/00 MMH, CSH
Date Verified/Analyst: 03/22/01 hoj, 04/03/01 kjh, 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC / 04/03/01 KJH

Covance# 6329-228

Study: TOX003, 104 Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats
Product Number(Test Substance): T-6316 (EtFOSE-OH)
Matrix: Rat Liver — Filename: See Attachments
Method/Revision: ETS-8-6.0 & ETS-8-7.0 — R-Squared Value: See Attachments
Analytical Equipment System Number: Amelia 062498, Davey 070799 — Slope: See Attachments
Instrument Software/Version: MassLynx 3.3 & 3.4 — Y-Intercept: See Attachments
Date of Extraction/Analyst: 4/14/00 SAL/RWW
Date of Analysis/Analyst: 4/17/00, 5/15/00 IAS/MMH/IAS
Date of Data Reduction/Analyst: 4/18/00, 5/16/00, 05/08/01 IAS/MMH

Sample Data

WEEK 105 RAT LIVER

Mean PFOSEA ug/g	RSD Std. Dev. MS/MSD RPD	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	PFOSA Calc. Conc. ug/g	Concentration of PFOSA ug/g or % Rec	Mean PFOSA ug/g	RSD Std. Dev. MS/MSD RPD	PFOSAA Calc. Conc. ug/g	Concentration of PFOSAA ug/g or % Rec	Mean PFOSAA ug/g	RSD Std. Dev. MS/MSD RPD	EtFOSE Calc. Conc. ug/g	Concentration of EtFOSE ug/g or % Rec		Mean EtFOSE ug/g	RSD Std. Dev. MS/MSD RPD
	NA	Method Blk	RBL041400-H2OBlk-1	0.00	< LOQ (0.0267 ug/g)			2.51	< LOQ (0.00644 ug/g)			0.00	< LOQ (0.0125 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
<LOQ	NA		RBL041400-H2OBlk-2	0.00	< LOQ (0.0107 ug/g)	<LOQ	NA	0.00	< LOQ (0.0322 ug/g)	<LOQ	NA	0.00	< LOQ (0.0314 ug/g)	<LOQ	NA	0.00	< LOQ (0.0559 ug/g)	A	<LOQ	NA
	NA	Matrix Blk	RBL041400-LiverBlk-1	0.00	< LOQ (0.0267 ug/g)			2.20	< LOQ (0.00644 ug/g)			0.00	< LOQ (0.0125 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
<LOQ	NA		RBL041400-LiverBlk-2	4.77	< LOQ (0.0107 ug/g)	<LOQ	NA	0.00	< LOQ (0.0322 ug/g)	<LOQ	NA	0.00	< LOQ (0.0314 ug/g)	<LOQ	NA	0.00	< LOQ (0.0559 ug/g)	A	<LOQ	NA
		QC - 300 ppb	C92197/MGR-MS-1	226	78%			224	78%			215	74%			165	92%	A		
77%	20%		C92197/MGR-MSD-1	224	78%	78%	1%	246	86%	82%	9%	249	86%	80%	15%	221	77%	A	84%	18%
			C92195/MGR-MS-1	403	137%			280	94%			240	82%			452	154%	A		
130%	22%		C92195/MGR-MSD-1	324	110%	124%	22%	188	63%	79%	39%	243	83%	82%	2%	439	149%	A	151%	3%
			RBL041400-MS-1	232	76%			282	92%			222	73%			NR	NR	A		
117%	9%		RBL041400-MSD-1	261	85%	80%	12%	259	85%	88%	9%	266	87%	80%	18%	NR	NR	A	NR	NR
		Group 8	C92197M	44.1	0.0441			0.00	< LOQ (0.0322 ug/g)			0.00	< LOQ (0.0314 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
		0.0 ppm	C92203M	2.94	< LOQ (0.0107 ug/g)			0.00	< LOQ (0.0322 ug/g)			0.00	< LOQ (0.0314 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
			C92208M	8.06	< LOQ (0.0107 ug/g)			0.00	< LOQ (0.0322 ug/g)			0.00	< LOQ (0.0314 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
			C92209M	0.00	< LOQ (0.0107 ug/g)			0.24	< LOQ (0.0322 ug/g)			0.00	< LOQ (0.0314 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
			C92218M	73.2	0.0732			0.00	< LOQ (0.0322 ug/g)			0.00	< LOQ (0.0314 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
			C92222M	3.43	< LOQ (0.0107 ug/g)			0.00	< LOQ (0.0322 ug/g)			0.00	< LOQ (0.0314 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
			C92231M	2.41	< LOQ (0.0107 ug/g)			0.00	< LOQ (0.0322 ug/g)			0.00	< LOQ (0.0314 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
			C92233M	0.00	< LOQ (0.0107 ug/g)			0.00	< LOQ (0.0322 ug/g)			0.00	< LOQ (0.0314 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
	NA		C92239M	225	0.225		141.4	0.00	< LOQ (0.0321 ug/g)		NA	0.00	< LOQ (0.0314 ug/g)		NA	0.00	< LOQ (0.0559 ug/g)	A		NA
