ANALYTICAL LABORATORY REPORT

ON THE

Determination of the Presence and Concentration

of Perfluorooctanesulfonate (PFOS)

(CAS Number: 2759-39-3)

in the Serum of Sprague-Dawley® Rats Exposed to Potassium Perfluorooctanesulfonate via Gavage

Laboratory Report No. U2779
Requester Project No. 3M Tox 6295.13

Study Dates

Study Initiation: 10 June 1999
Sample Analysis Initiation: 22 June 1999
Sample Analysis Completion: 28 June 1999
Study Completion: At signing

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STUDY PERSONNEL AND CONTRIBUTORS

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Raymond G. York, Ph.D. DABT, *Study Director*

Sponsor

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

STATEMENT OF COMPLIANCE

Study Title: Analytical Laboratory Report on the Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in the Serum of Sprague-Dawley® Rats Exposed to Potassium Perfluorooctanesulfonate via Gavage

Study Identification Number: FACT Tox-108

This study was conducted in compliance with Food and Drug Administration Good Laboratory Practice (GLP) Regulations for Nonclinical Laboratory Studies [Data Requirement(s): 21 CFR (Part 58)], with the exceptions in the bulleted list below. In addition, the present study has been audited retrospectively by an independent quality assurance unit. Audit procedures and findings for audits performed at the 3M Environmental Laboratory and at participating contract laboratories are housed with documentation pertinent to this study in archives at the 3M laboratory and will be retained for a period not to exceed ten years. The analytical portion completed at the 3M Environmental Lab was performed in accordance with 3M Environmental Technology and Safety Services Standard Operating Procedures.

Exceptions to GLP compliance:

- Two separate study directors were assigned to the in-life phase and the analytical phase of this study
- The ABS final report does not have a Statement of Compliance
- The QAU statement in the ABS final report indicates compliance with EPA 40 CFR Part 792, rather than FDA 21 CFR Part 58
- Dose confirmation analyses were not conducted according to the GLP regulations; analytical method was not fully validated
- Not all raw data were verified by the group leader or designee

Kristen He — 11-Feb-00
Study Director — Date

Maureen T Case — 10 February 2000
Sponsor Representative — Date

GLP STUDY
QUALITY ASSURANCE STATEMENT

Study Title: Analytical Laboratory Report on the Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in the Serum of Sprague-Dawley® Rats Exposed to Potassium Perfluorooctanesulfonate via Gavage

Study Identification Number: FACT Tox-108

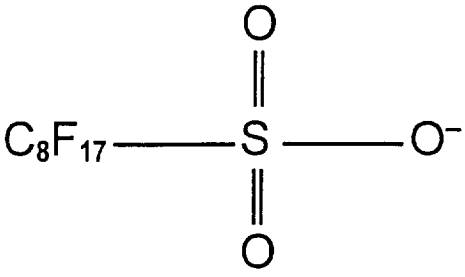
This study has been inspected by the 3M Environmental Laboratory Quality Assurance Unit (QAU) as indicated in the following table. The findings were reported to the study director and management.

INSPECTION DATES		PHASE	DATE REPORTED TO	
From	*To*		*Management*	*Study Director*
12-30-99	01-06-00	Draft Final Report	01-11-00	01-07-00

Kathleen M Stock — 2-11-00

QAU Representative — Date

INTRODUCTION



Perfluorooctanesulfonate (PFOS)

CAS Number: 2759-39-3

Chemical Formula: $C_8F_{17}SO_3^-$ Molecular Weight: 499

Purpose

The purpose of the study is to determine the presence and concentration of PFOS ($C_8F_{17}SO_3^-$) in rat serum samples collected during the study of F1 offspring of F0 generation female rats exposed to potassium perfluorooctanesulfonate via gavage.

Data quality objectives for this analytical study (outlined in the 3M Environmental Technology and Safety Services protocol for FACT Tox-108) were met.

Test System

In the present study, two groups of female rats were used as the test system. Group I consisted of control F0 female rats that were administered only Tween 80 (vehicle). Group II F0 female rats were administered 1.6 mg of potassium perfluorooctanesulfonate per kg of body weight/day in 0.5% Tween 80 (test article). The dose corresponds to a concentration of 0.32 mg/mL. Table 1 outlines the F0 generation female rat population demographics for study T-6295.13. Group II F0 female rats were treated with the test article 42 days prior to cohabitation, and through day 14 (pharmacokinetic subgroup) or day 21 postpartum (lactation and delivery). F1 male and female rat pups were not directly administered the test article, but were exposed to the chemical through maternal gestation (*in utero* exposure) and during lactation. F1 pups were identified as being from Group I or II. Cross fostering was randomized; half of the pups from each group were cross fostered with Group I or II females creating four F1 animal subgroups (A-D). The identification and cross-fostering procedure is documented in the in-life study report (Final Report 418-014 for study T-6295.13 page II-10). Various physical and biological parameters were monitored in test animals.

Table 1. F0 Generation Rat Population Demographics for Study T-6295.13

POPULATION	TOTAL	SELECTED FOR STUDY	SELECTED FOR CROSS FOSTERING
Initial Quantity Acclimated	86 virgin female rats	78 virgin female rats	—
Control (Group I)	42 female rats	42 mated female rats	25 mated female rats
Exposed or Treated (Group II)	36 female rats	36 mated female rats	25 mated female rats
Pharmacokinetic Sample Subgroup	10 female rats	10 mated females (8 control, 2 treated rats)	None

The test system species and strain selected was the Crl:CD®BR VAF/Plus® (Sprague-Dawley) rat received from Charles River Laboratories, Inc., permanently identified using a Monel® self-piercing ear tag. F0 generation rats were identified with ear tags. F1 generation rats were identified as part of either Group I or II litters, but were not individually tagged, and all parameters were evaluated in terms of the litter (dam and litter numbers are in Attachment E, Table 4). F0 virgin female rats were at least 60 days of age and weighed approximately 200–225 g when received (Weight data are included in Argus Research Laboratories, Inc., final report for study T-6295.13 on file at the 3M archives). Male rats of the same source and strain were used only as breeders and were not administered the test article or considered part of the test system.

Sample Collection and Analysis

F0 and F1 animals assigned to the control pool were culled on day 1 postpartum. On day 14 postpartum, samples were collected from the F0 mated female rats and F1 pups assigned to the pharmacokinetic subgroup. On day 22 postpartum, samples were collected from the remaining animals in the test system. All collected samples were immediately frozen on dry ice and maintained frozen (-70°C or below) until shipped to the 3M Environmental Laboratories.

In the analytical study reported here, sera samples collected from the population of exposed animals (generation F0) and their offspring (generation F1) were analyzed for the presence of PFOS.

Sera samples were extracted by a liquid-liquid extraction procedure using an ion pairing agent and ethyl acetate. The extracts were quantitatively analyzed using turbo ion spray liquid chromatography/mass spectrometry (LC/MS) in selected ion monitoring (SIM) mode, and PFOS levels were evaluated against extracted standards. Analytical details are included in this report; further details are available in the study binder maintained by the 3M Fluorine Analytical Chemistry Team (FACT); the binder is located in the 3M archives.

Analyses assessing the presence and concentration of PFOS in the sera of Sprague-Dawley rats were conducted in compliance with Good Laboratory Practice Regulations (21 CFR 58). Validated methods and standard operating procedures were followed during the preparation and analysis of the samples associated with this study.

SAMPLE RECEIPT

Samples were received from Argus Research Laboratories sporadically, during and at the end of the in-life phase of study, from November 1998, through February, 1999. Samples received were packaged in dry ice. Specimens were registered with the 3M Environmental Laboratory and transferred to a freezer for storage.

Samples of body tissues and fluids other than rat serum were collected and received from Argus Research Laboratories (Protocol 418-014, Study T-6295.13), but were not part of the scope of analysis in the protocol determined by the study director.

Specimens sent to Advanced BioAnalytical Services, Inc., (ABS) for analysis were received and tracked according to the Sample Tracking System Standard Operating Procedure. Specimens sent to ABS will be returned to the 3M Environmental Laboratory upon completion of analysis and submission of the subcontract laboratory(s) final report. Specimens will be maintained in the 3M Environmental Laboratory specimen archives. Sample receipt, identification, storage, and chain of custody protocols and data are located in the study binder for this report (FACT Tox-108); the binder is located in the 3M archives.

MATERIALS AND METHODS

Chemical Characterization

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

Table 2. Characterization and Treatment of the Test Substance, Control, and Reference Materials in Study FACT Tox-108

	ANALYSES PERFORMED AT ABS
Test Substance	Potassium Perfluorooctanesulfonate
Source	3M Specialty Chemicals
Preparation	Daily—Records maintained in the raw data
Maximum Storage Time	Expiration May 2000
Storage Conditions	Room temperature
Chemical Lot Number	217
Physical Description and Identity Analyses	FC-95, White powder
Purity	99.28%
Stability	48-hr data of prepared formulations on file with the sponsor
Control Material	Deionized Water
Source	J.T. Baker, Phillipsburg NJ
Preparation	Suspensions were prepared daily
Maximum Storage Time	Unknown
Storage Conditions	Room temperature
Chemical Lot Number	Tween® 80—M03H05, L06662, AND M29477
Physical Description and Identity Analyses	0.5% Tween® 80 in Reverse Osmosis Deionized Water
Purity	There are no known contaminants in the diet that would interfere with this study.
Stability	48-hr data of prepared formulations on file with the sponsor

Table 2. Characterization and Treatment of the Test Substance, Control, and Reference Materials in Study FACT Tox-108 (continued)

	ANALYSES PERFORMED AT ABS
Analytical Reference Material	PFOS (Perfluorooctanesulfonate)
Source	3M Specialty Chemicals
Preparation	Analytical procedure in the ABS final report (Attachment F)
Maximum Storage Time	Unused reference material will be retained for use by the 3M Environmental Laboratory and will be discarded when the quality of preparation no longer affords evaluation
Storage Conditions	Room temperature
Chemical Lot Number	171
Physical Description and Identity Analyses	Light-colored powder
Purity	99.49%
Stability	Undetermined

Dose analyses were performed on test substance samples taken at various times during the in-life phase of the study: data are located in the study binder for this report (FACT Tox-108) and are presented in Attachment D.

Reserve samples of control materials from the 3M Environmental Laboratory analyses will be stored at the 3M Environmental Laboratory for a period not to exceed 10 years following the effective date of the final test rule, or until the quality of the preparation no longer affords evaluation, as will any reserve samples of test substance returned from the in-life phase of the study. Reserve samples of control material from ABS will be stored at the subcontract laboratories in such a manner that the control materials will be preserved for use in ensuing studies.

Method Summaries

Following is a brief description of the methods used during this analytical study by ABS. Detailed descriptions of these methods are presented in Attachment C.

- ABS Preparatory and Analytical Method: Method Validation for the Quantitation of Perfluorooctanesulfonate (PFOS) in Rat Serum by Turbo Ion Spray LC/MS.

  Analytical standard stock solution, standard spiking solutions, internal standard stock solutions, internal standard working solutions, control blanks, quality control stock solutions, quality control samples, and study samples are prepared in dilutions with appropriate volumes of methanol or purified water in preparation for extraction. Tetrabutyl ammonium hydrogen sulfate (1mL of 0.5 molar solution) is added to all quality control and study samples, along with 2mL of 0.25 molar sodium carbonate/ 0.25 molar sodium bicarbonate. Water (500µL) is added to the control blank, and 500µL of the internal standard working solution is added to all other tubes. Five mL of ethyl acetate is then added to each tube, and the tubes are placed on a reciprocal shaker at medium speed for 20 minutes. The tubes are then centrifuged, and the organic layer is transferred to a clean polypropylene tube. The organic layer is evaporated to dryness in a TurboVap™ at about 20°C under nitrogen. The dried extracts are then reconstituted with acetonitrile and water, and 200µL of the extract is transferred to an autosampler tube for analysis.

Equipment used at ABS:

LC/MS/MS System:

Autosampler: Waters 717plus
Mass spectrometer: PE SCIEX API 365 with TurboIonSpray™ interface
Analytical column: Keystone Betasil™ C_{18}, 2.1x50 mm (5µm)
Mobile phase components:
Component A—10:90 methanol:2 mM ammonium acetate
Component B—90:10 methanol:2 mM ammonium acetate
Injection volume: 3-5µL
Column temperature: Ambient
Curtain gas: UHP nitrogen
Nebulizer gas: UHP nitrogen
Ion spray voltage: -3000 V
Declustering potential: -35 V
Negative ions monitored:
m/z=499.0 for PFOS $[M-K]^-$
m/z=427.0 for tetra-H-PFOS $[M-H]^-$
Total run time: 8.0 minutes

Protocol Deviations

There were no deviations from the original protocol.

DATA SUMMARY, ANALYSES, AND RESULTS

Summary of Quality Control Analyses Results (ABS)

- The inter-assay precision (relative standard deviation–RSD) data from daily quality control samples ranged from 1.57–4.01% for PFOS, and the inter-assay accuracy (relative error–RE) ranged from -7.32–10.1%. Both are acceptable ranges.
- The inter-assay precision (RSD) of standards ranged from 1.11–5.83% for PFOS. The inter-assay accuracy (RE) from standards ranged from -5.86–11.0%. All are acceptable ranges.
- Coefficients of determination were ≥0.9954.
- Refer to the ABS final report (Attachment F, Table 2) for precision and accuracy data on the analyses of quality control partial volume (one-to-ten dilutions).

