Study Title

Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats

Analytical Laboratory Report Title

Determination of the Presence and Concentration of Perfluorooctanesulfonate, Perfluorooctanesulfonylamide, M556 and M570 in Liver and Sera Samples

Data Requirement

Not Applicable

Author

3M Environmental Laboratory

Study Completion Date

February 23, 2001

Performing Laboratories

Liver and Sera Analyses

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

Liver and Sera Extractions

Pace Analytical Tier II Laboratory, Inc.
1700 Elm Street, Suite 100
Minneapolis, MN 55414

Project Identification

3M Study: T-6314.1
Covance In-Life Study: 6329-225
Analytical Report: FACT-TOX-028
3M Laboratory Request No. U2636

Total Number of Pages

130

This page has been reserved for specific country requirements.

GLP Compliance Statement

Analytical Laboratory Report Title: Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats

Study Identification Numbers: T-6314.1, FACT-TOX-028, LRN-U2636

The determination of M570 in liver and sera specimens will be conducted according to documented, but not validated methods. This study was conducted in compliance with United States Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations for Nonclinical Laboratory Studies, Final Rule 21 CFR Part 58, with the exceptions in the bulleted list below. Exceptions to GLP compliance:

- Analytical standard stability was not yet determined.
- Purity of the following analytical reference materials have not been completed at this time; PFOSA, M556 and M570.
- Occasionally, corrections were not made according to 21 CFR 58.130 (e).
- The electronic data systems have not been validated and there is not an electronic audit trail of corrections currently available. Authenticated hard copies of chromatography and associated documents will be considered as the original raw data.
- Results of purity and stability analysis of the test material have not been completed by the sponsor. This information will be included in the final toxicology report.

Andrew M. Seacat — 2/23/01

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

John T. Butenhoff — 9:28 February 23, 2001

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

Kristen Hansen — 02/22/01

Kristen Hansen Ph.D., *Principal Analytical Investigator* — Date

GLP Study—Quality Assurance Statement

Analytical Laboratory Report Title: Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats

Study Identification Numbers: T-6314.1, FACT-TOX-028, LRN-U2636

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
10/30/00	Analysis	10/31/00	10/31/00
10/10/00	Extraction	10/11/00	10/11/00
11/13-17/00, 11/20/00, 11/27-28/00	Data	11/28/00	11/28/00
12/19/00, 12/21/00 12/27-12/28/01	Draft report #1	01/02/01	01/02/01
02/05-02/06/01	Draft report #2	02/07/01	02/07/01

QAU Representative

02/22/01
Date

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Location of Archives

All original raw data, protocol, and analytical report have been archived at the 3M Environmental Laboratory. The test substance and 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 for a minimum of 10 years.

Introduction and Purpose

The purpose of the analytical phase of this study is to evaluate levels of PFOS, PFOSA, M556, and M570 in liver and sera specimens of the rats exposed to N-MeFOSE-OH (T-6314). The in-life phase of this study was conducted at Covance Laboratories, Inc., (Covance Study Number 6329-225). The in-life data, dose preparation and analysis are reported in Covance Study Number Report 6329-225. The analytical portion of the study was initiated on 23 August 2000.

Test System

The test system species and strain selected was the Crl:CD®(SD) IGS BR rats received from Charles River Laboratories, Inc.

Four dose groups comprised of 20 male and 20 female rats each (80 males and 80 females total) were fed daily nominal doses of 0-(control), 3-(low dose), 30-(mid dose), or 100-(high dose) ppm of N-MeFOSE-OH (T-6314) in their feed for at least 13 weeks. After 4 weeks, liver and sera specimens were collected from five-animals/sex/dose group. At the end of the study (after week 13), liver and sera specimens were collected from all remaining animals in the test system. All liver and sera specimens were sent to the 3M Environmental Laboratory for analysis.

Table 1. Dosage Levels, Dose Groups and Group Composition for Study 6329-225

Dose Group	Number of Animals	Dietary Levels[a]
1 (Control [b])	20 male / 20 female	0 ppm
2 (Low Dose)	20 male / 20 female	3 ppm
3 (Mid Dose)	20 male / 20 female	30 ppm
4 (High Dose)	20 male / 20 female	100 ppm

[a] Dose Levels are expressed as ppm of N-MeFOSE-OH.
[b] Control Group animals received a basal diet only.

Specimen Collection and Analyses

In the analytical study reported here, 160 liver (80 male and 80 female) and 80 (40 male and 40 female) sera specimens were collected during the course of the in-life phase of Covance Study 6329-225 and sent to the 3M Environmental Laboratory to be extracted and analyzed for PFOS, PFOSA, M556 and M570. The study director determined that it was appropriate only to analyze the liver samples that have corresponding sera samples. There was a total of 80 liver analyses and a total of 80 sera analyses for each of the four compounds.

Blood specimens were allowed to clot at room temperature and centrifuged. Serum was then harvested and stored in a freezer set to maintain specimens at -60°C to -80°C until shipped to the 3M Environmental Laboratory. Liver specimens collected from each animal were flash frozen in liquid nitrogen and then stored in a freezer set to maintain specimens at -60°C to -80°C until shipped to the 3M Environmental Laboratory. Liver and sera specimens were shipped to the 3M Environmental Laboratory frozen and on dry ice 22 December 1998 and 13 October 1998, respectively.

Liver and Sera samples were sent to Pace Analytical Tier II Laboratory, Inc. on 21 September 2000. The sera and livers were extracted using 3M Environmental Laboratory analytical methods beginning on 25 September 2000 and 10 October 2000, respectively. Analytical samples were extracted using an ion-pairing procedure. An ion-pairing reagent was added to the laboratory sample and the analyte ion pair was partitioned into methyl-*tert*-butyl ether (MtBE). The MtBE extract was removed and put onto a nitrogen evaporator until dry. Each extracted laboratory sample was then reconstituted in 1.0 mL of methanol and filtered through a 3 cc plastic syringe attached to a 0.2 µm nylon filter into a glass autovial. Liver samples were homogenized prior to the extraction procedure. The extracts were returned 11 October 2000 to the 3M Environmental Laboratory after the extractions were completed.

Sample analyses were performed by monitoring one product ion selected from a single primary ion characteristic of a particular fluorochemical using HPLC-ES/MS/MS in the multiple reaction monitoring. Analytical details are included in this report.

The PFOS data reported has been adjusted to reflect the purity determined for the analytical reference material. (See table 2).

Specimen Receipt and Maintenance

The 3M Environmental Laboratory received liver and sera specimens on 13 October 1998 and 22 December 1998 that were collected during and at the end of the in-life phase of Covance Study 6329-225. All specimens were received frozen on dry ice and were immediately transferred to storage at –20°C ±10°C.

Chemical Characterization of Reference Materials

Table 2. Characterization of the Analytical Reference Materials in Study FACT-TOX-028

Material	KPFOS[a]	PFOSA	M556	M570
Identification	Potassium Perfluorooctane-sulfonate	Perfluorooctane-sulfonylamide	M556	M570
Chemical Formula	$C_8F_{17}SO_3^-K^+$	$C_8F_{17}SO_2NH_2$	$C_8F_{17}SO_2N(H)(CH_2COOH)$	$C_8F_{17}SO_2N(CH_3)(CH_2COOH)$
Source	3M Specialty Chemicals	3M Specialty Chemicals	3M Specialty Chemicals	3M Specialty Chemicals
Expiration Date	01/01/2010	01/01/2010	01/01/2010	01/01/2010
Storage Conditions	Room Temperature	Room Temperature	Room Temperature	Room Temperature
Chemical Lot Number	Lot 171	L-15709	3M# 113047-80	3M# 118506-26
Physical Description	Light colored powder	Light yellow waxy solid	White powder	White powder
Purity	86.4%	TBD[b]	TBD[b]	TBD[b]

TBD – To be determined

[a] The target analyte is PFOS (Perfluorooctanesulfonate), $C_8F_{17}SO_3^-$

[b] The purity of the substances listed above were nominally determined by NMR analyses. Subsequent chemical characterization are occurring and this analytical report will be amended to indicate the purity of these substances when a certificate of analysis are issued.

Method Summaries

Following is a brief description of the methods used for extraction and analyses during this analytical study by the 3M Environmental Laboratory. Detailed descriptions of the methods used in this study are located in Appendix C. The preparatory methods used by the technicians at the Pace Analytical Tier II Laboratory, Inc. are methods from and developed by the 3M Environmental Laboratory.

PREPARATORY METHODS

- **ETS-8-4.1,** "Extraction of Potassium Perfluorooctanesulfonate or other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"
- **ETS-8-6.0,** "Extraction of Potassium Perfluorooctane-sulfonate or other Fluorochemical Compounds from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry"

  Both extraction methods use an ion-pairing procedure. An ion-pairing reagent was added to the laboratory sample and the analyte ion pair was partitioned into methyl-*tert*-butyl ether (MtBE). The MtBE extract was removed and put onto a nitrogen evaporator until dry. Each extracted laboratory sample was then reconstituted in 1.0 mL of methanol and filtered through a 3 cc plastic syringe attached to a 0.2 µm nylon filter into a glass autovial. Liver samples were homogenized prior to the extraction procedure.

ANALYTICAL METHODS

- **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"

  These methods describe the analysis of fluorochemical surfactants from liver and serum extracts using HPLC-ES/MS/MS. The analysis was performed by monitoring a product ion selected from a single primary ion characteristic of a particular fluorochemical. For example, molecular ion 499, selected as the primary ion for PFOS ($C_8F_{17}SO_3^-$) analysis, was fragmented to produce ion 99 (FSO_3^-). The characteristic ion 99 was monitored for quantitative analysis.

ANALYTICAL EQUIPMENT

The analytical equipment settings used in the present analytical phase of this study may have varied slightly during 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: 10.0 minutes

Time (minutes)	*%B*
0.0	10%
1.0	10%
5.5	95%
7.5	95%
8.0	10%

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

Table 3. Negative Ions Monitored in 3M Laboratory Analyses

Target Analyte	Primary Ion (AMU)	Product Ion (AMU)
PFOS	499	99
PFOSA	498	78
M556	556	169
M570	570	169
THPFOS *	427	80

*Internal Standard

Data Quality Objectives and Data Integrity

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

- **Linearity:** The coefficient of determination (r^2) must be equal to or greater than 0.985 using either no weighting or 1/x weighting as appropriate.
- **Limits of Quantitation (LOQ):** The LOQ was equal to the lowest acceptable standard in the calibration curve.
- **Acceptable Precision:** Precision should be better than 30% for the method.
- **Acceptable Spike Recoveries:** 70–130% unless otherwise documented.
- **Demonstration of Specificity:** Compound identification will be substantiated by chromatographic retention time (within 5%) and by selection of a characteristic product ion from a characteristic primary ion. See table 3.

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-028 were met with the exceptions noted in this report (see Appendix B).

Summary of Quality Control Analyses Results

- **Linearity:** The coefficient of determination (r^2) of the standard curves was ≥0.985.
- **Calibration Standards:** Calibration curves were prepared at concentrations within the linear range of the instrument and were not diluted. Quantitation of the target analyte was based on linear regression analysis (1/x weighting) of one or two extracted matrix curves bracketing each group of samples. 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 specific product ion using the multiple response-monitoring mode of the instrument (see Appendix C, Analytical Methods).
- **Limits of Quantitation (LOQ):** The LOQ is equal to the lowest acceptable standard in the calibration curve (defined as a standard within ±30% of the theoretical value), and is at least two times the analyte peak area detected in the extraction blanks. (Appendix D, Table 16).
- **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 a suitable surrogate matrices for the preparation of calibration curves and continuing calibration verification standards. Non-exposed rat sera was used as the control matrix for matrix spike studies.

- **Precision:** Precision was determined by a triplicate analysis of one extract and was reproducible to within 5% for all analytes.
- **Reproducibility:** At the request of the study sponsor, data was collected to assess the reproducibility of extraction/homogeneity of distribution of the target analytes in liver of dosed test animals. This was conducted by extracting duplicate liver samples for each of six, group 2 animals; a separate homogenate was prepared for each of the 12 extractions. The samples were evaluated against an extracted curve and the percent difference in the levels of each analyte was calculated. Results of this analysis are presented in Appendix D, tables 17 and 18. Note that the samples were not spiked. Reproducibility was assessed based on the non-spiked levels of analytes.
- **Matrix Spikes:** Matrix spike studies were prepared to approximate concentrations in Group I samples. Matrix spikes and matrix spike duplicates (MS/MSD) were extracted with each set of samples and analyzed during analytical runs at the 3M Environmental Laboratory.

  Sera Spike Recoveries: Two sets of MS/MSD were prepared with the sera samples. All matrix spikes were within acceptance criteria, with the exception of RTS09250-250 ppb-MS-5 and -MSD-5 for M556. These spikes were evaluated at 135 and 140% recovery. Because all the other analyte spikes were within acceptance criteria, it is unlikely that these high recoveries are a result of spiking error. The high recovery results were confirmed, but cannot be explained. An additional four matrix spikes were prepared and analyzed. These four matrix spikes were within acceptance criteria. (See table 4).

Table 4. Matrix Spike/Matrix Spike Duplicate results for M556 in Sera

Matrix/Compound	Original MS/MSD Recoveries (%)	Re-extract MS/MSD Recoveries (%)
Sera/M556	135	118
	140	118
	128	124
	116	128

Liver Spike Recoveries: Two sets of MS/MSD were prepared with the liver samples. Of the four spikes, a single spike, C95991F/G1/wk5-MSD-250 ppb was low for PFOSA and M556. An additional four matrix spikes were prepared and analyzed. These four matrix spikes were within acceptance criteria for PFOSA. Some responses for M556 were still lower than expected. The average recovery for the 8 prepared matrix spikes was 66%. (See table 5).

Table 5. Matrix Spike/Matrix Spike Duplicate results for PFOSA and M556 in Liver

Matrix/Compound	Original MS/MSD Recoveries (%)	Re-extract MS/MSD Recoveries (%)
Liver/PFOSA	92	86
	89	99
	78	90
	58	107
Liver/M556	79	41
	84	63
	74	71
	42	70

Internal Standards: The internal standard (IS), THPFOS was added to all samples and standards. THPFOS was not used for quantitation, but was used to monitor for gross instrument failure. The IS response of each analytical run was verified to determine that it did not vary more than ±50% from the mean within each analytical run. IS responses deviated by > ± 50% in several sera and liver analyses. These responses were confirmed but cannot be attributed to a specific problem. This data is flagged in the raw data table but no further action is being taken, as more suitable internal standards (e.g. radio labeled material) are not available.

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, indicates the data are quantitative to ±30%. Determination of M556 levels in liver may be biased low and should be considered quantitative to ± 50%.

Summary of Analytical Sample Results

- **Samples from Control Animals:** Low levels of some analytes were often detected in the sera and liver of some the control animals. These levels were significantly lower than those found in the low dose test animals.
- **Samples from Dosed Animals:** In general, analyte levels found in the sera and liver of the test animals increased with dose group. Analyte levels increased as dosage increased. 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-028. When one or more sample results within a group was below the LOQ, calculations for average were done using the LOQ value in the calculation

Statement of Conclusion

Under the conditions of the present studies, the fluorochemicals PFOS, PFOSA, M556 and M570 were observed in the liver and sera of all rats dosed with the N-MeFOSE-OH during the in-life phase of the study.

Appendix A: Control Matrices and Test Substance Characterization

Table 6. Characterization of the Control Matrices Used for Liver and Sera Analyses in Study FACT-TOX-028

Control Matrix	Rabbit Liver	Rat Serum	Rabbit Serum
Source	Covance Laboratory	Sigma Chemicals	Sigma Chemicals
Expiration Date	01/01/2010	01/01/2010	01/01/2010
Storage Conditions	-20°C ±10°C	-20°C ±10°C	-20°C ±10°C
Chemical Lot #	F04051-Female	19H89292	118H8418
Physical Description	Rabbit Liver	Rat Serum	Rabbit Serum

Test Substance Characterization

N-Methyl Perfluorooctanesulfonamido Ethanol
(N-MeFOSE-OH)

CAS Number: 24448-09-7

Chemical Formula: $C_8F_{17}SO_2N$ ($(CH_3)(CH_2CH_2OH)$) Molecular Weight: 557.0

Table 7. Characterization of Test Substance in Study FACT-TOX-028

Test Substance	
Chemical Name	N-Methyl Perfluorooctanesulfonamido -ethanol (N-MeFOSE-OH)
Source	3M Specialty Chemicals
Expiration Date	unknown
Storage Conditions	Ambient temperature
Chemical Lot #	T-6314

Based on information obtained from the in-life protocol.

Appendix B: Protocol, Amendments and Deviations

Study Title

Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats

ANALYTICAL PHASE PROTOCOL

An FDA Good Laboratory Practice (GLP) Compliant Study

Date:

15 August 2000

Performing Laboratories

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

Pace Analytical Services, Inc.
1700 Elm Street SE, Suite 100
Minneapolis, MN 55414

Laboratory Project Identification

FACT Tox Number: Tox-028
LRN Number: U2636

Protocol Number: FACT-Tox-028
Lab Request Number (LRN): U2636

Study Identification

Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats

Test Article	N-Methyl Perfluorooctanesulfonamido Ethanol (N-MeFOSE, T-6314)
Sponsor	3M Toxicology Services–Medical Department 3M Center, Building 220-2E-02 St. Paul, MN 55144-1000
Sponsor Representative	John L. Butenhoff, Ph.D. 3M Toxicology Services Telephone: (612) 733-1962
Study Director	Andrew M. Seacat, Ph.D. 3M Toxicology Services Telephone: (612) 575-3161 Facsimile: (612) 733-1773
Study Location(s)	
In vivo *Testing Facility*	Covance Laboratories Inc. 3301 Kinsman Boulevard Madison, WI 53704
Analytical Testing Laboratories	3M Environmental Laboratory Building 2-3E-09 935 Bush Avenue St. Paul, MN 55106
	Pace Analytical Services, Inc. 1700 Elm Street SE, Suite 100 Minneapolis, MN 55414
Proposed Study Timetable	
In-Life Experimental Start Date	01 September 1998
In-Life Termination Date	03 December 1998
Analytical Start Date	1 September 2000
Analytical Termination Date	1 December 2000
Study Completion Date	1 March 2001

Protocol Number: FACT-Tox-028
Lab Request Number (LRN): U2636

1. STUDY

Groups of male and female Crl:CD (SD) IGS BR rats were exposed daily to basal diet, or N-MeFOSE (T-6314) in feed for 13 weeks. At the end of the in-life study phase, liver and sera specimens were sent to the 3M Environmental Laboratory for analyses to evaluate the presence and concentration of T-6314 metabolites; specifically, PFOS, PFOSA, M556, and M570 (see Table 1). The determination of M570 in sera and liver specimens will be conducted according to documented, but not validated, methods.

COMPOUND	PFOS	PFOSA	M556	M570
Identification	Perfluorooctane-sulfonate	Perfluorooctane-sulfonylamide	M556	M570
Chemical Formula	$C_8F_{17}SO_3$-	$C_8F_{17}SO_2N(H_2)$	$C_8F_{17}SO_2N(H)(CH_2COOH)$	$C_8F_{17}SO_2N(CH_3)(CH_2COOH)$
CAS Number	2795-39-3	754-91-6	N/A	N/A
Molecular Weight (amu)	498.9	498.9	556.96	570.97

Table 1. Metabolites of N-MeFOSE (Test Article) to be Analyzed in the Present Study

2. PURPOSE

The analytical phase of this study is designed to evaluate levels of PFOS, PFOSA, M556, and M570 in liver and sera specimens of the rats exposed to T-6314. The in-life phase of the study was conducted at Covance Laboratories, Inc., (Covance Study number 6329-225). All liver and sera specimens will be extracted by Pace Analytical Services, Inc., and analyzed at the 3M Environmental Laboratory.

Specimen matrices for this study will be serum and liver; data quality objectives for this study are indicated in section 12.

3. REGULATORY COMPLIANCE

With the initial exception of M570 determination in sera and liver samples, this study will be conducted in accordance with the United States Food and Drug Administration Good Laboratory Practice Regulations for Nonclinical Laboratory Studies, Final Rule 21 CFR Part 58.

4. QUALITY ASSURANCE

The 3M Environmental Laboratory Quality Assurance Unit (QAU) will audit the study conduct, raw data, and final report to determine compliance with Good Laboratory Practice Regulations, this protocol, and 3M Environmental Laboratory Standard Operating Procedures.

5. TEST ARTICLE

5.1 Identification
N-Methyl Perfluorooctanesulfonamido Ethanol (N-MeFOSE T-6314, CAS 24448-09-7)

Note: Acetone was used as the vehicle to dissolve the test article before mixing with the diet.

5.2 Physical Description
Amber waxy solid

5.3 Purity and Stability
Results of purity and stability analyses completed by the sponsor will be presented in the final report.

5.4 Storage Conditions
T-6314 was stored at room temperature at Covance Laboratories during the in-life phase of the study.

5.5 Reserve Samples
A reserve sample (approximately 5g) from each lot was set aside and stored at room temperature. Reserve samples were shipped to the Sponsor at the end of the in-life phase of the study.

5.6 Disposal
Unused test article was returned to the sponsor at the end of the in-life phase of the study. The test article will be maintained in the 3M Environmental Laboratory specimen archives for a period of time specified by GLP regulation, or as long as the quality of the preparation affords evaluation.

6. CONTROL ARTICLE

6.1 Identification
Certified Rodent Diet #5002 meal

6.2 Source
PMI Nutritional International

6.3 Physical Description
Meal (*ad libitum*, unless otherwise specified)

6.4 Purity and Stability
There are no known contaminants in the diet that would interfere with this study.

6.5 Storage Conditions
Room temperature

6.6 Reserve Samples
Approximately 100g was taken from each dose preparation and stored at room temperature.

6.7 Disposal
Unless used for dose analyses, samples were discarded one month after the completion of the in-life phase of the study by Covance Laboratories.

7. REFERENCE MATERIALS

	PFOS	PFOSA	M556	M570
Identification	Perfluorooctanesulfonate	Perfluorooctane-sulfonylamide	M556	M570
TCR Substance #	SD009, SD018, SD046 (equivalent lots)	SE027 or SD029 (equivalent lots)	SD014	SD040
Source	3M Specialty Chemicals	3M Specialty Chemicals (R. Buckanin)	3M Specialty Chemicals (T. Spawn)	3M Specialty Chemicals (G. Moore)
Physical Description	Light-colored powder	White powder/chunks/ Light-yellow waxy solid	White Powder	White powder
Purity	Purity of standards is currently under determination. Purity values will be included in the final report or in an amendment to the final report.			
Stability	unknown	unknown	unknown	unknown
Storage Conditions	Room temperature	Room temperature	Room temperature	Room temperature
Chemical Lot #	Batch 171 or 217	L-15709	3M# 113047-80	3M# NB118506-26
Reserve Samples	A reserve sample from each batch/lot of reference material will be retained for 5 years after submission of the final report or two years after completion if not submitted, but not longer than 10 years following the effective date of the final test rule (if applicable).			
Disposal	Unused reference material will be retained for use by 3M Environmental Laboratory and will be discarded when the quality of preparation no longer affords evaluation.			
Safety Precautions (Attachment A)	Refer to the MSDS for PFOS	Refer to the MSDS for PFOSA	Refer to the MSDS for M556	Refer to the MSDS for M570

Table 2. Reference Materials for FACT Tox-028

8. TEST SYSTEM

Four dose groups comprised of 20 male and 20 female Crl:CD® (SD) IGS BR rats each, were exposed daily to 0- (control), 3- (low exposure), 30- (mid exposure), or 100- (high exposure) µg/g N-MeFOSE (T-6314) in their feed for at least 13 weeks (Table 3). Individuals in the control group were identified with an implantable microchip identification device.

After 4 weeks, liver and sera specimens were collected from five animals/sex/dose group. At the end of the study (after week 13), liver and sera specimens were collected from all remaining animals in the test system. All liver and sera specimens were sent to the 3M Environmental Laboratory for analysis.

DOSE GROUP	NUMBER OF ANIMALS	DIETARY LEVELS[a]
1 Control[b]	20 male/ 20 female animals	0 ppm
2 Low Exposure	20 male/ 20 female animals	3 ppm
3 Mid Exposure	20 male/ 20 female animals	30 ppm
4 High Exposure	20 male/ 20 female animals	100 ppm

a Dose Levels are expressed as ppm of N-MeFOSE
b Control group animals will receive a basal diet only

Table 3. Dosage Levels, Dose Groups and Group Composition in the 13-Week Dietary Study of T-6314 in Rats

9. *SPECIMEN RECEIPT*

Tissue specimens were received from Covance Laboratories during and at the end of the in-life phase of study, on October 13, and December 22, 1998. Liver and sera specimens were shipped by overnight courier and were received frozen on dry ice. Specimens were identified with specimen numbers (lab request numbers or numbers assigned by Covance Laboratories) upon receipt, registered with the 3M Environmental Laboratory and transferred to a freezer for storage. All specimens sent to the 3M Environmental Laboratory were received and tracked according to the Sample Tracking System Standard Operating Procedures. Details of specimen inspection for damage, receipt, storage, identification, chain of custody protocols, and data will be presented in the final report.

10. *PREPARATORY METHODS*

Method numbers:

10.1 **ETS-8-4,** "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Serum for Analysis Using HPLC-Electrospray/Mass Spectrometry"

10.2 **ETS-8-6,** "Extraction of Potassium Perfluorooctane-sulfonate or other Fluorochemical Compounds from Liver for Analysis using HPLC-Electrospray/Mass Spectrometry"

Analytical samples are extracted using an ion-pairing extraction procedure. An ion pairing reagent is added to a laboratory sample and the analyte ion pair partitioned into MtBE. The MtBE extract is then removed and put onto a nitrogen evaporator until dry. Each extracted laboratory sample is reconstituted in 1.0 mL of methanol and filtered through a 3 cc plastic syringe attached to a 0.2 µm nylon filter into glass autovials.

A modification will be made to the extraction method prior to the commencement of this study; an internal standard (THPFOS) will be added after the extracted laboratory sample is reconstituted in 1.0 mL of methanol.

11. *ANALYTICAL METHODS*

11.1 **ETS-8-5,** "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical in Serum Extracts Using HPLC-Electrospray/Mass Spectrometry"

11.2 **ETS-8-7,** "Analysis of Potassium Perfluorooctane-sulfonate or other Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

Analyses are performed by monitoring one or more product ions selected from a single primary ion characteristic of a particular fluorochemical using HPLC-ESMSMS. For example, molecular ion 499, selected as the primary ion for PFOS ($C_8F_{17}SO_3$-) analysis, is fragmented further to produce ion 99 (FSO_3-). The characteristic ion 99 is monitored for quantitative analysis.

The analysis of M570 in sera and liver matrix samples will be conducted according to the methods used by 3M Environmental Laboratory for the other target analytes. However, these methods have not been specifically validated for M570.

12. DATA QUALITY OBJECTIVES

The number of spikes/duplicates, use of internal standards, and information on other quality indicators are included in the analytical methods (Attachment B). As there were no sponsor-recommended data quality objectives, the following will be used:

12.1 Linearity

The coefficient of determination (r^2) of the standard curve must be equal to or greater than 0.985 using either no weighting or 1/x weighting, as appropriate.

12.2 Limits of Quantitation (LOQ)

The LOQ may be determined according to 40 CFR Part 136, Appendix B, or alternatively, the LOQ may be equal to the lowest acceptable standard in the calibration curve and the low curve point, which is within ±30% of the calculated calibration curves, as appropriate.

12.3 Acceptable Precision

<30% for the method

12.4 Acceptable Recoveries

70–130%. Removal of individual outliers from calculations may be done if it is documented with a reasonable explanation.

12.5 Use of Confirmatory Methods

None. If a confirmatory method is used, an amendment to this protocol will be written.

12.6 Demonstration of Specificity, etc.

For HPLC/MS/MS, compound identification will be substantiated by chromatographic retention time and by selection of a characteristic product ion from a characteristic primary ion (see Table 4).