<LOQ	NA		C92252M	246	0.246	0.065	0.092	0.00	< LOQ (0.0322 ug/g)	<LOQ	NA	0.00	< LOQ (0.0314 ug/g)	<LOQ	NA	0.00	< LOQ (0.0559 ug/g)	A	<LOQ	NA
			C92333F	109	0.109			0.00	< LOQ (0.0322 ug/g)			0.00	< LOQ (0.0314 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
			C92334F	93.0	0.0930			0.37	< LOQ (0.0322 ug/g)			0.00	< LOQ (0.0314 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
			C92348F	153	0.153			0.09	< LOQ (0.0322 ug/g)			0.00	< LOQ (0.0314 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
			C92354F	13.8	0.0138			0.00	< LOQ (0.0322 ug/g)			0.00	< LOQ (0.0314 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
			C92358F	6.42	< LOQ (0.0107 ug/g)			0.09	< LOQ (0.0322 ug/g)			0.00	< LOQ (0.0314 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
			C92360F	88.4	0.0884			0.26	< LOQ (0.0322 ug/g)			0.00	< LOQ (0.0314 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
			C92362F	29.9	0.0299			0.54	< LOQ (0.0322 ug/g)			0.00	< LOQ (0.0314 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
			C92369F	112	0.112			0.00	< LOQ (0.0322 ug/g)			0.00	< LOQ (0.0314 ug/g)			0.00	< LOQ (0.0559 ug/g)	A		
	NA		C92374F	111	0.111		59.9	0.46	< LOQ (0.0322 ug/g)		NA	0.00	< LOQ (0.0314 ug/g)		NA	0.00	< LOQ (0.0559 ug/g)	A		NA
<LOQ	NA		C92383F	150	0.150	0.08717	0.0522	0.32	< LOQ (0.0322 ug/g)	<LOQ	NA	0.00	< LOQ (0.0314 ug/g)	<LOQ	NA	0.00	< LOQ (0.0559 ug/g)	A	<LOQ	NA
		Group 9	C92261M	5037	5.04			422	0.422			591	0.591			0.00	< LOQ (0.0559 ug/g)	A		
		1.0 ppm	C92266M	7515	7.51			280	0.280			396	0.396			0.00	< LOQ (0.0559 ug/g)	A		
			C92270M	12702	12.7			829	0.829			2279	2.279			0.00	< LOQ (0.0559 ug/g)	A		
			C92278M	3781	3.78			505	0.505			778	0.778			0.00	< LOQ (0.0559 ug/g)	A		
			C92281M	4660	4.66			246	0.246			612	0.612			0.00	< LOQ (0.0559 ug/g)	A		
			C92283M	10841	10.8			352	0.352			531	0.531			0.00	< LOQ (0.0559 ug/g)	A		
			C92309M	568	0.568			180	0.180			518	0.518			0.00	< LOQ (0.0559 ug/g)	A		
			C92311M	2084	2.08			378	0.378			565	0.565			0.00	< LOQ (0.0559 ug/g)	A		
	NA		C92318M	8678	8.68		61.2	265	0.265		50.4	496	0.496		74.9	0.00	< LOQ (0.0559 ug/g)	A		NA
<LOQ	NA		C92321M	6249	6.25	6.21	3.80	258	0.258	0.371	0.187	593	0.593	0.736	0.551	0.00	< LOQ (0.0559 ug/g)	A	<LOQ	NA
			C92410F	10717	10.7			546	0.546			545	0.545			0.00	< LOQ (0.0559 ug/g)	A		
			C92416F	10911	10.9			773	0.773			1831	1.831			0.00	< LOQ (0.0559 ug/g)	A		
			C92419F	2177	2.18			512	0.512			424	0.424			0.00	< LOQ (0.0559 ug/g)	A		
			C92423F	5114	5.11			624	0.624			835	0.835			55.1	< LOQ (0.0559 ug/g)	A		
			C92430F	8351	8.35			402	0.402			496	0.496			0.00	< LOQ (0.0559 ug/g)	A		
			C92431F	6178	6.18			484	0.484			707	0.707			0.00	< LOQ (0.0559 ug/g)	A		
			C92439F	9900	9.90			551	0.551			414	0.414			0.00	< LOQ (0.0559 ug/g)	A		
			C92443F	16395	16.4			671	0.671			780	0.780			0.00	< LOQ (0.0559 ug/g)	A		
	NA		C92451F	11614	11.6		45.2	490	0.490		20.3	885	0.885		53.2	0.00	< LOQ (0.0559 ug/g)	A		NA
<LOQ	NA		C92452F	14968	15.0	9.63	4.35	446	0.446	0.550	0.112	796	0.796	0.771	0.411	0.00	< LOQ (0.0559 ug/g)	A	<LOQ	NA

A = Qualitative only

NR = Not Reported
NA = Not Applicable
LOQ = Limit of Quantitation

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol
PFOSEA = Perfluorooctane sulfonyl ethylamide
M556 = C8F17SO2N((H)CH2COO)

Date Entered/Analyst: 05/01/00, 05/26/00 MMH, CSH
Date Verified/Analyst: 03/22/01 hoj, 04/03/01 kjh, 5/22/01 mmh
Purity Entered/Verified: 03/29/01 LAC / 04/03/01 KJH