Summary of Sample Results (ABS)

Following is information regarding sample analyses in the present study:

- The samples in analytical run numbers 1 and 3 were reassayed because the measured concentrations exceeded the calibration range and upper limit of quantitation (ULQ) (refer to Table 6 in attachment F). Smaller volumes were reextracted from the samples and reanalyzed. Reassayed samples resulted in measured concentrations within the calibration range, and the data were reported (refer to Table 6 in attachment F).

Original data or copies thereof are available at the 3M Environmental Laboratory.

STATISTICAL METHODS

At ABS, data from peak areas were integrated by the PE SCIEX program MacQuan™, version 1.4, on a Macintosh computer. Following peak area integration, MacQuan results data were converted to text files, moved to the ABS file server, and analyzed with the Watson™ software package (version 5.3.1.01 from PSS, Inc., Wayne, PA). The Watson analysis applied a weighted ($1/y^2$) quadratic regression to the data, and calculations were performed on unrounded numbers; however, standard and quality control data were rounded to three significant figures prior to reporting the data.

DATA QUALITY OBJECTIVES AND DATA INTEGRITY

No circumstances existed during the present study that would have affected the quality or integrity of the data. The following data quality objectives (DQOs) were followed during the study:

- Linearity—The coefficient of determination (r^2) of the standard curve was equal to or greater than 0.98
- Limits of Quantitation (LOQ)—Equal to the lowest acceptable standard in the calibration curve
- Duplicate acceptable precision— <30%
- Spike acceptable recoveries— 70% to 130%
- Use of confirmatory methods—no confirmatory methods were used
- Demonstration of specificity—specificity was demonstrated by chromatographic retention time, mass spectral [M-K] product ion characterization

Assuming that spike recovery studies form a suitable indication of endogenous analyte recovery, data are quantitative to ± 30%. The validity of this assumption has not been verified by other techniques. In serum analyses, the limit of detection (LOD) for PFOS is approximately 1.75ng/mL and the limit of quantitation (LOQ) is 0.05µg/mL PFOS.

STATEMENT OF CONCLUSION

Statement of Conclusion:

Under the conditions of the present studies, PFOS was observed in the sera of F0 female rats exposed during the in-life phase of the study. Additionally, PFOS was observed in sera tissues taken from F1 generation pups from female rats exposed to the test substance, and in F1 generation pups exposed to the test substance via lactation, but not exposed *in utero*.

REFERENCES

None.

ATTACHMENTS

- Attachment A: Sample Receipt and Chain of Custody Documentation
- Attachment B: Protocol
- Attachment C: Extraction and Analytical Methods
- Attachment D: Dose Confirmation Analyses
- Attachment E: Data Summary and Tables
- Attachment F: Analytical Reports from Contributing Laboratories
- Attachment G: Report Signature Page

ATTACHMENT A
SAMPLE RECEIPT AND CHAIN OF CUSTODY DOCUMENTATION

Sample receipt and chain of custody information is documented in the binder for this report (FACT Tox-108); the binder is located in the 3M archives.

ATTACHMENT B
PROTOCOL

3M

Study Title

Oral (Gavage) Cross-Fostering Study of PFOS in Rats

PROTOCOL

Author
Lisa Clemen

Date:
June 8, 1999

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

Laboratory Project Identification
FACT-TOX-108
U2779

Study Identification

Oral (Gavage) Cross-Fostering Study of PFOS in Rats

Test Material	Perfluorooctane sulfonic acid potassium salt (T-6295)
Sponsor	3M Toxicology Services - Medical Department 3M Center, Building 220-2E-02 St. Paul, MN 55144-1000
Sponsor Representative	Marvin T. Case, D.V.M., Ph.D. 3M Toxicology Services Telephone: 651-733-5180 Facsimile: 651-733-1773
Study Director	Kristen J. Hansen, Ph.D. 3M Environmental Technology and Safety Services Building 2-3E-09 651-778-6018
Study Location(s)	
In vivo Testing Facility	Argus Research Laboratories, Inc. 905 Sheehy Drive, Building A Horsham, PA 19044
Analytical Testing Laboratory	3M Environmental Laboratory Building 2-3E-09 935 Bush Avenue St. Paul, MN 55106

Sub-Contract Laboratory	Advanced Bioanalytical Services, Inc. 15 Catherwood Road Ithaca, NY 14850 Battelle Memorial Institute 505 King Avenue Columbus, Ohio 43201-2693
Proposed Study Timetable	
Study Initiation Date	June 08, 1999
Study Completion Date	June 08, 2000

1. STUDY

Oral (gavage) cross-fostering study of potassium perfluorooctane sulfonic acid (PFOS) in rats.

2. PURPOSE

This analytical study is designed to determine levels of potassium perfluorooctanesulfonate (PFOS) in specimens of liver and serum of rats. The in-life portion of this study was conducted at Argus Research Laboratories, study #418-014. All serum samples will be extracted and analyzed at Advanced Bioanalytical Services, Inc. and all liver samples extracted and analyzed at Battelle Memorial Institute. Additional analyses may be performed at the 3M Environmental Laboratory as methods are developed and validated. If additional analyses are performed an amendment to this protocol will be written.

3. REGULATORY COMPLIANCE

This study will be conducted in accordance with the United States Food and Drug Administration, Good Laboratory Practices Standards, Final Rule 21 CFR 58, with the exception that analysis of the test material mixture for concentration, solubility, homogeneity, and stability will not be conducted, and is the responsibility of the Sponsor.

4. QUALITY ASSURANCE

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

The QA Unit at the sub-contract laboratory will audit their study conduct, data, and results report prior to submitting to the 3M Environmental Laboratory.

5. TEST MATERIAL

5.1 Refer to Argus Research Laboratory protocol for study #418-014.

6. CONTROL MATRICES

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

6.2 *Source* Argus Research and/or Sigma Chemical

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

6.4 *Purity and Stability* Not applicable

6.5 *Storage Conditions* Frozen at -20 °C ± 10 °C or –50 °C ± 10 °C

6.6 *Reserve Matrix* A portion of the control matrix will be retained in the 3M archives for as long as the quality of the preparation affords evaluation, but not longer than ten years following the effective date of the final test rule (if applicable).

6.7 *Disposition* Matrices will be retained at the 3M Environmental Laboratory per GLP regulation. Certain matrices (feces, urine, and blood) may be disposed after QAU verification.

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

7. REFERENCE MATERIAL

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

7.2 *Source* 3M Specialty Chemicals

7.3 *Physical Description* White powder

7.4 *Purity and Stability* Purity of PFOS is 99% or greater. Stability has not been determined.

7.5 *Storage Conditions* Room temperature

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

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

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

8. TEST SYSTEM

Female rats were used as the test system and were maintained and dosed as described in Argus Research protocol #418-014. Group 1 control animals did not receive the test substance while the group 2 treated animals received the test substance at a concentration of 1.6 mg/kg/day for 6 weeks. Refer to Argus Research protocol #418-014 for tabular presentation of data. Dosing of the female animals in group 2 was continued during mating, gestation, and lactation. Cross-fostering of the pups born to the control dams were randomized, half of the pups were placed on control dams while the other half were placed on the PFOS dosed dams. Likewise, the same was done with the group 2 pups. Each of these pups will be marked to identify whether they are from control or PFOS dosed dams.

9. SPECIMEN AND SAMPLE RECEIPT

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

Body tissue/fluid	Collected	Expected # of specimens
Serum – Dam and Pup animals	At lactation day 14 and termination of the study	60 Dam and 34 Pup
Urine and feces – Dam animals	After termination of the study	48 Urine and 48 Feces
Liver – Dam and Pup animals	At lactation day 14 and termination of the study	60 Dam and 34 Pup
Milk Secreting Glands – Dam animals	At lactation day 14 and termination of the study	58
Milk – Dam animals	At lactation day 14	10

Total number of expected specimens: 352
Total number of test animals: 36
Total number of control animals: 42

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

10. PREPARATORY METHODS

10.1 FACT-M-1.1, Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactant from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry

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

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

10.4 If analyses are sub-contracted to other laboratories, an amendment will be written to include their methods and copies of each method will be attached to this protocol.

11. ANALYTICAL METHODS

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

11.2 ETS-8-5.1, Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemicals in Serum or Other Fluid Extracts Using HPLC-Electrospray/Mass Spectrometry

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

11.4 If analyses are sub-contracted to other laboratories, an amendment will be written to include their methods and copies of each method will be attached to this protocol.

12. DATA QUALITY OBJECTIVES

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

12.1 *Linearity* $r^2 \geq 0.98$

12.2 *Limits of detection / quantitation*

12.2.1 Method Detection Limit (MDL) for PFOS

a) Serum: 1.75 ppb

b) Liver: 15 ppb

12.2.2 Limit of Quantitation (LOQ) – Equal to the lowest acceptable standard in the calibration curve

12.3 Duplicate acceptable precision < 30% for the method

12.4 Spike acceptable recoveries 70% - 130%

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

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

13. Sub-Contracted Analysis

13.1 All analyses as detailed in this protocol will be performed at 3M Environmental Laboratories, Building 2-3E-09, 935 Bush Avenue, St. Paul, MN 55106, at Advanced Bioanalytical Services, Inc., 15 Catherwood Road, Ithaca, NY 14850, or at Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio 43201-2693.

13.2 An amendment to this protocol will be written if analyses are performed at laboratories other than 3M Environmental Laboratories, Advanced Bioanalytical Services, Inc., or Battelle Memorial Institute.

14. Statistical Analysis

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

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

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

15. Report

A report containing all the results of the study will be prepared by the 3M Environmental Laboratory. If analyses are sub-contracted to other laboratories, each laboratory will prepare a report and submit it to the 3M Environmental Laboratory for inclusion in the 3M Environmental Laboratory 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 changes in the original protocol

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

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

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

15.8 A description of the test system

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

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

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

15.12 Statistical methods used to evaluate the data, if applicable

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

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

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

If it is necessary to make corrections or additions to a 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 report that is being amended, the reasons for the amendment, and will be signed by the Study Director.

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

Original data, or copies thereof, will be available at 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 those items listed below, will be retained in the archives of 3M Environmental Laboratory for at least a period of time as specified by regulation, and as established by 3M Environmental Laboratory Standard Operating Procedures.

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

16.1.1 Approved protocol and amendments

16.1.2 Study correspondence

16.1.3 Shipping records

16.1.4 Raw data

16.1.5 Approved final report (original signed copy)

16.1.6 Electronic copies of data

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

16.2.1 Training records

16.2.2 Calibration records

16.2.3 Instrument maintenance logs

16.2.4 Standard Operating Procedures, Equipment Procedures, and Methods

17. SPECIMEN RETENTION

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

18. PROTOCOL AMENDMENTS AND DEVIATIONS

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

19. ATTACHMENTS

19.1 Attachment A Preparatory and analytical methods

20. SIGNATURES

Marvin T Case 9 June 1999

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

Kristen Hz 6/10/99

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

Study Title

Oral (Gavage) Cross-Fostering Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 1

Amendment Date:

August 12, 1999

Performing Laboratory

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

Laboratory Project Identification

ET&SS FACT-TOX108
LIRN U2779

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

1. ***PROTOCOL READS:*** Section 2.0 states that all liver samples will be extracted and analyzed at Battelle Memorial Institute.

AMEND TO READ: All liver samples will be extracted and analyzed at the 3M Environmental Laboratory.

REASON: Due to time constraints, the liver extraction and analysis will be performed at the 3M Environmental Laboratory.

2. ***PROTOCOL READS:*** Section 10.0 and 11.0 list the following methods to use for extraction and analysis:

FACT-M-1.1 "Extraction of Potassium Perfluorooctanesulfonate or Other Anionic Fluorochemical Surfactant from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"
FACT-M-2.1 "Analysis of Fluorochemicals in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

AMEND TO READ: The extraction and analytical methods to follow at the 3M Environmental Laboratory are:

ETS-8-6.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Liver for Analysis Using HPLC-Electrospray/Mass Spectrometry"
ETS-8-7.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"

REASON: The extraction and analytical methods FACT-M-1.1 and FACT-M-2.1, respectively, were updated on 07/22/99 to ETS-8-6.0 and ETS-8-7.0. These methods were updated to replace the extraction solvent ethyl acetate with a different extraction solvent MTBE (methyl *tert* butyl ether), POAA and Monoester were removed from the standard mix, and M556 was added to the standard mix.
The analytical method was updated to include linear regression with 1/x weighting and a few minor changes in the HPLC 1100 instrument parameters.

3. ***PROTOCOL READS:*** Section 10.4 and 11.4 state that if analyses are sub-contracted to other laboratories an amendment will be written to include their methods.

AMEND TO READ: The extraction and analytical methods to follow at Advanced Bioanalytical Services will be attached to the protocol.

REASON: The analytical methods at the sub-contract laboratory were not included in the original protocol.