COMPOUND	PRIMARY ION	PRODUCT ION	APPROXIMATE RETENTION TIME (MIN)
THPFOS (SS)	427	80	7.2
PFOS	499	99	7.3
M556	556	169	7.5
M570	570	169	7.7
PFOSA	498	78	8.0

Table 4. Compound Identification with HPLC/MS/MS

13. *STATISTICAL METHODS AND CALCULATIONS*

Statistical methods will be limited to the calculation of means and standard deviations. Examples of the calculations used in this study will be included in the final report.

14. *SUBCONTRACT LABORATORY*

Pace Analytical Services, Inc. (Pace—Tier II Facility, Minneapolis, MN) will be performing the extraction of samples for analyses of liver and serum specimens. All extracted samples will be shipped to the 3M Environmental Laboratory for analysis. Pace will follow all applicable 3M Environmental Laboratory Standard Operating Procedures and the following Pace Standard Operating Procedures:

PSS-Admin-01	Quality System
PSS-DC-05	Lab Notebooks, Logbooks, and Phone Logs
PSS-OPS-01	Use and Maintenance of Ultra-Turrax T25 Homogenizer
PSS-OPS-02	Raw Data
PSS-OPS-03	Hazardous Waste Disposal
PSS-OPS-04	Use and Maintenance of N-Evap Analytical Evaporator
PSS-OPS-05	Use of Compressed Gases in the Laboratory
PSS-OPS-06	Use and Maintenance of NANOpure II Water Purification System
PSS-OPS-07	Cleaning Non-disposable Volumetric Pipettes
PSS-OPS-08	Laboratory Calculations
PSS-OPS-13	Use and Maintenance of Lab Refrigerators, Freezers, and Ovens
PSS-OPS-15	Glassware Cleaning
PSS-OPS-16	Use and Maintenance of the Fisher Scientific Isotemp Freezer
PSS-OPS-31	Use and Maintenance of Shakers (Use for Shaker Only)
PSS-OPS-32	Operation, Maintenance and Calibration of Laboratory Centrifuges
ETS-9-40 (3M Lab)	Operation and Maintenance of Shakers and Vortex Mixers (Use for Vortex Mixer Only)

PSS-OPS-37	Operation, Maintenance, and Calibration of pH Meters and pH Electrodes
PSS-MTR-01	Calibration of Certified Weight Set
PSS-MTR-02	Calibration of Certified Thermometers/Thermocouples
PSS-MTR-06	Verification of Calibration of the Eppendorf Pipettor
PSS-MTR-08	Verification of Calibration and Use of Analytical Balances
PSS-MC-01	Purchasing of Laboratory Supplies
PSS-ARC-02	Disposition of Archive Materials
PSS-DM-03	Statistical Evaluation of Data

15. REPORT

A report of the results of the study will be prepared by 3M Environmental Laboratory. When analyses are subcontracted to other laboratories, each laboratory will prepare an analytical report and submit a copy of it to the 3M Environmental Laboratory for inclusion as an attachment in the 3M Environmental Laboratory final report. Each report will include, but not be limited to, the following, when applicable:

15.1 Name and address of the facility performing the study.

15.2 Dates upon which the study was initiated and completed.

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

15.4 Objectives and procedures as stated in the approved protocol, including any amendments to the original protocol.

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

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

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

15.8 A description of the test system (not required in the contract lab analytical report).

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

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

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

15.12 Statistical methods used to evaluate the data, if applicable.

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

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

15.15 A statement prepared by the quality assurance unit listing the dates that study inspections and audits were made and the dates of any findings reported to the Study Director and Management.

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

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

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.

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

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

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

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

17. Data / Specimen Retention

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

applicable), and as established by 3M Environmental Laboratory Standard Operating Procedures.

18. PROTOCOL AMENDMENTS AND DEVIATIONS

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

19. ATTACHMENTS

19.1 Attachment A: Material Safety Data Sheets (MSDS) for Reference Materials
19.2 Attachment B: Extraction and Analytical Methods

20. SIGNATURES

John L. Butenhoff 8/23/00

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

Andrew M. Seacat 8/23/00

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

Protocol Number: FACT-Tox-028
Lab Request Number (LRN): U2636

ATTACHMENT A
MATERIAL SAFETY DATA SHEETS (MSDS) FOR REFERENCE MATERIALS

Protocol Number: FACT-Tox-028
Lab Request Number (LRN): U2636

ATTACHMENT B
EXTRACTION AND ANALYTICAL METHODS

Study Title

Covance 6329-225
13-Week Dietary Toxicity Study with N-Methyl Perfluorooctanesulfonamido Ethanol (N-MeFOSE, T-6314) in Rats

PROTOCOL AMENDMENT NO. 4

Amendment Date:

August 17, 2000

Performing Laboratory

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

Laboratory Project Identification

Covance 6329-225
ET&SS FACT TOX-028
LRN Number: U2636

3M Environmental Laboratory

Covance Protocol 6329-225
Amendment 4

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

1. ***PROTOCOL READS:***
Andrew M. Seacat is assigned to the role of sponsor representative (study monitor).

AMEND TO READ:
The role of sponsor representative has been reassigned to John Butenhoff.

REASON: The role of sponsor representative must be assigned to one person for both the analytical and in-life protocols. Since 3M is the sponsor for the study, the sponsor representative must be from 3M.

2. ***PROTOCOL READS:***
The study director role is assigned to Peter J. Thomford from Covance Laboratories.

AMEND TO READ:
The role of study director has been reassigned to Andrew Seacat. The role of principal in-life investigator has been reassigned to Peter Thomford.

REASON:
Per GLP regulations, only one study director may be assigned to a study. Since 3M is the sponsor of the study, the study director should be from 3M.

3. ***PROTOCOL READS:***
The performing laboratory is listed as Covance Laboratories Inc., 3301 Kinsman Boulevard, Madison WI 53704-2595.

AMEND TO READ:
Change the performing laboratory to 3M Environmental Laboratory, Building 2-3E-09, 935 Bush Avenue, St. Paul MN 55106. Selected extractions will be performed at Pace Analytical Service, Inc. as per the PAI.

REASON:
All analytical testing will be done at 3M Environmental Laboratory or PACE.

4. ***PROTOCOL READS:***
The study location is specified as Covance Laboratories, Inc., 3301 Kinsman Boulevard, Madison WI 53704-2595.

AMEND TO READ:
Change the study location to 3M Toxicology Services—Medical Department, 3M Center, Building 220-2E-02, St. Paul MN 55144-1000.

3M Environmental Laboratory

Covance Protocol 6329-225
Amendment 4

REASON:
3M Toxicology Services is the sponsor of the study.

5. ***PROTOCOL READS:***
There is no principal analytical investigator (PAI) listed for 3M Environmental Laboratory for FACT Tox-028.

AMEND TO READ:
Kristen J. Hansen has been assigned to the role of principal analytical investigator for the 3M Environmental Laboratory.

REASON:
No PAI had previously been assigned.

6. ***PROTOCOL READS:***
The analytical phase protocol has not been written or approved.

AMEND TO READ:
Add that the analytical phase protocol is attached to this list of protocol amendments.

REASON:
There are two phases to the study; the in-life phase and the analytical phase. The analytical phase protocol FACT TOX-028 is an amendment to the Covance protocol 6329-225.

Covance Protocol 6329-225
Amendment 4

Amendment Approval

John 2. Butenhoff 8/23/00

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

Andrew M. Seacat 8/23/00

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

Kristen Hansen 8/23/00

Kristen J. Hansen, Ph.D., Principal Analytical Investigator — Date

3M Environmental Laboratory

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

3M

Study Title

Determination of the Presence and Concentration of PFOS, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats

PROTOCOL AMENDMENT NO. 5

Amendment Date:

January 05, 2001

Performing Laboratory

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

Laboratory Project Identification

FACT-TOX-028
LRN Number U2636

3M Environmental Laboratory

FACT-TOX-028
Amendment 5

This amendment clarifies identification of the test article.

1. PROTOCOL READS:
This study uses different abbreviations to identify the test article, N-Methyl Perfluoroctanesulfonamido Ethanol. The abbreviations include N-MeFOSE-OH, N-MeFOSE, MeFOSE, MeFOS, and MeFOSE alcohol.

AMEND TO:
The abbreviations N-MeFOSE-OH, N-MeFOSE, MeFOSE, MeFOS, and MeFOSE alcohol are deemed to be equivalent for this study.

REASON:
To clarify the use of these different abbreviations.

Amendment Approval

Kristen Hansen 01/05/01

Kristen J. Hansen Ph.D., Principal Analytical Investigator Date

Andrew Seacat 01/08/01

Andrew Seacat, Ph.D., Study Director Date

Record of Deviation

I. Identification

Study / Project No.
FACT-TOX-28

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

Document Number	Date(s) of occurrence
Analytical Phase Protocol — 15 Aug 2000	

II. Description:

Required Procedure/process:
Protocol requires M570-SD040 - remaining sample archived - not available for use

Actual Procedure/process:
Standard preparation - 9/19/00 - used FCR00017-5C

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

This deviation written
Lot will be characterized at future date. kjh 09/20/00

Recorded By	Date
R. Wynne	9/19/00

IV. Impact on Study / Project

Characterization will be provided. No adverse impact. kjh 09/21/00

Authorized By *(Study Director / Project Lead)*: John R. Butenhoff 10/12/2000 Andrew M. Seacat 10/16/00

Sponsor Rep - John Butenhoff Study Director - Andrew Seacat

3M Environmental Laboratory
Form ETS-4-8.0

Deviation No. 1
(assigned by Study Director or Project Lead at the end of study or project)

Record of Deviation

I. Identification

Study / Project No.
TOX-028

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

Document Number	Date(s) of occurrence
ETS-8-4.1	9/25/00

II. Description:

Required Procedure/process:
The extraction sheet for TOX-028 Rat Serum indicates the CCV's were spiked at 250ppb using 5ul of a 5ppm standard.

Actual Procedure/process:
Using 5ul of a 5ppm standard gives a concentration of 25ppb for the CCV's not 250ppb.

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

Deviation was documented on this form.

Recorded By	Date
Kelly J. Kuehlwein	10/4/00

IV. Impact on Study / Project

These CCVs can be used as low-level QC. No impact on the study. kjk 10/04/00

Authorized By (Study Director / Project Lead)	Date
John Z. Butenhoff 10/12/2000 Andrew M. Seacat	10/16/00

Sponsor Rep - John Butenhoff Study Director - Andrew Seacat

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.
TOX-028 Covance # 6329-225

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

Document Number	Date(s) of occurrence
ETS-8-4.1, ETS-8-6.0	9/25/00, 10/9/00

II. Description:

Required Procedure/process:
Type I water is required for GLP studies.

Actual Procedure/process:
The type of nanopure water at Tier II is unknown.

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

The Tier II nanopure water was sent in for testing.
The results will be added to this study when they come back.

Recorded By	Date
Kelly Kuehlwein	10/30/00

IV. Impact on Study / Project

Type 2 water will not adversely affect the study.
kh 11/17/00

Authorized By (Study Director / Project Lead)	Date
Andrew M. Seacat 30 Nov 2000	11/28/00

Sponsor: John Butenhoff Study Director: Andrew Seacat

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.
TOX0028 (LIMS #U2636)

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

Document number	Date(s) of occurrence
ETS-8-5.1 and ETS-8-7.0	10/09/00, 10/18/00 10/23/00 10/24/00 11/02/00

II. Description

Required procedure/process:

Section 14.2.1: Solvent blanks, method blanks, and matrix blanks must be below the lowest standard on the calibration curve.

Actual procedure/process:

Occasionally, the first solvent blank injected for a run was above the LOQ.

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

Deviation written.

Recorded by kjh 12/05/00 Date

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

In most places a high solvent blank was analyzed, additional solvent blanks or method blanks were analyzed immediately following the high blank. This second injection was typically well below the LOQ. Occasionally, the first injection of a run is high because it may immediately follow injection of a high standard from a previous run. For this reason, more than one blank is usually analyzed prior to the start of a calibration curve. These second and third, etc. blanks are below the LOQ and are more representative of the analytical conditions of the samples; the study data is not adversely affected. kjh 12/05/00

Authorized by John Z. Butenhoff 14 DEC 2000 Andrew Stewart Date 12/15/00

Sponsor: John Butenhoff Study Director: Andrew Stewart **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. TOX028

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

Document Number: ETS-8-7.0

Date(s) of occurrence: 10/23/00

II. Description:

Required Procedure/process: Analyze a mid-range calibration standard....

Actual Procedure/process: A 306 ppb calibration standard was used for sample run D001023a. Although 306 ppb is in the mid-range of the entire curve, many of the high curve points were deactivated in order to optimize a linear range appropriate to the data. As a result, the 306 ppb CCV was no longer at the mid-range, but at the same level as the highest point in the calibration curve.

III. Actions Taken:

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

A deviation was written.

Recorded By: kjh 11/30/00

Date: November 30, 2000

IV. Impact on Study / Project

The CCVs were acceptable. Although this use of the high CCVs is not ideal, given the QC followed for construction of the calibration curve, this deviation will not have an adverse impact on the data. kjh 11/30/00

Not (added kjh 02/12/01)

AB 2/23/01

Authorized By *(Study Director / Project Lead)*: Andrew M. Seacat 5 DEC 2000

Date: 12/4/00

Sponsor: John Butenhoff Study Director: Andrew Seacat

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. TOX028

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

Document Number: ETS-8-5.1

Date(s) of occurrence: 11/6/00

II. Description:

Required Procedure/process: Continuing calibration verification percent recoveries must be within ±30% of the spiked concentration.

Actual Procedure/process: A calibration curve was followed by 5 samples, a CCV and a closing calibration curve. TheCCV was at 25ppb and the samples were at 400+ ppb. The linear range of the of the curve optimized to the data was 49.6 to 740 ppb; as a result, the CCV was out of the calibration range. Since the five samples were in the mid range of the curve and were immediately surrounded by two calibration curves, the data is sufficently bracketed.

III. Actions Taken:

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

A deviation was written.

Recorded By: Harold Johnson

Date: November 30, 2000

IV. Impact on Study / Project

No adverse impact on the data. KH 11/30/00

Authorized By *(Study Director / Project Lead)*: Andrew M Seacat 12/4/00 John T. Butenhoff 5 DEC 2000

Date: 12/4/00

Sponsor: John Butenhoff Study Director: Andrew Seacat

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)

Appendix C: Extraction and Analytical Methods

This appendix includes the following methods:

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)

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

ETS-8-7.0, "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in LiverUsing HPLC-Electrospray/Mass Spectrometry," (10 pages)

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: ETS-8-4.1

Adoption Date: 03/01/99

Revision Date: 4/27/99

Author: Lisa Clemen, Glenn Langenburg

Approved By:

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

1.0 SCOPE AND APPLICATION

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

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

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

2.0 Summary of Method

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

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

3.0 Definitions

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

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

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

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

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

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

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

4.0 Warnings and Cautions

4.1 **Health and safety warnings**

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

5.0 Interferences

5.1 There are no interferences known at this time.

6.0 Equipment

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

6.1.1 Vortex mixer, VWR, Vortex Genie 2

6.1.2 Centrifuge, Mistral 1000 or IEC

6.1.3 Shaker, Eberbach or VWR

6.1.4 Nitrogen evaporator, Organomation

6.1.5 Balance (± 0.100 g)

7.0 Supplies and Materials

7.1 Gloves

7.2 Eppendorf or disposable pipettes

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

7.4 Volumetric flasks, glass, type A

7.5 I-CHEM vials, glass, 40 mL glass

7.6 Centrifuge tubes, polypropylene, 15 mL

7.7 Labels

7.8 Oxford Dispenser – 3.0 to 10.0 mL

7.9 Syringes, capable of measuring 5 µL to 50 µL

7.10 Graduated pipettes

7.11 Syringes, disposable plastic, 3 cc

7.12 Syringe filters, nylon, 0.2 µm, 25 mm

7.13 Timer

7.14 Crimp cap autovials and caps

7.15 Crimpers

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

8.0 Reagents and Standards

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

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

8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent

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

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

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

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

8.8 Serum or blood, frozen from supplier

8.9 **Fluorochemical standards**

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

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

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

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

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

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

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

8.9.8 Other fluorochemicals, as appropriate

8.10 Reagent preparation

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

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

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

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

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

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

8.11 Standards preparation

8.11.1 Prepare PFOS standards for the standard curve.

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

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

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

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

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

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

8.12 Surrogate stock standard preparation

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

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

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

9.0 Sample Handling

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

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

10.0 Quality Control

10.1 Solvent Blanks, Method blanks and matrix blanks

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

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

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

10.2 Matrix spikes

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

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

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

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

10.3 Continuing calibration checks

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

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

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

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

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

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

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

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

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

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

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

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

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

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

12.0 PROCEDURE

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

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

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

12.4 Record the initial volume on the extraction worksheet.

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

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

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

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

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

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

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

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

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

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

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

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

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

12.18 Vortex mix for 30 seconds.

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

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

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

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

13.0 Data Analysis and Calculations

13.1 Calculations

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

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

Final Concentration (µg/mL) of PFOS in matrix

14.0 Method Performance

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

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

14.2.1 Method blanks and matrix blanks.

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

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

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

15.0 Pollution Prevention and Waste Management

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

16.0 RECORDS

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

17.0 ATTACHMENTS

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values and summary

17.3 Attachment C, Calibration standard concentration worksheet

18.0 REFERENCES

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

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

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

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

Extraction Worksheet ETS-8-4.1

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

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

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

MDL/LOQ values for rabbit serum

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

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

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

Compound: PFOS

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

Compound: PFOSA

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

Compound: PFOSAA

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

Compound: EtFOSE-OH

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

Compound: PFOSEA

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

Compound: M556

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

Ion Pair Standard Curves – Fluids

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

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

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

Actual concentrations of standards in the FC mix

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

Calculated concentrations of standards in the sample matrix

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

Validated ranges – approximate concentrations

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

3M ENVIRONMENTAL LABORATORY

METHOD

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

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

Revision Date: NR

Author: Lisa Clemen, Robert Wynne

Approved By:

Laboratory Manager — Date: 7/22/99

Group Leader — Date: 7/14/99

Technical Reviewer — Date: 07/14/99

1.0 SCOPE AND APPLICATION

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

1.2 Applicable Compounds: Fluorochemical surfactants or other fluorinated compounds.

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

2.0 Summary of Method

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

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

3.0 Definitions

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

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

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

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

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

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

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

4.0 Warnings and Cautions

4.1 Health and Safety Warnings:

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

5.0 Interferences

5.1 There are no interferences known at this time.

6.0 Equipment

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

6.1.1 Ultra-Turrax T25 Grinder for grinding liver samples

6.1.2 Vortex mixer, VWR, Vortex Genie 2

6.1.3 Centrifuge, Mistral 1000 or IEC

6.1.4 Shaker, Eberbach or VWR

6.1.5 Nitrogen Evaporator, Organomation

6.1.6 Balance (sensitivity to 0.100 g)

7.0 SUPPLIES AND MATERIALS

7.1 Gloves

7.2 Dissecting scalpels

7.3 Eppendorf or disposable pipettes

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

7.5 Volumetric flasks, glass, type A

7.6 I-CHEM vials, 40 mL glass

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

7.8 Centrifuge tubes, polypropylene, 15 mL

7.9 Labels

7.10 Oxford Dispensor – 3.0 to 10.0 ml

7.11 Syringes, capable of measuring 5 µL to 50 µL

7.12 Graduated pipettes

7.13 Syringes, disposable plastic, 3 cc

7.14 Syringe filters, nylon, 0.2 µm, 25 mm

7.15 Timer

7.16 Crimp cap autovials and caps

7.17 Crimpers

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

8.0 REAGENTS AND STANDARDS

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

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

8.3 Tetrabutylammonium hydrogen sulfate(TBA), Kodak or equivalent

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

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

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

8.7 Methanol, Omnisolv, glass distilled or HPLC grade

8.8 Liver, frozen from supplier

8.9 Dry ice from supplier

8.10 **Fluorochemical standards**

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

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

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

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

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

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

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

8.10.8 Other fluorochemicals, as appropriate

8.11 Reagent preparation

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

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

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

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

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

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

8.12 Standards preparation

8.12.1 Prepare PFOS standards for the standard curve.

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

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

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

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

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

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

8.13 Surrogate stock standard preparation

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

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

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

9.0 Sample Handling

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

10.0 Quality Control

10.1 Matrix blanks and method blanks

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

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

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

10.2 Matrix spikes

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

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

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

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

10.3 Continuing calibration verifications

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

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

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

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

11.0 CALIBRATION AND STANDARDIZATION

11.1 Prepare matrix calibration standards

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

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

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

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

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

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

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

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

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

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

Table 1
Approximate Spiking Amounts for Calibration Standards

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

12.0 PROCEDURE

12.1 Obtain frozen liver samples.

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

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

12.4 Record the liver weight in the study notebook.

12.5 Return unused liver portions to freezer.

12.6 Add 2.5 mLs of water to sampule vial.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

12.25 Vortex mix for 30 seconds.

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

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

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

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

13.0 Data Analysis and Calculations

13.1 Calculations:

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

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

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

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

* refer to **13.1.1** for details.

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

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

*refer to **13.1.2** for details.

14.0 Method Performance

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

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

14.2.1 Method blanks and matrix blanks.

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

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

14.3 Refer to section 14 of **ETS-8-7.0** for method performance criteria.

15.0 Pollution Prevention and Waste Management

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

16.0 RECORDS

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

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

17.1 Attachment A, Extraction worksheet

17.2 Attachment B, MDL/LOQ values and summary

17.3 Attachment C, Calibration standard calculation and concentration worksheet

18.0 REFERENCES

18.1 The validation report associated with this method is **ETS-8-6.0 & 7.0-V-1.**

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

18.3 FACT-M-1.1, "Extraction of PFOS or Other Anionic Fluorochemical Surfactants from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

Revision Number.	Reason For Revision	Revision Date

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

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

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

MDL/LOQ values for rabbit liver

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

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

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

Compound: PFOS

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

Compound: PFOSA

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

Compound: PFOSAA

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

Compound: EtFOSE-OH

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

Compound: PFOSEA

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

Compound: M556

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

Ion Pair Standard Curves – Tissue

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

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

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

Actual concentrations of standards in the FC mix

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

Calculated concentrations of standards in the sample matrix

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

Validated ranges – approximate concentrations

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

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: ETS-8-5.1

Adoption Date: 03/01/99

Revision Date: 4/26/99

Author: Lisa Clemen, Robert Wynne

Approved By:

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

1.0 SCOPE AND APPLICATION

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

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

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

2.0 SUMMARY OF METHOD

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

3.0 DEFINITIONS

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

3.2 Electrospray Ionization (ES, ESI): a method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These charged droplets are produced by the application of a strong electrical field.

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

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

3.5 Mass Lynx Software: System software designed for the specific operation of these Quattro II triple quadrupole systems. Currently MassLynx has Windows 95 and WindowsNT 4.0 versions. All versions are similar. For more details see the manual specific to the instrument (Micromass Quattro II triple quadrupole MassLynx or MassLynx NT User's Guide).

4.0 WARNINGS AND CAUTIONS

4.1 Health and Safety Warnings:

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

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

4.2 Cautions:

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

4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

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

6.0 EQUIPMENT

6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.

6.1.1 Micromass Quattro II triple quadrupole Mass Spectrometer equipped with an electrospray ionization source

6.1.2 HP1100 low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

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

7.1.2 HPLC analytical column, specifics to be determined by the analyst and documented in the raw data.

7.1.3 Capped autovials or capped 15 mL centrifuge tubes

8.0 REAGENTS AND STANDARDS

8.1 Reagents

8.1.1 Methanol, HPLC grade or equivalent

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

8.1.3 Ammonium acetate, reagent grade or equivalent

8.2 Standards

8.2.1 Typically two method blanks, two matrix blanks, and eighteen matrix standards are prepared during the extraction procedure. See **ETS-8-4.1**.

9.0 SAMPLE HANDLING

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

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

10.0 QUALITY CONTROL

10.1 Solvent Blanks, Method Blanks and Matrix Blanks

10.1.1 Solvent blanks, method blanks and matrix blanks are prepared and analyzed with each batch to determine contamination or carryover.

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

10.2 Matrix Spikes

10.2.1 Matrix spikes are prepared and analyzed to determine the matrix effect on the recovery efficiency.

10.2.2 Matrix spike duplicates are prepared and analyzed to measure the precision and the recovery for each analyte.

10.2.3 Analyze a matrix spike and matrix spike duplicate per forty samples, with a minimum of 2 spikes per batch.

10.2.4 Matrix spike and matrix spike duplicate concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.3 Continuing Calibration Verifications

10.3.1 Continuing calibration verifications are analyzed to verify the continued accuracy of the calibration curve.

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

11.0 CALIBRATION AND STANDARDIZATION

11.1 Analyze the extracted matrix standards prior to and following each set of extracts. The average of two standard curves will be plotted by linear regression ($y = my + b$), weighted $1/x$, not forced through zero, using MassLynx or other suitable software.

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

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

12.0 PROCEDURES

12.1 Acquisition Set up

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

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

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

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

12.2 Using the Autosampler

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

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

12.2.2.1 Sample size = 10 µL injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 13.5 minutes

12.2.2.4 Solvent ramp =

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

12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

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

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

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

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

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

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

12.3.6.1 Drying gas 250-400 liters/hour

12.3.6.2 ESI nebulizing gas 10-15 liters/hour

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

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

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

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

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

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

13.0 DATA ANALYSIS AND CALCULATIONS

13.1 Calculations:

13.1.4 Calculate matrix spike percent recoveries using the following equation:

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

13.1.5 Calculate percent difference using the following equation:

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

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

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

14.0 Method Performance

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

14.2 Solvent Blanks, Method Blanks, and Matrix Blanks

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

14.3 Calibration Curves

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

14.4 Matrix Spikes

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

14.5 Continuing Calibration Verifications

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

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

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

15.0 Pollution Prevention and Waste Management

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

16.0 Records

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

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

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

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

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

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

17.0 TABLES, DIAGRAMS, FLOWCHARTS, AND VALIDATION DATA

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

18.0 REFERENCES

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

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

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

19.0 AFFECTED DOCUMENTS

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

20.0 REVISIONS

Revision Number.	Reason For Revision	Revision Date
1	Section 6.1.2 Clarification of HP1100 system components. 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

Study #: FACT-TOX-028

3M Environmental Lab -- Method Modification

Method:

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

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

Effective date of modifications: April 26, 1999

Section 10.3.2

Method reads:

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

Modify method to read:

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

Section 14.5.1

Method reads:

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

Modify method to read:

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

Section 14.3.2

Method reads:

NA

Modify method to read:

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

Study #: FACT-TOX-028

Section 14.3.3

Method reads:

NA

Modify method to read:

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

Section 14.3.4

Method reads:

NA

Modify method to read:

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

Section 14.3.5

Method reads:

NA

Modify method to read:

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

Section 14.3.6

Method reads:

NA

Modify method to read:

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

11/29/00
Signature of PAI and date

5 DEC 2000
Signature of Sponsor and date

12/4/00
Signature of Study Director and date

3M ENVIRONMENTAL LABORATORY

METHOD

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

Method Number: ETS-8-7.0

Adoption Date: 07/22/99

Revision Date: NA

Author: Lisa Clemen, Glenn Langenburg

Approved By:

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

1.0 SCOPE AND APPLICATION

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

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

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

2.0 Summary of Method

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

3.0 Definitions

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

3.2 **Electrospray Ionization (ES, ESI):** a method of ionization performed at atmospheric pressure, whereby ions in solution are transferred to the gas phase via tiny charged droplets. These charged droplets are produced by the application of a strong electrical field.

3.3 **Mass Spectrometry, Mass Spectrometer (MS), Tandem Mass Spectrometer (MS/MS):** The API Quattro II triple quadrupole mass spectrometer is equipped with two quadrupole mass selective detectors and a collision cell. Ions are selectively discriminated by mass to charge ratio (m/z) and subsequently detected. A single MS may be employed for ion detection or an ion may be selected in the first quadrupole, fragmented in the collision cell, and these fragments may be analyzed in the second quadrupole.