3M Medical Department Study: T-6316.1 Analytical Report: FACT TOX-003
LRN-U2104

Appendix F: Example Calculations

Formula Used for Sera Analyses in Study FACT TOX-003

$$\text{AR (ng/mL)} \times \text{DF} \times \frac{\text{PC2}}{\text{PC1}} \times \frac{\text{FV (mL)}}{\text{EV (mL)}} \times \frac{1.0\ \mu\text{g}}{1000\ \text{ng}} = \textbf{Reported Concentration (µg/mL)}$$

Calculation Used for Group 9, Week 105, Animal ID C92430/F

$$199\ \text{ng/mL} \times 10 \times \frac{0.8640}{0.9949} \times \frac{1\ \text{mL}}{1\ \text{mL}} \times \frac{1.0\ \mu\text{g}}{1000\ \text{ng}} = \textbf{1.73 µg/mL}$$

AR— Analytical result from MassLynx summary
DF— Dilution factor
PC1—Purity Correction–1
PC2—Purity Correction–2
FV—Final extract volume (1.0 mL unless otherwise noted)
EV—Volume of sera extracted

Formula Used for Liver Analyses in Study FACT TOX-003

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

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

Calculation Used for Group 9, Week 53, Animal ID C92267M

$$497\ \text{ng/g} \times \frac{1\ \text{g/}\ 5\ \text{mL}}{1.0137\ \text{g/}\ 5\text{mL}} \times \frac{0.8640}{0.9949} \times 100 \times \frac{1.0\ \mu\text{g}}{1000\ \text{ng}} = \textbf{42.6 µg/g}$$

AR— Analytical result from MassLynx summary
∂ curve—Density of the liver standard curve, assumed to be 1g liver/ 5 ml water
∂ sample—Density of the liver sample (g sample/ 5 mL H_2O)
PC1—Purity Correction–1
PC2—Purity Correction–2
DF— Dilution factor

3M Medical Department Study: T-6316.1 Analytical Report: FACT TOX-003
LRN-U2104

Appendix F: Example Calculations

Formula Used for Sera Analyses in Study FACT TOX-003

$$\text{AR (ng/mL)} \times \text{DF} \times \frac{\text{PC2}}{\text{PC1}} \times \frac{\text{FV (mL)}}{\text{EV (mL)}} \times \frac{1.0\ \mu g}{1000\ ng} = \textbf{Reported Concentration (µg/mL)}$$

Calculation Used for Group 9, Week 105, Animal ID C92430/F

$$199\ \text{ng/mL} \times 10 \times \frac{0.8640}{0.9949} \times \frac{1\ \text{mL}}{1\ \text{mL}} \times \frac{1.0\ \mu g}{1000\ ng} = \textbf{1.73 µg/mL}$$

AR— Analytical result from MassLynx summary
DF— Dilution factor
PC1—Purity Correction–1
PC2—Purity Correction–2
FV—Final extract volume (1.0 mL unless otherwise noted)
EV—Volume of sera extracted

Formula Used for Liver Analyses in Study FACT TOX-003

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

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

Calculation Used for Group 9, Week 53, Animal ID C92267M

$$497\ \text{ng/g} \times \frac{1\ \text{g}/\ 5\ \text{mL}}{1.0137\ \text{g}/\ 5\text{mL}} \times \frac{0.8640}{0.9949} \times 100 \times \frac{1.0\ \mu g}{1000\ ng} = \textbf{42.6 µg/g}$$

AR— Analytical result from MassLynx summary
∂ curve—Density of the liver standard curve, assumed to be 1g liver/ 5 ml water
∂ sample—Density of the liver sample (g sample/ 5 mL H_2O)
PC1—Purity Correction–1
PC2—Purity Correction–2
DF— Dilution factor

3M Medical Department Study: T-6316.1 Analytical Report: FACT TOX-003
LRN-U2104

Appendix G: Interim Certificates of Analysis

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

INTERIM CERTIFICATE OF ANALYSIS

Revision 1(9/7/00)

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

Test Name	Specifications	Result
Purity[1]		**86.4%**
Appearance	White Crystalline Powder	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 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.%

Centre Analytical Laboratories, Inc.
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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018B

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/01

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

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

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

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

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

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

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

Centre Analytical Laboratories, Inc.
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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018B

LC/MS Purity Profile:

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

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

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

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

Centre Analytical Laboratories, Inc.
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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-022-2
3M Product: EtFOSE-OH
Test Control Reference #: TCR-00017-52
Purity: 97.4%

Test Name	Specifications	Result
Purity[1]		**97.4%**
Appearance	Yellow-white, waxy solid	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium 4. Potassium 5. Nickel 6. Iron 7. Manganese		 1. <0.001 wt./wt.% 2. <0.001 wt./wt.% 3. <0.001 wt./wt.% 4. <0.001 wt./wt.% 5. <0.001 wt./wt.% 6. <0.001 wt./wt.% 7. <0.001 wt./wt.%
Total % Impurity (NMR)		1.26 wt./wt.%
Total % Impurity (LC/MS)		None Quantified
Total % Impurity (GC/MS)		1.29 wt./wt.%
Related Compounds – POAA		0.10 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		90.3 wt./wt.%.
Inorganic Anions (IC) 1. Chloride 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate		 1. <0.015 wt./wt.% 2. <0.005 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.007 wt./wt.% 7. <0.154 wt./wt.%
Organic Acids[2] (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[3]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	 1. Theoretical Value = 25.2% 2. Theoretical Value = 1.75% 3. Theoretical Value = 2.45% 4. Theoretical Value = 5.60% 5. Theoretical Value = 56.6%	 1. 25.04 wt./wt.% 2. 1.69 wt./wt.% 3. 2.61 wt./wt.% 4. 8.88 wt./wt.% 5. 56.8 wt./wt.%