4. ***PROTOCOL READS:*** Section 12.2.1 b) lists the liver method detection limit as 15 ppb.

AMEND TO READ: The liver method detection limit is 8.50 ppb (ng/g).

REASON: The validation supporting methods ETS-8-6.0 and ETS-8-7.0 includes a lower method detection limit for PFOS.

Amendment Approval

Marvin T Case — 17 August 1999

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

Kris J. Hansen — 8/18/99

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

Study Title

Oral (Gavage) Cross-Fostering Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 2

Amendment Date:

September 30, 1999

Performing Laboratory

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

Laboratory Project Identification

ET&SS FACT-TOX108
LIRN U2779

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

1. ***PROTOCOL READS:***
Section 2.0 states that all liver samples will be extracted and analyzed at the 3M Environmental Laboratory.

AMEND TO READ:
No liver samples will be extracted and analyzed.

REASON:
Liver results are no longer required.

2. ***PROTOCOL READS:***
Section 16 states that 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, electronic copies of data, training records, calibration records, instrument maintenance logs and standard operating procedures, equipment procedures, and methods will be retained in the archives of the 3M Environmental Laboratory.

AMEND TO READ:
Section 16 states that the original data, or copies thereof, will be available at the 3M Environmental Laboratory to facilitate audits of the study during its progress and before acceptance of the final report. When the final report is completed, all original paper data, including: approved protocol and amendments, study correspondence, shipping records, raw data, approved final report, and electronic copies of data will be retained in the archives of the 3M Environmental Laboratory. All corresponding training records, calibration records, instrument maintenance logs, standard operating procedures, equipment procedures, and methods will be retained in the archives of the facility performing each analysis.

REASON:
Clarification of the disposition of archived records if analyses are performed at a sub-contract laboratory.

3. ***PROTOCOL READS:***
Section 17 states that specimens will be maintained in the 3M Environmental Laboratory specimen archives.

AMEND TO READ:
Specimens will be maintained in the 3M Environmental Laboratory specimen archives. All specimens sent to sub-contract laboratories will be returned to the 3M Environmental Laboratory upon completion of analysis and submission of the sub-contract laboratory(s) final report. The specimens will be returned with the following documentation: the signed original chain of custody and records of storage conditions while at the sub-contract facility.

REASON:
Clarification of the disposition of specimens and documentation for analyses performed by a sub-contract laboratory.

Amendment Approval

Marvin T Case — 4 Oct 1999
Marvin Case, D.V.M., Ph.D., Sponsor Representative — Date

Kristen Hansen — 10/5/99
Kristen J. Hansen, Ph.D., Study Director — Date

ATTACHMENT C
EXTRACTION AND ANALYTICAL METHODS

ADVANCED
BIOANALYTICAL
SERVICES, INC.

METHOD VALIDATION REPORT

TITLE: METHOD VALIDATION FOR THE QUANTITATION OF PERFLUOROOCTANESULFONATE (PFOS) IN RAT SERUM BY TURBO ION SPRAY LC/MS

DATE: 26 August 1999

REPORT: 99VDJA01.MI.DOC

AUTHORS: David J. Anderson, M.S.
Amie J. Prince, B.S.
Holly D. Ross, M.S.

PREPARED FOR: 3M Environmental Technology and Safety Services
St. Paul, MN 55133-3331

NUMBER OF PAGES: 50

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AUTHORS: **David J. Anderson, M.S.**
Amie J. Prince, B.S.
Holly D. Ross, M.S.

FOR: **3M Environmental Technology and Safety Services**

DATE: **26 August 1999**

TITLE: **METHOD VALIDATION FOR THE QUANTITATION OF PERFLUOROOCTANESULFONATE (PFOS) IN RAT SERUM BY TURBO ION SPRAY LC/MS**

ABSTRACT

A sensitive, specific, accurate, and reproducible analytical method was developed by Advanced BioAnalytical Services, Inc., Ithaca, New York to quantitate perfluorooctanesulfonate (PFOS) in rat serum samples. Serum samples (50 µL) were extracted by a liquid-liquid extraction procedure to isolate the analyte from rat serum. Sample extracts were reduced to dryness, reconstituted, and analyzed by turbo ion spray liquid chromatography/mass spectrometry (LC/MS) in the negative ion mode. The assay demonstrated a lower limit of quantitation (LLQ) of 0.05 µg/mL using 50-µL sample aliquots. The calibration curves were fit from 0.05 µg/mL to 20 µg/mL for PFOS by a weighted ($1/y^2$) quadratic equation. The coefficients of determination of the calibration curves ranged from 0.9962 to 0.9965.

Precision and accuracy quality control (QC) samples were prepared at concentrations of 0.2, 6, and 18 µg/mL PFOS. Quality control (QC) samples were prepared at a concentration of 100 µg/mL PFOS for partial volume analysis. The intra- and inter-assay precision (RSD) results calculated from all QC samples ranged from 1.92% to 4.87% for PFOS. The intra- and inter-assay accuracies (RE) calculated from QC samples ranged from -3.58% to 5.58% for PFOS. The mean extraction recoveries were from 87.6% to 100% for PFOS and 89.9% for the internal standard (IS).

PFOS was measured as stable in rat serum for up to 24 hours at ambient temperature. PFOS was measured as stable in rat serum at –20 °C, currently for up to 33 days, and

after three freeze/thaw cycles. Reliable results were obtained for sample extracts reinjected 27 hours after initial reconstitution.

QAU STATEMENT

Periodic inspections of the method validation for the quantitation of PFOS in rat serum were conducted by the Quality Assurance Unit of Advanced BioAnalytical Services (ABS) for compliance with EPA GLP regulations (40 CFR Part 792).

The study was inspected on the following dates:
13, 21 May 1999; 7, 8, 14, 15 June 1999; 1, 2 July 1999; 3, 4 August 1999.

Results of the inspections were reported to ABS Management on:
13, 21 May 1999; 7, 8, 14, 15 June 1999; 1, 2 July 1999; 3, 4 August 1999.

Results of the inspections were reported to the Study Director on 5 August 1999.

Based on the inspections and the data reviewed, this report is a complete and accurate representation of the data.

Kathleen Cormack
Kathleen Cormack, B.S.
Quality Auditor

26 Aug 99
Date

SIGNATURE PAGE

TITLE: METHOD VALIDATION FOR THE QUANTITATION OF PERFLUOROOCTANESULFONATE (PFOS) IN RAT SERUM BY TURBO ION SPRAY LC/MS

Report Number: 99VDJA01.MI.DOC

Reported by: _____ 26 August 1999
David J. Anderson, M.S. Date
Research Scientist

Reviewed by: Kathleen Cormack 26 Aug 99
Kathleen Cormack, B.S. Date
Associate Auditor

Authorized for Release by: _____ 26 AUGUST 99
John R. Perkins, Ph.D. Date
Assistant Scientific Director

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1. INTRODUCTION

The purpose of this report is to describe the method validation for the quantitative determination of perfluorooctanesulfonate (PFOS) in rat serum samples after modification of a previous method (1). The objectives of the method validation were to validate a simple extraction procedure, determine the lower limit of quantitation for routine analysis, determine the extraction recoveries of the analyte and internal standard (IS), determine the analyte stability in rat serum at ambient temperature, long-term freezer storage, and over three freeze/thaw cycles, and to provide specific, accurate, and reproducible quantitative results by turbo ion spray liquid chromatography/mass spectrometry (LC/MS).

2. EXPERIMENTAL

2.1. CHEMICALS AND MATERIALS

Perfluorooctanesulfonate (PFOS, Lot# 171) was obtained from 3M, Inc. The internal standard (IS) for PFOS was 1H,1H,2H,2H-perfluorooctane sulfonic acid (Tetra-H-PFOS). The internal standard (Lot# 59909) was obtained from 3M, Inc. A detailed list of chemicals and materials is found in Appendix A.

Stock and working solutions which were used to prepare the calibration curves for the analytes were prepared as described in Appendix A. Stock solutions used in the preparation of quality control (QC) samples were prepared separately from those used in preparation of the calibration curves.

Preparation of all solutions used during extraction and analysis are described in Appendix A.

2.2. LC/MS INSTRUMENTATION

The liquid chromatography/mass spectrometry system consisted of two LC-10AD pumps (Shimadzu, Columbia, MD 21046), a SCL-10A pump controller (Shimadzu,

Columbia, MD 21046), a WISP 717plus autosampler (Waters Associates, Millipore Corporation, Milford, MA 01757), a Betasil C_{18} (2 x 50 mm, 5 μm) column (Keystone Scientific, Inc., Bellefonte, PA 16823), and a PE SCIEX API 365 mass spectrometer (PE SCIEX, Concord, Ontario). A detailed list of the instrumentation and instrument conditions is found in Appendix A.

2.3. SAMPLE PREPARATION AND EXTRACTION PROCEDURE

For each analytical run (tray), duplicate 50-μL aliquots of the calibration curve samples were prepared as described in Appendix A. The nominal (theoretical) concentrations of PFOS in the calibration curves were 0.05, 0.1, 0.25, 0.5, 1, 2, 4, 8, 16, and 20 μg/mL.

Rat serum quality control samples were prepared in advance of the validation study at nominal (theoretical) concentrations of 0.2, 6, and 18 μg/mL for QC1, QC2, and QC3, respectively, as detailed in Appendix A. A dilution QC (QC4, 100 μg/mL) was prepared at a concentration exceeding the upper limit of the calibration curve range (20 μg/mL), and was assayed using a 10-fold dilution for partial volume analysis.

Calibration standards and QC samples were extracted by the procedure detailed in Appendix A.

2.4. RAT SERUM VALIDATION DATA

The data were collected using selected ion monitoring (SIM) turbo ion spray LC/MS in the negative ion mode. Peak areas were integrated by the PE SCIEX program MacQuan, version 1.4, residing on a Macintosh computer. Following peak area integration, the results tables from MacQuan were saved as text files and uploaded to the Advanced BioAnalytical Services (ABS) file server where a weighted ($1/y^2$) quadratic regression was performed using the software package Watson v 5.3.1.01 (PSS, Inc., Wayne, PA 19087). All data were rounded to no less than three significant figures by ABS prior to reporting in Tables 1 through 11. The data for the rat serum validation are stored in ABS Notebook 2304.

All calculations were based on the peak area ratio of the analyte to the internal standard. Concentrations of each analyte in quality control samples were determined by inverse prediction from the calibration curve.

2.5. ASSAY EVALUATION

2.5.1. Intra- and Inter-Assay Accuracy

The intra- and inter-assay accuracy of the method was assessed by determining the relative error observed in the analysis of quality control samples. The mean concentration for each quality control level was divided by the theoretical concentration. One was subtracted from the result, converted to percent and expressed as RE. QC1 through QC3 were assayed in replicates of five. In addition, the RE was reported for standards at all levels over three runs.

2.5.2. Intra- and Inter-Assay Precision

The intra- and inter-assay precision of the method was assessed by determining the Relative Standard Deviation (RSD) observed for quality control sample data. The mean concentration and RSD were calculated for the first run and over three runs for intra-assay and inter-assay precision, respectively. QC1 through QC3 were assayed in replicates of five. In addition, the RSD was reported for standards at all levels over three runs.

2.5.3. Partial Volume Analysis

The effect of dilution on the analysis of PFOS in rat serum was determined by partial volume analysis. QC4 (100 μg/mL) was prepared containing PFOS at approximately five times the upper limit of quantitation (ULQ). Five replicates of QC4 were diluted ten-fold and analyzed. The RSD and RE were reported.

2.5.4. Lower Limit of Quantitation (LLQ)

Rat serum control samples from six separate individuals were spiked with PFOS at a concentration of 0.05 µg/mL. The LLQ was determined by obtaining the back-calculated concentrations from these control samples. The overall mean, RSD, and RE were also calculated.

2.5.5. Selectivity

The selectivity of the assay was determined by LC/MS. To monitor for interference from the biological matrix, rat serum samples containing neither the analyte nor the internal standard (control blank) were assayed with all experiments.

2.5.6. Carryover Evaluation

The carryover of analyte from one injection to the next was assessed by analyzing a control blank injected immediately after a high calibration standard (STD10, 20 µg/mL).

2.6. STABILITY OF PFOS IN QUALITY CONTROL SAMPLES

QC1 through QC3 were used to determine the stability of the analyte during sample storage, extraction, and analysis.

2.6.1. Ambient-Temperature Stability of PFOS in Rat Serum

The stability of PFOS was evaluated by storing QC samples at each concentration level at ambient temperature (ca. 25 °C) for nominal timepoints of 0, 2.5, 6, and 24 hours after thawing. All QCs were assayed in replicates of five.

2.6.2. Freezer Stability of PFOS in Rat Serum at –20 °C

The freezer stability of PFOS was evaluated by storing QC samples at each concentration level at –20 °C for 7, 13, and 33 days. Additional freezer stability timepoints will be reported in an addendum to this report. All QCs were assayed in replicates of four.