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

3.5 **Mass Lynx Software:** System software designed for the specific operation of these Quattro II triple quadrupole systems. Currently MassLynx has Windows 95 and WindowsNT 4.0 versions. All versions are similar. For more details refer to the manual specific to the instrument (Micromass Quattro II triple quadrupole MassLynx or MassLynx NT User's Guide).

4.0 Warnings and Cautions

4.1 **Health and Safety Warnings:**

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

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

4.2 Cautions:

4.2.1 Operate the solvent pumps below a back pressure of 400 bar (5800 psi). If the back pressure exceeds 400 bar, the HP1100 will initiate automatic shutdown.

4.2.2 Do not run solvent pumps to dryness.

5.0 INTERFERENCES

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

6.0 EQUIPMENT

6.1 Equipment listed below may be modified in order to optimize the system. Document any modifications in the raw data as method deviations.

6.1.1 Micromass Quattro II triple quadrupole Mass Spectrometer equipped with an electrospray ionization source.

6.1.2 HP1100 low pulse solvent pumping system, solvent degasser, column compartment, and autosampler

7.0 SUPPLIES AND MATERIALS

7.1 Supplies

7.1.1 High purity grade air regulated to approximately 100 psi (house air system)

7.1.2 HPLC analytical column, specifics to be determined by the analyst and documented in the raw data

7.1.3 Capped autovials or capped 15 ml centrifuge tubes

8.0 REAGENTS AND STANDARDS

8.1 Reagents

8.1.1 Methanol, HPLC grade or equivalent

8.1.2 Milli-Q™ water (ASTM type I), all water used in this method should be ATSM type I, or equivalent, and be provided by a Milli-Q TOC Plus system or other vendor

8.1.3 Ammonium acetate, reagent grade or equivalent

8.1.3.1 When preparing different amounts than those listed, adjust accordingly.

8.1.3.2 2.0 mM ammonium acetate solution: Weigh approximately 0.300 g ammonium acetate. Pour into a 2000 mL volumetric container containing 2000 mL Milli-Q™ water, mix until all solids are dissolved. Store at room temperature.

8.2 Standards

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

9.0 Sample Handling

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

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

10.0 Quality Control

10.1 Method Blanks and Matrix Blanks

10.1.1 Solvent blanks, method blanks, and matrix blanks are prepared and analyzed with each batch to determine contamination or carryover.

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

10.2 Matrix Spikes

10.2.1 Matrix spikes are prepared and analyzed to determine the matrix effect on the recovery efficiency.

10.2.2 Matrix spike duplicates are prepared and analyzed to measure the precision and the recovery for each analyte.

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

10.2.4 Matrix spike and matrix spike duplicate concentrations will fall in the mid-range of the initial calibration curve. Additional spike concentrations may fall in the low-range of the initial calibration curve.

10.3 Continuing Calibration Checks

10.3.1 Continuing calibration verifications are analyzed to verify the continued accuracy of the calibration curve.

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

11.0 Calibration and Standardization

11.1 Analyze the extracted matrix standards prior to and following each set of sample extracts. The average of two standard curves will be plotted by linear regression ($y = mx + b$), weighted 1/x, not forced through the origin, using MassLynx or other suitable software.

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

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

12.0 PROCEDURES

12.1 Acquisition Set up

12.1.1 Set up the sample list.

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

12.1.1.2 Assign a method (MS file) for acquiring

12.1.1.3 Assign an HPLC program (Inlet file)

12.1.1.4 Type in sample descriptions and vial position numbers

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

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

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

12.2 Using the Autosampler

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

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

12.2.2.1 Sample size = 10 µL injection

12.2.2.2 Inject/sample = 1

12.2.2.3 Cycle time = 9 minutes

12.2.2.4 Solvent ramp conditions

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

12.2.2.5 Press the "Start" button.

12.3 Instrument Set-up

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

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

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

12.3.4 Turn on the nitrogen.

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

12.3.6 Open the Inlet Editor.

12.3.6.1 Set HPLC pump to "On"

12.3.6.2 Set the flow to 10 - 500 uL/min or as appropriate

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

12.3.6.4 Allow to equilibrate for approximately 10 minutes.

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

12.3.7.1 Drying gas 250-400 liters/hour

12.3.7.2 ESI nebulizing gas 10-15 liters/hour

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

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

12.3.7.5 Source block temperature 150°

12.3.7.6 Desolvation temperature 250°

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

12.3.9 Click on start button in the Acquisition Control Panel (this may vary among MassLynx versions, refer to appropriate MassLynx User's Guide). Ensure start and end sample number includes all samples to be analyzed.

13.0 Data Analysis and Calculations

13.1 Calculations:

13.1.4 Calculate matrix spike percent recoveries using the following equation:

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

13.1.5 Calculate percent difference using the following equation:

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

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

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

Final Volume (mL)

14.0 Method Performance

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

14.2 Solvent Blanks, Method Blanks and Matrix Blanks

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

14.3 Calibration Curves

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

14.4 Matrix Spikes

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

14.5 Continuing Calibration Verification

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

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

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

15.0 Pollution Prevention and Waste Management

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

16.0 Records

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

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

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

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

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

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

17.0 Tables, Diagrams, Flowcharts, and Validation Data

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

18.0 References

18.1 FACT-M-2.1, "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"

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

18.3 The validation report associated with this method is **ETS-8-6.0 & 7.0-V-1**

19.0 Affected Documents

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

20.0 REVISIONS

Revision Number	Reason For Revision	Revision Date

Laboratory Study

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

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

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

Study #: FACT-TOX-028

3M Environmental Lab -- Method Modification

Method:

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

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

Effective date of modifications: July 22, 1999

Section 10.3.2

Method reads:

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

Modify method to read:

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

Section 14.5.1

Method reads:

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

Modify method to read:

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

Section 14.3.2

Method reads:

NA

Modify method to read:

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

Study #: FACT-TOX-028

Section 14.3.3

Method reads:

NA

Modify method to read:

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

Section 14.3.4

Method reads:

NA

Modify method to read:

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

Section 14.3.5

Method reads:

NA

Modify method to read:

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

Section 14.3.6

Method reads:

NA

Modify method to read:

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

Signature of PAI and date 11/29/00

Signature of Sponsor and date 5 DEC 2000

Signature of Study Director and date 12/4/00

Appendix D: Data Tables

Table 8. Rat Serum Week 5 Data for FACT-TOX-028

Group Dose	Sample #	PFOS µg/mL	PFOSA µg/mL	M556 µg/mL	M570 µg/mL
Group 1 0 ppm	C95911M	0.00937	<LOQ (0.00980)	<LOQ (0.00980)	0.00864
	C95912M	<LOQ (0.00848)	<LOQ (0.00980)	<LOQ (0.00980)	<LOQ (0.00495)
	C95913M	<LOQ (0.00848)	<LOQ (0.00980)	<LOQ (0.00980)	<LOQ (0.00495)
	C95914M	<LOQ (0.00848)	<LOQ (0.00980)	<LOQ (0.00980)	<LOQ (0.00495)
	C95915M	0.0111	<LOQ (0.00980)	<LOQ (0.00980)	0.00963
	C95991F	0.0298	<LOQ (0.00980)	<LOQ (0.00980)	0.00802
	C95992F	0.0486	<LOQ (0.00980)	<LOQ (0.00980)	0.00862
	C95993F	0.0431	<LOQ (0.00980)	<LOQ (0.00980)	0.00971
	C95994F	0.0422	<LOQ (0.00980)	<LOQ (0.00980)	0.00949
	C95995F	0.0387	<LOQ (0.00980)	<LOQ (0.00980)	0.0185
Group 2 3 ppm	C95931M	1.90	0.0196	0.674	4.29
	C95932M	2.30	0.0179	0.440	3.18
	C95933M	1.94	0.0211	0.524	3.63
	C95934M	1.88	0.0210	0.743	3.98
	C95935M	2.36	0.0279	0.943	4.19
	C96011F	3.12	0.0448	0.442	2.29
	C96012F	3.65	0.0394	0.323	2.29
	C96013F	4.25	0.0676	0.628	4.39
	C96014F	4.66	0.0618	0.448	3.22
	C96015F	4.62	0.0682	0.590	5.11
Group 3 30 ppm	C95951M	24.3	0.420	5.98	39.5
	C95952M	27.9	0.471	6.42	40.4
	C95953M	30.1	0.547	10.1	45.1
	C95954M	28.8	0.451	8.07	43.3
	C95955M	34.0	0.503	6.78	44.2
	C96031F	41.0	0.649	4.34	33.4
	C96032F	52.8	0.775	3.40	31.8
	C96033F	41.6	0.869	7.57	33.1
	C96034F	38.7	0.498	7.98	35.0
	C96035F	50.2	0.857	5.87	36.0
Group 4 100 ppm	C95971M	48.2	0.599	10.2	51.4
	C95972M	47.1	0.660	11.3	57.4
	C95973M	56.2	0.745	15.9	54.9
	C95974M	53.2	0.777	14.5	62.5
	C95975M	55.8	0.658	12.0	61.3
	C96051F	63.8	1.27	10.6	64.6
	C96052F	69.5	1.20	20.1	68.4
	C96053F	75.2	1.23	17.0	51.2
	C96054F	50.3	1.20	14.2	51.3
	C96055F	58.1	1.41	12.3	62.5

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data quantitative to ±30%.

Table 9. Rat Serum Week 14 Data for FACT-TOX-028

Group Dose	Sample #	PFOS µg/mL	PFOSA µg/mL	M556 µg/mL	M570 µg/mL
Group 1 0 ppm	C95907M	<LOQ (0.0215)	<LOQ (0.00980)	<LOQ (0.0249)	<LOQ (0.00980)
	C95908M	0.0226	<LOQ (0.00980)	<LOQ (0.0249)	<LOQ (0.00980)
	C95910M	<LOQ (0.0215)	<LOQ (0.00980)	<LOQ (0.0249)	<LOQ (0.00980)
	C95916M	<LOQ (0.0215)	<LOQ (0.00980)	<LOQ (0.0249)	<LOQ (0.00980)
	C95917M	<LOQ (0.0215)	<LOQ (0.00980)	<LOQ (0.0249)	<LOQ (0.00980)
	C95981F	0.0370	<LOQ (0.00980)	<LOQ (0.0249)	<LOQ (0.00980)
	C95982F	0.0908	<LOQ (0.00980)	<LOQ (0.0249)	<LOQ (0.00980)
	C95989F	0.0809	<LOQ (0.00980)	<LOQ (0.0249)	<LOQ (0.00980)
	C95998F	0.0701	<LOQ (0.00980)	<LOQ (0.0249)	<LOQ (0.00980)
	C95999F	0.0701	<LOQ (0.00980)	<LOQ (0.0249)	<LOQ (0.00980)
Group 2 3 ppm	C95923M	3.45	0.0598	1.07	3.07
	C95929M	4.38	0.0348	1.25	2.99
	C95930M	4.12	0.0367	0.764	4.35
	C95938M	4.10	0.0308	0.820	3.54
	C95940M	3.65	0.0364	0.960	3.90
	C96004F	6.89	0.0415	0.709	3.75
	C96005F	9.41	0.0618	0.547	3.28
	C96009F	10.2	0.0688	0.637	3.05
	C96017F	8.57	0.0523	0.672	4.06
	C96018F	11.3	0.0668	1.01	3.53
Group 3 30 ppm	C95945M	44.7	0.663	9.00	23.4
	C95946M	38.9	0.497	6.94	20.1
	C95956M	36.9	0.453	8.22	22.7
	C95957M	33.8	0.486	8.22	20.5
	C95959M	46.6	0.571	6.82	16.4
	C96028F	56.0	0.742	5.95	24.1
	C96029F	79.9	0.780	7.08	21.3
	C96036F	74.7	0.726	7.14	21.4
	C96037F	65.6	0.649	7.30	18.2
	C96039F	66.8	1.05	7.29	22.2
Group 4 100 ppm	C95966M	104	1.27	26.7	32.6
	C95970M	134	0.784	22.7	16.7
	C95976M	109	0.946	26.8	20.6
	C95978M	110	0.890	26.9	22.2
	C95979M	101	0.905	22.6	26.3
	C96041F	178	1.25	23.9	25.0
	C96043F	135	1.55	24.7	27.0
	C96057F	238	1.33	21.3	23.2
	C96058F	185	1.19	29.4	26.7
	C96059F	262	0.969	30.0	21.0

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 data quantitative to ±30%.

Table 10. Rat Liver Week 5 Data for FACT-TOX-028

Group Dose	Sample #	PFOS µg/g	PFOSA µg/g	M556 µg/g	M570 µg/g
Group 1 0 ppm	C95911M	0.134	<LOQ (0.0122)	0.00989	0.0318
	C95912M	0.134	<LOQ (0.0122)	0.00683	0.0213
	C95913M	0.124	<LOQ (0.0122)	0.00654	0.0324
	C95914M	0.104	<LOQ (0.0122)	<LOQ (0.00611)	<LOQ (0.0122)
	C95915M	0.158	<LOQ (0.0122)	<LOQ (0.00611)	0.0159
	C95991F	0.0589	<LOQ (0.0122)	<LOQ (0.00611)	0.0198
	C95992F	0.0399	<LOQ (0.0122)	<LOQ (0.00611)	0.0129
	C95993F	0.0705	<LOQ (0.0122)	<LOQ (0.00611)	0.0157
	C95994F	0.0741	<LOQ (0.0122)	<LOQ (0.00611)	0.0238
	C95995F	0.0839	<LOQ (0.0122)	0.0102	0.0442
Group 2 3 ppm	C95931M	17.9	1.15	2.00	6.88
	C95932M	19.9	0.733	1.83	6.97
	C95933M	18.6	1.05	2.41	8.39
	C95934M	15.7	1.15	1.94	6.89
	C95935M	15.0	1.61	1.86	6.90
	C96011F	18.7	2.10	1.99	5.97
	C96012F	11.2	0.877	1.03	3.09
	C96013F	8.50	1.54	0.917	4.35
	C96014F	8.45	0.555	0.846	2.22
	C96015F	7.95	0.777	0.667	2.98
Group 3 30 ppm	C95951M	82.0	8.67	23.4	85.5
	C95952M	98.9	6.70	21.1	78.4
	C95953M	72.1	8.01	21.9	72.4
	C95954M	88.6	8.66	21.7	99.8
	C95955M	84.1	11.3	21.5	88.2
	C96031F	70.3	5.92	10.8	30.4
	C96032F	146	7.47	17.6	47.8
	C96033F	77.4	6.97	11.5	27.3
	C96034F	80.4	6.16	15.9	42.6
	C96035F	96.0	4.25	11.6	33.3
Group 4 100 ppm	C95971M	224	15.3	54.4	163
	C95972M	141	10.6	45.7	138
	C95973M	159	12.0	50.8	132
	C95974M	221	15.4	62.7	176
	C95975M	216	13.3	55.8	157
	C96051F	197	12.3	34.7	114
	C96052F	218	14.2	48.5	141
	C96053F	275	14.4	46.0	124
	C96054F	208	15.4	46.1	138
	C96055F	218	14.5	29.4	123

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 data quantitative to ±30%. Determination of M556 levels in liver may be biased low and should be considered quantitative to ±50%.

Table 11. Rat Liver Week 14 Data for FACT-TOX-028

Group Dose	Sample #	PFOS µg/g	PFOSA µg/g	M556 µg/g	M570 µg/g
Group 1 0 ppm	C95907M	0.216	<LOQ (0.0122)	0.0164	0.0466
	C95908M	0.249	<LOQ (0.0122)	0.0196	0.0388
	C95910M	0.320	<LOQ (0.0122)	0.0151	0.0566
	C95916M	0.107	0.0125	<LOQ (0.0122)	0.0827
	C95917M	0.0945	<LOQ (0.0122)	<LOQ (0.0122)	0.0209
	C95981F	0.0555	0.0122	<LOQ (0.0122)	0.0183
	C95982F	0.160	0.0164	<LOQ (0.0122)	0.0242
	C95989F	0.159	0.0153	<LOQ (0.0122)	0.0606
	C95998F	0.146	0.0237	0.0246	0.125
	C95999F	0.0768	<LOQ (0.0122)	<LOQ (0.0122)	0.0248
Group 2 3 ppm	C95923M	23.8	1.81	1.94	7.27
	C95929M	25.7	10.8	1.45	4.08
	C95930M	22.3	5.88	1.66	5.48
	C95938M	29.5	5.01	1.39	5.09
	C95940M	29.3	7.88	1.88	5.56
	C96004F	17.8	1.47	1.03	3.83
	C96005F	42.7	2.67	1.30	6.08
	C96009F	45.6	2.25	1.42	4.86
	C96017F	31.7	2.70	1.00	4.67
	C96018F	47.0	2.07	1.94	4.51
Group 3 30 ppm	C95945M	233	27.6	45.4	171
	C95946M	234	18.9	31.5	162
	C95956M	213	20.8	34.7	152
	C95957M	195	16.7	37.8	166
	C95959M	311	24.2	50.4	196
	C96028F	203	14.2	11.4	93.7
	C96029F	403	13.4	19.2	86.3
	C96036F	336	11.7	15.3	88.1
	C96037F	309	26.8	13.1	75.6
	C96039F	215	14.7	13.6	71.4
Group 4 100 ppm	C95966M	651	30.4	118	347
	C95970M	822	25.8	90.4	229
	C95976M	729	32.3	103	278
	C95978M	665	29.6	95.3	259
	C95979M	783	30.8	115	346
	C96041F	717	26.0	35.4	136
	C96043F	610	24.7	39.5	148
	C96057F	820	27.0	39.0	139
	C96058F	759	24.6	30.8	106
	C96059F	800	23.9	34.4	108

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 data quantitative to ±30%. Determination of M556 levels in liver may be biased low and should be considered quantitative to ±50%.

Table 12. Data Summary of Average Week 5 Rat Serum Concentration (µg/mL) and Standard Deviation (±SD) for FACT-TOX-028

Group Dose	Sex (n=5)	PFOS Average ±SD	PFOSA Average ±SD	M556 Average ±SD	M570 Average ±SD
Group 1 0 ppm	Male	0.00918 ±0.00113	<LOQ NA	<LOQ NA	0.00662 ±0.00232
	Female	0.0405 ±0.00691	<LOQ NA	<LOQ NA	0.0109 ±0.00430
Group 2 3 ppm	Male	2.08 ±0.232	0.0215 ±0.00380	0.665 ±0.196	3.85 ±0.453
	Female	4.06 ±0.664	0.0563 ±0.0134	0.486 ±0.123	3.46 ±1.26
Group 3 30 ppm	Male	29.0 ±3.52	0.478 ±0.0488	7.47 ±1.66	42.5 ±2.44
	Female	44.9 ±6.24	0.730 ±0.157	5.83 ±1.99	33.9 ±1.68
Group 4 100 ppm	Male	52 ±4.23	0.688 ±0.0722	12.8 ±2.34	57.5 ±4.59
	Female	63.4 ±9.71	1.26 ±0.0889	14.9 ±3.77	59.6 ±7.92

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 data quantitative to ±30%. NA = Not Applicable

Table 13. Data Summary of Average Week 14 Rat Serum Concentration (µg/mL) and Standard Deviation (±SD) for FACT-TOX-028

Group Dose	Sex (n=5)	PFOS Average ±SD	PFOSA Average ±SD	M556 Average ±SD	M570 Average ±SD
Group 1 0 ppm	Male	0.0217 ±0.000512	<LOQ NA	<LOQ NA	<LOQ NA
	Female	0.0698 ±0.0202	<LOQ NA	<LOQ NA	<LOQ NA
Group 2 3 ppm	Male	3.94 ±0.377	0.0397 ±0.0115	0.973 ±0.197	3.57 ±0.571
	Female	9.30 ±1.67	0.0582 ±0.0113	0.714 ±0.174	3.53 ±0.393
Group 3 30 ppm	Male	40.2 ±5.38	0.534 ±0.0840	7.84 ±0.936	20.6 ±2.72
	Female	68.6 ±9.14	0.790 ±0.154	6.95 ±0.568	21.4 ±2.11
Group 4 100 ppm	Male	111 ±13.0	0.958 ±0.182	25.2 ±2.26	23.7 ±6.06
	Female	200 ±50.5	1.26 ±0.212	25.9 ±3.73	24.6 ±2.51

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 data quantitative to ±30%. NA = Not Applicable

Table 14. Data Summary of Average Week 5 Rat Liver Concentration (µg/g) and Standard Deviation (±SD) for FACT-TOX-028

Group Dose	Sex (n=5)	PFOS Average ±SD	PFOSA Average ±SD	M556 Average ±SD	M570 Average ±SD
Group 1 0 ppm	Male	0.131 ±0.0197	<LOQ NA	0.00710 ±0.00159	0.0227 ±0.00915
	Female	0.0655 ±0.0169	<LOQ NA	0.00693 ±0.00184	0.0233 ±0.0124
Group 2 3 ppm	Male	17.4 ±2.03	1.14 ±0.313	2.01 ±0.235	7.20 ±0.664
	Female	11.0 ±4.50	1.17 ±0.637	1.09 ±0.521	3.72 ±1.47
Group 3 30 ppm	Male	85.2 ±9.77	8.67 ±1.67	21.9 ±0.878	84.9 ±10.4
	Female	94.0 ±30.6	6.15 ±1.23	13.5 ±3.08	36.3 ±8.62
Group 4 100 ppm	Male	192 ±39.2	13.3 ±2.09	53.9 ±6.29	153 ±18.2
	Female	223 ±30.4	14.1 ±1.14	40.9 ±8.39	128 ±11.5

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data quantitative to ±30%. Determination of M556 levels in liver may be biased low and should be considered quantitative to ±50%. NA = Not Applicable

Table 15. Data Summary of Average Week 14 Rat Liver Concentration (µg/g) and Standard Deviation (±SD) for FACT-TOX-028

Group Dose	Sex	PFOS Average ±SD	PFOSA Average ±SD	M556 Average ±SD	M570 Average ±SD
Group 1 0 ppm	Male	0.197 ±0.0960	0.0123 ±0.000114	0.0151 ±0.00310	0.0491 ±0.0229
	Female	0.120 ±0.0498	0.0160 ±0.00470	0.0147 ±0.00556	0.0506 ±0.0448
Group 2 3 ppm	Male	26.1 ±3.23	6.28 ±3.35	1.67 ±0.249	5.50 ±1.15
	Female	36.9 ±12.3	2.23 ±0.505	1.34 ±0.381	4.79 ±0.820
Group 3 30 ppm	Male	237 ±44.1	21.6 ±4.32	39.9 ±7.79	169 ±16.4
	Female	293 ±84.0	16.2 ±6.1	14.5 ±2.98	83.0 ±9.23
Group 4 100 ppm	Male	730 ±73.9	29.8 ±2.43	104 ±12.0	292 ±53.0
	Female	741 ±83.3	25.3 ±1.24	35.8 ±3.55	128 ±19.3

It is not possible to verify true recovery of endogenous analyte from tissues without radio-labeled reference material. The only measurement of accuracy available at this time, matrix spike studies, indicate that the data quantitative to ±30%. Determination of M556 levels in liver may be biased low and should be considered quantitative to ±50%. NA = Not Applicable

Table 16. Approximate LOQ Values Used in FACT-TOX-028 Analyses by Matrix, Compound and Usage Dates

Matrix	Compound	LOQ	Usage Dates
Liver	PFOS	0.0122 / 0.0306 µg/g	10/23 to 11/02/00
	PFOSA	0.00611 / 0.0306 / 0.0122 µg/g	10/23 to 11/02/00
	M556	0.00611 / 0.0306 / 0.0122 µg/g	10/23 to 11/02/00
	M570	0.0122 / 0.0306 µg/g	10/23 to 11/02/00
Sera	PFOS	0.00848 / 0.0215 µg/mL	10/02 to 11/02/00
	PFOSA	0.00445 / 0.00980 µg/mL	10/02 to 11/02/00
	M556	0.00980 / 0.0249 µg/mL	10/02 to 11/02/00
	M570	0.00495 / 0.00980 µg/mL	10/02 to 11/02/00

Table 17. FACT-TOX-028 Rat Liver Week 5 Precision Data for PFOS and PFOSA

Group Dose	Sample #	PFOS µg/g	PFOS Mean	PFOS Relative % Difference	PFOSA µg/g	PFOSA Mean	PFOSA Relative % Difference
Group 2 3 ppm	C95931M-1	15.5	15.7	1.92	0.754	0.935	38.6
	C95931M-2	15.8			1.12		
	C95932M-1	17.9	19.2	13.1	0.732	0.668	19.0
	C95932M-2	20.4			0.605		
	C95934M-1	15.5	15.0	6.67	1.01	1.00	0.400
	C95934M-2	14.5			1.00		
	C96011F-1	21.6	21.3	2.82	0.764	0.860	22.3
	C96011F-2	21.0			0.956		
	C96014F-1	20.1	18.7	15.0	0.511	0.452	26.2
	C96014F-2	17.3			0.392		
	C96015F-1	17.5	17.6	1.14	0.940	0.880	13.5
	C96015F-2	17.7			0.821		
	Average Percent Difference			6.76			20.0

Table 18. FACT-TOX-028 Rat Liver Week 5 Precision Data for M556 and M570

Group Dose	Sample #	M556 µg/g	M556 Mean	M556 Relative % Difference	M570 µg/g	M570 Mean	M570 Relative % Difference
Group 2 3 ppm	C95931M-1	1.82	1.99	16.7	7.44	8.20	18.4
	C95931M-2	2.16			8.95		
	C95932M-1	1.73	1.75	2.83	7.90	7.86	1.12
	C95932M-2	1.77			7.81		
	C95934M-1	1.91	1.74	20.1	6.99	6.87	3.45
	C95934M-2	1.56			6.75		
	C96011F-1	1.60	1.75	17.9	6.00	6.18	5.77
	C96011F-2	1.91			6.36		
	C96014F-1	1.85	1.56	37.4	5.94	5.43	18.9
	C96014F-2	1.27			4.92		
	C96015F-1	0.955	1.05	18.4	5.75	5.89	4.75
	C96015F-2	1.15			6.03		
	Average Percent Difference			18.9			8.72

Appendix E: Data Spreadsheets

FACT-TOX-028
Covance# 6329-225

Study: Covance 6329-225, FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats
Product Number(Test Substance): T-6314
Matrix: Rat Serum
Method/Revision: ETS-8-4.1 & ETS-8-5.1
Analytical Equipment System Number: Amelie 063498
Instrument Software/Version: Masslynx 3.4
Filename: See Below
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments
Dates of Extraction/Analyst: 09/25/00 SAL/KJK/RWW
Dates of Analysis/Analyst: 10/02/00, 10/05/00, 10/10/00, 10/12/00, 10/23/00 MAH/KJK
Date of Data Reduction/Analyst: 10/05/00, 10/09/00, 10/12/00, 10/13/00, 10/24/00, 11/06/00 MAH/KJK