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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-022-2
3M Product: EtFOSE-OH
Test Control Reference #: TCR-00017-52

Date of Last Analysis: 11/26/00

Expiration Date: 11/26/01

Storage Conditions: <-10 °C

Re-assessment Date: 11/26/01

[1]Purity = 100% - (total NMR impurities, 1.26% + GC/MS impurities, 1.29 + POAA, 0.10%)

Total impurity from all tests = 2.65%
Purity = 100% - 2.65% = 97.4%

[2]TFA	Trifluoroacetic acid
HFBA	Heptafluorobutyric acid
NFPA	Nonafluoropentanoic acid
PFPA	Pentafluoropropanoic acid

[3]Theoretical value calculations based on the empirical formula, $C_{12}H_{10}F_{17}NO_3S$ (MW=571)

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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-022-2
3M Product: EtFOSE-OH
Test Control Reference #: TCR-00017-52

GC/MS Purity Profile

Peak #	Retention Time (min)	Identity	% Impurity
1	13.934	PFOSDEA	0.36
2	17.307	C7	0.93
Total	-	-	**1.29**

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

Prepared By: _____ 11/27/00
David S. Bell Date
Scientist
Centre Analytical Laboratories

Reviewed By: _____ 11/27/00
John Flaherty Date
Laboratory Manager
Centre Analytical Laboratories

Centre Analytical Laboratories, Inc.
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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-022-1
3M Product: EtFOSE-OH
Test Control Reference #: SD-013
Purity: 88.9%

Test Name	Specifications	Result
Purity[1]		**88.9%**
Appearance	Yellow-white, waxy solid	Conforms
Identification NMR		Positive
Metals (ICP/MS) 1. Calcium 2. Magnesium 3. Sodium 4. Potassium 5. Nickel 6. Iron 7. Manganese		 1. <0.001 wt./wt.% 2. <0.001 wt./wt.% 3. <0.001 wt./wt.% 4. 0.002 wt./wt.% 5. <0.001 wt./wt.% 6. <0.001 wt./wt.% 7. <0.001 wt./wt.%
Total % Impurity (NMR)		0.90 wt./wt.%
Total % Impurity (LC/MS)		None Quantified
Total % Impurity (GC/MS)		10.21 wt./wt.%
Related Compounds – POAA		0.03 wt./wt.%
Residual Solvents (TGA)		None Detected
Purity by DSC		87.6 wt./wt.%.
Inorganic Anions (IC) 1. Chloride 2. Fluoride 3. Bromide 4. Nitrate 5. Nitrite 6. Phosphate 7. Sulfate		 1. <0.015 wt./wt.% 2. <0.005 wt./wt.% 3. <0.040 wt./wt.% 4. <0.009 wt./wt.% 5. <0.006 wt./wt.% 6. <0.007 wt./wt.% 7. <0.040 wt./wt.%
Organic Acids[2] (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[3]: 1. Carbon 2. Hydrogen 3. Nitrogen 4. Sulfur 5. Fluorine	 1. Theoretical Value = 25.2% 2. Theoretical Value = 1.75% 3. Theoretical Value = 2.45% 4. Theoretical Value = 5.60% 5. Theoretical Value = 56.6%	 1. 24.42 wt./wt.% 2. 1.78 wt./wt.% 3. 2.72 wt./wt.% 4. 9.34 wt./wt.% 5. 58.4 wt./wt.%

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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-022-1
3M Product: EtFOSE-OH
Test Control Reference #: SD-013

Date of Last Analysis: 11/26/00

Expiration Date: 11/26/01

Storage Conditions: <-10 °C

Re-assessment Date: 11/26/01

[1]Purity = 100% - (total metal impurities, 0.002% + total NMR impurities, 0.90% + GC/MS impurities, 10.21 + POAA, 0.03%)

Total impurity from all tests = 11.14%
Purity = 100% - 11.14% = 88.9%

[2]TFA	Trifluoroacetic acid
HFBA	Heptafluorobutyric acid
NFPA	Nonafluoropentanoic acid
PFPA	Pentafluoropropanoic acid

[3]Theoretical value calculations based on the empirical formula, $C_{12}H_{10}F_{17}NO_3S$ (MW=571)

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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-022-1
3M Product: EtFOSE-OH
Test Control Reference #: SD-013

GC/MS Purity Profile

Peak #	Retention Time (min)	Identity	% Impurity
1	6.163	Unknown	0.12
2	8.011	Unknown	0.23
3	8.206	Unknown	0.51
4	9.065	Unknown	0.21
5	9.844	Unknown	0.34
6	13.93	Unknown	0.62
7	14.238	Unknown	0.11
8	15.130	C2	0.11
9	15.52	C3	1.11
10	15.941	C4	1.55
11	16.379	C5	1.07
12	16.801	C6	3.30
13	17.222	C7	0.93
Total	-	-	**10.21**