2.6.3. Freeze/Thaw Stability in Rat Serum

The stability of PFOS was evaluated after three freeze/thaw cycles. The mean concentrations of the QC samples after three freeze/thaw cycles (i.e., at least 2 hours frozen storage at the nominal temperature of –20 °C followed by thawing at ambient temperature for 30 minutes) were compared to the mean concentrations of freshly thawed QC samples. All QCs were assayed in replicates of five.

2.7. Reproducibility of Reinjecting Extracted Samples

The reproducibility of reinjecting reconstituted serum extracts was investigated by reinjecting a set of previously-assayed standards and QC samples which had been stored after injection at approximately 25 °C for 27 hours. The RE of the QC samples was used to assess processed sample stability in the reconstitution solution. All QCs were assayed in replicates of five.

2.8. Extraction Recovery

The extraction recovery of PFOS from rat serum was determined by comparing the peak area ratio (PAR) of samples (0.25, 4, and 16 µg/mL) spiked after extraction (post-extract) with the PAR of samples spiked before extraction (pre-extract). The internal standard was spiked post-extraction for all samples. The recovery of the internal standard (4 µg/mL) was assessed following a similar approach using PFOS as the reference. The extraction recovery (% Recovery) was determined by dividing the pre-extract PAR by the post-extract PAR and expressing the result as a percentage. Five replicates were used at each concentration level.

3. RESULTS AND DISCUSSION

A quantitative analytical procedure using turbo ion spray LC/MS in the negative ion mode was developed to meet the high sensitivity, specificity, and reproducibility requirements for the determination of PFOS in rat serum. The full-scan single MS mass spectrum of PFOS showed an abundant $[M-K]^-$ ion at m/z = 499 (Figure 1). The full-scan single MS mass spectrum of Tetra-H-PFOS showed an abundant $[M-H]^-$ ion at m/z = 427 (Figure 2).

The following selected ion monitoring (SIM) was used to quantify the analytes in rat serum:

PFOS	m/z = 499.0
Tetra-H-PFOS (IS)	m/z = 427.0

The peak labeling of full-scan data does not accurately represent the performance of the instrument in SIM mode. The SIM ions were derived from separate experiments using narrow-range scanning, which more accurately depicts the operation of the instrument in the SIM mode. The mass chromatograms of a representative control blank rat serum extract are shown in Figure 3. Mass chromatograms from a representative control rat serum extract containing only the internal standard (zero sample), are shown in Figure 4.

Figures 5 and 6 are mass chromatograms of representative extracts from calibration standards 1 and 10, respectively.

3.1. ASSAY EVALUATION RESULTS

3.1.1. Intra- and Inter-Assay Accuracy

The intra-assay accuracy (RE) data from QC samples ranged from -2.35 to 5.58% for PFOS (Table 1). The inter-assay accuracy ranged from -3.58 to 4.95% for PFOS (Table 2). These data indicate acceptable intra- and inter-assay accuracy for the determination of PFOS in rat serum.

3.1.2. Intra- and Inter-Assay Precision

The intra-assay precision data (RSD) ranged from 1.99 to 3.28% for PFOS at all QC concentration levels (Table 1). The inter-assay precision results from QC samples ranged from 1.92 to 4.87% for PFOS (Table 2). The RSD ranged from 0.613 to 4.38% for calibration standards at all levels (Table 3). These data indicate acceptable intra-and inter-assay precision for the determination of PFOS in rat serum.

3.1.3. Linearity

The calibration curves were fit by a weighted ($1/y^2$) quadratic regression. Coefficients of determination (r^2) were ≥0.9962 for PFOS in rat serum. The calibration curve statistics are shown in Table 4.

3.1.4. Partial Volume Analysis

The results of partial volume analysis of QC4 is shown in Table 5. The precision (RSD) of QC4 samples diluted 1 in 10 was 2.71%. The accuracy was 5.89%. These data indicate acceptable accuracy and precision for partial volume analysis for the determination of PFOS in rat serum.

3.1.5. Lower Limit of Quantitation (LLQ)

Table 6 shows the lower limit of quantitation (LLQ) data. The serum LLQ experiment demonstrated that 0.05 µg/mL is an acceptable LLQ for PFOS. The precision (RSD) was 4.95% for PFOS. The RE was 4.57%.

3.1.6. Selectivity

The assay was specific for PFOS. No chromatographic interferences were observed in any of the control serum samples analyzed (Figure 3). The control blanks and zero samples did show some evidence of small chromatographic peaks at the retention times of the analyte. These peaks were not quantifiable as they were below the lower limit of quantitation (LLQ).

3.1.7. Carryover Evaluation

Carryover of PFOS and the IS was evaluated by injection of an extracted rat serum control blank following an injection of an extracted high standard (STD 10). The response for the small chromatographic peak at the retention time for PFOS was comparable to the response of a rat serum control blank injected before the high standard. Thus, carryover is negligible for PFOS. There was no evidence of carryover for the internal standard.

3.2. Stability of PFOS in Quality Control Samples

3.2.1. Ambient Temperature Stability of PFOS in Rat Serum

The results of the ambient stability of PFOS in QC samples are shown in Table 7. The mean predicted concentrations deviated from -4.45 to 10.5% from the 0-hour values for all QC levels and time points. Based on these data, PFOS was stable in rat serum for up to 24 hours at ambient temperature.

3.2.2. Freezer Stability of PFOS in Rat Serum at –20 °C

The results of the long-term stability of PFOS in QC samples stored at –20 °C are presented in Table 8. The PFOS concentrations deviated from 0-hour values from -2.76 to 12.3% for all QC levels stored up to 33 days.

Results of freezer storage after two, four, and eight months will be reported as an addendum to this report.

3.2.3. Freeze/Thaw Stability of PFOS in Rat Serum

The results of the freeze/thaw stability of PFOS in QC samples after three freeze/thaw cycles is shown in Table 9. PFOS was stable after three freeze/thaw cycles with deviations ranging from –1.38% to 10.1% from the 0-cycle samples for all QC levels (Table 9).

3.3. Reproducibility of Reinjecting Extracted Samples

The results of reinjecting reconstituted samples containing PFOS after storage for 27 hours at approximately 25 °C in reconstitution solution are shown in Table 10. Reinjecting processed samples after 27 hours in reconstitution solution was appropriate with RE values ranging from -6.33 to 4.59% at 27 hours for all QC levels.

3.4. Extraction Recovery

The mean recoveries of PFOS and Tetra-H-PFOS are shown in Table 11. The mean recoveries ranged from 87.6 to 100% for PFOS. The mean recovery for Tetra-H-PFOS was 89.9%.

4. CONCLUSIONS

The turbo ion spray LC/MS assay procedure for the determination of PFOS in rat serum has proven to be sensitive, specific, accurate, and reproducible. Its high sensitivity allows reliable and reproducible quantitation of PFOS down to a level of 0.05 μg/mL in rat serum based on 50-μL samples.

5. DATA RETRIEVAL

The data for the rat serum validation are stored in ABS Notebook 2304 and in the ABS Archives.

6. REFERENCES

1. Advanced BioAnalytical Services, Inc. Report 98AGKP02.MMM. Analytical Report for the Determination of Perfluorooctanoate and Perfluorooctanesulfonate in Human Serum by LC/MS. Grace K. Poon, Ph.D., David Hardwick, B.S., and Ellen Pace, M.A.T., 6 February 1998.

7. TABLES

Table 1: Intra-Assay Accuracy and Precision of the PFOS Assay in Rat Serum for QC Samples

	PFOS Concentration (µg/mL)		
	QC1	QC2	QC3
Theoretical Conc.	0.2	6	18
Run 9	0.210	6.52	17.9
	0.202	6.31	18.0
	0.206	6.17	16.7
	0.205	6.32	18.0
	0.194	6.35	17.3
Mean	0.204	6.34	17.6
RSD (%)	2.93	1.99	3.28
RE (%)	1.77	5.58	-2.35

RSD = (SD/Mean) x 100

RE = [(Mean/Theoretical)-1] x 100

Table 2: Inter-Assay Accuracy and Precision of the PFOS Assay in Rat Serum for QC Samples from Three Validation Runs

	PFOS Concentration (µg/mL)		
	QC1	QC2	QC3
Theoretical Conc.	0.2	6	18
Run 9	0.210	6.52	17.9
	0.202	6.31	18.0
	0.206	6.17	16.7
	0.205	6.32	18.0
	0.194	6.35	17.3
Run 10	0.187	6.44	16.9
	0.186	6.44	17.3
	0.182	6.32	17.5
	0.189	6.19	18.0
	0.182	6.31	17.4
Run 11	0.194	6.17	18.2
	0.197	6.13	17.1
	0.184	6.41	17.6
	0.192	6.15	17.7
	0.184	6.24	17.0
Mean	0.193	6.30	17.5
RSD (%)	4.87	1.92	2.66
RE (%)	-3.58	4.95	-2.73

RSD = (SD/Mean) x 100
RE = [(Mean/Theoretical)-1] x 100

Table 3: Accuracy and Precision of the PFOS Assay in Rat Serum for Calibration Standards from Three Validation Runs

	PFOS Calibration Standard Concentration (µg/mL)									
Theoretical Conc.	STD1 0.05	STD2 0.1	STD3 0.25	STD4 0.5	STD5 1	STD6 2	STD7 4	STD8 8	STD9 16	STD10 20
Run 9	0.0483	0.0972	0.251	0.480	0.942	2.10	4.48	7.61	15.8	20.0
	0.0530	0.101	0.252	0.483	0.938	2.13	4.55	7.70	16.2	19.9
Run 10	0.0491	0.0951	0.239	0.469	0.954	2.16	4.58	8.05	15.9	19.6
	0.0542	0.101	0.251	0.479	0.942	2.11	4.43	7.87	15.7	20.0
Run 11	0.0510	0.0960	0.251	0.469	0.945	2.21	4.40	7.64	15.8	20.3
	0.0510	0.0995	0.252	0.477	0.948	2.13	4.53	7.78	15.6	20.0
Mean	0.0511	0.0983	0.249	0.476	0.945	2.14	4.50	7.78	15.8	20.0
RSD (%)	4.38	2.65	2.07	1.15	0.613	1.90	1.62	2.13	1.22	1.08
RE (%)	2.20	-1.67	-0.207	-4.78	-5.53	7.05	12.4	-2.80	-1.00	-0.0764

RSD = (SD/Mean) x 100

RE = [(Mean/Theoretical)-1] x 100

Table 4: Calibration Curve Parameters for PFOS in Rat Serum

Run #	Quadratic Coefficient (A)[a]	Coefficient Linear (B)	Intercept (C)	Coefficient of Determination (r^2)
9	-0.0010769	0.13730	0.00042009	0.9965
10	-0.0010516	0.14535	0.0014910	0.9962
11	-0.0012359	0.15173	0.00088515	0.9964
Mean	-0.0011215	0.14479	0.00093206	0.9964

a: $y = Ax^2 + Bx + C$, weighted $1/y^2$

Table 5: Partial Volume Analysis of PFOS in Rat Serum

	PFOS Conc. (µg/mL)
Theoretical Conc.	QC4 100 (1 in 10 dilution)
Run 9	111 105 105 104 104
Mean	106
RSD (%)	2.71
RE (%)	5.89

RSD = (SD/Mean) x 100
RE = [(Mean/Theoretical)-1] x 100

Table 6: Lower Limit of Quantitation of PFOS in Rat Serum

Run 11 Theoretical Concentration	PFOS Conc. Found (µg/mL)
LLQ (0.05 µg/mL)	0.0548
	0.0489
	0.0499
	0.0554
	0.0527
	0.0520
Mean	0.0523
RSD (%)	4.95
RE (%)	4.57

RSD = (SD/Mean) x 100

RE = [(Mean/Theoretical)-1] x 100

Table 7: Ambient-Temperature Stability of PFOS in Rat Serum After 24 Hours

Run 11 QC (Theoretical Conc.)	PFOS (µg/mL)			
	0 Hour	2.5 Hour	6 Hour	24 Hour
QC 1 (0.2 µg/mL)	0.194	0.187	0.177	0.190
	0.197	0.181	0.179	0.187
	0.184	0.184	0.180	0.191
	0.192	0.180	0.183	0.182
	0.184	0.178	0.189	0.190
Mean	0.190	0.182	0.182	0.188
RSD (%)	3.03	1.91	2.52	2.01
%DEV from 0 Hour	NA	-4.20	-4.45	-1.16
QC 2 (6 µg/mL)	6.17	6.17	6.36	6.55
	6.13	6.30	6.38	6.53
	6.41	6.26	6.35	6.85
	6.15	6.28	6.22	6.66
	6.24	6.24	6.11	6.81
Mean	6.22	6.25	6.28	6.68
RSD (%)	1.82	0.797	1.86	2.16
%DEV from 0 Hour	NA	0.525	1.05	7.42
QC 3 (18 µg/mL)	18.2	18.0	18.1	19.1
	17.1	18.0	18.0	19.4
	17.6	17.7	17.6	19.2
	17.7	17.9	18.2	19.4
	17.0	18.0	18.0	19.7
Mean	17.5	17.9	18.0	19.4
RSD (%)	2.84	0.719	1.26	1.19
%DEV from 0 Hour	NA	2.33	2.39	10.5