Sample Data
RAT SERUM WEEK 5

lot 171

Group Dose	Sample #	Extraction Vol. mL	Surrogate Verified	PFOS Purity Correction Factor	PFOS Dilution Factor	PFOS Conc. ng/mL	Filename (optional)	Concentration of PFOS ng/mL or % Rec	Mean PFOS ng/mL	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	PFOSA Dilution Factor	PFOSA Conc. ng/mL	Filename (optional)	Concentration of PFOSA ng/mL or % Rec	Mean PFOSA ug/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS09250-1IO Blk-5-1	1.00	NA	0.8640	1	0.00	A001005005	<LOQ (9.82 ng/mL)			NA	1	0.00	A001002005	<LOQ (9.80 ng/mL)		NA
	RBS09250-1IO Blk-5-2	1.00	NA	0.8640	1	0.00	A001005019	<LOQ (9.82 ng/mL)	<LOQ (9.80 ng/mL)	NA	NA	1	0.810	A001002019	<LOQ (9.80 ng/mL)	<LOQ	NA
Matrix Blk	RBS09250-Sera Blk-5-1	1.00	NA	0.8640	1	0.00	A001005006	<LOQ (9.82 ng/mL)			NA	1	0.300	A001002006	<LOQ (9.80 ng/mL)		NA
	RBS09250-Sera Blk-5-2	1.00	NA	0.8640	1	0.00	A001005020	<LOQ (9.82 ng/mL)	<LOQ (9.80 ng/mL)	NA	NA	1	0.850	A001002020	<LOQ (9.80 ng/mL)	<LOQ	NA
	RTS09250-Sera Blk-5-1	1.00	NA	0.8640	1	0.970	A001005007	<LOQ (9.82 ng/mL)			NA	1	0.00	A001002007	<LOQ (9.80 ng/mL)		NA
	RTS09250-Sera Blk-5-2	1.00	NA	0.8640	1	0.00	A001005021	<LOQ (9.82 ng/mL)	<LOQ (9.80 ng/mL)	NA	NA	1	0.740	A001002021	<LOQ (9.80 ng/mL)	<LOQ	NA
QC	RTS09250-250 ppb-MS-5	1.00	NA	NA	1	256	A001005022	103%			NA	1	301	A001002022	121%		
	RTS09250-250 ppb-MSD-5	1.00	NA	NA	1	265	A001005023	106%	104%	4%	NA	1	310	A001002023	124%	123%	3%
Group 1 0 ppm	C95911M	1.00	NA	0.8640	1	10.8	A001005026	0.00937			NA	1	0.700	A001002026	<LOQ (9.80 ng/mL)		
	C95912M	1.00	NA	0.8640	1	2.34	A001005027	<LOQ (9.82 ng/mL)			NA	1	0.130	A001002027	<LOQ (9.80 ng/mL)		
	C95913M	1.00	NA	0.8640	1	3.19	A001005028	<LOQ (9.82 ng/mL)			NA	1	0.410	A001002028	<LOQ (9.80 ng/mL)		
	C95914M	1.00	NA	0.8640	1	4.66	A001005029	<LOQ (9.82 ng/mL)		NA	NA	1	0.00	A001002029	<LOQ (9.80 ng/mL)		NA
	C95915M	1.00	NA	0.8640	1	12.8	A001005030	0.0111	<LOQ - 2 Anomalies	NA	NA	1	0.870	A001002030	<LOQ (9.80 ng/mL)	<LOQ	NA
	C95991F	1.00	NA	0.8640	1	34.5	A001005033	0.0298			NA	1	0.00	A001002033	<LOQ (9.80 ng/mL)		
	C95992F	1.00	NA	0.8640	1	56.2	A001005034	0.0486			NA	1	0.900	A001002034	<LOQ (9.80 ng/mL)		
	C95993F	1.00	NA	0.8640	1	49.3	A001005035	0.0431			NA	1	0.100	A001002035	<LOQ (9.80 ng/mL)		
	C95994F	1.00	NA	0.8640	1	48.8	A001005036	0.0422		17.1	NA	1	0.700	A001002036	<LOQ (9.80 ng/mL)		NA
	C95995F	1.00	NA	0.8640	1	44.8	A001005037	0.0387	0.0405	0.00691	NA	1	0.00	A001002037	<LOQ (9.80 ng/mL)	<LOQ	NA
Group 2 3 ppm	C95931M	1.00	2nd analysis OK	0.8640	10	220	A001023017	1.90			NA	1	19.6	A001005054	0.0196		
	C95932M	1.00	2nd analysis OK	0.8640	10	367	A001023018	2.30			NA	1	17.9	A001005055	0.0179		
	C95933M	1.00	2nd analysis OK	0.8640	10	225	A001023019	1.94			NA	1	21.1	A001005056	0.0211		
	C95934M	1.00	2nd analysis OK	0.8640	10	218	A001023020	1.88		11.2	NA	1	21.0	A001005057	0.0210		17.7
	C95935M	1.00	2nd analysis OK	0.8640	10	273	A001023021	2.36	2.08	0.232	NA	1	27.9	A001005058	0.0279	0.0215	0.00380
	C96011F	1.00	2nd analysis OK	0.8640	10	361	A001023025	3.12			NA	1	44.8	A001005061	0.0448		
	C96012F	1.00	2nd analysis OK	0.8640	10	422	A001023026	3.65			NA	1	39.4	A001005062	0.0394		
	C96013F	1.00	2nd analysis OK	0.8640	10	492	A001023027	4.25			NA	1	67.6	A001005063	0.0676		
	C96014F	1.00	2nd analysis OK	0.8640	10	540	A001023028	4.66		16.4	NA	1	61.8	A001005064	0.0618		23.8
	C96015F	1.00	2nd analysis OK	0.8640	10	535	A001023029	4.62	4.06	0.664	NA	1	68.2	A001005065	0.0682	0.0563	0.0134
Group 3 30 ppm	C95931M	1.00	NA	0.8640	100	282	A001010019	24.3			Confirmed Low	1	420	A001010082	0.420		
	C95932M	1.00	NA	0.8640	100	323	A001010020	27.9			Confirmed Low	1	471	A001010083	0.471		
	C95933M	1.00	NA	0.8640	100	348	A001010021	30.1			Confirmed Low	1	547	A001010084	0.547		
	C95934M	1.00	NA	0.8640	100	333	A001010022	28.8		12.1	Confirmed Low	1	451	A001010085	0.451		10.2
	C95935M	1.00	NA	0.8640	100	394	A001010023	34.0	29.0	2.52	Confirmed Low	1	503	A001010086	0.503	0.478	0.0488
	C96031F	1.00	NA	0.8640	100	475	A001010026	41.0			NA	1	649	A001010089	0.649		
	C96032F	1.00	NA	0.8640	100	611	A001010027	52.8			Confirmed Low	1	775	A001010090	0.775		
	C96033F	1.00	NA	0.8640	100	482	A001010028	41.6			Confirmed Low	1	869	A001010091	0.869		
	C96034F	1.00	NA	0.8640	100	448	A001010029	38.7		13.9	NA	1	498	A001010092	0.498		21.5
	C96035F	1.00	NA	0.8640	100	581	A001010030	50.1	44.9	6.24	Confirmed Low	1	857	A001010093	0.857	0.730	0.157
Group 4 100 ppm	C95971M	1.00	NA	0.8640	100	358	A001010033	48.2			Confirmed Low	1	599	A001010096	0.599		
	C95972M	1.00	NA	0.8640	100	545	A001010034	47.1			Confirmed Low	1	660	A001010097	0.660		
	C95973M	1.00	NA	0.8640	100	650	A001010035	56.2			Confirmed Low	1	745	A001010098	0.745		
	C95974M	1.00	NA	0.8640	100	616	A001010036	53.2		8.13	Confirmed Low	1	777	A001010099	0.777		10.5
	C95975M	1.00	NA	0.8640	100	645	A001010037	55.8	52.1	4.23	Confirmed Low	1	658	A001010100	0.658	0.688	0.0722
	C96051F	1.00	NA	0.8640	100	738	A001010040	63.8			Confirmed Low	5	254	A001010075	1.27		
	C96052F	1.00	NA	0.8640	100	804	A001010041	69.5			Confirmed Low	5	240	A001010076	1.20		
	C96053F	1.00	NA	0.8640	100	871	A001010042	75.2			Confirmed Low	5	247	A001010077	1.23		
	C96054F	1.00	NA	0.8640	100	582	A001010043	50.3		15.3	Confirmed Low	5	240	A001010078	1.20		7.04
	C96055F	1.00	NA	0.8640	100	672	A001010044	58.1	63.4	9.71	Confirmed Low	5	282	A001010079	1.41	1.36	0.0889

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL, PFOSA = 4.79 ppb, M556 = 19.2 ppb, M570 = 4.95 ppb
* High recovery confirmed with 10/05/00 analysis. LAC 10/13/00

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
M556 = $C_8F_{17}SO_2N(H)CH_2COOH$
M570 = $C_8F_{17}SO_2N(CH_3)CH_2COOH$

Date Entered/By: 10/11/00, 10/18/00, 10/19/00, 10/26/00, 11/06/00 LAC
Date Verified/ By: 11/06/00, 11/07/00 KJH
Purity Entered/Verified: 12/20/00 LAC, 12/27/00 mmh

FACT-TOX-028
Covance# 6329-225

Study: Covance 6329-225, FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats
Product Number(Test Substance): T-6314
Matrix: Rat Serum
Method/Revision: ETS-8-4.1 & ETS-8-5.1
Analytical Equipment System Number: Amelia 062498
Instrument Software/Version: Masslynx 3.4
Filename: See Below
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments
Dates of Extraction/Analyst: 09/25/00 SAL/KJK/RWW
Dates of Analysis/Analyst: 10/02/00, 10/05/00, 10/10/00, 10/12/00, 10/23/00 MB-GH/KJH
Date of Data Reduction/Analyst: 10/05/00, 10/09/00, 10/12/00, 10/13/00, 10/24/00, 11/06/00 MB-GH/KJH

Sample Data
RAT SERUM WEEK 5

Group Dose	Sample #	Surrogate Verified	M556 Dilution Factor	M556 Conc. ng/mL	Filename (optional)	Concentration of M556 ng/mL or % Rec	Mean M556 ng/mL	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	M570 Dilution Factor	M570 Conc. ng/mL	Filename (optional)	Concentration of M570 ng/mL or % Rec	Mean M570 ng/mL	RSD Std. Dev. MS/MSD RPD
Method HR	RHS09250-H2O Blk-5-1	NA	1	0.00	A001002005	<LOQ (9.80 ng/mL)		NA	NA	1	0.00	A001002005	<LOQ (4.95 ng/mL)		NA
	RHS09250-H2O Blk-5-2	NA	1	0.00	A001002019	<LOQ (9.80 ng/mL)	<LOQ	NA	NA	1	0.00	A001002019	<LOQ (4.95 ng/mL)	<LOQ	NA
Matrix Blk	RHS09250-Sera Blk-5-1	NA	1	0.00	A001002006	<LOQ (9.80 ng/mL)		NA	NA	1	0.00	A001002006	<LOQ (4.95 ng/mL)		NA
	RHS09250-Sera Blk-5-2	NA	1	0.00	A001002020	<LOQ (9.80 ng/mL)	<LOQ	NA	NA	1	0.00	A001002020	<LOQ (4.95 ng/mL)	<LOQ	NA
	RTS09250-Sera Blk-5-1	NA	1	0.00	A001002007	<LOQ (9.80 ng/mL)		NA	NA	1	0.00	A001002007	<LOQ (4.95 ng/mL)		NA
	RTS09250-Sera Blk-5-2	NA	1	0.00	A001002021	<LOQ (9.80 ng/mL)	<LOQ	NA	NA	1	0.00	A001002021	<LOQ (4.95 ng/mL)	<LOQ	NA
QC	RTS09250-250 ppb-MS-5	NA	1	336	A001002022	135% *			NA	1	281	A001005022	113%		
	RTS09250-250 ppb-MSD-5	NA	1	350	A001002023	140% *	138%	4%	NA	1	285	A001005023	114%	114%	1%
Group 1	C95911M	NA	1	2.94	A001002026	<LOQ (9.80 ng/mL)			NA	1	8.64	A001002026	0.00864		
0 ppm	C95912M	NA	1	0.00	A001002027	<LOQ (9.80 ng/mL)			NA	1	3.22	A001002027	<LOQ (4.95 ng/mL)		
	C95913M	NA	1	0.00	A001002028	<LOQ (9.80 ng/mL)			NA	1	3.14	A001002028	<LOQ (4.95 ng/mL)		
	C95914M	NA	1	0.00	A001002029	<LOQ (9.80 ng/mL)		NA	NA	1	3.33	A001002029	<LOQ (4.95 ng/mL)		NA
	C95915M	NA	1	0.00	A001002030	<LOQ (9.80 ng/mL)	<LOQ	NA	NA	1	9.63	A001002030	0.00963	<LOQ - 2 Anomalies	NA
	C95991F	NA	1	0.00	A001002033	<LOQ (9.80 ng/mL)			NA	1	8.02	A001002033	0.00802		
	C95992F	NA	1	0.00	A001002034	<LOQ (9.80 ng/mL)			NA	1	8.62	A001002034	0.00862		
	C95993F	NA	1	0.00	A001002035	<LOQ (9.80 ng/mL)			NA	1	9.71	A001002035	0.00971		
	C95994F	NA	1	0.00	A001002036	<LOQ (9.80 ng/mL)		NA	NA	1	9.49	A001002036	0.00949		39.6
	C95995F	NA	1	0.00	A001002037	<LOQ (9.80 ng/mL)	<LOQ	NA	NA	1	18.5	A001002037	0.0185	0.0109	0.00430
Group 2	C95931M	2nd analysis OK	10	67.4	A001023017	0.674			2nd analysis OK	10	429	A001023017	4.29		
3 ppm	C95932M	2nd analysis OK	10	44.0	A001023018	0.440			2nd analysis OK	10	318	A001023018	3.18		
	C95933M	2nd analysis OK	10	52.4	A001023019	0.524			2nd analysis OK	10	363	A001023019	3.63		
	C95934M	2nd analysis OK	10	74.3	A001023020	0.743		29.5	2nd analysis OK	10	398	A001023020	3.98		11.7
	C95935M	2nd analysis OK	10	94.3	A001023021	0.943	0.665	0.196	2nd analysis OK	10	419	A001023021	4.19	3.85	0.453
	C96011F	2nd analysis OK	10	44.2	A001023025	0.442			2nd analysis OK	10	329	A001023025	3.29		
	C96012F	2nd analysis OK	10	32.3	A001023026	0.323			2nd analysis OK	10	229	A001023026	2.29		
	C96013F	2nd analysis OK	10	62.8	A001023027	0.628			2nd analysis OK	10	439	A001023027	4.39		
	C96014F	2nd analysis OK	10	44.8	A001023028	0.448		25.4	2nd analysis OK	10	322	A001023028	3.22		36.5
	C96015F	2nd analysis OK	10	59.0	A001023029	0.590	0.486	0.123	2nd analysis OK	10	511	A001023029	5.11	3.46	1.26
Group 3	C95951M	NA	100	59.8	A001010019	5.98			NA	100	395	A001012019	39.5		
30 ppm	C95952M	NA	100	64.2	A001010020	6.42			NA	100	404	A001012020	40.4		
	C95953M	NA	100	101.0	A001010021	10.1			NA	100	451	A001012021	45.1		
	C95954M	NA	100	80.7	A001010022	8.07		22.3	NA	100	433	A001012022	43.3		5.74
	C95955M	NA	100	67.8	A001010023	6.78	7.47	1.66	NA	100	442	A001012023	44.2	42.5	2.44
	C96031F	NA	100	43.4	A001010026	4.34			NA	100	334	A001012027	33.4		
	C96032F	NA	100	34.0	A001010027	3.40			NA	100	318	A001012028	31.8		
	C96033F	NA	100	75.7	A001010028	7.57			NA	100	331	A001012029	33.1		
	C96034F	NA	100	79.8	A001010029	7.98		34.1	NA	100	350	A001012030	35.0		4.96
	C96035F	NA	100	58.7	A001010030	5.87	5.83	1.99	NA	100	359	A001012031	36.0	35.9	1.68
Group 4	C95971M	NA	100	102	A001010033	10.2			NA	100	514	A001012035	51.4		
100 ppm	C95972M	NA	100	113	A001010034	11.3			NA	100	574	A001012036	57.4		
	C95973M	NA	100	159	A001010035	15.9			NA	100	549	A001012037	54.9		
	C95974M	NA	100	145	A001010036	14.5		18.3	NA	100	625	A001012038	62.5		7.98
	C95975M	NA	100	120	A001010037	12.0	12.8	2.34	NA	100	613	A001012039	61.3	57.5	4.59
	C96051F	NA	100	106	A001010040	10.6			NA	100	646	A001012043	64.6		
	C96052F	NA	100	201	A001010041	20.1			NA	100	684	A001012044	68.4		
	C96053F	NA	100	170	A001010042	17.0			NA	100	512	A001012045	51.3		
	C96054F	NA	100	142	A001010043	14.2		25.4	NA	100	513	A001012046	51.3		13.3
	C96055F	NA	100	123	A001010044	12.3	14.9	3.77	NA	100	625	A001012047	62.5	59.6	7.92

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL, PFOSA = 4.79 ppb, M556 = 19.2 ppb, M570 = 4.95 ppb
* High recovery confirmed with 10/05/00 analysis. LAC 10/11/00

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
M556 = C8F17SO2N(H)CH2COOH
M570 = C8F17SO2N(CH3)CH2COOH

Date Entered/By: 10/11/00, 10/18/00, 10/19/00, 10/26/00, 11/06/00 LAC
Date Verified/ By: 11/06/00, 11/07/00 KJH
Purity Entered/Verified: 12/20/00 LAC, 12/27/00 mmh

BACK TO MAIN

FACT-TOX-028
Covance# 6329-225

Study: Covance 6329-225, FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats
Product Number(Test Substance): T-6314
Matrix: Rat Serum
Method/Revision: ETS-8-4.1 & ETS-8-5.1
Analytical Equipment System Number: Astelia 062498
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: 09/25/00 SAL/KJK/RWW
Dates of Analysis/Analyst: 10/02/00, 10/05/00, 10/10/00, 10/12/00, 10/23/00 MMH/KJH
Date of Data Reduction/Analyst: 10/05/00, 10/09/00, 10/13/00, 10/13/00, 10/24/00, 11/06/00 MMH/KJH

Sample Data

RAT SERUM WEEK 5

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ng/mL or % Rec	Mean PFOS ng/mL	RSD Std. Dev. MS/MSD RPD	PFOSA Conc. ng/mL	Concentration of PFOSA ng/mL or % Rec	Mean PFOSA ng/mL	RSD Std. Dev. MS/MSD RPD	M556 Conc. ng/mL	Concentration of M556 ng/mL or % Rec	Mean M556 ng/mL	RSD Std. Dev. MS/MSD RPD	M570 Conc. ng/mL	Concentration of M570 ng/mL or % Rec	Mean M570 ng/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS09250-HDO Blk-5-1	0.00	<LOQ (9.82 ng/mL)			0.00	<LOQ (9.80 ng/mL)			0.00	<LOQ (9.80 ng/mL)			0.00	<LOQ (4.95 ng/mL)		
	RBS09250-HDO Blk-5-2	0.00	<LOQ (9.82 ng/mL)	<LOQ (9.80 ng/mL)	NA	0.810	<LOQ (9.80 ng/mL)	<LOQ	NA	0.00	<LOQ (9.80 ng/mL)	<LOQ	NA	0.00	<LOQ (4.95 ng/mL)	<LOQ	NA
Matrix Blk	RBS09250-Sera Blk-5-1	0.00	<LOQ (9.82 ng/mL)			0.300	<LOQ (9.80 ng/mL)			0.00	<LOQ (9.80 ng/mL)			0.00	<LOQ (4.95 ng/mL)		
	RBS09250-Sera Blk-5-2	0.00	<LOQ (9.82 ng/mL)	<LOQ (9.80 ng/mL)	NA	0.850	<LOQ (9.80 ng/mL)	<LOQ	NA	0.00	<LOQ (9.80 ng/mL)	<LOQ	NA	0.00	<LOQ (4.95 ng/mL)	<LOQ	NA
	RTS09250-Sera Blk-5-1	0.970	<LOQ (9.82 ng/mL)			0.00	<LOQ (9.80 ng/mL)			0.00	<LOQ (9.80 ng/mL)			0.00	<LOQ (4.95 ng/mL)		
	RTS09250-Sera Blk-5-2	0.00	<LOQ (9.82 ng/mL)	<LOQ (9.80 ng/mL)	NA	0.740	<LOQ (9.80 ng/mL)	<LOQ	NA	0.00	<LOQ (9.80 ng/mL)	<LOQ	NA	0.00	<LOQ (4.95 ng/mL)	<LOQ	NA
QC	RTS09250-250 ppb-MS-5	256	103%			301	121%			336	135% *			281	113%		
	RTS09250-250 ppb-MSD-5	265	106%	104%	4%	310	124%	123%	3%	350	140% *	138%	4%	285	114%	114%	1%
Group 1 0 ppm	C95911M	10.8	0.00937			0.700	<LOQ (9.80 ng/mL)			2.94	<LOQ (9.80 ng/mL)			8.64	0.00864		
	C95912M	2.24	<LOQ (9.82 ng/mL)			0.130	<LOQ (9.80 ng/mL)			0.00	<LOQ (9.80 ng/mL)			3.22	<LOQ (4.95 ng/mL)		
	C95913M	3.19	<LOQ (9.82 ng/mL)			0.410	<LOQ (9.80 ng/mL)			0.00	<LOQ (9.80 ng/mL)			3.14	<LOQ (4.95 ng/mL)		
	C95914M	4.66	<LOQ (9.82 ng/mL)		NA	0.00	<LOQ (9.80 ng/mL)		NA	0.00	<LOQ (9.80 ng/mL)		NA	3.33	<LOQ (4.95 ng/mL)		NA
	C95915M	12.8	0.0111	<LOQ - 2 Anomalies	NA	0.870	<LOQ (9.80 ng/mL)	<LOQ	NA	0.00	<LOQ (9.80 ng/mL)	<LOQ	NA	9.63	0.00963	<LOQ - 2 Anomalies	NA
	C95991F	34.5	0.0296			0.00	<LOQ (9.80 ng/mL)			0.00	<LOQ (9.80 ng/mL)			8.02	0.00802		
	C95992F	56.2	0.0486			0.900	<LOQ (9.80 ng/mL)			0.00	<LOQ (9.80 ng/mL)			8.62	0.00862		
	C95993F	49.8	0.0431			0.100	<LOQ (9.80 ng/mL)			0.00	<LOQ (9.80 ng/mL)			9.71	0.00971		
	C95994F	48.8	0.0422		17.1	0.700	<LOQ (9.80 ng/mL)		NA	0.00	<LOQ (9.80 ng/mL)		NA	9.49	0.00949		39.6
	C95995F	44.8	0.0387	0.0405	0.00691	0.00	<LOQ (9.80 ng/mL)	<LOQ	NA	0.00	<LOQ (9.80 ng/mL)	<LOQ	NA	18.5	0.0185	0.0109	0.00430
Group 2 3 ppm	C95931M	220	1.90			19.6	0.0196			67.4	0.674			429	4.29		
	C95932M	267	2.30			17.9	0.0179			44.0	0.440			318	3.18		
	C95933M	235	1.94			21.1	0.0211			52.4	0.524			363	3.63		
	C95934M	218	1.88		11.2	21.0	0.0210		17.7	74.3	0.743		29.5	398	3.98		11.7
	C95935M	273	2.36	2.08	0.232	27.9	0.0279	0.0215	0.00380	94.3	0.943	0.665	0.196	419	4.19	3.85	0.453
	C96011F	361	3.12			44.8	0.0448			44.2	0.442			229	2.29		
	C96012F	422	3.65			39.4	0.0394			32.3	0.323			229	2.29		
	C96013F	492	4.25			67.6	0.0676			62.8	0.628			439	4.39		
	C96014F	540	4.66		16.4	61.8	0.0618		23.8	44.8	0.448		25.4	322	3.22		36.5
	C96015F	535	4.62	4.06	0.664	68.2	0.0682	0.0563	0.0134	59.0	0.590	0.486	0.123	511	5.11	3.46	1.26
Group 3 30 ppm	C95951M	282	24.3			420	0.420			59.8	5.98			395	39.5		
	C95952M	323	27.9			471	0.471			64.2	6.42			404	40.4		
	C95953M	348	30.1			547	0.547			101	10.1			451	45.1		
	C95954M	333	28.8		12.1	451	0.451		10.2	80.7	8.07		22.3	433	43.3		5.74
	C95955M	396	34.0	29.0	3.52	503	0.503	0.478	0.0488	67.8	6.78	7.47	1.66	442	44.2	42.5	2.44
	C96031F	475	41.0			649	0.649			43.4	4.34			334	33.4		
	C96032F	611	52.8			775	0.775			34.0	3.40			318	31.8		
	C96033F	482	41.6			869	0.869			75.7	7.57			331	33.1		
	C96034F	448	38.7		13.9	498	0.498		21.5	79.8	7.98		34.1	350	35.0		4.96
	C96035F	581	50.2	44.9	6.24	857	0.857	0.730	0.157	58.7	5.87	5.83	1.98	360	36.0	35.9	1.68
Group 4 100 ppm	C95971M	558	48.2			599	0.599			102	10.2			514	51.4		
	C95972M	545	47.1			660	0.660			113	11.3			574	57.4		
	C95973M	650	56.2			745	0.745			159	15.9			549	54.9		
	C95974M	616	53.2		8.13	777	0.777		10.5	145	14.5		18.3	625	62.5		7.98
	C95975M	645	55.8	52.1	4.23	658	0.658	0.688	0.0722	120	12.0	12.8	2.34	613	61.3	57.5	4.59
	C96051F	738	63.8			254	1.27			106	10.6			646	64.6		
	C96052F	804	69.5			240	1.20			201	20.1			684	68.4		
	C96053F	871	75.2			247	1.23			170	17.0			512	51.2		
	C96054F	583	50.3		15.3	240	1.20		7.04	142	14.2		25.4	513	51.3		13.3
	C96055F	672	58.1	63.4	9.71	282	1.41	1.26	0.0839	123	12.3	14.9	3.77	625	62.5	59.6	7.92

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL, PFOSA = 4.79 ppb, M556 = 19.2 ppb, M570 = 4.95 ppb
* High recovery confirmed with 10/05/00 analysis. LAC 10/11/00

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
M556 = $C_8F_{17}SO_2N(H)CH_2COOH$
M570 = $C_8F_{17}SO_2N(CH_3)CH_2COOH$

Data Entered/By: 10/11/00, 10/18/00, 10/19/00, 10/26/00, 11/06/00 LAC
Data Verified/ By: 11/06/00, 11/07/00 KJH
Purity Entered/Verified: 12/20/00 LAC, 12/27/00 mmh

FACT-TOX-028
Covance# 6329-225

Study: Covance 6329-225, FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats
Product Number/Test Substance): T-6314
Matrix: Rat Serum
Method/Revision: ETS-8-4.1 & ETS-8-5.1
Analytical Equipment System Number: Soup 020199, Dave 070799, Amelia 062498
Instrument Software/Version: Masslynx 3.4
Filename: See Below
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments
Dates of Extraction/Analyst: 09/25/00 SAL/KJK/RWW
Dates of Analysis/Analyst: 10/09/00, 10/11/00, 10/13/00, 10/18/00, 10/27/00, 11/02/00 MAH/KJH
Date of Data Reduction/Analyst: 10/16/00, 10/18/00, 10/23/00, 10/30/00, 11/06/00 MAH/KJH