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

Prepared By: _____ 11/27/00
David S. Bell Date
Scientist
Centre Analytical Laboratories

Reviewed By: _____ 11/27/00
John Flaherty Date
Laboratory Manager
Centre Analytical Laboratories

ANALYTICAL REPORT

STUDY TITLE

PURITY DETERMINATION OF SAMPLE LOTS OF PFOS
Sample # TCR-00065-022

DATA REQUIREMENTS

Test Article Characterization

STUDY DIRECTOR

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BY STE DATE 10/25/00

Kevin Lloyd

ANALYTICAL REPORT COMPLETION DATE

October 25, 2000

PERFORMING LABORATORIE / TESTING FACILITIES

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

STUDY SPONSOR

3M Environmental Technology and Safety Services
Building 2-3E-09
PO Box 33331
St. Paul, MN 55133-3331

PROJECT IDENTIFICATION

Centre Study Number: 023-045

Total Pages: 15

Centre Study No.: 023-045

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Centre Study Number 023-045, entitled "Purity Determination of Sample Lots of PFOS, Sample # TCR-00065-022" conducted for 3M Environmental Laboratory, was performed in compliance with US EPA Good Laboratory Practice Standards (40 CFR Part 160) by Centre Analytical Laboratories, Inc. with the following exceptions:

The automated data collection systems used in this study were not fully compliant with 21 CFR 58.130 (e).

Kevin Lloyd
Study Director
Centre Analytical Laboratories, Inc.

10/25/00
Date

James K. Lundberg, Ph.D.
Sponsor Representative
3M Environmental Technology and Safety Services

10/27/00
Date

STL 10/25/00

Centre Study No.: 023-045

QUALITY ASSURANCE STATEMENT

Centre Study Number 023-045, entitled "Purity Determination of Sample Lots of PFOS, Sample # TCR-00065-022" was reviewed by Centre Analytical Laboratories' Quality Assurance Unit. All reviewed phases were reviewed for conduct according to Centre Analytical Laboratories' Standard Operating Procedures, the Study Protocol, and all applicable Good Laboratory Practice Standards. All findings were reported to the Study Director and to management.

Phase	Date Inspected	Date Reported to Study Director and Centre Management	Date Reported to Sponsor Management
1. Protocol Review	10/19/00	10/25/00	10/25/00
2. Raw Data Review	10/19/00	10/25/00	10/25/00
3. Report Review	10/24/00	10/25/00	10/25/00

William C Spare
William Spare
Quality Assurance Officer

10/25/00
Date

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Centre Study No.: 023-045

CERTIFICATION OF AUTHENTICITY

This report, for Centre Study Number 023-045, is a true and complete representation of the raw data for the study.

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

Study Director, Centre:

10/25/00

Kevin Lloyd
Study Director
Centre Analytical Laboratories, Inc.

Date

Centre Analytical Laboratories, Inc. Facility Management:

25-OCT-00

Richard A. Grazzini, Ph.D.
President
Centre Analytical Laboratories, Inc.

Date

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Centre Study No.: 023-045

STUDY IDENTIFICATION

PURITY DETERMINATION OF SAMPLE LOTS OF PFOS
Sample # TCR-00065-022

TYPE OF STUDY:	Characterization
TEST SYSTEM:	Not Applicable
TEST ARTICLE:	PFOS, Test Control Reference # TCR-00065-022
SPONSOR:	3M Environmental Technology and Safety Services Building 2-3E-09 PO Box 33331 St. Paul, MN 55133-3331
STUDY DIRECTOR:	Kevin Lloyd Center Analytical Laboratories, Inc. Phone: (814) 231-8032
TESTING FACILITIES:	Centre Analytical Laboratories, Inc. (Centre) 3048 Research Drive State College, PA 16801 Phone: 814-231-8032
ANALYTICAL PHASE TIMETABLE:	Study Initiation Date: 09/28/00 Analytical Start Date: 10/05/00 Analytical Termination Date: 10/24/00

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Centre Study No.: 023-045

PROJECT PERSONNEL

The Study Director for this project was Kevin Lloyd at Centre Analytical Laboratories, Inc. The following personnel from Centre Analytical Laboratories, Inc., were associated with various phases of the study:

Name	Title
Gerry Shero	Scientist
David S. Bell	Scientist
Emily R. Stauffer	Scientist
Mark Ammerman	Sample Custodian

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TABLE OF CONTENTS

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1.0 SUMMARY

The purity of PFOS from sample # TCR-00065-022, was determined from the LC/MS test data to be 88.0% pure as compared with the PFOS control (TCR-00017-046, 97.9% purity).

2.0 INTRODUCTION

This report details the results of the analysis of PFOS, Test Control Reference# TCR-00065-022. The test was analysis by liquid chromatography – mass spectrometry (LC/MS). The LC/MS was conducted at Centre.

The study was initiated on September 28, 2000 when the Study Director signed protocol number 00P-023-045. The analytical start date was October 5, 2000, and the experimental termination date was October 9, 2000.