NA: Not Applicable
RSD = (SD/Mean) x 100
%DEV from 0 Hour = [(X Hour Conc. - 0 Hour Conc.)/0 Hour Conc.] x 100

Table 8: Freezer Stability of PFOS in Rat Serum at -20 °C

QC Nominal Concentration	PFOS (ug/mL)			
	0 Hour (Run 2)*	7 Days (Run 6)*	13 Days (Run 3)**	33 Days (Run 16)
QC 1 (0.2 ug/mL)	0.213	0.225	0.211	0.245
	0.209	0.222	0.210	0.234
	0.208	0.218	0.217	0.228
	0.208	0.219	0.214	0.232
	0.207			
Mean	0.209	0.221	0.213	0.235
RSD (%)	1.24	1.32	1.55	3.17
%DEV from 0 Hour	NA	5.72	1.84	12.3
QC 2 (6 ug/mL)	6.02	6.22	5.97	6.45
	5.71	6.15	5.84	6.13
	6.00	6.39	6.07	6.58
	6.06	6.27	5.99	6.37
	5.69			
Mean	5.89	6.26	5.97	6.38
RSD (%)	3.08	1.62	1.58	2.97
%DEV from 0 Hour	NA	6.19	1.20	8.28
QC 3 (18 ug/mL)	20.2	19.1	18.8	18.6
	18.6	19.5	18.2	18.8
	18.9	19.8	18.8	18.7
	18.2	19.3	17.9	18.7
	18.9			
Mean	18.9	19.4	18.4	18.7
RSD (%)	3.96	1.37	2.42	0.395
%DEV from 0 Hour	NA	2.52	-2.76	-1.30

NA: Not Applicable
RSD = (SD/Mean) x 100
%DEV from 0 Hour = [(Conc. - 0 Hour Conc.)/0 Hour Conc.] x 100
*From Protocol FACT-TOX-110
**From Protocol FACT-TOX-111

Table 9: Stability of PFOS in Rat Serum After Three Freeze/Thaw Cycles

Run 10 QC (Theoretical Conc.)	PFOS (µg/mL)	
	0 Cycle	3 Cycles
QC 1 (0.2 µg/mL)	0.187	0.177
	0.186	0.177
	0.182	0.185
	0.189	0.185
	0.182	0.189
Mean	0.185	0.182
RSD (%)	1.70	3.12
%DEV from 0 Cycle	NA	-1.38
QC 2 (6 µg/mL)	6.44	6.59
	6.44	6.61
	6.32	6.44
	6.19	6.39
	6.31	6.40
Mean	6.34	6.49
RSD (%)	1.66	1.63
%DEV from 0 Cycle	NA	2.35
QC 3 (18 µg/mL)	16.9	19.2
	17.3	19.1
	17.5	19.2
	18.0	19.6
	17.4	18.7
Mean	17.4	19.2
RSD (%)	2.31	1.80
%DEV from 0 Cycle	NA	10.1

NA: Not Applicable
RSD = (SD/Mean) x 100
%DEV from 0 Cycle = [(3 Cycle - 0 Cycle)/0 Cycle] x 100

Table 10: Reproducibility of Reinjecting Extracted Samples Containing PFOS after 27 Hours in Reconstitution Solution

	PFOS (µg/mL)	
	t=0 Hours (Run 10)	t=27 Hours (Run 12)
QC 1 (0.2 µg/mL)	0.187	0.187
	0.186	0.182
	0.182	0.188
	0.189	0.188
	0.182	0.190
Mean	0.185	0.187
RSD (%)	1.70	1.61
RE (%)	-7.50	-6.33
QC 2 (6 µg/mL)	6.44	6.39
	6.44	6.02
	6.32	6.19
	6.19	6.55
	6.31	6.22
Mean	6.34	6.28
RSD (%)	1.66	3.23
RE (%)	5.62	4.59
QC 3 (18 µg/mL)	16.9	17.3
	17.3	18.2
	17.5	18.1
	18.0	17.3
	17.4	17.6
Mean	17.4	17.7
RSD (%)	2.31	2.40
RE (%)	-3.29	-1.73

NA: Not Applicable
RSD = (SD/Mean) x 100
RE = [(Mean/Theoretical)-1] x 100

Table 11: Extraction Recovery of PFOS from Rat Serum

Theoretical Conc. in serum (µg/mL)	Pre-Extraction Response (Area Ratio)	Post-Extraction Response (Area Ratio)	% Recovery*
PFOS	0.03160	0.02880	110
Spike 1	0.02850	0.03010	94.7
(0.25 µg/mL)	0.02930	0.02950	99.3
Run 14	0.02790	0.02930	95.2
	0.03050	0.02960	103
Mean	0.02956	0.02946	100
PFOS	0.4597	0.5270	87.2
Spike 2	0.4751	0.5304	89.6
(4 µg/mL)	0.4776	0.5335	89.5
Run 14	0.4724	0.5298	89.2
	0.4772	0.5392	88.5
Mean	0.4724	0.5320	88.8
PFOS	1.649	1.874	88.0
Spike 3	1.679	1.857	90.4
(16 µg/mL)	1.719	1.869	92.0
Run 14	1.535	1.887	81.4
	1.612	1.864	86.5
Mean	1.639	1.870	87.6
Tetra-H-PFOS	0.4068	0.4460	91.2
Spike IS	0.3884	0.4423	87.8
(4 µg/mL)	0.4043	0.4445	91.0
Run 13	0.3921	0.4512	86.9
	0.4078	0.4412	92.4
Mean	0.3999	0.4450	89.9