Sample Data
RAT SERUM WEEK 14

lot 171

Group Dose	Sample #	Extraction Vol. mL	Surrogate Verified	PFOS Purity Correction Factor	PFOS Dilution Factor	PFOS Conc. ng/mL	Filename (optional)	Concentration of PFOS ng/mL or % Rec	Mean PFOS ng/mL	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	PFOSA Dilution Factor	PFOSA Conc. ng/mL	Filename (optional)	Concentration of PFOSA ng/mL or % Rec	Mean PFOSA ng/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS09250-H2O Blk-1-1	1.00	NA	0.8640	1	0.00	D001009005	<LOQ (9.82 ng/mL)		NA	NA	1	1.25	D001018005	<LOQ (4.95 ng/mL)		NA
	RBS09150-H2O Blk-1-2	1.00	NA	0.8640	1	0.00	D001009019	<LOQ (9.82 ng/mL)	<LOQ	NA	NA	1	0.00	D001018019	<LOQ (4.95 ng/mL)	<LOQ	NA
Matrix Blk	RBS09250-Sera Blk-1-1	1.00	NA	0.8640	1	0.00	D001009006	<LOQ (9.82 ng/mL)		NA	NA	1	0.00	D001018006	<LOQ (4.95 ng/mL)		NA
	RBS09250-Sera Blk-1-2	1.00	NA	0.8640	1	0.00	D001009020	<LOQ (9.82 ng/mL)	<LOQ	NA	NA	1	0.00	D001018020	<LOQ (4.95 ng/mL)	<LOQ	NA
	RTS09250-Sera Blk-1-1	1.00	NA	0.8640	1	0.00	D001009007	<LOQ (9.82 ng/mL)		NA	NA	1	0.00	D001018007	<LOQ (4.95 ng/mL)		NA
	RTS09250-Sera Blk-1-2	1.00	NA	0.8640	1	0.00	D001009021	<LOQ (9.82 ng/mL)	<LOQ	NA	NA	1	0.00	D001018021	<LOQ (4.95 ng/mL)	<LOQ	NA
QC	RTS09250-250 ppb-MS-1	1.00	NA		1	224	D001009022	90%			NA	1	237	D001009022	95%		
	RTS09250-250 ppb-MSD-1	1.00	NA		1	222	D001009023	89%	90%	1%	NA	1	211	D001009023	85%	90%	12%
Group 1 0 ppm	C95907M	1.00	2nd Analysis OK	0.8640	1	7.80	A001027117	<LOQ (24.9 ng/mL)			NA	1	0.710	A001027117	<LOQ (9.80 ng/mL)		
	C95908M	1.00	2nd Analysis OK	0.8640	1	36.2	A001027118	0.0226			NA	1	0.860	A001027118	<LOQ (9.80 ng/mL)		
	C95910M	1.00	2nd Analysis OK	0.8640	1	7.68	A001027119	<LOQ (24.9 ng/mL)			NA	1	0.620	A001027119	<LOQ (9.80 ng/mL)		
	C95916M	1.00	2nd Analysis OK	0.8640	1	0.600	A001027120	<LOQ (24.9 ng/mL)	0.0226	NA	NA	1	0.520	A001027120	<LOQ (9.80 ng/mL)		NA
	C95917M	1.00	2nd Analysis OK	0.8640	1	14.9	A001027121	<LOQ (24.9 ng/mL)	<LOQ - 1 Anomaly	NA	NA	1	0.170	A001027121	<LOQ (9.80 ng/mL)	<LOQ	NA
	C95981F	1.00	NA	0.8640	1	42.9	D001018033	0.0370			NA	1	0.0300	A001027124	<LOQ (9.80 ng/mL)		
	C95982F	1.00	NA	0.8640	1	105	D001018034	0.0906			NA	1	0.00	A001027125	<LOQ (9.80 ng/mL)		
	C95989F	1.00	NA	0.8640	1	93.6	D001018035	0.0809			NA	1	0.00	A001027126	<LOQ (9.80 ng/mL)		
	C95998F	1.00	NA	0.8640	1	81.1	D001018036	0.0701		29.0	NA	1	0.00	A001027127	<LOQ (9.80 ng/mL)		NA
	C95999F	1.00	NA	0.8640	1	81.2	D001018037	0.0701	0.0698	0.0202	NA	1	0.0200	A001027128	<LOQ (9.80 ng/mL)	<LOQ	NA
Group 2 3 ppm	C95923M	1.00	NA	0.8640	10	399	A001027139	3.45			NA	1	59.8	A001027153	0.0398		
	C95929M	1.00	NA	0.8640	10	507	A001027136	4.38			NA	1	14.6	A001027152	0.0348		
	C95930M	1.00	NA	0.8640	10	477	A001027137	4.12			NA	1	36.7	A001027151	0.0367		
	C95938M	1.00	NA	0.8640	10	475	A001027138	4.10		9.58	NA	1	30.8	A001027150	0.0308		28.9
	C95940M	1.00	NA	0.8640	10	423	A001027139	3.65	3.94	0.377	NA	1	36.4	A001027149	0.0364	0.0397	0.0115
	C96004F	1.00	NA	0.8640	20	599	A001102016	6.89			NA	1	41.5	A001027161	0.0415		
	C96005F	1.00	NA	0.8640	20	545	A001102017	9.41			NA	1	61.8	A001027160	0.0618		
	C96009F	1.00	NA	0.8640	20	589	A001102018	10.2			NA	1	68.8	A001027159	0.0688		
	C96017F	1.00	NA	0.8640	20	496	A001102019	8.57		18.0	NA	1	52.3	A001027158	0.0523		19.4
	C96018F	1.00	NA	0.8640	20	654	A001102020	11.3	9.3	1.67	NA	1	66.8	A001027157	0.0668	0.0582	0.0113
Group 3 30 ppm	C95945M	1.00	Confirmed Low	0.8640	100	518	S001011050	44.7			Confirmed Low	20	33.2	S001013051	0.663		
	C95946M	1.00	Confirmed Low	0.8640	100	450	S001011031	38.9			Confirmed Low	20	24.9	S001013052	0.497		
	C95956M	1.00	Confirmed Low	0.8640	100	427	S001011032	36.9			Confirmed Low	20	22.7	S001013053	0.453		
	C95957M	1.00	Confirmed Low	0.8640	100	391	S001011033	33.8		13.4	Confirmed Low	20	24.3	S001013054	0.486		15.7
	C95959M	1.00	Confirmed Low	0.8640	100	539	S001011034	46.6	40.2	5.38	Confirmed Low	20	28.6	S001013055	0.571	0.534	0.0840
	C96028F	1.00	Confirmed Low	0.8640	100	648	S001011037	56.0			Confirmed Low	20	37.1	S001013058	0.742		
	C96029F	1.00	Confirmed Low	0.8640	100	924	S001011038	79.9			Confirmed Low	20	39.0	S001013059	0.780		
	C96036F	1.00	Confirmed Low	0.8640	100	864	S001011039	74.7			Confirmed Low	20	36.3	S001013060	0.726		
	C96037F	1.00	Confirmed Low	0.8640	100	760	S001011040	65.6		13.3	Confirmed Low	20	32.5	S001013061	0.649		19.5
	C96039F	1.00	Confirmed Low	0.8640	100	773	S001011041	66.8	68.6	9.14	Confirmed Low	20	52.6	S001013062	1.05	0.790	0.154
Group 4 100 ppm	C95966M	1.00	Confirmed Low	0.8640	200	599	S001011072	104			Confirmed Low	5	253	S001013079	1.27		
	C95970M	1.00	Confirmed Low	0.8640	200	775	S001011073	134			Confirmed Low	5	157	S001013080	0.784		
	C95976M	1.00	Confirmed Low	0.8640	200	629	S001011074	109			Confirmed Low	5	189	S001013081	0.946		
	C95978M	1.00	Confirmed Low	0.8640	200	634	S001011075	110		11.7	Confirmed Low	5	178	S001013082	0.890		19.0
	C95979M	1.00	Confirmed Low	0.8640	200	587	S001011076	101	111	13.0	Confirmed Low	5	181	S001013083	0.905	0.958	0.182
	C96041F	1.00	Confirmed Low	0.8640	200	1029	S001011079	178			Confirmed Low	5	249	S001013086	1.25		
	C96043F	1.00	Confirmed Low	0.8640	200	784	S001011080	135			Confirmed Low	5	310	S001013087	1.55		
	C96057F	1.00	Confirmed Low	0.8640	1000	276	S001013046	238			Confirmed Low	5	265	S001013088	1.33		
	C96058F	0.97	Confirmed Low	0.8640	200	1039	S001011082	185		25.3	Confirmed Low	5	230	S001013089	1.19		16.9
	C96059F	1.00	Confirmed Low	0.8640	1000	303	S001013048	262	200	50.5	Confirmed Low	5	194	S001013090	0.969	1.26	0.212

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL (ppb), PFOSA = 4.79 ppb, M556 = 19.2 ppb, M570 = 9.80 ppb

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
M556 = C8F17SO2N(H)CH2COOH
M570 = C8F17SO2N(CH3)CH2COOH

Date Entered/By: 10/18/00, 10/19/00, 10/26/00, 10/31/00, 11/07/00 LAC
Date Verified/By: 11/07/00 KJH
Purity Entered/Verified: 12/20/00 LAC, 12/27/00 mmh

FACT-TOX-028
Covance# 6329-225

Study: Covance 6329-225, FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats
Product Number(Test Substance): T-6314
Matrix: Rat Serum
Method/Revision: ETS-8-4.1 & ETS-8-5.1
Analytical Equipment System Number: Setup 020199, Davey 070799, Amelia 062498
Instrument Software/Version: Masslynx 3.4
Filenames: See Below
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments
Dates of Extraction/Analyst: 09/25/00 SAL/KJK/RWW
Dates of Analysis/Analyst: 10/09/00, 10/11/00, 10/13/00, 10/18/00, 10/27/00, 11/02/00 MAH/KJH
Date of Data Reduction/Analyst: 10/16/00, 10/18/00, 10/23/00, 10/30/00, 11/06/00 MAH/KJH

Sample Data
RAT SERUM WEEK 14

Group Dose	Sample #	Surrogate Verified	M556 Dilution Factor	M556 Conc. ng/mL	Filename (optional)	Concentration of M556 ng/mL or % Rec	Mean M556 ng/mL	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	M570 Dilution Factor	M570 Conc. ng/mL	Filename (optional)	Concentration of M570 ng/mL or % Rec	Mean M570 ng/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS09250-H2O Blk-1-1	NA	1	0.00	D001009005	<LOQ (24.9 ng/mL)			NA	1	0.00	D001009005	<LOQ (9.80 ng/mL)		NA
	RBS09250-H2O Blk-1-2	NA	1	4.48	D001009019	<LOQ (24.9 ng/mL)	<LOQ	NA	NA	1	0.00	D001009019	<LOQ (9.80 ng/mL)	<LOQ	NA
Matrix Blk	RBS09250-Sera Blk-1-1	NA	1	0.00	D001009006	<LOQ (24.9 ng/mL)			NA	1	0.00	D001009006	<LOQ (9.80 ng/mL)		NA
	RBS09250-Sera Blk-1-2	NA	1	0.00	D001009020	<LOQ (24.9 ng/mL)	<LOQ	NA	NA	1	0.00	D001009020	<LOQ (9.80 ng/mL)	<LOQ	NA
	RTS09250-Sera Blk-1-1	NA	1	0.00	D001009007	<LOQ (24.9 ng/mL)			NA	1	0.00	D001009007	<LOQ (9.80 ng/mL)		NA
	RTS09250-Sera Blk-1-2	NA	1	0.00	D001009021	<LOQ (24.9 ng/mL)	<LOQ	NA	NA	1	0.00	D001009021	<LOQ (9.80 ng/mL)	<LOQ	NA
QC	RTS09250-250 ppb-MS-1	NA	1	318	D001009022	128%			NA	1	253	D001009022	102%		
	RTS09250-250 ppb-MSD-1	NA	1	289	D001009023	116%	122%	9%	NA	1	260	D001009033	104%	103%	3%
Group 1 0 ppm	C959078M	NA	1	0.00	D001009026	<LOQ (24.9 ng/mL)			NA	1	12.0	D001018026	<LOQ (9.80 ng/mL)		
	C959084M	NA	1	0.00	D001009027	<LOQ (24.9 ng/mL)			NA	1	13.7	D001018027	<LOQ (9.80 ng/mL)		
	C959108M	NA	1	0.00	D001009028	<LOQ (24.9 ng/mL)			NA	1	17.8	D001018028	<LOQ (9.80 ng/mL)		
	C959168M	NA	1	0.00	D001009029	<LOQ (24.9 ng/mL)		NA	NA	1	10.9	D001018029	<LOQ (9.80 ng/mL)		NA
	C959178M	NA	1	0.00	D001009030	<LOQ (24.9 ng/mL)	<LOQ	NA	NA	1	12.6	D001018030	<LOQ (9.80 ng/mL)	<LOQ	NA
	C959081F	NA	1	0.00	D001009033	<LOQ (24.9 ng/mL)			NA	1	11.4	D001018033	<LOQ (9.80 ng/mL)		
	C959082F	NA	1	0.00	D001009034	<LOQ (24.9 ng/mL)			NA	1	13.1	D001018034	<LOQ (9.80 ng/mL)		
	C959089F	NA	1	0.00	D001009035	<LOQ (24.9 ng/mL)			NA	1	23.5	D001018035	<LOQ (9.80 ng/mL)		
	C959098F	NA	1	0.00	D001009036	<LOQ (24.9 ng/mL)		NA	NA	1	18.0	D001018036	<LOQ (9.80 ng/mL)		NA
	C959999F	2nd Analysis OK	1	0.00	D001018037	<LOQ (24.9 ng/mL)	<LOQ	NA	2nd Analysis OK	1	15.2	D001018037	<LOQ (9.80 ng/mL)	<LOQ	NA
Group 2 3 ppm	C959238M	2nd Analysis OK	10	107	A001027139	1.07			2nd Analysis OK	10	307	A001027139	3.07		
	C959198M	2nd Analysis OK	10	125	A001027138	1.25			2nd Analysis OK	10	299	A001027138	2.99		
	C959308M	NA	10	76.4	D001018042	0.764			NA	10	435	D001018042	4.35		
	C959388M	2nd Analysis OK	10	82.0	A001027136	0.820		20.2	2nd Analysis OK	10	354	A001027136	3.54		16.01
	C959408M	NA	10	96.0	D001018044	0.960	0.973	0.197	NA	10	390	D001018044	3.90	3.57	0.571
	C96004F	NA	10	70.9	D001018047	0.709			NA	10	375	D001018047	3.75		
	C96005F	NA	10	54.7	D001018048	0.547			NA	10	328	D001018048	3.28		
	C96009F	NA	10	63.7	D001018049	0.637			NA	10	305	D001018049	3.05		
	C96017F	NA	10	67.2	D001018050	0.672		24.3	NA	10	406	D001018050	4.06		11.1
	C96018F	NA	10	101	D001018051	1.01	0.714	0.174	NA	10	353	D001018051	3.53	3.53	0.393
Group 3 30 ppm	C959458M	Confirmed Low	20	450	S001013051	9.00			Confirmed Low	100	234	S001013017	23.4		
	C959468M	Confirmed Low	20	347	S001013052	6.94			Confirmed Low	100	201	S001013018	20.1		
	C959568M	Confirmed Low	20	411	S001013053	8.22			Confirmed Low	100	227	S001013019	22.7		
	C959578M	Confirmed Low	20	411	S001013054	8.23		11.9	Confirmed Low	100	205	S001013020	20.5		13.2
	C959598M	Confirmed Low	20	341	S001013055	6.82	7.84	0.936	Confirmed Low	100	164	S001013021	16.4	20.6	2.72
	C96028F	Confirmed Low	20	297	S001013058	5.95			Confirmed Low	100	241	S001013024	24.1		
	C96029F	Confirmed Low	20	354	S001013059	7.08			Confirmed Low	100	213	S001013025	21.3		
	C96036F	Confirmed Low	20	357	S001013060	7.14			Confirmed Low	100	214	S001013026	21.4		
	C96037F	Confirmed Low	20	365	S001013061	7.30		8.17	Confirmed Low	100	182	S001013027	18.2		9.83
	C96039F	Confirmed Low	20	364	S001013062	7.29	6.95	0.568	Confirmed Low	100	222	S001013028	22.2	21.4	2.11
Group 4 100 ppm	C959668M	Confirmed Low	200	134	S001013031	26.7			Confirmed Low	200	163	S001013031	32.6		
	C959708M	Confirmed Low	200	114	S001013032	22.7			Confirmed Low	200	83.5	S001013032	16.7		
	C959768M	Confirmed Low	200	134	S001013033	26.8			Confirmed Low	200	103	S001013033	20.6		
	C959788M	Confirmed Low	200	134	S001013034	26.9		9.00	Confirmed Low	200	111	S001013034	22.2		25.6
	C959798M	Confirmed Low	200	113	S001013035	22.6	25.2	2.26	Confirmed Low	200	131	S001013035	26.3	23.7	6.06
	C96041F	Confirmed Low	200	120	S001013038	23.9			Confirmed Low	200	125	S001013038	25.0		
	C96043F	Confirmed Low	200	123	S001013039	24.7			Confirmed Low	200	135	S001013039	27.0		
	C96057F	Confirmed Low	200	107	S001013040	21.3			Confirmed Low	200	116	S001013040	23.2		
	C96058F	Confirmed Low	200	143	S001013041	29.4		14.4	Confirmed Low	200	129	S001013041	26.7		10.3
	C96059F	Confirmed Low	200	150	S001013042	30.0	25.9	3.73	Confirmed Low	200	105	S001013042	21.0	24.6	2.51

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL (ppb), PFOSA = 4.79 ppb, M556 = 19.2 ppb, M570 = 9.80 ppb

Date Entered/By: 10/18/00, 10/19/00, 10/26/00, 10/31/00, 11/07/00 LAC
Date Verified/By: 11/07/00 KJH
Purity Entered/Verified: 12/20/00 LAC, 12/27/00 mmh

FACT-TOX-028
Covance# 6329-225

Study: Covance 6329-225, FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats
Product Number(Test Substance): T-6314
Matrix: Rat Serum
Method/Revision: ETS-8-4.1 & ETS-8-5.1
Analytical Equipment System Number: Soup 020199, Davey 070799, Amelia 062498
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: 09/25/00 SAL/KJK/RWW
Dates of Analysis/Analyst: 10/09/00, 10/11/00, 10/13/00, 10/18/00, 10/27/00, 11/02/00 MAH/KJH
Date of Data Reduction/Analyst: 10/16/00, 10/18/00, 10/23/00, 10/30/00, 11/06/00 MAH/KJH

Sample Data
RAT SERUM WEEK 14

Group Dose	Sample #	PFOS Conc. ng/mL	Concentration of PFOS ng/mL or % Rec	Mean PFOS ng/mL	RSD Std. Dev. MS/MSD RPD	PFOSA Conc. ng/mL	Concentration of PFOSA ng/mL or % Rec	Mean PFOSA ng/mL	RSD Std. Dev. MS/MSD RPD	M556 Conc. ng/mL	Concentration of M556 ng/mL or % Rec	Mean M556 ng/mL	RSD Std. Dev. MS/MSD RPD	M570 Conc. ng/mL	Concentration of M570 ng/mL or % Rec	Mean M570 ng/mL	RSD Std. Dev. MS/MSD RPD
Method Blk	RBS09250-1DO Blk-1-1	0.00	<LOQ (9.82 ng/mL)			1.25	<LOQ (4.95 ng/mL)			0.00	<LOQ (24.9 ng/mL)			0.00	<LOQ (9.80 ng/mL)		
	RBS09250-1DO Blk-1-2	0.00	<LOQ (9.82 ng/mL)	<LOQ	NA	0.00	<LOQ (4.95 ng/mL)	<LOQ	NA	4.48	<LOQ (24.9 ng/mL)	<LOQ	NA	0.00	<LOQ (9.80 ng/mL)	<LOQ	NA
Matrix Blk	RBS09250-Sera Blk-1-1	0.00	<LOQ (9.82 ng/mL)			0.00	<LOQ (4.95 ng/mL)			0.00	<LOQ (24.9 ng/mL)			0.00	<LOQ (9.80 ng/mL)		
	RTS09250-Sera Blk-1-2	0.00	<LOQ (9.82 ng/mL)	<LOQ	NA	0.00	<LOQ (4.95 ng/mL)	<LOQ	NA	0.00	<LOQ (24.9 ng/mL)	<LOQ	NA	0.00	<LOQ (9.80 ng/mL)	<LOQ	NA
	RTS09250-Sera Blk-1-1	0.00	<LOQ (9.82 ng/mL)			0.00	<LOQ (4.95 ng/mL)			0.00	<LOQ (24.9 ng/mL)			0.00	<LOQ (9.80 ng/mL)		
	RTS09250-Sera Blk-1-2	0.00	<LOQ (9.82 ng/mL)	<LOQ	NA	0.00	<LOQ (4.95 ng/mL)	<LOQ	NA	0.00	<LOQ (24.9 ng/mL)	<LOQ	NA	0.00	<LOQ (9.80 ng/mL)	<LOQ	NA
QC	RTS09250-250 ppb-MS-1	224	90%			237	95%			318	128%			253	102%		
	RTS09250-250 ppb-MSD-1	222	89%	90%	1%	211	85%	90%	12%	289	116%	122%	9%	260	104%	103%	3%
Group 1	C95907M	7.80	<LOQ (24.9 ng/mL)			0.7	<LOQ (9.80 ng/mL)			0.00	<LOQ (24.9 ng/mL)			12.0	<LOQ (9.80 ng/mL)		
0 ppm	C95908M	26.2	0.0226			0.9	<LOQ (9.80 ng/mL)			0.00	<LOQ (24.9 ng/mL)			13.7	<LOQ (9.80 ng/mL)		
	C95910M	7.68	<LOQ (24.9 ng/mL)			0.6	<LOQ (9.80 ng/mL)			0.00	<LOQ (24.9 ng/mL)			17.8	<LOQ (9.80 ng/mL)		
	C95916M	0.600	<LOQ (24.9 ng/mL)		NA	0.52	<LOQ (9.80 ng/mL)		NA	0.00	<LOQ (24.9 ng/mL)		NA	10.9	<LOQ (9.80 ng/mL)		NA
	C95917M	14.9	<LOQ (24.9 ng/mL)	<LOQ - 1 Anomaly	NA	0.2	<LOQ (9.80 ng/mL)	<LOQ	NA	0.00	<LOQ (24.9 ng/mL)	<LOQ	NA	12.6	<LOQ (9.80 ng/mL)	<LOQ	NA
	C95981F	42.9	0.0370			0.0300	<LOQ (9.80 ng/mL)			0.00	<LOQ (24.9 ng/mL)			11.4	<LOQ (9.80 ng/mL)		
	C95982F	105	0.0908			0.00	<LOQ (9.80 ng/mL)			0.00	<LOQ (24.9 ng/mL)			13.1	<LOQ (9.80 ng/mL)		
	C95989F	93.6	0.0809			0.00	<LOQ (9.80 ng/mL)			0.00	<LOQ (24.9 ng/mL)			23.5	<LOQ (9.80 ng/mL)		
	C95998F	81.1	0.0701		29.0	0.00	<LOQ (9.80 ng/mL)		NA	0.00	<LOQ (24.9 ng/mL)		NA	18.0	<LOQ (9.80 ng/mL)		NA
	C95999F	81.2	0.0701	0.0698	0.0202	0.0200	<LOQ (9.80 ng/mL)	<LOQ	NA	0.00	<LOQ (24.9 ng/mL)	<LOQ	NA	15.2	<LOQ (9.80 ng/mL)	<LOQ	NA
Group 2	C95923M	399	3.45			59.8	0.0598			107	1.07			307	3.07		
3 ppm	C95929M	507	4.38			34.8	0.0348			125	1.25			299	2.99		
	C95930M	477	4.12			36.7	0.0367			76.4	0.764			435	4.35		
	C95938M	475	4.10		9.58	30.8	0.0308		28.9	82.0	0.820		20.2	354	3.54		16.0
	C95940M	423	3.65	3.94	0.377	36.4	0.0364	0.0397	0.0115	96.0	0.960	0.973	0.197	390	3.90	3.57	0.571
	C96004F	399	6.89			41.5	0.0415			70.9	0.709			375	3.75		
	C96005F	545	9.41			61.8	0.0618			54.7	0.547			328	3.28		
	C96009F	589	10.2			68.8	0.0688			63.7	0.637			305	3.05		
	C96017F	496	8.57		18.0	52.3	0.0523		19.4	67.2	0.672		24.3	406	4.06		11.1
	C96018F	654	11.3	9.27	1.67	66.8	0.0668	0.0582	0.0113	101	1.01	0.714	0.174	353	3.53	3.53	0.393
Group 3	C95945M	518	44.7			31.2	0.663			450	9.00			334	33.4		
30 ppm	C95946M	450	38.9			24.9	0.497			347	6.94			201	20.1		
	C95956M	427	36.9			22.7	0.453			411	8.23			227	22.7		
	C95957M	391	33.8		13.4	24.3	0.486		15.7	411	8.23		11.9	205	20.5		13.2
	C95959M	539	46.6	40.2	5.38	28.6	0.571	0.534	0.0840	341	6.82	7.84	0.936	164	16.4	20.6	2.72
	C96028F	648	56.0			37.1	0.742			297	5.95			241	24.1		
	C96029F	924	79.9			39.0	0.780			354	7.08			213	21.3		
	C96036F	864	74.7			36.3	0.726			357	7.14			214	21.4		
	C96037F	760	65.6		13.3	32.5	0.649		19.5	365	7.30		8.17	182	18.2		9.83
	C96039F	773	66.8	68.6	9.14	52.6	1.053	0.790	0.154	364	7.29	6.95	0.568	222	22.2	21.4	2.11
Group 4	C95966M	599	104			253	1.27			134	26.7			163	32.6		
100 ppm	C95970M	775	134			157	0.784			114	22.7			84	16.7		
	C95976M	629	109			189	0.946			134	26.8			103	20.6		
	C95978M	634	110		11.7	178	0.890		19.0	134	26.9		9.00	111	22.2		25.6
	C95979M	587	101	111	13.0	181	0.905	0.958	0.182	113	22.6	25.3	2.26	131	26.3	23.7	6.06
	C96041F	1029	178			249	1.25			120	23.9			125	25.0		
	C96043F	784	135			310	1.55			123	24.7			135	27.0		
	C96057F	276	238			265	1.33			107	21.3			116	23.2		
	C96058F	1039	185		25.3	230	1.19		16.9	143	29.4		14.4	129	26.7		10.2
	C96059F	303	262	200	50.5	194	0.969	1.26	0.212	150	30.0	25.9	3.73	105	21.0	24.6	2.51

Limit of Quantitation (LOQ): PFOS = 5.55 ng/mL (ppb), PFOSA = 4.79 ppb, M556 = 19.2 ppb, M570 = 9.80 ppb

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
M556 = $C_8F_{17}SO_2N(H)CH_2COOH$
M570 = $C_8F_{17}SO_2N(CH_3)CH_2COOH$

Date Entered/By: 10/18/00, 10/19/00, 10/26/00, 10/31/00, 11/07/00 LAC
Date Verified/ By: 11/07/00 KJH
Purity Entered/Verified: 12/20/00 LAC, 12/27/00 mmh

FACT-TOX-028
Covance# 6329-225

Study:	Covance 6329-225, FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS Rat Rats
Product Number(Test Substance):	T-6314
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:	Aquila 062498 — Slope: See Attachments
Instrument Software/Version:	Mondyne 3.4 — Y-Intercept: See Attachments
Date of Extraction/Analyst:	10/10/00 SAL/KJK — Box #: 00-98
Date of Analysis/Analyst:	10/23/00, 10/24/00, 10/27/00, 10/30/00, 11/02/00, 12/01/00, 12/04/00 KJK/MMH/HOJ
Date of Data Reduction/Analyst:	10/24/00, 10/25/00, 10/30/00, 10/31/00, 11/06/00, 12/04/00, 12/05/00 MMH