3.0 TEST SYSTEM

There is no test system associated with this characterization study, therefore the GLP requirement for test system description, justification, and identification do not apply. The route of administration, levels and frequency of administration also do not apply to characterization studies.

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4.0 TEST ARTICLE

The test article was PFOS, Test Control Reference# TCR-00065-022. 3M Environmental Laboratory supplied the test article and it was logged at Centre Analytical Laboratories, Inc. as follows:

Compound	Lot or NB Number	Centre Control No.	Purity	Date Received
PFOS TCR-00065-022	193	00-023-068	TBD	10/05/00

The sample received was a white, crystalline solid. It was received under frozen conditions and was stored in a temperature-monitored freezer kept at <-10°C.

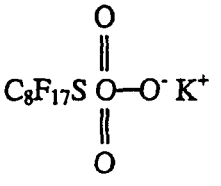
The analytical/control standard PFOS was:

Control Article	Lot Number	Centre Control No.	Purity	Expiration Date
PFOS	TCR-00017-046	00-023-042	97.9%	8/31/2001

The chemical and physical data for PFOS is as follows.

Common Name: PFOS
Molecular weight: 499 ($C_8F_{17}SO_3^-$)
CAS Number: 2795-39-3

Structure:



Note: the neutral molecule and standard form that the PFOS (anion) is derived from is potassium perfluorooctane sulfonate ($C_8F_{17}SO_3K$), molecular weight 538.

5.0 EXPERIMENTAL PROCEDURES

LC/MS Spectral Analysis

PFOS sample TCR-00065-022 was analyzed for purity using liquid chromatography/mass spectrometry (LC/MS). The sample was analyzed according to the following procedure.

The sample was prepared at about 250 µg/mL in methanol and analyzed by LC/MS using a Hewlett-Packard 1100 HPLC system interfaced with a Hewlett-Packard 1100 mass selective detector. The mass spectrometer was run in negative ionization mode using electrospray ionization (ESI) using Selective Ionization Monitoring for ion m/z 499. The liquid chromatography system was operated in reversed-phase mode using a C18 silica-based column.

Each resulting chromatogram was calculated against the analytical control article (TCR-00017-046) calibration curve.

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6.0 RESULTS AND DISCUSSION

LC/MS Spectral Analysis

Responses were observed for the PFOS sample in negative ion mode using an ESI interface. Quantitation was performed using SIM mode, m/z 499, and PFOS was detected at 7.8 minutes. TH-PFOS was used as the internal standard, m/z 427, and was detected at 7.5 minutes. The total percent purity, calculated against the analytical control article (TCR-00017-046) calibration curve was determined to be 88.0% from the average of three replicate analyses.

8.0 CIRCUMSTANCES THAT MAY HAVE AFFECTED THE DATA

Electronic records are not fully compliant with 21 CFR 11, "Electronic records; Electronic Signature." However, approved SOPs were in place and all instrumentation used in this study was fully calibrated and operational. All original raw data were printed as hard copies and fully audited by quality assurance. Verified exact copies and the electronic data will be stored in the archives at Centre Analytical Laboratories. Original raw data will be returned to the Sponsor.

9.0 RETENTION OF DATA AND SAMPLES

When the final report is final, all original paper data generated by Centre Analytical Laboratories, Inc. will be shipped to the sponsor. This does not include facility-specific raw data such as instrument logs, however exact copies of temperature logs will be submitted. Exact copies of all raw data, as well as a signed copy of the final analytical report and all original facility-specific raw data, will be retained in the Centre Analytical Laboratories, Inc. archives for the period of time specified in 40 CFR Part 160. Retained samples of reference substances are archived by the sponsor.

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Table I. Results From LC/MS Of TCR-00065-022, ESI Negative mode

3M ID	Retention Time (min)	Mass	Value µg/L	Determined µg/L	% Recovery
TCR-00065-022	7.79	499	250	219	88.0
TCR-00065-022	7.80	499	250	220	88.0
TCR-00065-022	7.80	499	250	220	88.0
				Average =	**88.0**
Standard	7.69	499	11	100*	*
Standard	7.73	499	11	17*	*
Standard	7.77	499	54	60	110
Standard	7.79	499	54	50	93
Standard	7.79	499	108	101	94
Standard	7.77	499	269	284	105
Standard	7.78	499	539	562	104
Standard	7.78	499	808	831	103
Standard	7.78	499	1078	1205	112
CCV	7.81	499	269	278	103
				Standards Average =	**103**
				Std Dev =	7

* First two injections during instrument warm up, not reported.
CCV = standard in run used to check calibration (C100500-4 at 269µg/L)

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Figure 1. PFOS Calibration Standard (C100500-4) at 269µg/L