*(Individual Response for Pre-Ext./Individual Response for Post-Ext.) x 100

8. FIGURES

Figure 1: Full-Scan Single MS Mass Spectrum of PFOS

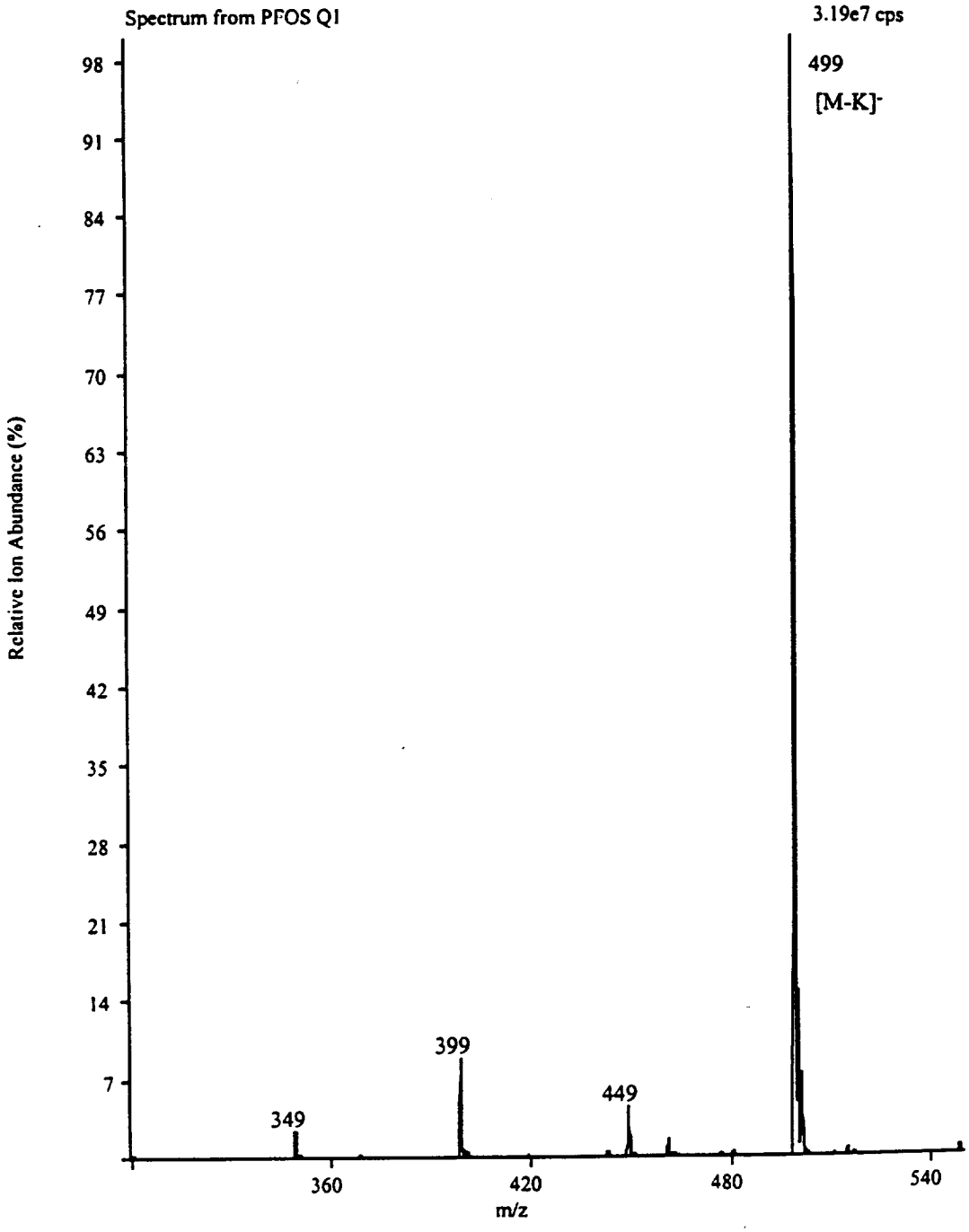


Figure 2: Full-Scan Single MS Mass Spectrum of Tetra-H-PFOS

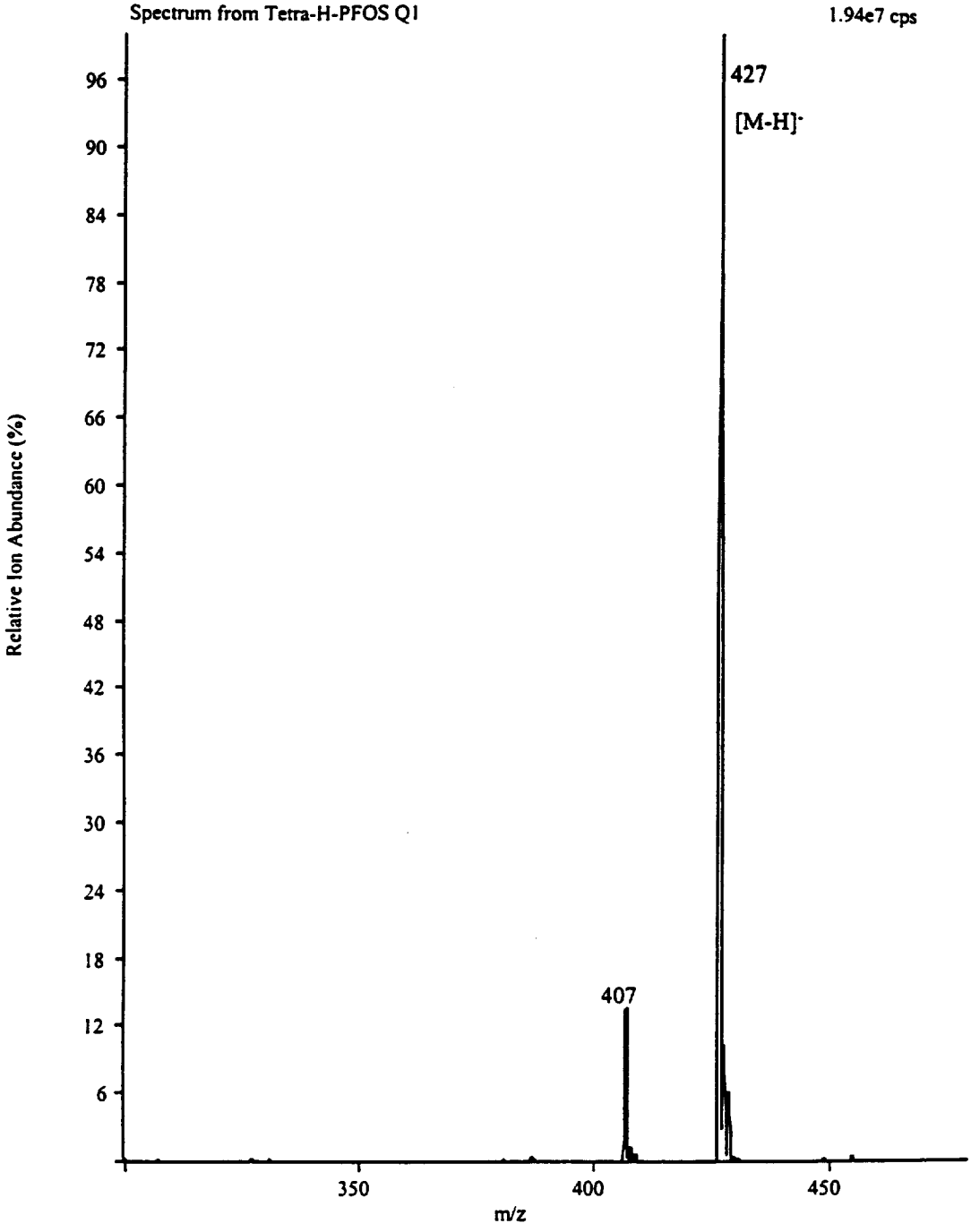
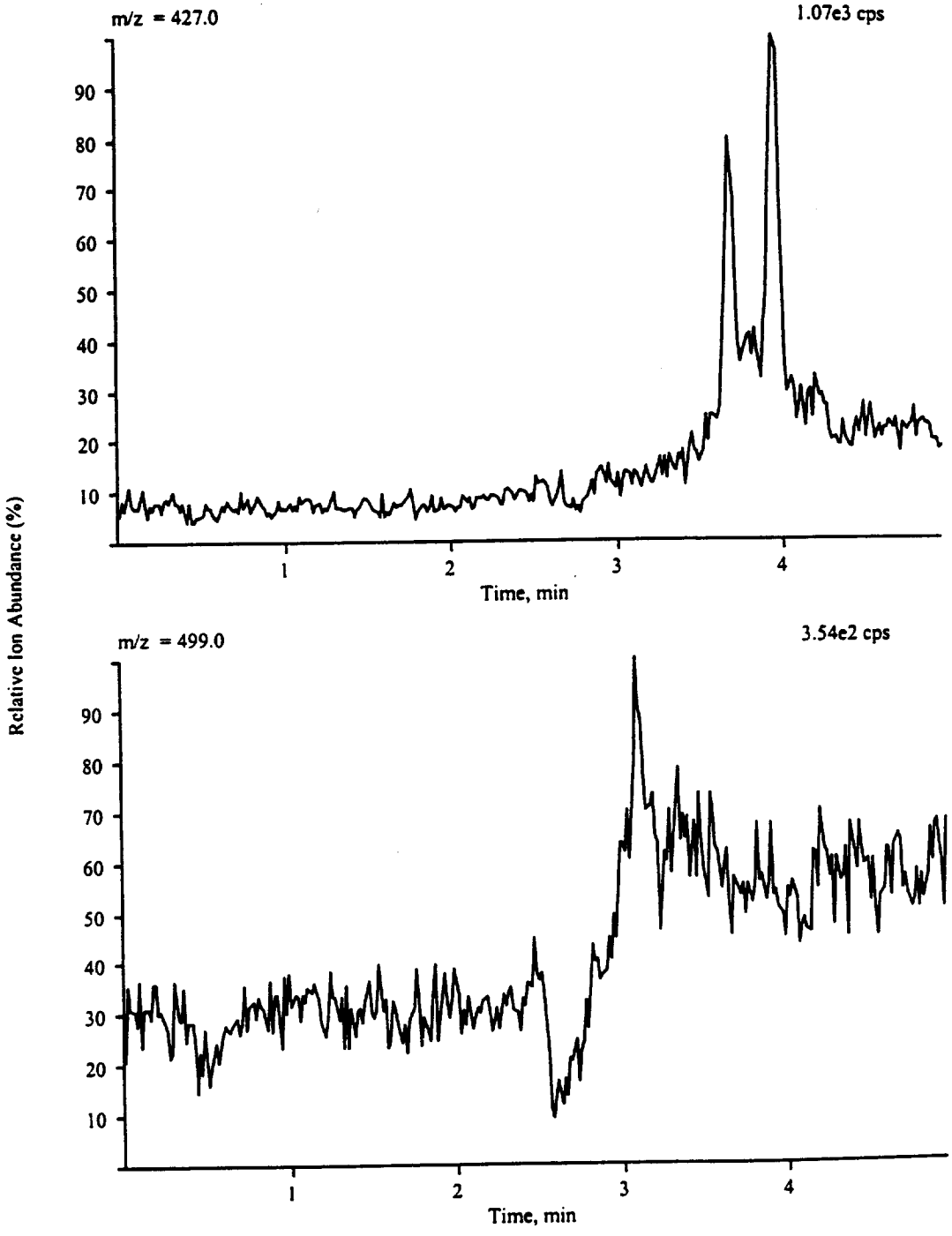


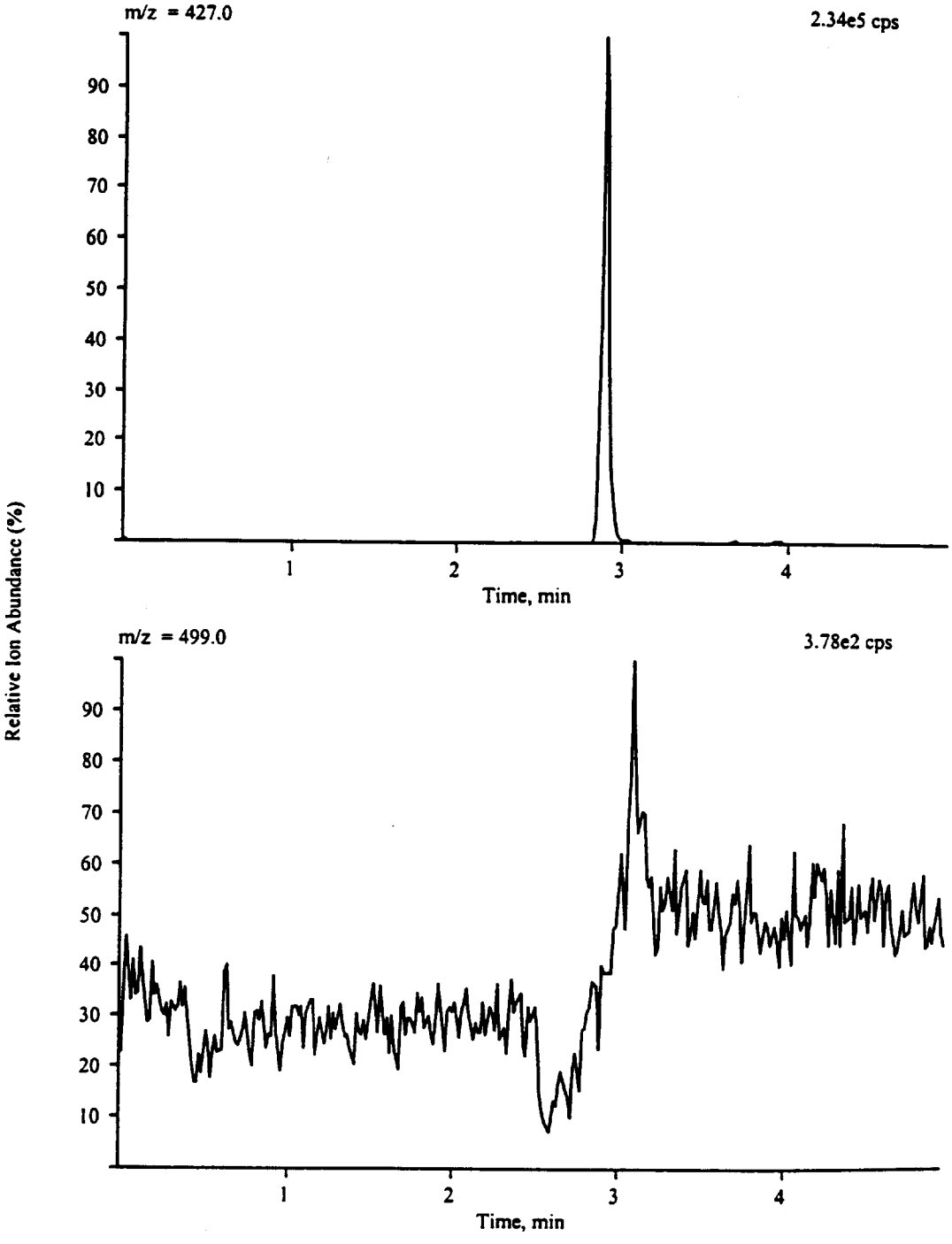
Figure 3: Mass Chromatograms of PFOS and its Internal Standard in Control Blank Rat Serum Extract



All quantitation results and figures were prepared from non-smoothed data.

Analyte	Ion	Retention Time
PFOS	m/z = 499.0	3.1
Tetra-H-PFOS (IS)	m/z = 427.0	2.9

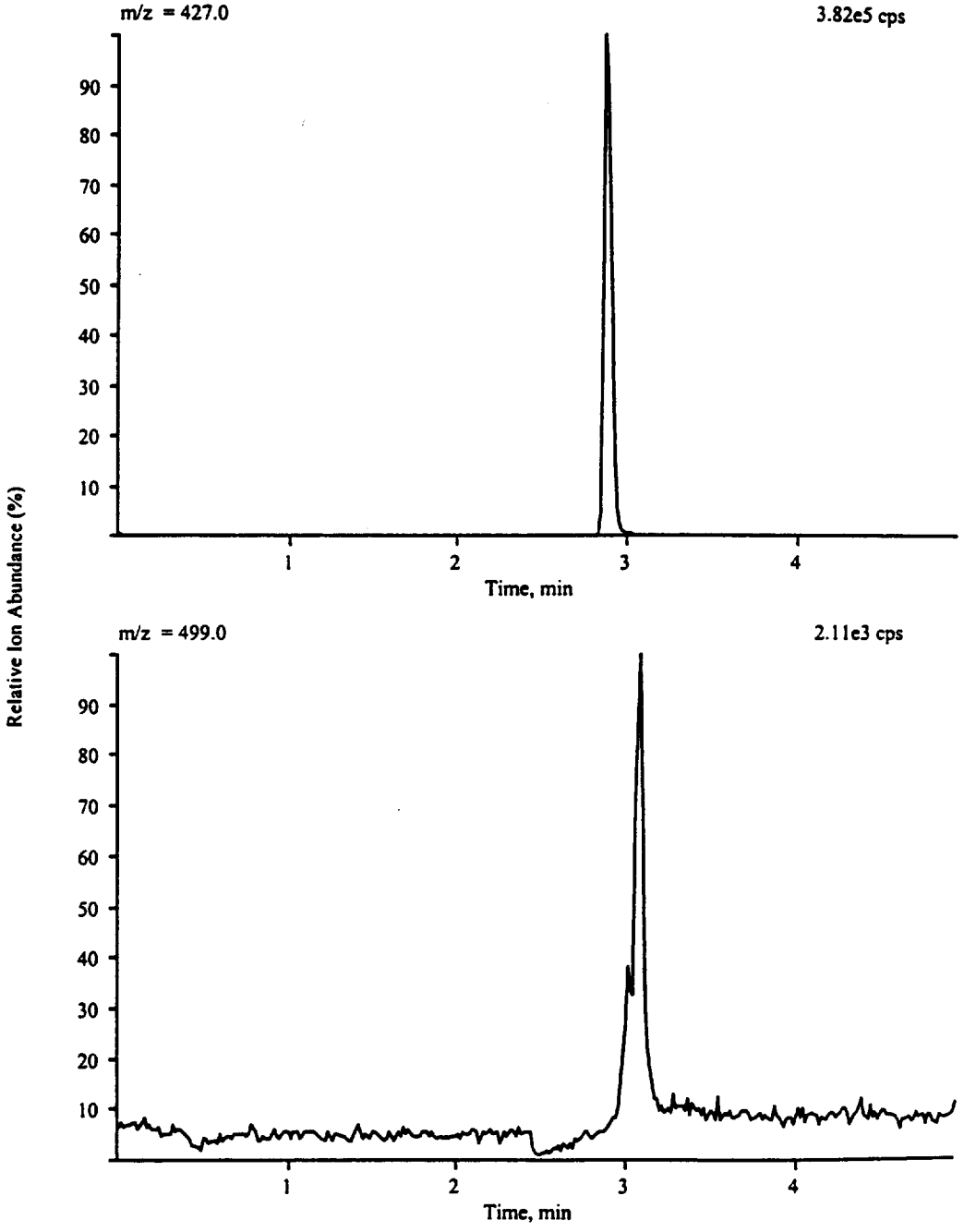
Figure 4: Mass Chromatograms of PFOS in a Rat Serum Extract Sample Containing Internal Standard Only (Zero Sample)



All quantitation results and figures were prepared from non-smoothed data.