Sample Data

RAT LIVER WEEK 5

Group / Dose	Sample #	Surrogate Verified	Initial Wt. g	Total Mass of Liver g	PFOS Purity Correction Factor	PFOS Conc. ug/g	PFOS Dilution Factor	PFOS Calc. Conc. ug/g	Filename (optional)	Concentration of PFOS ug/g or % Rec	Mean PFOS ug/g	RSD Std. Dev. MB/MSD RPD	Surrogate Verified	PFOSA Conc. ug/g	PFOSA Dilution Factor	PFOSA Calc. Conc. ug/g	Filename (optional)	Concentration of PFOSA ug/g or % Rec	Mean PFOSA ug/g	RSD Std. Dev. MB/MSD RPD
																		lot L-15709		
						lot 171														
Method Blk	10100-H2O BR-5	NA	1.0000	NA	0.8640	0.00	1	0.00	D001023006	<LOQ (0.00105 ug/g)			NA	0.00	1	0.00	D001023006	<LOQ (0.00611 ug/g)		
	10100-H2O-BR-6	NA	1.0000	NA	0.8640	0.00	1	0.00	A001023052	<LOQ (0.0264 ug/g)	<LOQ	NA	NA	3.63	1	3.63	A001024004	<LOQ (0.0306 ug/g)	<LOQ	NA
Matrix Blk	RBL10100-Liver BR-5	NA	1.0000	NA	0.8640	0.00	1	0.00	D001023007	<LOQ (0.00105 ug/g)			NA	0.210	1	0.210	D001023007	<LOQ (0.00611 ug/g)		
	RBL10100-Liver BR-6	NA	1.0000	NA	0.8640	0.00	1	0.00	A001023053	<LOQ (0.0264 ug/g)	<LOQ	NA	NA	0.00	1	0.00	A001024005	<LOQ (0.0306 ug/g)	<LOQ	NA
QC	C959911MGI/WLS-MS-250 ppb	NA	1.0051	NA	NA	306	1	348	A001201015	116%			NA	277	1	276	D001027034	92%		
	C959911MGI/WLS-MSD-250 ppb	NA	1.0051	NA	NA	430	1	272	A001201016	91%	104%	25%	NA	269	1	268	D001027035	89%	91%	3%
	C959991FGI/WLS-MS-250 ppb	NA	1.0592	NA	NA	311	1	225	A001201017	79%			NA	234	1	223	D001027036	79%		
	C959991FGI/WLS-MSD-250 ppb	Confirmed Low	1.0592	NA	NA	333	1	246	A001201018	86%	82%	9%	NA	176	1	165	D001027038	59% **	68%	30%
Group 1 0 ppm	C959111M	NA	1.0051	NA	0.8640	156	1	134	D001027020	0.134			NA	0.00	1	0.00	D001027020	<LOQ (0.0122 ug/g)		
	C959123M	NA	1.0107	NA	0.8640	156	1	134	D001027021	0.134			NA	0.00	1	0.00	D001027021	<LOQ (0.0122 ug/g)		
	C959133M	NA	1.0484	NA	0.8640	151	1	124	D001027022	0.124			NA	0.00	1	0.00	D001027022	<LOQ (0.0122 ug/g)		
	C959146M	NA	0.9967	NA	0.8640	120	1	104	D001027023	0.104		15.0	NA	0.00	1	0.00	D001027023	<LOQ (0.0122 ug/g)		NA
	C959158M	NA	1.0617	NA	0.8640	194	1	158	D001027024	0.158	0.131	0.0197	NA	0.00	1	0.00	D001027024	<LOQ (0.0122 ug/g)	<LOQ	NA
	C95991F	NA	1.0592	NA	0.8640	72.2	1	58.9	D001027027	0.0589			NA	1.88	1	1.77	D001027027	<LOQ (0.0122 ug/g)		
	C95992F	NA	1.0115	NA	0.8640	47.6	1	39.9	D001027028	0.0399			NA	0.00	1	0.00	D001027028	<LOQ (0.0122 ug/g)		
	C95993F	NA	1.0571	NA	0.8640	86.2	1	70.5	D001027029	0.0705			NA	0.00	1	0.00	D001027029	<LOQ (0.0122 ug/g)		
	C95994F	NA	0.9933	NA	0.8640	85.2	1	74.1	D001027030	0.0741		25.8	NA	0.00	1	0.00	D001027030	<LOQ (0.0122 ug/g)		NA
	C95995F	NA	1.0472	NA	0.8640	102	1	83.9	D001027031	0.0839	0.0655	0.0169	NA	3.24	1	3.09	D001027031	<LOQ (0.0122 ug/g)	<LOQ	NA
Group 2 3 ppm	C959311M	Confirmed High	1.0031	NA	0.8640	1040	20	17915	A001030030	17.9			NA	57.7	20	1149	D001027041	1.15		
	C959323M	Confirmed High	1.0105	NA	0.8640	1164	20	19097	A001030031	19.9			NA	37.0	20	733	D001027042	0.733		
	C959333M	Confirmed High	0.9932	NA	0.8640	1069	20	18594	A001030032	18.6			NA	52.1	20	1050	D001027043	1.05		
	C959341M	Confirmed High	1.0479	NA	0.8640	952	20	15700	A001030033	15.7		11.7	NA	60.2	20	1149	D001027044	1.15		27.5
	C959350M	Confirmed High	1.0518	NA	0.8640	914	20	15017	A001030034	15.0	17.4	2.03	NA	84.5	20	1607	D001027045	1.61	1.14	0.313
	C96011F	NA	0.9978	NA	0.8640	427	50	18475	D001023047	18.7			NA	104	20	2103	D001027048	2.10		
	C96012F	NA	1.0071	NA	0.8640	261	50	11210	D001023048	11.2			NA	44.2	20	877	D001027049	0.877		
	C96013F	NA	1.0027	NA	0.8640	197	50	8502	D001023049	8.50			NA	77.0	20	1536	D001027050	1.54		
	C96014F	NA	0.9898	NA	0.8640	194	50	8451	D001023050	8.45		41.1	NA	27.5	20	535	D001027051	0.535		54.4
	C96015F	NA	1.0302	NA	0.8640	190	50	7947	D001023051	7.95	11.0	4.50	NA	40.0	20	777	D001027052	0.777	1.17	0.637
Group 3 30 ppm	C959511M	NA	1.0164	NA	0.8640	193	500	82031	D001023019	82.0			NA	17.6	500	8673	D001023019	8.67		
	C959523M	NA	1.0057	NA	0.8640	230	500	98943	D001023020	98.9			NA	13.5	500	6702	D001023020	6.70		
	C959533M	NA	1.0195	NA	0.8640	170	500	72137	D001023021	72.1			NA	16.3	500	8014	D001023021	8.01		
	C959543M	NA	0.9829	NA	0.8640	201	500	88358	D001023022	88.6		11.5	NA	17.0	500	8663	D001023022	8.66		19.3
	C959553M	NA	1.0246	NA	0.8640	208	500	84140	D001023023	84.1	85.2	9.77	NA	23.1	500	11287	D001023023	11.3	8.67	1.67
	C96031F	NA	1.0256	NA	0.8640	167	500	70239	D001023026	70.3			NA	12.1	500	5918	D001023026	5.92		
	C96032F	NA	1.0443	NA	0.8640	353	500	146010	D001023027	146			NA	15.6	500	7469	D001023027	7.47		
	C96033F	NA	0.9863	NA	0.8640	177	500	77386	D001023028	77.4			NA	13.8	500	6970	D001023028	6.97		
	C96034F	NA	0.9871	NA	0.8640	184	500	80356	D001023029	80.4		32.3	NA	12.2	500	6159	D001023029	6.16		20.0
	C96035F	NA	1.0416	NA	0.8640	232	500	96030	D001023030	96.0	94.0	30.6	NA	8.85	500	4248	D001023030	4.25	6.15	1.23
Group 4 100 ppm	C959711M	NA	1.0108	NA	0.8640	525	500	224219	D001023033	224			NA	31.0	500	13310	D001023033	15.3		
	C959723M	NA	0.9764	NA	0.8640	319	500	141323	D001023034	141			NA	20.7	500	10616	D001023034	10.6		
	C959733M	NA	0.9850	NA	0.8640	362	500	158735	D001023035	159			NA	23.7	500	12015	D001023035	12.0		
	C959743M	NA	1.0120	NA	0.8640	519	500	221345	D001023036	221		30.4	NA	31.3	500	15445	D001023036	15.4		15.7
	C959753M	NA	1.0592	NA	0.8640	530	500	216024	D001023037	216	192	39.2	NA	28.2	500	13317	D001023037	13.3	13.3	2.09
	C96051F	NA	1.0305	NA	0.8640	480	500	197433	D001023040	197			NA	25.8	500	12270	D001023040	12.3		
	C96052F	NA	1.0497	NA	0.8640	529	500	217597	D001023041	218			NA	29.8	500	14180	D001023041	14.2		
	C96053F	NA	0.9833	NA	0.8640	627	500	275376	D001023042	275			NA	28.3	500	14365	D001023042	14.4		
	C96054F	NA	1.0139	NA	0.8640	488	500	207802	D001023043	208		13.6	NA	31.2	500	15376	D001023043	15.4		8.05
	C96055F	NA	0.9883	NA	0.8640	498	500	217713	D001023044	218	223	30.4	NA	28.6	500	14454	D001023044	14.5	14.1	1.14

Limit of Quantitation (LOQ) updated for purity on 01/31/01 PFOS = 12.2 ug/g, 30.6 ug/g. LAC 01/31/01

** Low recovery confirmed with 10/27/00 and 11/02/00 analyses. LAC 11/07/00

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
M556 = $C_8F_{17}SO_2N(H)CH_2COOH$
M570 = $C_8F_{17}SO_2N(CH_3)CH_2COOH$

Data Entered/Analyst: 10/26/00, 10/31/00, 11/07/00, 12/08/00 LAC/KJH
Data Verified/Analyst: 11/08/00, 11/09/00, 12/11/00 KJH/LAC
Purity Entered/Verified: 12/20/00 LAC, 12/27/00 mmh

FACT-TOX-028
Covance# 6329-225

Study: Covance 6329-225, FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats
Product Number(Test Substance): T-6314
Matrix: Rat Liver
Method/Revision: ETS-8-6.0 & ETS-8-7.0
Analytical Equipment System Number: Aquila 062498
Instrument Software/Version: Masslynx 3.4
Date of Extraction/Analyst: 10/10/00 SAL/KJK
Date of Analysis/Analyst: 10/23/00, 10/24/00, 10/27/00, 10/30/00, 11/02/00, 12/01/00, 12/04/00 KJK/MMH/HOJ
Date of Data Reduction/Analyst: 10/24/00, 10/25/00, 10/30/00, 10/31/00, 11/06/00, 12/04/00, 12/05/00 MMH

Filename: See Below
R-Squared Value: See Attachments
Slope: See Attachments
Y-Intercept: See Attachments

Sample Data

RAT LIVER WEEK 5

Group Dose	Sample #	Surrogate Verified	lot NB113047-80 M556 Conc. ng/g	M556 Dilution Factor	M556 Calc. Conc. ng/g	Filename (optional)	Concentration of M556 ng/g or % Rec	Mean M556 ng/g	RSD Std. Dev. MS/MSD R/PD	Surrogate Verified	lot TC000017-56 M570 Conc. ng/g	M570 Dilution Factor	M570 Calc. Conc. ng/g	Filename (optional)	Concentration of M570 ng/g or % Rec	Mean M570 ng/g	RSD Std. Dev. MS/MSD R/PD
Method Blk	10100-H2O BR-5	NA	0.00	1	0.00	D001023006	<LOQ (0.0122 ng/g)			NA	0.00	1	0.00	D001023006	<LOQ (0.0306 ng/g)		
	10100-H2O-BR-6	NA	0.00	1	0.00	A001023052	<LOQ (0.0306 ng/g)	<LOQ	NA	NA	0.00	1	0.00	A001023052	<LOQ (0.0122 ng/g)	<LOQ	NA
Matrix Blk	RBL10100-Liver Blk-5	NA	4.16	1	4.16	D001023007	<LOQ (0.0122 ng/g)			NA	0.00	1	0.00	D001023007	<LOQ (0.0306 ng/g)		
	RBL10100-Liver Blk-6	NA	0.00	1	0.00	A001023053	<LOQ (0.0306 ng/g)	<LOQ	NA	NA	0.00	1	0.00	A001023052	<LOQ (0.0122 ng/g)	<LOQ	NA
QC	C95911I6/G1/WL5-MS-250 ppb	NA	248	1	237	D001027034	79%			NA	325	1	292	A001102076	98%		
	C95911M/G1/WL5-MSD-250 ppb	NA	261	1	250	D001027035	84%	81%	5%	NA	331	1	297	A001102077	99%	98%	2%
	C95991FG1/WL5-MS-250 ppb	NA	226	1	210	A001102078	74%			NA	327	1	280	A001102078	101%		
	C95991FG1/WL5-MSD-250 ppb	NA	131	1	120	D001027038	42% **	58%	55%	NA	263	1	228.4	A001102079	80%	90%	23%
Group 1 0 ppm	C95911M	NA	9.94	1	9.88	D001027028	0.00988			NA	32.8	1	31.8	D001027028	0.0318		
	C95912M	NA	6.90	1	6.83	D001027021	0.00683			NA	21.6	1	21.3	D001027021	0.0213		
	C95913M	NA	6.86	1	6.54	D001027022	0.00654			NA	34.0	1	32.4	D001027022	0.0324		
	C95914M	NA	3.69	1	3.69	D001027023	<LOQ (0.00611 ng/g)		22.4	NA	8.88	1	8.88	D001027023	<LOQ (0.0122 ng/g)		40.2
	C95915M	NA	1.55	1	1.46	D001027024	<LOQ (0.00611 ng/g)	0.00710	0.00159	NA	16.9	1	15.9	D001027024	0.0159	0.0227	0.00915
	C95991F	NA	3.30	1	3.12	D001027027	<LOQ (0.00611 ng/g)			NA	21.0	1	19.8	D001027027	0.0198		
	C95992F	NA	1.39	1	1.35	D001027028	<LOQ (0.00611 ng/g)			NA	13.3	1	12.9	D001027028	0.0129		
	C95993F	NA	0.00	1	0.00	D001027029	<LOQ (0.00611 ng/g)			NA	16.6	1	15.7	D001027029	0.0157		
	C95994F	NA	3.87	1	3.98	D001027030	<LOQ (0.00611 ng/g)		26.6	NA	23.7	1	23.8	D001027030	0.0238		53.3
	C95995F	NA	10.7	1	10.2	D001027031	0.0102	0.00693	0.00184	NA	46.3	1	44.2	D001027031	0.0442	0.0233	0.0124
Group 2 3 ppm	C95931M	NA	100	20	1999	D001027041	2.00			NA	345	20	6882	D001027041	6.88		
	C95932M	NA	92.3	20	1826	D001027042	1.83			NA	352	20	6967	D001027042	6.97		
	C95933M	NA	120	20	2412	D001027043	2.41			NA	417	20	8390	D001027043	8.39		
	C95934M	NA	102	20	1945	D001027044	1.94		11.7	NA	361	20	6885	D001027044	6.89		9.22
	C95935M	NA	98.1	20	1865	D001027045	1.86	2.01	0.235	NA	363	20	6896	D001027045	6.90	7.20	0.664
	C96011F	NA	39.3	50	1991	D001023047	1.99			NA	118	50	5975	D001023047	5.97		
	C96012F	NA	20.8	50	1032	D001023048	1.03			NA	62.3	50	3094	D001023048	3.09		
	C96013F	NA	18.4	50	917	D001023049	0.917			NA	87.3	50	4354	D001023049	4.35		
	C96014F	NA	16.7	50	846	D001023050	0.846		47.8	NA	43.9	50	2219	D001023050	2.22		39.6
	C96015F	NA	13.7	50	667	D001023051	0.667	1.09	0.521	NA	61.3	50	2976	D001023051	2.98	3.72	1.47
Group 3 30 ppm	C95951M	2nd Analysis OK	476	50	23410	D001027055	23.4			NA	174	500	85537	D001023019	85.5		
	C95952M	2nd Analysis OK	425	50	21120	D001027056	21.1			NA	158	500	78405	D001023020	78.4		
	C95953M	2nd Analysis OK	446	50	21892	D001027057	21.9			NA	148	500	72359	D001023021	72.4		
	C95954M	2nd Analysis OK	428	50	21747	D001027058	21.7		4.00	NA	196	500	99817	D001023022	99.8		12.3
	C95955M	2nd Analysis OK	440	50	21483	D001027059	21.5	21.9	0.878	NA	181	500	88239	D001023023	88.2	84.9	10.4
	C96031F	2nd Analysis OK	221	50	10735	D001027062	10.8			NA	63.3	500	30382	D001023026	30.4		
	C96032F	2nd Analysis OK	368	50	17619	D001027063	17.6			NA	99.9	500	47822	D001023027	47.8		
	C96033F	2nd Analysis OK	227	50	11486	D001027064	11.5			NA	53.8	500	27294	D001023028	27.3		
	C96034F	2nd Analysis OK	314	50	15921	D001027065	15.9		22.8	NA	84.1	500	42600	D001023029	42.6		23.8
	C96035F	2nd Analysis OK	243	50	11642	D001027066	11.6	13.5	3.08	NA	69.4	500	33314	D001023030	33.3	36.3	8.62
Group 4 100 ppm	C95971M	NA	110	500	54363	D001023033	54.4			NA	330	500	163074	D001023033	163		
	C95972M	NA	89.3	500	45719	D001023034	45.7			NA	269	500	137823	D001023034	138		
	C95973M	NA	100	500	50777	D001023035	50.8			NA	259	500	131558	D001023035	132		
	C95974M	NA	127	500	62727	D001023036	62.7		11.7	NA	356	500	175791	D001023036	176		11.9
	C95975M	NA	118	500	55802	D001023037	55.8	53.9	6.29	NA	332	500	156689	D001023037	157	153	18.2
	C96031F	NA	73.0	500	34722	D001023040	34.7			NA	240	500	114108	D001023040	114		
	C96032F	NA	102	500	48504	D001023041	48.5			NA	297	500	141493	D001023041	141		
	C96033F	NA	90.4	500	45983	D001023042	46.0			NA	243	500	123614	D001023042	124		
	C96034F	NA	93.4	500	46060	D001023043	46.1		20.3	NA	281	500	138480	D001023043	138		9.02
	C96035F	NA	58.1	500	29374	D001023044	29.4	40.9	8.30	NA	242	500	122574	D001023044	123	128	11.5

Limit of Quantitation (LOQ) updated for purity on 01/31/01 PFOS = 12.2 ng/g, 30.6 ng/g LAC 01/31/01
** Low recovery confirmed with 10/27/00 and 11/02/00 analyses. LAC 11/07/00

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
M556 = $C_8F_{17}SO_2N(H)CH_2COOH$
M570 = $C_8F_{17}SO_2N(CH_3)CH_2COOH$

Data Entered/Analyst: 10/26/00, 10/31/00, 11/07/00, 12/06/00 LAC/KJK
Data Verified/Analyst: 11/08/00, 11/09/00, 12/11/00 KJK/LAC
Purity Entered/Verified: 12/20/00 LAC, 12/27/00 mmh

FACT-TOX-028
Covance# 6329-225

Study: Covance 6329-225, FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of CrlCD® (SD) IGS BR Rats
Product Number(Test Substance): T-6314
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: Aquila 062498 — Slope: See Attachments
Instrument Software/Version: Masslynx 3.4 — Y-Intercept: See Attachments
Date of Extraction/Analyst: 10/10/00 KAL/KJK
Date of Analysis/Analyst: 10/23/00, 10/24/00, 10/27/00, 10/30/00, 11/02/00, 12/01/00, 12/04/00 KJH/MMH/HOJ
Date of Data Reduction/Analyst: 10/24/00, 10/25/00, 10/30/00, 10/31/00, 11/06/00, 12/04/00, 12/05/00 MMH

Sample Data

RAT LIVER WEEK 5

Group Dose	Sample #	PFOS Calc. Conc. ng/g	Concentration of PFOS ng/g or % Rec	Mean PFOS ng/g	RSD Std. Dev. MS/MSD RPD	PFOSA Calc. Conc. ng/g	Concentration of PFOSA ng/g or % Rec	Mean PFOSA ng/g	RSD Std. Dev. MS/MSD RPD	M556 Calc. Conc. ng/g	Concentration of M556 ng/g or % Rec	Mean M556 ng/g	RSD Std. Dev. MS/MSD RPD	M570 Calc. Conc. ng/g	Concentration of M570 ng/g or % Rec	Mean M570 ng/g	RSD Std. Dev. MS/MSD RPD
Method Blk	10100-H2O BR-5	0.00	<LOQ (0.00105 ng/g)			0.00	<LOQ (0.00611 ng/g)			0.00	<LOQ (0.0122 ng/g)			0.00	<LOQ (0.0306 ng/g)		
	10100-H2O-BR-6	0.00	<LOQ (0.0264 ng/g)	<LOQ	NA	3.63	<LOQ (0.0306 ng/g)	<LOQ	NA	0.00	<LOQ (0.0306 ng/g)	<LOQ	NA	0.00	<LOQ (0.0122 ng/g)	<LOQ	NA
Matrix Blk	RBL10100-Liver Blk-5	0.00	<LOQ (0.00105 ng/g)			0.210	<LOQ (0.00611 ng/g)			4.16	<LOQ (0.0122 ng/g)			0.00	<LOQ (0.0306 ng/g)		
	RBL10100-Liver Blk-6	0.00	<LOQ (0.0264 ng/g)	<LOQ	NA	0.00	<LOQ (0.0306 ng/g)	<LOQ	NA	0.00	<LOQ (0.0306 ng/g)	<LOQ	NA	0.00	<LOQ (0.0122 ng/g)	<LOQ	NA
QC	C95911M/GI/WL5-MS-250 ppb	348	116%			276	92%			237	79%			292	98%		
	C95911M/GI/WL5-MSD-250 ppb	272	91%	104%	25%	268	89%	91%	3%	250	84%	81%	5%	297	99%	98%	2%
	C95991FGI/WL5-MS-250 ppb	225	79%			223	78%			210	74%			289	101%		
	C95991FGI/WL5-MSD-250 ppb	246	86%	82%	9%	165	58% **	68%	30%	120	42% **	58%	55%	228	80%	90%	23%
Group 1 0 ppm	C95911M	134	0.134			0.00	<LOQ (0.0122 ng/g)			9.89	0.00989			31.8	0.0318		
	C95912M	134	0.134			0.00	<LOQ (0.0122 ng/g)			6.83	0.00683			21.3	0.0213		
	C95913M	124	0.134			0.00	<LOQ (0.0122 ng/g)			6.54	0.00654			32.4	0.0324		
	C95914M	104	0.104		15.0	0.00	<LOQ (0.0122 ng/g)		NA	3.69	<LOQ (0.00611 ng/g)		22.4	8.89	<LOQ (0.0122 ng/g)		40.2
	C95915M	158	0.158	0.131	0.0197	0.00	<LOQ (0.0122 ng/g)	<LOQ	NA	1.46	<LOQ (0.00611 ng/g)	0.00710	0.00159	15.9	0.0159	0.0227	0.00915
	C95991F	58.9	0.0589			1.77	<LOQ (0.0122 ng/g)			3.12	<LOQ (0.00611 ng/g)			19.8	0.0198		
	C95992F	39.9	0.0399			0.00	<LOQ (0.0122 ng/g)			1.35	<LOQ (0.00611 ng/g)			12.9	0.0129		
	C95993F	70.5	0.0705			0.00	<LOQ (0.0122 ng/g)			0.00	<LOQ (0.00611 ng/g)			15.7	0.0157		
	C95994F	74.1	0.0741		25.8	0.00	<LOQ (0.0122 ng/g)		NA	3.90	<LOQ (0.00611 ng/g)		26.6	23.8	0.0238		53.3
	C95995F	83.9	0.0839	0.0655	0.0169	3.09	<LOQ (0.0122 ng/g)	<LOQ	NA	10.2	0.01023	0.00693	0.00184	44.2	0.0442	0.0233	0.0124
Group 2 3 ppm	C95931M	17915	17.9			1149	1.15			1999	2.00			6682	6.68		
	C95932M	19097	19.9			733	0.733			1826	1.83			6967	6.97		
	C95933M	18594	18.6			1050	1.05			2412	2.41			8390	8.39		
	C95934M	15700	15.7		11.7	1149	1.15		27.5	1945	1.94		11.7	6885	6.89		9.22
	C95935M	15017	15.0	17.4	2.81	1607	1.61	1.14	0.313	1865	1.86	2.01	0.235	6896	6.90	7.20	0.664
	C96011F	18675	18.7			2103	2.10			1991	1.99			5975	5.97		
	C96012F	11210	11.2			877	0.877			1032	1.03			3094	3.09		
	C96013F	8502	8.50			1536	1.54			917	0.917			4354	4.35		
	C96014F	8451	8.45		41.1	555	0.555		54.4	846	0.846		47.8	2219	2.22		39.6
	C96015F	7947	7.95	11.0	4.50	777	0.777	1.17	0.637	667	0.667	1.09	0.521	2976	2.98	3.72	1.47
Group 3 30 ppm	C95951M	82031	82.0			8673	8.67			23410	23.4			85537	85.5		
	C95952M	98943	98.9			6702	6.70			21120	21.1			78403	78.4		
	C95953M	72137	72.1			8014	8.01			21892	21.9			72359	72.4		
	C95954M	88558	88.6		11.5	8663	8.66		19.3	21747	21.7		4.00	99817	99.8		12.3
	C95955M	84140	84.1	85.2	8.77	11287	11.3	9.67	1.67	21483	21.5	21.9	0.878	88239	88.2	84.9	10.4
	C96031F	70259	70.3			5918	5.92			10755	10.8			30382	30.4		
	C96032F	146010	146			7468	7.47			17619	17.6			47822	47.8		
	C96033F	77386	77.4			6970	6.97			11486	11.5			27294	27.3		
	C96034F	80356	80.4		32.5	6159	6.16		20.0	15921	15.9		22.8	42600	42.6		23.8
	C96035F	96030	96.0	94.0	30.6	4248	4.25	6.15	1.23	11642	11.6	13.5	3.08	33314	33.3	36.3	8.62
Group 4 100 ppm	C95971M	224219	224			15310	15.3			54363	54.4			163074	163		
	C95972M	141325	141			10616	10.6			45719	45.7			137823	138		
	C95973M	158735	159			12015	12.0			50777	50.8			131558	132		
	C95974M	221345	221		30.4	15445	15.4		15.7	62727	62.7		11.7	175791	176		11.9
	C95975M	216024	216	192	39.2	13317	13.3	13.3	2.09	55802	55.8	53.9	6.29	156689	157	153	16.2
	C96051F	197433	197			12270	12.3			34722	34.7			114108	114		
	C96052F	217597	218			14180	14.2			48304	48.5			141493	141		
	C96053F	275376	275			14365	14.4			45983	46.0			123614	124		
	C96054F	207502	208		12.6	15376	15.4		0.93	46060	46.1		20.5	138480	138		9.02
	C96055F	217713	218	223	30.4	14454	14.5	14.1	1.14	29374	29.4	40.9	8.39	122574	123	128	11.5

Limit of Quantitation (LOQ) updated for purity on 01/31/01 PFOS = 12.2 ng/g, 30.6 ng/g. LAC 01/31/01
** Low recovery confirmed with 10/27/00 and 11/02/00 analyses. LAC 11/07/00

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
M556 = $C_8F_{17}SO_2N(H)CH_2COOH$
M570 = $C_8F_{17}SO_2N(CH_3)CH_2COOH$

Data Entered/Analyst: 10/26/00, 10/31/00, 11/07/00, 12/08/00 LAC/KJH
Data Verified/Analyst: 11/08/00, 11/09/00, 12/11/00 KJH/LAC
Purity Entered/Verified: 12/20/00 LAC, 12/27/00 mmh

FACT-TOX-028
Covance# 6329-225

Study: Covance 6329-225, FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats
Product Number(Test Substance): T-6314
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: Aaalin 062498 — Slope: See Attachments
Instrument Software/Version: Masslynx 3.4 — Y-intercept: See Attachments
Date of Extraction/Analyst: 10/10/00 SAL/KJK — Box #: 00-98
Date of Analysis/Analyst: 10/23/00, 10/24/00, 10/27/00, 10/30/00, 10/31/00, 11/02/00, 12/01/00, 12/04/00 KJH/MMH/HOJ
Date of Data Reduction/Analyst: 10/24/00, 10/25/00, 10/30/00, 10/31/00, 11/01/00, 11/06/00, 12/04/00, 12/05/00 MMH