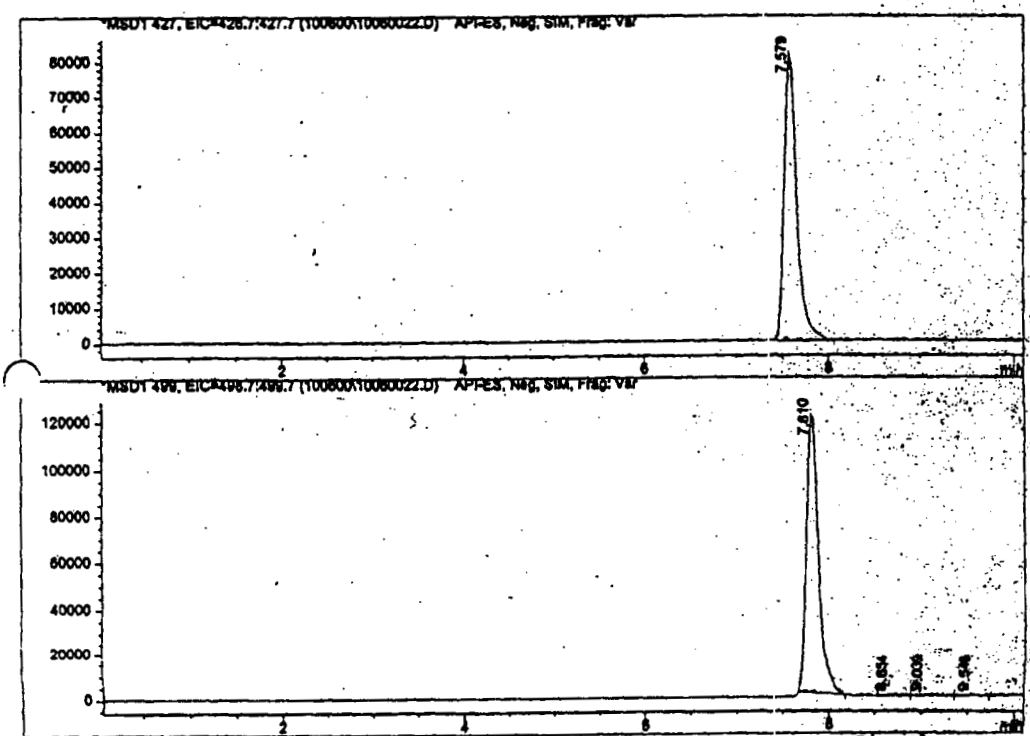
Sample Name:C100500-4
Sample Info: 1

Data file :C:\HPCHEM\1\DATA\100600\10060022.D

Injection Date	: 10/6/2000 3:11:22 PM	Seq Line	: 20
Acq Operator	: GS	Vial No.	: 14
Instrument	: Instrument 1	Inj. No.	: 1
Dilution	: 1	Inj. Vol.	: 5 µl

Acq. Method : PFOS.M
Analysis Method : C:\HPCHEM\1\METHODS\100600.M
Last Changed : 10/23/2000 11:44:26 am

Analysis for PFOS



Compound Name	Amount	R.T.	Area	Signal
TH-PFOS	500.0	7.58	785730	MSD1 427, EIC=426.7:42
PFOS	261.5	7.81	1078963	MSD1 499, EIC=498.7:49

*** End of Report ***

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Figure 2. PFOS Calibration Standard (C100500-3) at 539µg/L

Sample Name:C100500-3
Sample Info: 1

Data file :C:\HPCHEM\1\DATA\100600\10060015.D

Injection Date	: 10/6/2000 1:13:21 PM	Seq Line	: 13
Acq Operator	: GS	Vial No.	: 15
Instrument	: Instrument 1	Inj. No.	: 1
Dilution	: 1	Inj. Vol.	: 5 µl

Acq. Method : PFOS.M
Analysis Method : C:\HPCHEM\1\METHODS\100600.M
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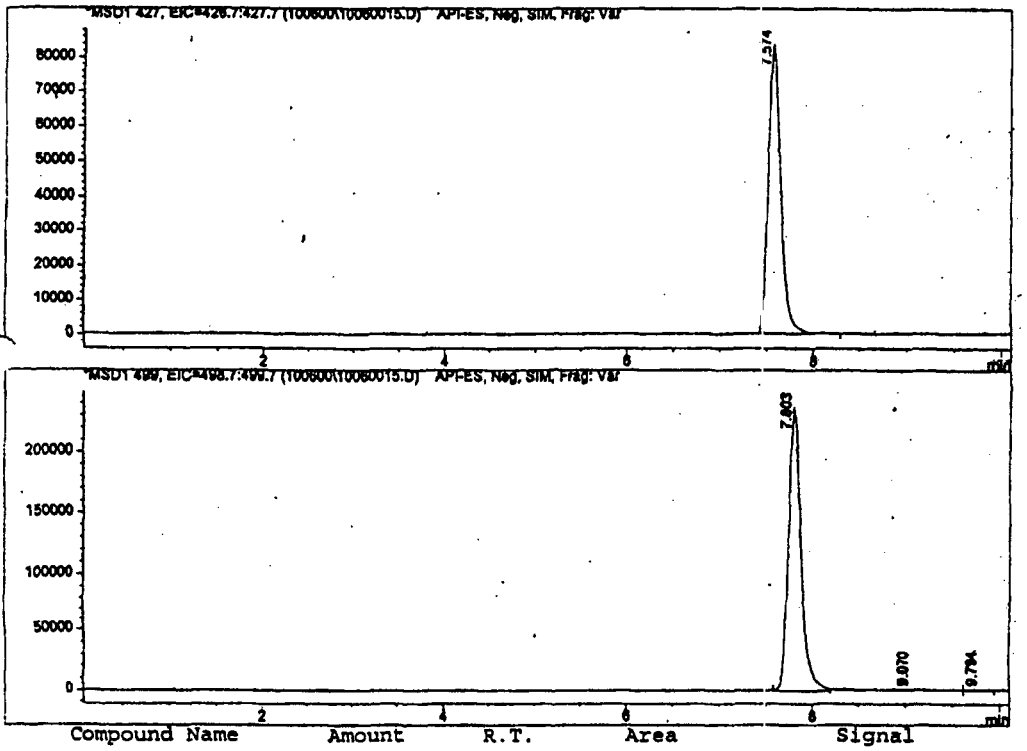
(modified after loading)
Analysis for PFOS