Analyte	Ion	Retention Time
PFOS	m/z = 499.0	3.1
Tetra-H-PFOS (IS)	m/z = 427.0	2.9

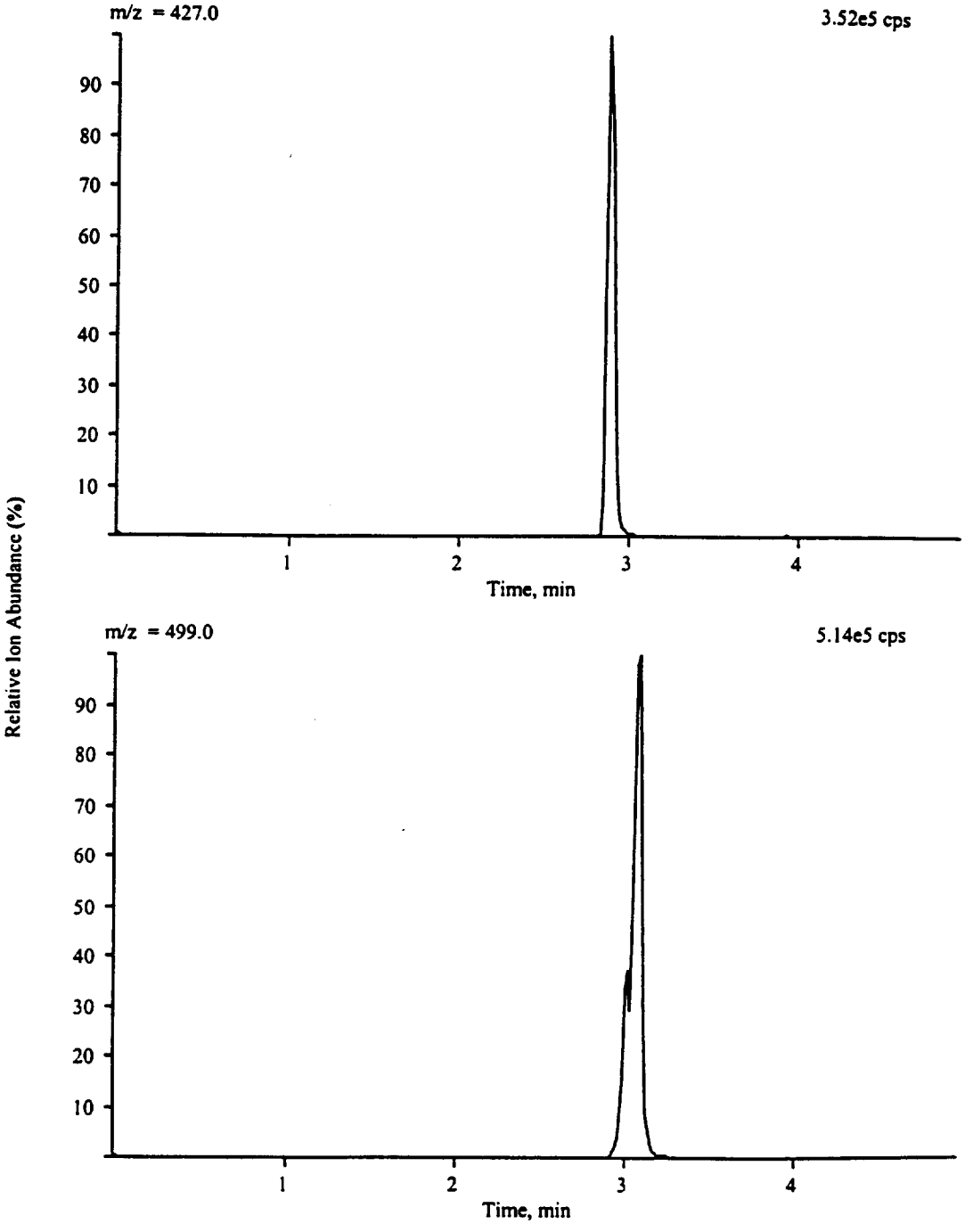
Figure 5: **Mass Chromatograms of Calibration Standard 1 in Rat Serum Extract Containing PFOS (0.05 µg/mL) and the Internal Standard**



All quantitation results and figures were prepared from non-smoothed data.

Analyte	Ion	Retention Time
PFOS	m/z = 499.0	3.1
Tetra-H-PFOS (IS)	m/z = 427.0	2.9

Figure 6: Mass Chromatograms of Calibration Standard 10 in Rat Serum Extract Containing PFOS (20 μg/mL) and the Internal Standard



All quantitation results and figures were prepared from non-smoothed data.

Analyte	Ion	Retention Time
PFOS	m/z = 499.0	3.1
Tetra-H-PFOS (IS)	m/z = 427.0	2.9

9. APPENDIX A: QUANTITATION OF PERFLUOROOCTANESULFONATE (PFOS) IN RAT SERUM BY TURBO ION SPRAY LC/MS

AUTHORS: **David J. Anderson, M.S.**
Amie J. Prince, B.S.
Holly D. Ross, M.S.

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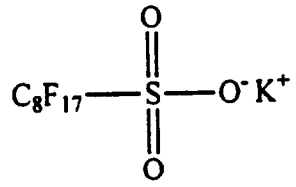
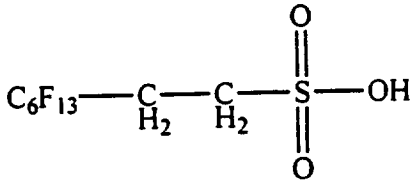
A.1.0 CHEMICAL STRUCTURE(S)



Potassium Perfluorooctanesulfonate
MW = 538



1H, 1H, 2H, 2H-Perfluorooctane Sulfonic Acid
MW = 428, Internal Standard

A.2.0 SPECIMEN(S)

This assay uses 50-µL aliquots of rat serum. Rat serum samples are stored at -20 °C.

A.3.0 ASSAY PRINCIPLE

PFOS and its internal standard, Tetra-H-PFOS, are extracted from rat serum samples (50 µL) using a liquid-liquid extraction procedure. The organic layer is evaporated to dryness and then reconstituted. An aliquot is then analyzed by turbo ion spray liquid chromatography/mass spectrometry (LC/MS) in the negative ion mode.

A.4.0 COMPOUNDS

PFOS: Lot# 171, 99.976% purity, 3M, Inc., Minneapolis, MN.

Tetra-H-PFOS: Lot# 59909, 90% purity, 3M, Inc., Minneapolis, MN.

A.5.0 CHEMICALS

All chemicals may be substituted with that of an equivalent manufacturer and grade of chemical.

Acetonitrile	Cat# 015-4, Burdick & Jackson, Muskegon, MI 49442
Ammonium Acetate	Cat# 24,019-2, Aldrich, Milwaukee, WI 53201
Ethyl Acetate	Cat# 100-4, Burdick & Jackson, Muskegon, MI 49442
Methanol	Cat# 230-4, Burdick & Jackson, Muskegon, MI 49442
Rhodapex mass calibration solution	A mixture of 0.11 mM trifluoroacetic acid (J.T. Baker, Phillipsburg, NJ 08865), 0.52 mM Rhodapex CO-436, 1 mM ABEX EP-110 and 1 mM ABEX EP-120 (all three supplied by Rhone-Poulenc, Inc., Cranbury, NJ 08512) in 1:1 methanol:water.
Sodium Bicarbonate	Cat# 3509-01, J.T. Baker, Phillipsburg, NJ 08865
Sodium Carbonate, Annhydrous	Cat# 3604-01, J.T. Baker, Phillipsburg, NJ 08865
Tetrabutylammonium Hydrogen Sulfate	HPLC Grade, Cat# J360-07, J.T. Baker, Phillipsburg, NJ 08865
Water	High Purity (NANOpure), Barnstead Model D7331 Ultrapure Water System, Dubuque, IA 52001
Rat Serum	Lampire Biological Laboratories, Piperville, PA 18947
Rat Serum	Harlan Bioproducts for Science, Inc., Indianapolis, IN 46229

A.6.0 MATERIALS AND EQUIPMENT

Mass Spectrometer	PE SCIEX API 365 atmospheric pressure ionization tandem triple quadrupole mass spectrometer equipped with TurboIonSpray™ interface, PE SCIEX, Concord, Ontario L4K 4V8
Data system	API Standard Software, MacQuan v 1.4, LC2Tune v 1.3, MacDAD v 1.3, Bundler v 1.3, Multiview v 1.3,

	and Sample Control v 1.3, on a Power Macintosh, PE SCIEX, Concord, Ontario L4K 4V8
HPLC Pump	Shimadzu LC-10AD pump, Shimadzu Co., Columbia, MD 21046
LC Pump Controller	Shimadzu SCL-10A, Shimadzu Co., Columbia, MD 21046
Autosampler	Waters 717plus, Waters Associates, Millipore Corporation, Milford, MA 01757
HPLC Column	Betasil C_{18}, 5 µm particle size, 2.1 x 50 mm, Cat #852055-701, Keystone Scientific, Inc., Bellefonte, PA 16823
Harvard syringe pump 11	Harvard Apparatus Inc., South Natick, MA 01760
Multi-tube vortexer	Cat# 58816-115, VWR Scientific, West Chester, PA 19380
Balance	Model FX-300, AND Ltd., Tokyo, Japan
pH Meter	Model 340, Corning Inc., Corning, NY 14830
Mechanical shaker	Cat# 6000, Eberbach Corp., Ann Arbor, MI 48106
NANOpure-UV water purification system	Barnstead, Dubuque, IA 52001
Microbalance	Model MT-5, Mettler-Toledo Inc., Hightstown, NJ 08520
Micro weigh boats	Cat# 0219-0041, Perkin-Elmer Corp., Norwalk, CT 06859
Weigh boats	Cat# 12577-025, VWR Scientific, West Chester, PA 19380
Sorvall RT-6000D refrigerated centrifuge	Cat# 83071, DuPont Co., Wilmington, DE 19898
Beckman GS6KR Refrigerated centrifuge	Beckman, Palo Alto, CA 94304

TurboVap LV Evaporator	Cat# 43750, Zymark Instruments, Hopkinton, MA 01748
Pipettes	Cat# P-5000, P-1000, P-200, P-100, Rainin Instrument Co., Woburn, MA 01888
Pipette tips	RT-20, RT-200, C-5000, CP-25, CP-50, CP-250, Rainin Instrument Co., Woburn, MA 01888
Polypropylene tubes	13 mm x 100 mm, Cat# 15070-574, VWR Scientific, West Chester, PA 19380
Screw-capped Polypropylene vials	16.5 x 57 mm, Cat# 60.542, Sarstedt, Inc., Newton, SC 28658
Screw-capped Polypropylene vials	16.5 x 101 mm, Cat# 60.541, Sarstedt, Inc., Newton, SC 28658
Plug Tite Multipurpose Caps	Cat# 78-127-0019-100, Elkay Products, Inc., Worcester, MA 01607
Pyrex volumetric flask	Cat# 28014P-10, 28014P-25, 28014P-100, 28014P-1000, Kimble/Kontes, Vineland, NJ 08360
Solvent Filtration Apparatus	Cat# 953781-0000, 953751-0000, 953753-0000, 953826-0000, 953827-0000, Kimble/Kontes, Vineland, NJ 08360
Nylon Titan Membrane Filters	0.45 µm Scientific Resources Inc., Cat# 74547-NN, Eatontown, NJ 07724
PEEK tubing	0.005" i.d. x 1/16" o.d., Cat# 1535, Upchurch Scientific Inc., Oak Harbor, WA 98277
Liquid Nitrogen	Supplied in-house from bulk tank, BOC Gasses, Buffalo, NY 14210-2005
Gastight Glass Syringes	Cat# 1750, Hamilton Company, Reno, NV 89510

Other general laboratory glassware and supplies were used.

A.7.0 PREPARATION OF SOLUTIONS

NANOpure water (18 megaohm-cm.) or equivalent should be used wherever water is called for. Mix all solutions well.

A.7.1 Preparation of Analytical Standard Stock Solutions

Analytical Standard Stock Solution, PFOS (1 mg/mL): Weigh approximately 5 mg of PFOS (after correction for purity) on a microbalance and transfer it to a polypropylene vial. Dilute with the appropriate volume of methanol to yield a 1 mg/mL solution. Prepare a fresh solution every three months. Store the solution at 4 °C and bring to ambient temperature before use.

Standard Spiking Solutions

Prepare Standard Working Solutions A through J in 5-mL class "A" volumetric flasks according to the following dilution scheme. Dilute to the 5-mL mark with water to yield the final concentration. Prepare fresh solutions every three months. Store the solutions at 4 °C and bring to ambient temperature before use.

Standard Working	Volume Spiked (µL)	Solution Used	PFOS Final Conc. (µg/mL)
A	1000	Stock	200
B	800	Stock	160
C	400	Stock	80
D	200	Stock	40
E	100	Stock	20
F	250	200 µg/mL (A)	10
G	125	200 µg/mL (A)	5
H	62.5	200 µg/mL (A)	2.5
I	500	10 µg/mL (F)	1.0
J	250	10 µg/mL (F)	0.5

A.7.2 Preparation of Internal Standard Stock Solutions

Internal Standard Stock Solution, Tetra-H-PFOS (1 mg/mL): Weigh approximately 5 mg of Tetra-H-PFOS on a microbalance and transfer it to a polypropylene vial. Dilute with an appropriate volume of methanol to yield a 1 mg/mL solution. Prepare a fresh solution every three months. Store the solution at 4 °C and bring to ambient temperature before use.

A.7.3 Preparation of Internal Standard Working Solution

Internal Standard Working Solution (4 µg/mL Tetra-H-PFOS): Add 400 µL of Internal Standard Stock Solution to a 100-mL class "A" volumetric flask. Dilute to the mark with water to give a 4 µg/mL Tetra-H-PFOS solution. Store the solution at 4 °C and bring to ambient temperature before use. Prepare fresh solution as needed.

A.7.4 Preparation of Standard Curve and Control Blank

Prepare fresh calibration standards for each analytical run by combining 360 µL of rat control serum and 40 µL of the analytical standard working solution (see table below) in labeled 1.7-mL microtubes. Prepare control blanks and zero samples by combining 360 µL of rat control serum with 40 µL of water. Vortex each for 60 seconds.