Sample Data

RAT LIVER WEEK 14

Group Dose	Sample #	Surrogate Verified	Initial Wt. g	Total Mass of Liver g	PFOS Purity Correction Factor	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Filename (optional)	Concentration of PFOS ng/g or % Rec	Mean PFOS ng/g	RSD Std. Dev. M/MSD RPD	Surrogate Verified	PFOSA Conc. ng/g	PFOSA Dilution Factor	PFOSA Calc. Conc. ng/g	Filename (optional)	Concentration of PFOSA ng/g or % Rec	Mean PFOSA ng/g
										lot 171								lot L-15709	
Method Blk	10100-H2O Blk-5	NA	1.0000	NA	0.8640	0.00	1	0.00	D001023006	<LOQ (0.00105 ug/g)			NA	0.00	1	0.00	D001023006	<LOQ (0.00611 ug/g)	
	10100-H2O-Blk-6	NA	1.0000	NA	0.8640	0.00	1	0.00	A001023052	<LOQ (0.0264 ug/g)	<LOQ	NA	NA	3.63	1	3.63	A001024004	<LOQ (0.0306 ug/g)	<LOQ
Matrix Blk	RBL10100-Liver Blk-5	NA	1.0000	NA	0.8640	0.00	1	0.00	D001023007	<LOQ (0.00105 ug/g)			NA	0.210	1	0.210	D001023007	<LOQ (0.00611 ug/g)	
	RBL10100-Liver Blk-6	NA	1.0000	NA	0.8640	0.00	1	0.00	A001023053	<LOQ (0.0264 ug/g)	<LOQ	NA	NA	0.00	1	0.00	A001024005	<LOQ (0.0306 ug/g)	<LOQ
QC	C95911M/G1/Wk5-MS-250 ppb	NA	1.0051	NA	NA	506	1	348	A001201015	116%			NA	277	1	276	D001027034	92%	
	C95911M/G1/Wk5-MSD-250 ppb	NA	1.0051	NA	NA	430	1	272	A001201016	91%	104%	25%	NA	269	1	268	D001027035	89%	91%
	C95991F/G1/Wk5-MS-250 ppb	NA	1.0592	NA	NA	311	1	225	A001201017	79%			NA	238	1	223	D001027036	78%	
	C95991F/G1/Wk5-MSD-250 ppb	Confirmed Low	1.0592	NA	NA	333	1	246	A001201018	86%	82%	9%	NA	176	1	165	D001027038	58% **	68%
Group 1 0 ppm	C95907M	NA	1.0158	NA	0.8640	254	1	216	A001102048	0.216			NA	12.0	1	11.8	A001102048	<LOQ (0.0122 ug/g)	
	C95908M	NA	0.9814	NA	0.8640	283	1	249	A001102049	0.249			NA	7.97	1	8.12	A001102049	<LOQ (0.0122 ug/g)	
	C95910M	NA	0.9960	NA	0.8640	369	1	320	A001102050	0.320			NA	10.8	1	10.8	A001102050	<LOQ (0.0122 ug/g)	
	C95916M	Confirmed Low	1.0037	NA	0.8640	124	1	107	A001102051	0.107		48.6	Confirmed Low	12.5	1	12.5	A001102051	0.0125	
	C95917M	NA	1.0530	NA	0.8640	115	1	94.5	A001102052	0.0945	0.197	0.096	NA	5.52	1	5.24	A001102052	<LOQ (0.0122 ug/g)	0.0123
	C95981F	NA	1.0133	NA	0.8640	65.1	1	55.5	A001102055	0.0555			NA	12.4	1	12.2	A001102055	0.0122	
	C95982F	NA	1.0103	NA	0.8640	188	1	160	A001102056	0.160			NA	16.6	1	16.4	A001102056	0.0164	
	C95989F	NA	1.0186	NA	0.8640	188	1	159	A001102057	0.159			NA	15.6	1	15.3	A001102057	0.0153	
	C95998F	NA	0.9845	NA	0.8640	167	1	146	A001102058	0.146		41.6	NA	23.3	1	23.7	A001102058	0.0237	
	C95999F	NA	1.0350	NA	0.8640	92.0	1	76.8	A001102059	0.0768	0.120	0.0498	NA	5.95	1	5.75	A001102059	<LOQ (0.0122 ug/g)	0.0160
Group 2 3 ppm	C95923M	2nd Analysis OK	0.9879	NA	0.8640	544	50	23770	A001024031	23.8			NA	89.3	20	1807	A01024045	1.81	
	C95929M	Confirmed Low	1.0090	NA	0.8640	600	50	25672	A001023094	25.7			NA	545	20	10807	A01024046	10.8	
	C95930M	2nd Analysis OK	1.0401	NA	0.8640	537	50	22319	A001024033	22.3			NA	306	20	5878	A01024047	5.88	
	C95938M	Confirmed Low	1.0538	NA	0.8640	719	50	29470	A001023096	29.5		12.4	NA	264	20	5014	A01024048	5.01	
	C95940M	Confirmed Low	1.0654	NA	0.8640	724	50	29337	A001023097	29.3	26.1	3.23	NA	420	20	7877	A01024049	7.88	6.28
	C96004F	2nd Analysis OK	1.0512	NA	0.8640	432	50	17763	A001024038	17.8			NA	77.3	20	1470	A01024053	1.47	
	C96005F	Confirmed Low	0.9951	NA	0.8640	984	50	42727	A001023101	42.7			NA	133	20	2672	A01024054	2.67	
	C96009F	NA	1.0224	NA	0.8640	1078	50	45557	A001024040	45.6			NA	115	20	2246	A01024055	2.25	
	C96017F	Confirmed Low	1.0187	NA	0.8640	748	50	31734	A001023103	31.7		33.2	NA	138	20	2702	A01024056	2.70	
	C96018F	Confirmed Low	1.0024	NA	0.8640	1090	50	46955	A001024042	47.0	36.9	12.3	NA	104	20	2073	A01024057	2.07	2.23
Group 3 30 ppm	C95945M	NA	0.9943	NA	0.8640	536	500	232806	A001023065	233			2nd Analysis OK	549	50	27604	S001031035	27.6 +	
	C95946M	NA	1.0351	NA	0.8640	561	500	233979	A001023066	234			NA	391	50	18888	S001031036	18.9 +	
	C95956M	NA	1.0049	NA	0.8640	496	500	213047	A001023067	213			Confirmed Low	418	50	20801	S001031037	20.8 +	
	C95957M	NA	1.0200	NA	0.8640	461	500	195332	A001023068	195		18.6	Confirmed Low	341	50	16702	S001031038	16.7 +	
	C95958M	NA	1.0196	NA	0.8640	733	500	310671	A001023069	311	237	44.1	Confirmed Low	493	50	24184	S001031039	24.2 +	21.6
	C96028F	NA	1.0386	NA	0.8640	489	500	203231	A001023072	203			NA	295	50	14178	S001031044	14.2 +	
	C96029F	NA	1.0118	NA	0.8640	944	500	402843	A001023073	403			Confirmed Low	271	50	13412	A001024062	13.4	
	C96036F	NA	1.0327	NA	0.8640	803	500	335782	A001023074	336			Confirmed Low	242	50	11709	A001024063	11.7	
	C96037F	Confirmed Low	1.0304	NA	0.8640	736	500	308777	A001023075	309		28.7	Confirmed Low	55.3	500	26810	A001024065	26.8	
	C96039F	NA	1.0255	NA	0.8640	511	500	215288	A001023076	215	293	84.0	Confirmed Low	302	50	14723	S001031048	14.7 +	16.2
Group 4 100 ppm	C95966M	Confirmed High	1.0019	NA	0.8640	754	1000	650557	A001030016	651			2nd Analysis OK	609	50	30402	S001031017	30.4 +	
	C95970M	Confirmed High	1.0409	NA	0.8640	991	1000	822348	A001030017	822			2nd Analysis OK	538	50	25836	S001031018	25.8 +	
	C95976M	Confirmed High	1.0242	NA	0.8640	864	1000	728917	A001030018	729			2nd Analysis OK	662	50	32341	S001031019	32.3 +	
	C95978M	Confirmed High	1.0319	NA	0.8640	795	1000	665261	A001030019	665		10.1	2nd Analysis OK	612	50	29630	S001031020	29.6 +	
	C95979M	NA	0.9962	NA	0.8640	963	1000	783402	A001030020	783	730	73.9	2nd Analysis OK	613	50	30782	S001031021	30.8 +	29.8
	C96041F	Confirmed High	0.9998	NA	0.8640	830	1000	717359	A001030023	717			2nd Analysis OK	520	50	25998	S001031026	26.0 +	
	C96043F	NA	1.0059	NA	0.8640	710	1000	609808	A001030024	610			Confirmed Low	497	50	24727	S001031027	24.7 +	
	C96057F	NA	1.0345	NA	0.8640	982	1000	820119	A001030025	820			NA	559	50	27040	S001031028	27.0 +	
	C96058F	NA	1.0466	NA	0.8640	919	1000	758530	A001030026	759		11.2	Confirmed Low	514	50	24571	S001031029	24.6 +	
	C96059F	NA	1.0231	NA	0.8640	947	1000	799658	A001030027	800	741	83.3	NA	490	50	23949	S001031030	23.9 +	25.3

Limit of Quantitation (LOQ) updated for purity on 01/31/01 PFOS = 12.2 ng/g, 30.6 ng/g. LAC 01/31/01
** Low recovery confirmed with 10/27/00 and 11/02/00 analysis. LAC 11/07/00
+ CCVs bracketing these data weren't within +/- 30%. Curve stds analyzed every 5 samples were within criteria. LAC 11/08/00

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
M556 = C8F17SO2N(H)CH2COOH
M570 = C8F17SO2N(CH3)CH2COOH

Date Entered/Analyst: 10/27/00, 11/03/00, 11/07/00, 12/08/00 LAC/KJH
Date Verified/Analyst: 11/08/00, 11/09/00, 12/11/00 KJH/LAC
Purity Entered/Verified: 12/28/00 LAC, 12/27/00 mmh

FACT-TOX-028
Covance# 6329-225

Study:	Covance 6329-225, FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats
Product Number(Test Substance):	T-6314
Matrix:	Rat Liver
Method/Revision:	ETS-8-6.0 & ETS-8-7.0
Analytical Equipment System Number:	Aurelia 06249E
Instrument Software/Version:	Masslynx 3.4
Date of Extraction/Analyst:	10/10/00 SAL/KJK
Date of Analysis/Analyst:	10/23/00, 10/24/00, 10/27/00, 10/30/00, 10/31/00, 11/02/00, 12/01/00, 12/04/00 KJH/MMH/HOJ
Date of Data Reduction/Analyst:	10/24/00, 10/25/00, 10/30/00, 10/31/00, 11/01/00, 11/06/00, 12/04/00, 12/05/00 MMH
Filename:	See Below
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments

Sample Data

RAT LIVER WEEK 14

lot NB113047-80 | lot TCR00017-56

RSD Std. Dev. MS/MSD RPD	Group Dose	Sample #	Surrogate Verified	M556 Conc. ug/g	M556 Dilution Factor	M556 Calc. Conc. ug/g	Filename (optional)	Concentration of M556 ug/g or % Rec	Mean M556 ug/g	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	M570 Conc. ug/g	M570 Dilution Factor	M570 Calc. Conc. ug/g	Filename (optional)	Concentration of M570 ug/g or % Rec	Mean M570 ug/g	RSD Std. Dev. MS/MSD RPD
NA	Method Blk	10100-H2O Blk-5	NA	0.00	1	0.00	D001023006	<LOQ (0.0122 ug/g)			NA	0.00	1	0.00	D001023006	<LOQ (0.0306 ug/g)		
		10100-H2O-Blk-6	NA	0.00	1	0.00	A001023052	<LOQ (0.0306 ug/g)	<LOQ	NA	NA	0.00	1	0.00	A001023052	<LOQ (0.0122 ug/g)	<LOQ	NA
NA	Matrix Blk	RBL10100-Liver Blk-5	NA	4.16	1	4.16	D001023007	<LOQ (0.0122 ug/g)			NA	0.00	1	0.00	D001023007	<LOQ (0.0306 ug/g)		
		RBL10100-Liver Blk-6	NA	0.00	1	0.00	A001023053	<LOQ (0.0306 ug/g)	<LOQ	NA	NA	0.00	1	0.00	A001023052	<LOQ (0.0122 ug/g)	<LOQ	NA
3%	QC	C95911M/G1/Wk5-MS-250 ppb	NA	248	1	237	D001027034	79%			NA	325	1	292	A001102076	98%		
		C95911M/G1/Wk5-MSD-250 ppb	NA	261	1	250	D001027035	84%	81%	5%	NA	331	1	297	A001102077	99%	98%	2%
30%		C95991F/G1/Wk5-MS-250 ppb	NA	226	1	210	A001102078	74%			NA	327	1	289	A001102078	101%		
		C95991F/G1/Wk5-MSD-250 ppb	NA	144	1	120	D001027038	42% **	58%	55%	NA	136	1	228.4	A001102079	80%	90%	23%
	Group 1	C95907M	NA	16.7	1	16.4	A001102048	0.0164			NA	47.3	1	46.6	A001102048	0.0466		
	0 ppm	C95908M	NA	19.2	1	19.6	A001102049	0.0196			NA	38.1	1	38.8	A001102049	0.0388		
		C95910M	NA	15.0	1	15.1	A001102050	0.0151			NA	56.4	1	56.6	A001102050	0.0566		
0.927		C95916M	Confirmed Low	9.47	1	9.44	A001102051	<LOQ (0.0122 ug/g)		20.6	Confirmed Low	83.0	1	82.7	A001102051	0.0827		46.6
0.000114		C95917M	NA	6.04	1	5.74	A001102052	<LOQ (0.0122 ug/g)	0.0151	0.00310	NA	22.0	1	20.9	A001102053	0.0209	0.0491	0.0229
		C95981F	NA	0.00	1	0.00	A001102055	<LOQ (0.0122 ug/g)			NA	18.6	1	18.3	A001102055	0.0183		
		C95982F	NA	4.95	1	4.90	A001102056	<LOQ (0.0122 ug/g)			NA	24.5	1	24.2	A001102056	0.0242		
		C95989F	NA	11.3	1	11.1	A001102057	<LOQ (0.0122 ug/g)			NA	61.7	1	60.6	A001102057	0.0606		
29.5		C95998F	NA	24.3	1	24.6	A001102058	0.0246		37.9	NA	123	1	125	A001102058	0.125		88.6
0.00470		C95999F	NA	3.18	1	3.07	A001102059	<LOQ (0.0122 ug/g)	0.0147	0.00556	NA	25.6	1	24.8	A001102059	0.0248	0.0506	0.0448
	Group 2	C95923M	NA	96.1	20	1945	A001024045	1.94			2nd Analysis OK	144	50	7272	A001024031	7.27		
	3 ppm	C95929M	NA	73.3	20	1450	A001024046	1.45			Confirmed Low	82.4	50	4083	A001023094	4.08		
		C95930M	NA	86.5	20	1663	A001024047	1.66			2nd Analysis OK	114	50	5477	A001024033	5.48		
53.3		C95938M	NA	73.1	20	1387	A001024048	1.39		15.0	Confirmed Low	107	50	5092	A001023096	5.09		21.8
3.35		C95940M	NA	100	20	1881	A001024049	1.88	1.67	0.249	Confirmed Low	118	50	5559	A001023097	5.56	5.50	1.15
		C96004F	NA	53.9	20	1026	A001024053	1.03			2nd Analysis OK	80.6	50	3831	A001024038	3.83		
		C96005F	NA	64.7	20	1301	A001024054	1.30			Confirmed Low	121	50	6084	A001023101	6.08		
		C96009F	NA	72.7	20	1422	A001024055	1.42			NA	99.3	50	4858	A001023102	4.86		
22.6		C96017F	NA	50.9	20	1000	A001024056	1.00		28.5	Confirmed Low	95.2	50	4674	A001023103	4.67		17.1
0.505		C96018F	NA	97.1	20	1938	A001024057	1.94	1.34	0.381	Confirmed Low	90.4	50	4510	A001023104	4.51	4.79	0.820
	Group 3	C95945M	NA	90.2	500	45379	A001023065	45.4			NA	341	500	171306	A001023065	171		
	30 ppm	C95946M	NA	65.2	500	31490	A001023066	31.5			NA	336	500	162318	A001023066	162		
		C95956M	NA	69.7	500	34685	A001023067	34.7			NA	305	500	151547	A001023067	152		
20.0		C95957M	NA	77.1	500	37784	A001023068	37.8		19.5	NA	340	500	166461	A001023068	166		9.67
4.32		C95959M	NA	103	500	50397	A001023069	50.4	39.9	7.79	NA	399	500	195724	A001023069	196	169	16.4
		C96028F	NA	23.6	500	11381	A001023072	11.4			NA	195	500	93698	A001023072	93.7		
		C96029F	NA	38.9	500	19218	A001023073	19.2			NA	175	500	86277	A001023073	86.3		
		C96036F	NA	31.6	500	15300	A001023074	15.3			NA	182	500	88143	A001023074	88.1		
37.5		C96037F	Confirmed Low	27.0	500	13092	A001023075	13.1		20.5	Confirmed Low	156	500	75611	A001023075	75.6		11.1
6.1		C96039F	NA	27.9	500	13593	A001023076	13.6	14.5	2.98	NA	146	500	71394	A001023076	71.4	83.0	9.23
	Group 4	C95966M	2nd Analysis OK	236	500	117766	A001024017	118			2nd Analysis OK	696	500	347101	A001024017	347		
	100 ppm	C95970M	2nd Analysis OK	188	500	90379	A001024018	90.4			2nd Analysis OK	477	500	229004	A001024018	229		
		C95976M	2nd Analysis OK	211	500	103227	A001024019	103			2nd Analysis OK	569	500	277583	A001024019	278		
8.14		C95978M	2nd Analysis OK	197	500	95251	A001024020	95.3		11.5	2nd Analysis OK	534	500	258853	A001024020	259		18.2
2.43		~~C95979M~~	~~2nd Analysis OK~~	~~229~~	~~500~~	~~114972~~	~~A001024021~~	~~115~~	106	12.0	2nd Analysis OK	690	500	346110	A001024021	346	292	53.0
		C96041F	2nd Analysis OK	70.7	500	35357	A001024024	35.4			2nd Analysis OK	273	500	136297	A001024024	136		
		C96043F	NA	79.4	500	39467	A001023087	39.5			NA	299	500	148390	A001023087	148		
		C96057F	2nd Analysis OK	80.7	500	38985	A001024026	39.0			2nd Analysis OK	288	500	139116	A001024026	139		
4.93		C96058F	Confirmed Low	64.5	500	30814	A001023089	30.8		9.9	Confirmed Low	222	500	105962	A001023089	106		15.1
1.24		C96059F	Confirmed Low	70.5	500	34439	A001023090	34.4	35.8	3.55	Confirmed Low	221	500	108117	A001023090	108	128	19.3

Limit of Quantitation (LOQ) updated for purity on 01/31/01 PFOS = 12.2 ng/g, 30.6 ng/g LAC 01/31/01

** Low recovery confirmed with 10/27/00 and 11/02/00 analysis. LAC 11/07/00

+ CCVs bracketing these data weren't within +/- 30%. Curve stds analyzed every 5 samples were within criteria. LAC 11/06/00

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
M556 = $C_8F_{17}SO_2N(H)CH_2COOH$
M570 = $C_8F_{17}SO_2N(CH_3)CH_2COOH$

Data Entered/Analyst: 10/27/00, 11/03/00, 11/07/00, 12/08/00 LAC/KJH
Data Verified/Analyst: 11/08/00, 11/09/00, 12/11/00 KJH/LAC
Purity Entered/Verified: 12/20/00 LAC, 12/27/00 mmh

FACT-TOX-028
Covance# 6329-225

Study:	Covance 6329-225, FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats
Product Number(Test Substance):	T-6314
Matrix:	Rat Liver
Method/Revision:	ETS-8-6.0 & ETS-8-7.0
Analytical Equipment System Number:	Aurora 062498
Instrument Software/Version:	Masslynx 3.4
Date of Extraction/Analyst:	10/10/00 SAL/KJK
Date of Analysis/Analyst:	10/23/00, 10/24/00, 10/27/00, 10/30/00, 10/31/00, 11/02/00, 12/01/00, 12/04/00 KJH/MMH/HOJ
Date of Data Reduction/Analyst:	10/24/00, 10/25/00, 10/30/00, 10/31/00, 11/01/00, 11/0600, 12/04/00, 12/05/00 MMH
Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-intercept:	See Attachments

Sample Data

RAT LIVER WEEK 14

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	M556 Calc. Conc. ug/g	Concentration of M556 ug/g or % Rec	Mean M556 ug/g	RSD Std. Dev. MS/MSD RPD	M570 Calc. Conc. ug/g	Concentration of M570 ug/g or % Rec	Mean M570 ug/g	RSD Std. Dev. MS/MSD RPD
Method Blk	10100-H2O Blk-5	0.00	<LOQ (0.00105 ug/g)			0.00	<LOQ (0.00611 ug/g)			0.00	<LOQ (0.0122 ug/g)			0.00	<LOQ (0.0306 ug/g)		
	10100-H2O-Blk-6	0.00	<LOQ (0.0264 ug/g)	<LOQ	NA	3.63	<LOQ (0.0306 ug/g)	<LOQ	NA	0.00	<LOQ (0.0306 ug/g)	<LOQ	NA	0.00	<LOQ (0.0122 ug/g)	<LOQ	NA
Matrix Blk	RBL10100-Liver Blk-5	0.00	<LOQ (0.00105 ug/g)			0.210	<LOQ (0.00611 ug/g)			4.16	<LOQ (0.0122 ug/g)			0.00	<LOQ (0.0306 ug/g)		
	RBL10100-Liver Blk-6	0.00	<LOQ (0.0264 ug/g)	<LOQ	NA	0.00	<LOQ (0.0306 ug/g)	<LOQ	NA	0.00	<LOQ (0.0306 ug/g)	<LOQ	NA	0.00	<LOQ (0.0122 ug/g)	<LOQ	NA
QC	C95911M/G1/WL5-MS-250 ppb	348	116%			276	92%			237	79%			292	98%		
	C95911M/G1/WL5-MSD-250 ppb	272	91%	104%	25%	268	89%	91%	3%	250	84%	81%	5%	297	99%	98%	2%
	C95991F/G1/WL5-MS-250 ppb	225	79%			223	78%			210	74%			289	101%		
	C95991F/G1/WL5-MSD-250 ppb	246	86%	82%	9%	165	58% **	68%	30%	120	42% **	58%	55%	228.4	80%	90%	23%
Group 1 0 ppm	C95907M	216	0.216			11.8	<LOQ (0.0122 ug/g)			16.4	0.0164			46.6	0.0466		
	C95908M	249	0.249			8.12	<LOQ (0.0122 ug/g)			19.6	0.0196			38.8	0.0388		
	C95910M	320	0.320			10.8	<LOQ (0.0122 ug/g)			15.1	0.0151			56.6	0.0566		
	C95916M	107	0.107		48.6	12.5	0.0125		0.927	9.44	<LOQ (0.0122 ug/g)		20.6	82.7	0.0827		46.6
	C95917M	95	0.095	0.20	0.10	5.24	<LOQ (0.0122 ug/g)	0.0123	0.000114	5.74	<LOQ (0.0122 ug/g)	0.0151	0.00310	20.9	0.0209	0.0491	0.0229
	C95981F	55.5	0.0555			12.2	0.0122			0.00	<LOQ (0.0122 ug/g)			18.3	0.0183		
	C95982F	160	0.160			16.4	0.0164			4.90	<LOQ (0.0122 ug/g)			24.2	0.0242		
	C95989F	159	0.159			15.3	0.0153			11.1	<LOQ (0.0122 ug/g)			60.6	0.0606		
	C95998F	146	0.146		41.6	23.7	0.0237		29.5	24.6	0.0246		37.9	123	0.123		88.6
	C95999F	76.8	0.0768	0.120	0.0498	5.75	<LOQ (0.0122 ug/g)	0.0160	0.00470	3.07	<LOQ (0.0122 ug/g)	0.0147	0.00556	24.8	0.0248	0.0506	0.0448
Group 2 3 ppm	C95923M	23770	23.8			1807	1.81			1945	1.94			7272	7.27		
	C95929M	25672	25.7			10807	10.8			1450	1.45			4083	4.08		
	C95930M	22319	22.3			5878	5.88			1663	1.66			5477	5.48		
	C95938M	29470	29.5		12.4	5014	5.01		53.3	1387	1.39		15.0	5092	5.09		21.0
	C95940M	29337	29.3	26.1	3.23	7877	7.88	6.28	3.35	1881	1.88	1.67	0.249	5559	5.56	5.50	1.15
	C96004F	17763	17.8			1470	1.47			1026	1.03			3831	3.83		
	C96005F	42727	42.7			2672	2.67			1301	1.30			6084	6.08		
	C96009F	45557	45.6			2246	2.25			1422	1.42			4858	4.86		
	C96017F	31734	31.7		33.2	2702	2.70		22.6	1000	1.00		28.5	4674	4.67		17.1
	C96018F	46955	47.0	36.9	12.3	2073	2.07	2.23	0.505	1938	1.94	1.34	0.381	4510	4.51	4.79	0.820
Group 3 30 ppm	C95945M	232806	233			27604	27.6 +			45379	45.4			171306	171		
	C95946M	233979	234			18888	18.9 +			31490	31.5			162318	162		
	C95956M	213047	213			20801	20.8 +			34685	34.7			151547	152		
	C95957M	195332	195		18.6	16702	16.7 +		20.0	37784	37.8		19.5	166461	166		9.67
	C95959M	310671	311	237	44.1	24184	24.2 +	21.6	4.32	50397	50.4	39.9	7.79	195724	196	169	16.4
	C96028F	203231	203			14178	14.2 +			11381	11.4			93698	93.7		
	C96029F	402843	403			13412	13.4			19218	19.2			86277	86.3		
	C96036F	335782	336			11709	11.7			15300	15.3			88143	88.1		
	C96037F	308777	309		28.7	26810	26.8		37.5	13092	13.1		20.5	75611	75.6		11.1
	C96039F	215288	215	293	84.0	14723	14.7 +	16.2	6.06	13593	13.6	16.5	2.98	71394	71.4	83.0	9.33
Group 4 100 ppm	C95966M	650557	651			30402	30.4 +			117766	118			347101	347		
	C95970M	822248	822			25836	25.8 +			90379	90.4			229004	229		
	C95976M	728917	729			32341	32.3 +			103227	103			277583	278		
	C95978M	665361	665		10.1	29630	29.6 +		8.14	95251	95.3		11.5	258853	259		18.2
	C95979M	782402	782	736	73.9	30782	30.8 +	29.8	2.43	114972	115	104	12.0	346110	346	292	53.0
	C96041F	717359	717			25998	26.0 +			35357	35.4			136297	136		
	C96043F	609808	610			24727	24.7 +			39467	39.5			148390	148		
	C96057F	820119	820			27040	27.0 +			38985	39.0			139116	139		
	C96058F	758530	759		11.2	24571	24.6 +		4.93	30814	30.8		9.9	105962	106		15.1
	C96059F	799658	800	741	83.3	23949	23.9 +	25.3	1.24	34439	34.4	35.8	3.55	108117	108	128	19.3

Limit of Quantitation (LOQ) updated for purity on 01/31/01 PFOS = 12.2 ng/g, 30.6 ng/g. LAC 01/31/01
** Low recovery confirmed with 10/27/00 and 11/02/00 analysis. LAC 11/07/00
+ CCVs bracketing these data weren't within +/- 30%. Curve stds analyzed every 5 samples were within criteria. LAC 11/08/00

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
M556 = C8F17SO2N(H)CH2COOH
M570 = C8F17SO2N(CH3)CH2COOH

Date Entered/Analyst: 10/27/00, 11/03/00, 11/07/00, 12/08/00 LAC/KJH
Data Verified/Analyst: 11/08/00, 11/09/00, 12/11/00 KJH/LAC
Purity Entered/Verified: 12/20/00 LAC, 12/27/00 mmh

FACT-TOX-028
Covance# 6329-225

Study:	Covance 6329-225, FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOSA, M356, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats
Product Number(Test Substance):	T-6314
Matrix:	Rat Liver
Method/Revision:	ETS-8-6.0 & ETS-8-7.0
Analytical Equipment System Number:	Aztec063498, Dawy070799
Instrument Software/Version:	Masslynx 3.4
Date of Extraction/Analyst:	11/21-00 KJE/SAL
Date of Analysis/Analyst:	11/22/00, 11/27/00 MMH
Date of Data Reduction/Analyst:	11/27/00, 11/28/00 MMH
Filename:	See Below
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments
Test # 00-109	