Compound Name	Amount	R.T.	Area	Signal
TH-PFOS	500.0	7.57	770327	MSD1 427, EIC=426.7:42
PFOS	499.8	7.80	2238071	MSD1 499, EIC=498.7:49

*** End of Report ***

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Centre Study No.: 023-045

Figure 3. PFOS (TCR-00065-022) at 250µg/L, ESI Negative Ion Mode

```
Sample Name:L29092-1
Sample Info:C100500-8                                              -> 1

Data file  :C:\HPCHEM\1\DATA\100600\10060019.D
================================================================================
Injection Date  : 10/6/2000     2:20:49 PM      Seq Line   :          17
Acq Operator    : GS   GS 10-23-00              Vial No.   :          21
Instrument      : Instrument 1                  Inj. No.   :           1
Dilution        : 1                             Inj. Vol.  :        5 µl

Acq. Method     : PFOS.M
Analysis Method : C:\HPCHEM\1\METHODS\100600.M
Last Changed    : 10/23/2000          11:44:26 am

Analysis for PFOS
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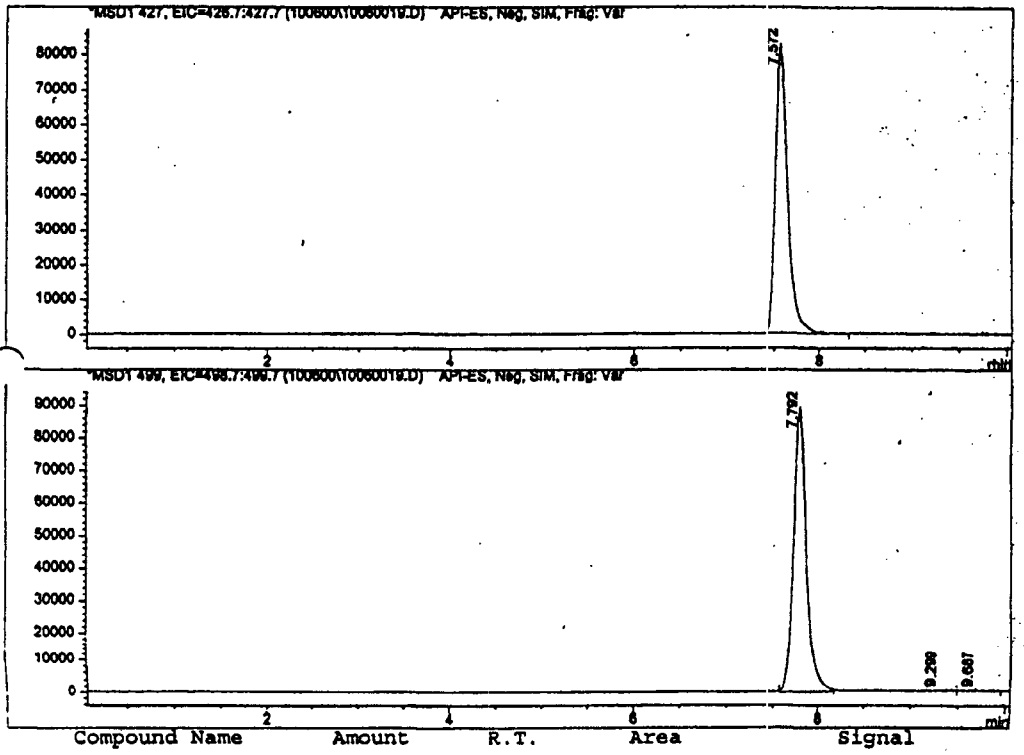

Compound Name	Amount	R.T.	Area	Signal
TH-PFOS	500.0	7.57	776592	MSD1 427, EIC=426.7:42
PFOS	218.7	7.79	894026	MSD1 499, EIC=498.7:49

```
================================================================================
                        *** End of Report ***

Instrument 1     Mon, 23. Oct. 2000     00:10:19 pm          Page 1 of 1
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APPENDIX A

Study Protocol

Purity Determination of Sample Lots of PFOS

Including Amendment 1

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3M Medical Department Study: T-6316.1 Analytical Report: FACT TOX-003
LRN-U2104

Appendix H: Report Signature Page

John L. Butenhoff, Ph.D., Ph.D., *Study Director* — Date: June 6, 2001

Marvin T. Case, D.V.M., Ph.D., *Sponsor Representative* — Date: 6 June 2001

Kristen J. Hansen, Ph.D., *Principal Analytical Investigator* — Date: 05/31/01

William K. Reagen, Ph.D., *Laboratory Manager* — Date: 05/31/01