Sample Data

RAT LIVER QC

Group Dose	Sample #	Surrogate Verified	Initial Wt. g	Total Mass of Liver g	PFOS Purity Correction Factor	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Filename (optional)	Concentration of PFOS ng/g or % Rec	Mean PFOS ng/g	RSD Std. Dev. MS/MSD RPD	Surrogate Verified	PFOSA Conc. ng/g	PFOSA Dilution Factor	PFOSA Calc. Conc. ng/g	Filename (optional)	Concentration of PFOSA ng/g or % Rec	Mean PFOSA ng/g	RSD Std. Dev. MS/MSD RPD
														Int 171						Int L-15709
Method Blk	10100-H2O Blk-5	NA	1.0000	NA	0.8640	0.00	1	0.00	A001123047	<LOQ (0.00528 ug/g)			NA	5.32	1	5.32	A001123047	<LOQ (0.0306 ug/g)		
	10100-H2O Blk-6	NA	1.0000	NA	0.8640	0.00	1	0.00	A001123060	<LOQ (0.00528 ug/g)	<LOQ	NA	NA	2.94	1	2.94	A001123060	<LOQ (0.0306 ug/g)	<LOQ	NA
Matrix Blk	RBL10100-Liver Blk-5	NA	1.0000	NA	0.8640	0.00	1	0.00	A001123048	<LOQ (0.00528 ug/g)			NA	6.23	1	6.23	A001123048	<LOQ (0.0306 ug/g)		
	RBL10100-Liver Blk-6	NA	1.0000	NA	0.8640	0.00	1	0.00	A001123061	<LOQ (0.00528 ug/g)	<LOQ	NA	NA	3.79	1	3.79	A001123061	<LOQ (0.0306 ug/g)	<LOQ	NA
QC	C959124A/G1/W15-MS-250 ppb	2nd Analysis OK	0.9971	NA	NA	378	1	223	A001127097	74%			2nd Analysis OK	260	1	260	A001127097	86%		
	C959124A/G1/W15-MSD-250 ppb	2nd Analysis OK	0.9971	NA	NA	435	1	282	A001127098	94%	84%	26%	2nd Analysis OK	297	1	298	A001127098	99%	93%	13%
	C959823FG1/W14-MS-250 ppb	2nd Analysis OK	1.0008	NA	NA	499	1	311	A001127100	104%			2nd Analysis OK	388	1	371	A001127101	90%		
	C959823FG1/W14-MSD-250 ppb	2nd Analysis OK	1.0008	NA	NA	483	1	295	A001127102	98%	101%	5%	2nd Analysis OK	336	1	321	A001127102	107%	99%	17%

Limit of Quantitation (LOQ) updated for purity on 01/31/01 PFOS = 6.11 ng/g LAC 01/31/01

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
M556 = $C_8F_{17}SO_2N(H)CH_2COOH$
M570 = $C_8F_{17}SO_2N(CH_3)CH_2COOH$

Data Entered/Analyst: 12/01/00 LAC
Data Verified/Analyst: 12/11/00 KJH
Purity Entered/Verified: 12/20/00 LAC

FACT-TOX-028
Covance# 6329-225

Study:	Covance 6329-225, FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats
Product Number(Test Substance):	T-6314
Matrix:	Rat Liver
Method/Revision:	ETS-8-6.0 & ETS-8-7.0
Analytical Equipment System Number:	Aurelia062498, Davey070799
Instrument Software/Version:	Masslynx 3.4
Date of Extraction/Analyst:	11/21/00 EJK/SAL
Date of Analysis/Analyst:	11/22/00, 11/27/00 MMH
Date of Data Reduction/Analyst:	11/27/00, 11/28/00 MMH

Filename:	See Below
R-Squared Value:	See Attachment
Slope:	See Attachment
Y-Intercept:	See Attachment

Sample Data

RAT LIVER QC

			lot NB113047-80								lot TCR00017-56					
Group Done	Sample #	Surrogate Verified	M556 Conc. ng/g	M556 Dilution Factor	M556 Calc. Conc. ng/g	Filename (optional)	Concentration of M556 ng/g or % Rec	Mean M556 ng/g	RSD Std. Dev. M5/MSD RPD	Surrogate Verified	M570 Conc. ng/g	M570 Dilution Factor	M570 Calc. Conc. ng/g	Filename (optional)	Concentration of M570 ng/g or % Rec	Mean M570 ng/g	RSD Std. Dev. M5/MSD RPD
Method Blk	10100-H2O Blk-5	NA	0.00	1	0.00	A001122047	<LOQ (0.0306 ng/g)			NA	4.33	1	4.33	A001122047	<LOQ (0.0122 ng/g)		
	10100-H2O-BB-6	NA	0.00	1	0.00	A001122060	<LOQ (0.0306 ng/g)	<LOQ	NA	NA	3.28	1	3.28	A001122060	<LOQ (0.0122 ng/g)	<LOQ	NA
Matrix Blk	RBL10100-Liver Blk-5	NA	0.00	1	0.00	A001122048	<LOQ (0.0306 ng/g)			NA	0.00	1	0.00	A001122048	<LOQ (0.0122 ng/g)		
	RBL10100-Liver Blk-6	NA	0.00	1	0.00	A001122061	<LOQ (0.0306 ng/g)	<LOQ	NA	NA	0.00	1	0.00	A001122061	<LOQ (0.0122 ng/g)	<LOQ	NA
QC	C959122M/G1/WL5-MS-250 ppb	2nd Analysis OK	130	1	123	A001127097	41%			2nd Analysis OK	197	1	176	A001127097	59%		
	C959122M/G1/WL5-MSD-250 ppb	2nd Analysis OK	195	1	188	A001127098	63%	52%	42%	2nd Analysis OK	249	1	229	A001127098	76%	68%	26%
	C959122F/G1/WL14-MS-250 ppb	2nd Analysis OK	218	1	213	A001127101	71%			2nd Analysis OK	243	1	218	A001127101	73%		
	C959122F/G1/WL14-MSD-250 ppb	2nd Analysis OK	215	1	210	A001127102	70%	71%	2%	2nd Analysis OK	283	1	238	A001127102	80%	76%	9%

Limit of Quantitation (LOQ) updated for purity on 01/31/01 PFOS = 6.11 ng/g, LAC 01/31/01

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
M556 = $C_8F_{17}SO_2N(H)CH_2COOH$
M570 = $C_8F_{17}SO_2N(CH_3)CH_2COOH$

Date Entered/Analyst: 12/01/00 LAC
Date Verified/Analyst: 12/11/00 KJH
Purity Entered/Verified: 12/20/00 LAC

FACT-TOX-028
Covance# 6329-225

Study:	Covance 6329-225, FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOSA, M556, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats
Product Number(Test Substance):	T-6314
Matrix:	Rat Liver
Method/Revision:	ETS-8-6.0 & ETS-8-7.0
Analytical Equipment System Number:	Aurelia062498, Davey070799
Instrument Software/Version:	Masslynx 3.4
Date of Extraction/Analyst:	11/21/00 EJK/SAL
Date of Analysis/Analyst:	11/22/00, 11/27/00 MMH
Date of Data Reduction/Analyst:	11/27/00, 11/28/00 MMH
Sample Data	

Filename:	See Attachments																
R-Squared Value:	See Attachments																
Slope:	See Attachments																
Y-Intercept:	See Attachments																

RAT LIVER QC

Group Dose	Sample #	PFOS Calc. Conc. ng/g	Concentration of PFOS ng/g or % Rec	Mean PFOS ng/g	RSD Std. Dev. MS/MSD RPD	PFOSA Calc. Conc. ng/g	Concentration of PFOSA ng/g or % Rec	Mean PFOSA ng/g	RSD Std. Dev. MS/MSD RPD	M556 Calc. Conc. ng/g	Concentration of M556 ng/g or % Rec	Mean M556 ng/g	RSD Std. Dev. MS/MSD RPD	M570 Calc. Conc. ng/g	Concentration of M570 ng/g or % Rec	Mean M570 ng/g	RSD Std. Dev. MS/MSD RPD
Method Blk	10100-H2O Blk-5	0.00	<LOQ (0.00528 ug/g)			5.32	<LOQ (0.0306 ug/g)			0.00	<LOQ (0.0306 ug/g)			4.33	<LOQ (0.0122 ug/g)		
	10100-H2O-Blk-6	0.00	<LOQ (0.00528 ug/g)	<LOQ	NA	2.94	<LOQ (0.0306 ug/g)	<LOQ	NA	0.00	<LOQ (0.0306 ug/g)	<LOQ	NA	3.28	<LOQ (0.0122 ug/g)	<LOQ	NA
Matrix Blk	RBL10100-Liver Blk-5	0.00	<LOQ (0.00528 ug/g)			6.230	<LOQ (0.0306 ug/g)			0.00	<LOQ (0.0306 ug/g)			0.00	<LOQ (0.0122 ug/g)		
	RBL10100-Liver Blk-6	0.00	<LOQ (0.00528 ug/g)	<LOQ	NA	3.79	<LOQ (0.0306 ug/g)	<LOQ	NA	0.00	<LOQ (0.0306 ug/g)	<LOQ	NA	0.00	<LOQ (0.0122 ug/g)	<LOQ	NA
QC	C959124M/G1/Wk5-MS-250 ppb	223	74%			360	86%			123	41%			176	59%		
	C959124M/G1/Wk5-MSD-250 ppb	282	94%	84%	24%	398	95%	93%	13%	189	63%	52%	42%	229	76%	68%	26%
	C959128F/G1/Wk14-MS-250 ppb	311	104%			271	90%			213	71%			218	73%		
	C959128F/G1/Wk14-MSD-250 ppb	295	98%	101%	5%	321	107%	99%	17%	210	70%	71%	2%	238	80%	76%	9%

Limit of Quantitation (LOQ) updated for purity on 01/31/01 PFOS = 6.11 ng/g. LAC 01/31/01

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
M556 = $C_8F_{17}SO_2N(H)CH_2COOH$
M570 = $C_8F_{17}SO_2N(CH_3)CH_2COOH$

Date Entered/Analyst: 12/01/00 LAC
Date Verified/Analyst: 12/11/00 EJH
Purity Entered/Verified: 12/20/00 LAC,

ETS-8-7.0
Excel 97

TOX-028-liver225-7E.xls

3/20/2001
12:36 PM

FACT-TOX-028
Covance# 6329-225

Study: Covance 6329-225, FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOSA, M356, and M570 in the 13-Week Dietary Study of Crl:CD® (SD) IGS BR Rats
Product Number(Test Substance): T-6314
Matrix: Rat Liver
Method/Revision: ETS-8-6.0 & ETS-8-7.0
Analytical Equipment System Number: Aquila 062496
Instrument Software/Version: Manlynx 3.4
Date of Extraction/Analyst: 12/07/00 RWW
Date of Analysis/Analyst: 12/13/00 MAH
Date of Data Reduction/Analyst: 12/14/00 KJH
Sample Data

Slope: See Attachments
Y-Intercept: See Attachments
Res #: 08-98

RAT LIVER Week 5 Precision — lot 171 — lot L-15709

Group Dose	Sample #	Surrogate Verified	Initial Wt. g	Total Mass of Liver g	PFOS Purity Correction Factor	PFOS Conc. ng/g	PFOS Dilution Factor	PFOS Calc. Conc. ng/g	Filename (optional)	Concentration of PFOS ng/g or % Rsd	Mean PFOS ng/g	Relative % Difference	Surrogate Verified	PFOSA Conc. ng/g	PFOSA Dilution Factor	PFOSA Calc. Conc. ng/g	Filename (optional)	Concentration of PFOSA ng/g or % Rsd	Mean PFOSA ng/g	Relative % Difference
Method Blk	RBL120700-H2O Blk-1-1	NA	1.0000	NA	0.8640	18.5	1	16.0	D001213010	0.0160			NA	0.00	1	0.00	D001213010	<LOQ (0.00611 ug/g)		
	RBL120700-H2O Blk-1-2	NA	1.0000	NA	0.8640	0.00	1	0.00	D001213022	<LOQ (0.0165 ug/g)	0.0133	0.80389	NA	0.00	1	0.00	D001213022	<LOQ (0.00611 ug/g)	<LOQ	NA
Matrix Blk	RBL120700-Liver Blk-1	NA	1.0000	NA	0.8640	0.00	1	0.00	D001213011	<LOQ (0.0165 ug/g)			NA	0.00	1	0.00	D001213011	<LOQ (0.00611 ug/g)		
	RBL120700-Liver Blk-2	NA/NE	1.0000	NA/NE	0.8640	NA/NE	1	NA/NE	NA/NE	NA/NE	<LOQ	NA	NA/NE	NA/NE	1	NA/NE	NA/NE	NA/NE	<LOQ	NA
QC	C95913M/G1-MB-300 ng/g	NA	1.0484	NA	NA	NE	1	NE	NA	NA			NA	NE	1	NE	NA	NA		
	C95915M/G1-MSD-300 ng/g	NA	1.0464	NA	NA	NE	1	NE	NA	NA	NA	NA	NA	NE	1	NE	NA	NA	NA	NA
Group 2 3 ppm	C99931M-1	NA	1.0072	NA	0.8640	361	50	15519	D001210016	15.5			NA	97.9	20	754	D001213024	0.754		
	C99951M-2	NA	1.0173	NA	0.8640	371	50	15762	D001210017	15.8	15.7	1.92%	NA	56.7	20	1115	D001213025	1.12	0.935	28.8%
	C99932M-1	NA	1.0295	NA	0.8640	427	50	17936	D001210018	17.9			NA	37.7	20	732	D001213026	0.732		
	C99932M-2	NA	1.0073	NA	0.8640	475	50	20390	D001210019	20.4	19.2	13.1%	NA	30.5	20	605	D001213027	0.605	0.668	19.0%
	C99934M-1	NA	1.0031	NA	0.8640	388	50	15471	D001210020	15.5			NA	54.5	20	1086	D001213030	1.08		
	C99934M-2	NA	1.0581	NA	0.8640	355	50	14498	D001210021	14.5	15.0	6.67%	NA	53.0	20	1082	D001213031	1.08	1.08	0.408%
	C96011F-1	NA	1.0461	NA	0.8640	522	50	21572	D001213032	21.6			NA	16.0	50	764	D001213032	0.764		
	C96011F-2	NA	1.0426	NA	0.8640	507	50	20999	D001213033	21.0	21.3	2.82%	NA	19.9	50	956	D001213033	0.956	0.860	22.3%
	C96014F-1	NA	1.0666	NA	0.8640	495	50	20075	D001213034	20.1			NA	10.9	50	511	D001213034	0.511		
	C96014F-2	NA	1.0410	NA	0.8640	417	50	17309	D001213035	17.3	18.7	15.0%	NA	8.17	50	392	D001213035	0.392	0.452	26.2%
	C96015F-1	NA	1.0796	NA	0.8640	434	50	17512	D001213038	17.5			NA	20.1	50	940	D001213038	0.940		
	C96015F-2	NA	1.0420	NA	0.8640	427	50	17688	D001213039	17.7	17.6	1.136%	NA	17.1	50	821	D001213039	0.821	0.880	13.5%
											Average difference:	6.76%							Average difference:	20.0%

Limit of Quantitation (LOQ) updated for purity on 01/31/01 PFOS = 12.3 ng/g LAC 01/31/01
NA - Not Applicable
NE - Not Spiked with this analyte. LAC 12/18/00
NE - Not Extracted
Data Entered/Analyst: 12/18/00, 12/27/00 LAC
Data Verified/Analyst:
Purity Entered/Verified: 12/20/00 LAC.

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
M356 = $C_8F_{17}SO_2N(CH_3)CH_2COOH$
M570 = $C_8F_{17}SO_2N(CH_2CH_3)CH_2COOH$

FACT-TOX-028
Covance# 6329-225

Study:	Covance 6329-225. FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOBA, M356, and M570 in the 13-Week Dietary Study of Cd:CD® (SD) IGS BR Rats
Product Number(Test Substance):	T-6314
Matrix:	Rat Liver
Method/Revision:	ETS-8-4.0 & ETS-8-7.0
Analytical Equipment System Number:	Amelie 062498
Instrument Software/Version:	Analyst 1.4
Date of Extraction/Analyst:	12/07/00 RWW
Date of Analysis/Analyst:	12/13/00 MMH
Date of Data Reduction/Analyst:	12/14/00 KJH
Sample Data	

Fitment:	See Below
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments

RAT LIVER Week 5 Precision

		lot NB113047-80								lot TCB00017-56						
Group Dose	Sample #	Surrogate Verified	M356 Conc. ng/g	M356 Dilution Factor	M356 Calc. Conc. ng/g	Fitment (optional)	Concentration of M356 ng/g or % Rec	Mean M356 ng/g	Relative % Difference	Surrogate Verified	M570 Conc. ng/g	M570 Dilution Factor	M570 Calc. Conc. ng/g	Fitment (optional)	Concentration of M570 ng/g or % Rec	Mean M570 ng/g	Relative % Difference
Method Blk	RBL120700-H2O Blk-1-1	NA	0.00	1	0.00	D001213010	<LOQ (0.00611 ng/g)			NA	0.00	1	0.00	D001213010	<LOQ (0.00611 ng/g)		
	RBL120700-H2O Blk-1-2	NA	0.00	1	0.00	D001213023	<LOQ (0.00611 ng/g)	<LOQ	NA	NA	0.00	1	0.00	D001213023	<LOQ (0.00611 ng/g)	<LOQ	NA
Matrix Blk	RBL120700-Liver Blk-1	NA	1.09	1	1.09	D001213011	<LOQ (0.00611 ng/g)			NA	0.00	1	0.00	D001213011	<LOQ (0.00611 ng/g)		
	RBL120700-Liver Blk-2	NA/NE	NA/NE	1	NA/NE	NA/NE	NA/NE	<LOQ	NA	NA/NE	NA/NE	1	NA/NE	NA/NE	NA/NE	<LOQ	NA
QC	C959134A01-MB-300 ng/g	NA	NS	1	NS	NA	NA			NA	NS	1	NS	NA	NA		
	C959134A01-MSD-300 ng/g	NA	NS	1	NS	NA	NA	NA	NA	NA	NS	1	NS	NA	NA	NA	NA
Group 2 2 MPK	C9991141-1	NA	91.7	20	1835	D001213024	1.82			NA	374	20	7485	D001213024	7.44		
	C9991141-2	NA	110	20	2197	D001213025	2.16	1.99	16.7%	NA	455	20	9050	D001213025	8.95	8.20	18.4%
	C9993241-1	NA	88.8	20	1775	D001213026	1.73			NA	407	20	7902	D001213026	7.90		
	C9993241-2	NA	89.4	20	1775	D001213027	1.77	1.75	2.83%	NA	394	20	7815	D001213027	7.81	7.86	1.12%
	C9993441-1	NA	104	20	1911	D001213030	1.91			NA	378	20	6989	D001213030	6.99		
	C9993441-2	NA	82.7	20	1563	D001213031	1.56	1.74	20.1%	NA	357	20	6752	D001213031	6.75	6.87	3.45%
	C96011F-1	NA	33.4	50	1598	D001213032	1.60			NA	126	50	6001	D001213032	6.00		
	C96011F-2	NA	39.9	50	1911	D001213033	1.91	1.75	17.9%	NA	133	50	6358	D001213033	6.36	6.18	5.77%
	C96014F-1	NA	39.4	50	1870	D001213034	1.85			NA	127	50	5944	D001213034	5.94		
	C96014F-2	NA	26.4	50	1266	D001213037	1.27	1.56	37.4%	NA	102	50	4920	D001213037	4.92	5.43	18.9%
	C96015F-1	NA	20.4	50	955	D001213038	0.955			NA	123	50	5748	D001213038	5.75		
	C96015F-2	NA	23.9	50	1149	D001213039	1.15	1.05	18.4%	NA	126	50	6027	D001213039	6.03	5.89	4.75%
Limit of Quantitation (LOQ) updated for purity on 01/31/01 PFOS = 12.2 ng/g. LAC 01/31/01								Average difference:	18.9%							Average difference:	8.72%

PFOS = Perfluorooctanesulfonate
PFOBA = Perfluorooctanesulfonamide
M356 = $C_8F_{17}SO_2N(H)CH_2COOH$
M570 = $C_8F_{17}SO_2N(CH_3)CH_2COOH$

Date Entered/Analyst: 12/18/00, 12/27/00 LAC
Date Verified/Analyst: 0
Purity Entered/Verified: 12/20/00 LAC,

FACT-TOX-028
Covance# 6329-225

Study:	Covance 6329-225, FACT-TOX-028 Determination of the Presence and Concentration of PFOS, PFOSA, M356, and M570 in the 13-Week Dietary Study of C6CCD® (SD) IGS BR Rats
Product Number(Test Substance):	T-6314
Matrix:	Rat Liver
Method/Revision:	ETS-8-6.0 & ETS-8-7.0
Analytical Equipment System Number:	Annala 062498
Instrument Software/Version:	Manhyen 3.4
Date of Extraction/Analyst:	12/07/00 RW/W
Date of Analysis/Analyst:	12/13/00 MJGH
Date of Data Reduction/Analyst:	12/14/00 LJH

Filename:	See Attachments
R-Squared Value:	See Attachments
Slope:	See Attachments
Y-Intercept:	See Attachments

Sample Data

RAT LIVER Week 5 Precision

Group Dose	Sample #	PFOS Calc. Conc. ng/g	Concentration of PFOS ng/g or % Rec	Mean PFOS ng/g	Relative % Difference	PFOSA Calc. Conc. ng/g	Concentration of PFOSA ng/g or % Rec	Mean PFOSA ng/g	Relative % Difference	M356 Calc. Conc. ng/g	Concentration of M356 ng/g or % Rec	Mean M356 ng/g	Relative % Difference	M570 Calc. Conc. ng/g	Concentration of M570 ng/g or % Rec	Mean M570 ng/g	Relative % Difference
Method Blk	RBL120700-H2O Blk-1-1	16.0	0.0160			0.00	<LOQ (0.00611 ng/g)			0.00	<LOQ (0.00611 ng/g)			0.00	<LOQ (0.00611 ng/g)		
	RBL120700-H2O Blk-1-2	0.00	<LOQ (0.0105 ng/g)	0.0133	0.00389	0.00	<LOQ (0.00611 ng/g)	<LOQ	NA	0.00	<LOQ (0.00611 ng/g)	<LOQ	NA	0.00	<LOQ (0.00611 ng/g)	<LOQ	NA
Matrix Blk	RBL120700-Liver Blk-1	0.00	<LOQ (0.0105 ng/g)			0.00	<LOQ (0.00611 ng/g)			1.09	<LOQ (0.00611 ng/g)			0.00	<LOQ (0.00611 ng/g)		
	RBL120700-Liver Blk-2	NA/NE	NA/NE	<LOQ	NA	NA/NE	NA/NE	<LOQ	NA	NA/NE	NA/NE	<LOQ	NA	NA/NE	NA/NE	<LOQ	NA
QC	C959133d/G1-MS-300 ng/g	NS	NA			NS	NA			NS	NA			NS	NA		
	C959133d/G1-MSD-300 ng/g	NS	NA	NA	NA	NS	NA	NA	NA	NS	NA	NA	NA	NS	NA	NA	NA
Group 2 3 ppm	C959313d-1	15519	15.5			754	0.754			1825	1.82			7445	7.46		
	C959313d-2	15762	15.8	15.7	1.92%	1115	1.12	0.935	38.6%	2157	2.16	1.99	16.7%	8950	8.95	8.20	18.4%
	C959324d-1	17936	17.9			732	0.732			1725	1.73			7902	7.90		
	C959324d-2	20795	20.4	19.2	13.87%	605	0.605	0.668	19.0%	1775	1.77	1.75	2.32%	7815	7.81	7.86	1.12%
	C959504d-1	15471	15.5			1006	1.01			1911	1.91			6909	6.90		
	C959504d-2	14498	14.5	15.0	6.67%	1002	1.00	1.00	0.400%	1563	1.56	1.74	20.1%	6752	6.75	6.87	3.43%
	C960113F-1	21572	21.6			764	0.764			1398	1.40			6801	6.80		
	C960113F-2	20999	21.0	21.3	2.82%	956	0.956	0.860	22.3%	1912	1.91	1.75	17.9%	6336	6.36	6.58	5.77%
	C960140F-1	20075	20.1			511	0.511			1830	1.83			5944	5.94		
	C960140F-2	17309	17.3	18.7	15.9%	392	0.392	0.452	26.2%	1266	1.27	1.56	27.4%	4920	4.92	5.43	18.9%
	C960150F-1	17512	17.5			940	0.940			953	0.953			5768	5.75		
	C960150F-2	17688	17.7	17.6	1.136%	821	0.821	0.880	13.5%	1149	1.15	1.05	18.4%	6027	6.03	5.89	4.77%
			Average difference:		6.76%		Average difference:		20.0%		Average difference:		18.9%		Average difference:		8.72%

Limit of Quantitation (LOQ) updated for purity on 01/31/01 PFOS = 12.2 ng/g. LAC 01/31/01

PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
M356 = $C_8F_{17}SO_2N(H)CH_2COOH$
M570 = $C_8F_{17}SO_2N(CH_3)CH_2COOH$

Data Entered/Analyst: 12/16/00, 12/27/00 LAC
Data Verified/Analyst: 0
Peaky Entered/Verified: 12/20/00 LAC

Appendix F: Example Calculations

Formula Used for Sera Analyses in Study FACT-TOX-028

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

Calculation Used for Group 3, Week 5, Animal ID C96031F (PFOS)

$$475\ \text{ng/mL} \times 100 \times 0.864 \times \frac{1.0\ \text{mL}}{1.0\ \text{mL}} \times \frac{1.0\ \mu\text{g}}{1000\ \text{ng}} = \textbf{41.0 µg/mL}$$

AR— Analytical result from MassLynx summary
DF— Dilution factor
FV—Final extract volume (1.0 mL unless otherwise noted)
EV—Volume of sera extracted
PC—Purity Correction (for PFOS, only)

Formula Used for Liver Analyses in Study FACT-TOX-028

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

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

Calculation Used for Group 3, Week 5, Animal ID C96032 (PFOS)

$$353\ \text{ng/g} \times \frac{1\ \text{g}/\ 5\ \text{mL}}{1.0443\ \text{g}/\ 5\text{mL}} \times 500 \times 0.864 \times \frac{1.0\ \mu\text{g}}{1000\ \text{ng}} = \textbf{146 µg/g}$$

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)
DF— Dilution factor
PC—Purity Correction (for PFOS, only)

Formula Used for Liver Precision Analyses in Study FACT-TOX-028

$$\frac{|(\text{Replicate 1(ng/g)} - \text{Replicate 2(ng/g)})|}{\text{Average of Replicates 1 and 2(ng/g)}} \times 100\% = \textbf{Relative Percent Difference}$$

Calculation Used for Group 2, Week 5, Animal ID C95931M (PFOS)

$$\frac{|(15.5\ \text{ng/g} - 15.8\ \text{ng/g})|}{(15.5\ \text{ng/g} + 15.8\ \text{ng/g}) / 2} \times 100\% = \mathbf{1.92\%}$$

Appendix G: Interim Certificate(s) 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.%

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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018B

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/01

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

Total impurity from all tests = 13.56%

Purity = 100% - 13.56% = 86.4%

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

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

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

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

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

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

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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018B

LC/MS Purity Profile:

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

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

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

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

Appendix H: Report Signature Page

Andrew M. Seacat — 2/23/01

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

John L. Butenhoff — FEB 23 2001

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

Kristen Hansen — 02/22/01

Kristen J. Hansen, Ph.D., *Principal Analytical Investigator* — Date

William K. Reagen — 02/22/01

William K. Reagen, Ph.D., *Laboratory Manager* — Date