Analytical Standard Dilution Table:

Standard #	Volume Spiked (µL)	Solution to use	µL control serum	Final Conc. (µg/mL)
10	40	A	360	20
9	40	B	360	16
8	40	C	360	8
7	40	D	360	4
6	40	E	360	2
5	40	F	360	1
4	40	G	360	0.5
3	40	H	360	0.25
2	40	I	360	0.1
1	40	J	360	0.05

A.7.5 Preparation of Other Solutions

0.25 M Sodium Carbonate/0.25 M Sodium Bicarbonate: Weigh 26.5 g of sodium carbonate and 21 g of sodium bicarbonate and dissolve in approximately 900 mL of HPLC-grade water. Transfer the solution to a 1000-mL volumetric flask and dilute to the mark with HPLC-grade water. Mix the solution thoroughly. Store at ambient temperature. Prepare a fresh solution every three months.

1 M Ammonium Acetate: Add 7.7 g of ammonium acetate to a 100-mL volumetric flask, dissolve in NANOpure water, and stir until completely dissolved. Dilute to the mark with NANOpure water. Store at ambient temperature. Prepare a fresh solution every three months.

2 mM Ammonium Acetate: Combine 2 mL of 1M ammonium acetate and 900 mL NANOpure water in a 1000-mL volumetric flask; stir until completely mixed. Dilute to the mark with NANOpure water. Store at ambient temperature. Prepare a fresh solution every three months.

10 M Sodium Hydroxide: Add 40 g of sodium hydroxide to a 100-mL volumetric flask, dissolve in NANOpure water, and stir until completely dissolved. Dilute to the mark with NANOpure water. Store at ambient temperature. Prepare a fresh solution every three months.

1 M Sodium Hydroxide: Add 10 mL of 10 M sodium hydroxide to a 100-mL volumetric flask. Dilute to the mark with NANOpure water; stir until completely mixed. Store at ambient temperature. Prepare a fresh solution every three months.

0.5 M Tetrabutylammonium Hydrogen Sulfate, pH 10: Weigh 16.98 g of tetrabutylammonium hydrogen sulfate and dissolve in 40 mL of NANOpure water. Adjusted the pH to 10.0 with 10 M and 1 M sodium hydroxide. Transfer the solution to a 100-mL volumetric flask and dilute to the mark with NANOpure water.

10:90 Methanol:2 mM Ammonium Acetate (Mobile Phase Eluent A): Add 100 mL of methanol and 900 mL of 2 mM ammonium acetate to a 1000-mL Pyrex bottle. Mix the solution thoroughly and filter the solution through a 0.45 µM filter with a vacuum flask. Store at ambient temperature. Prepare a fresh solution every three months.

90:10 Methanol:2 mM Ammonium Acetate (Mobile Phase Eluent B): Add 900 mL of methanol and 100 mL of 2 mM ammonium acetate to a 1000-mL Pyrex bottle. Mix the solution thoroughly and filter the solution through a 0.45 µM filter with a vacuum flask. Store at ambient temperature. Prepare a fresh solution every three months.

A.8.0 PREPARATION OF QUALITY CONTROL SAMPLES

A.8.1 Preparation of Quality Control Stock Solutions

QC Stock Solution, PFOS (1 mg/mL): Weigh approximately 5 mg of PFOS (after correction for purity) on a microbalance and transfer it to polypropylene vial. Dilute

with the appropriate volume of methanol to yield a 1 mg/mL solution. Store the solution at 4 °C and bring to ambient temperature before use. Prepare a fresh solution every three months.

A.8.2 Preparation of Serum QC Samples

QC4 (Dilution QC, 100 µg/mL PFOS): Add 1000 µL QC Stock Solution to a 10-mL class "A" volumetric flask to yield a 100-µg/mL solution. Dilute to the mark with control serum, cap and mix. Aliquots of approximately 0.2 mL are placed in polypropylene vials and stored frozen at –20 °C.

QC3 (18 µg/mL PFOS): Add 450 µL QC Stock Solution to a 25-mL class "A" volumetric flask to yield a 18-µg/mL solution. Dilute to the mark with control serum, cap and mix. Aliquots of approximately 0.5 mL are placed in polypropylene vials and stored frozen at –20 °C.

QC2 (6 µg/mL PFOS): Add 150 µL of QC Stock Solution to a 25-mL class "A" volumetric flask to yield a 6-µg/mL solution. Dilute to the mark with control serum, cap and mix. Aliquots of approximately 0.5 mL are placed in polypropylene vials and stored frozen at –20 °C.

QC1 (0.2 µg/mL PFOS): Add 111.1 µL of QC3 to a 10-mL class "A" volumetric flask to yield a 0.2-µg/mL solution. Dilute to the mark with control serum, cap and mix. Aliquots of approximately 0.5 mL are placed in polypropylene vials and stored frozen at –20 °C.

A.9.0 LIQUID-LIQUID EXTRACTION PROCEDURE

1. Prepare fresh calibration standards for each run according to Section A.7.4.
2. Thaw QC samples.
3. Prepare QC4 samples (diluted 1 in 10) in two steps:
   a) Add 180 µL of control serum and 20 µL of QC4 into 1.7-mL microtubes and

vortex.

b) Aliquot 50 µL of diluted QC4 into labeled 16 x 100 mm polypropylene tubes.

4. Aliquot 50 µL of each control blank, zero sample, calibration standard, and quality control samples 1-3 into labeled 16 x 100 mm polypropylene tubes. Standards and blanks are analyzed in duplicate. All QC samples are analyzed in replicates of five.
5. Add 1 mL of 0.5 M tetrabutylammonium hydrogen sulfate, pH 10 to each tube.
6. Add 2 mL of 0.25 M sodium carbonate/0.25 M sodium bicarbonate to each tube.
7. Add 500 µL of the Internal Standard Working Solution to each tube (except control blank, add 500 µL water).
8. Add 5 mL ethyl acetate to each tube.
9. Shake the tubes on a reciprocal shaker at medium speed for 20 minutes.
10. Centrifuge the tubes at 3000 rpm for 20 minutes at 20 °C.
11. Freeze the aqueous phase in an acetone/dry ice bath and keep the tubes in the bath an additional 5 minutes.
12. Transfer the ethyl acetate layer to a fresh, labeled 13 x 100 mm polypropylene tube.
13. Evaporate the ethyl acetate layer to dryness in a TurboVap™ at approximately 20 °C under nitrogen.
14. Reconstitute the dried extracts in two steps:
    Add 500 µL acetonitrile and vortex for 60 seconds.
    Then add 500 µL water and vortex for 60 seconds.
15. Transfer 200 µL of the extract to a labeled polypropylene autosampler vial.

A.10.0 INSTRUMENT CONDITIONS

HPLC Conditions:

Eluent (gradient)	Mobile Phase A: 10:90 methanol:2 mM ammonium acetate (v/v) Mobile Phase B: 90:10 methanol:2 mM ammonium acetate (v/v)

Eluent Gradient Conditions:

Time (min)	%B
0	45
1.0	100
5	100
5.5	45%
8	Stop

Flow Rate	300 µL/min.
Autosampler	Waters 717plus
Autosampler Temperature	Ambient
Injection Volume	3 to 5 µL
HPLC Column	Keystone Betasil C_{18}, 5 µm particle size, 2.1 mm x 50 mm
HPLC Column Temperature	Ambient
Typical Initial Column Pressure	90 bar
Autosampler Run Time	8.0 minutes

Analyte	Representative Retention Time (minutes)
PFOS	3.1
Tetra-H-PFOS (IS)	2.9

Mass Spectrometer Conditions:

Curtain Gas	UHP Nitrogen
Nebulizer Gas	UHP Nitrogen
TurboIonSpray™ Temperature	400 °C
TurboIonSpray™ Auxiliary Gas	UHP Nitrogen at 8 L/min

Ions Monitored:

Analyte	Ion Monitored	Dwell Time
PFOS	m/z = 499.0	500 ms
Tetra-H-PFOS (IS)	m/z = 427.0	500 ms

Ionization Mode	Negative Ion
Ion Spray Voltage	-3000 V
Declustering Potential	-35 V
MS Acquisition Time	5 minutes
Pause Time	2 ms

Calibrate the mass axis of the instrument by infusion of Rhodapex mass calibration solution (Section A.5.0) at a flow rate of 10 µL/min. Optimize the sensitivity of the instrument using an infusion of a 10 µg/mL solution of the analyte at 10 µL/min into a flow of 190 µL/min of mobile phase using the gradient condition at which the analyte elutes from the LC column (100% eluent B). Mass spectral peak widths should be approximately 0.6 amu at half-height.

One set of calibration standards was injected at the beginning and one set of calibration standards was injected at the end of each analytical run (tray).

A.11.0 CALCULATIONS

Calculated concentrations are based on peak area ratios of PFOS to Tetra-H-PFOS. The peak area for the analyte ion is divided by the peak area for the corresponding internal standard ion.

Data generated from samples in this study were acquired and integrated using PE SCIEX software applications Sample Control (v. 1.3) and MacQuan (v. 1.4). The validation data were formatted in the ABS laboratory information management system (Watson, v.5.3.1.01). All data were rounded to no less than three significant figures by ABS prior to reporting.

Acceptance Criteria for Sample Analysis

Calibration standards: The coefficient of determination (r^2) must be ≥0.98. At least three-fourths of the individual calibration standard replicates will have deviations within ±15% from their nominal concentrations.

LLQ acceptance criteria: At least one replicate at the LLQ must exhibit a deviation within ±20% from its nominal concentration. If this criterion is not met, both replicates are rejected and the standards at the next higher level are subjected to the same test.

Quality control samples: At least two-thirds of the individual QC sample replicates will have deviations within ±15% from their nominal concentrations, with at least one replicate at each QC concentration meeting this criterion.

ATTACHMENT D
DOSE CONFIRMATION ANALYSES

Study:	Argus 418-014, Oral (Gavage) Cross-Fostering Study of PFOS in Rats		
Product Number(Test Substance):	PFOS (T-6295.9)		
Matrix:	Tween Dosing Vehicle	See list to right	0
Method/Revision:	ETS-8-4.1 & ETS-8-5.1	See Attachments	0
Analytical Equipment System Number:	Madeline041098	See Attachments	0
Instrument Software/Version:	MassLynx 3.2	See Attachments	Grp 1
Date of Extraction/Analyst:	9/13/99 IAS		
Date of Analysis/Analyst:	9/15/99 IAS		
Date of Data Reduction/Analyst:	9/16/99 IAS		

Sample Data

Tween Dosing Confirmation

Group Dose	Sample #	Expected Conc. PFOS ng/ml	PFOS Conc. ng/ml	PFOS % Recovery Accuracy
QC	B-418-014-B, 11/1/98 (302ppb MS)	201	199	99%
	B-418-014-B, 1/27/99 (302ppb MS)	201	141	70%
Group 1 Control 0.0 mg/kg/day	B-418-014-A (11/01/98) Diluted 1/1	0	0	<LOD
	B-418-014-A (1/27/99) Diluted 1/1	0	0	<LOD
Group 2 1.6 mg/kg/day 0.32 mg/ml	B-418-014-B (11/01/98) Diluted 1/808	320000	252516	79%
	B-418-014-B (1/27/99) Diluted 1/808	320000	276069	86%

Limit of Quantitation Limit (LOQ) = PFOS = 30 ng/g
Method Detection Limit (MDL): PFOS = 15 ng/g
PFOS = Perfluorooctanesulfonate

NR = Sample not received nor reported.
NA = Not Applicable

Date Entered/Analyst: 9/27/99 GML
Date Verified/Analyst:

ATTACHMENT E
DATA SUMMARY TABLES

Table 1. Concentrations of PFOS in Rat Serum Samples from Study FACT Tox-108

<LLQ: Less than the Lower Limit of Quantitation (0.05µg/mL)

RAT NUMBER (RAT NUMBER WITH A "P" INDICATES A PUP SAMPLE)	PFOS CONCENTRATION (µg/mL)
18901	0.0507
18901P	<LLQ[0.0323]<(0.05)
18903	4.80
18903P	35.7
18904	<LLQ[0.0462]<(0.05)
18906	<LLQ[0.0329]<(0.05)
18907	1.35
18907P	33.5
18908	2.41
18909	1.06
18910	<LLQ[0.0316]<(0.05)
18910P	<LLQ[0.0382]<(0.05)
18911	0.0798
18911P	0.123
18912	<LLQ[0.0376]<(0.05)
18913	<LLQ[0.0341]<(0.05)
18913P	<LLQ[0.0398]<(0.05)
18915	<LLQ[0.0221]<(0.05)
18915P	<LLQ[0.0242]<(0.05)
18917	<LLQ[0.0355]<(0.05)
18917P	<LLQ[0.0221]<(0.05)
18918	<LLQ[0.0323]<(0.05)
18918P	<LLQ[0.0247]<(0.05)
18919	<LLQ[0.0340]<(0.05)
18920	1.96
18922	<LLQ[0.0412]<(0.05)
18922P	<LLQ[0.0367]<(0.05)
18923	<LLQ[0.0291]<(0.05)
18923P	<LLQ[0.0331]<(0.05)
18924	2.72
18924P	30.7
18926	<LLQ[0.0323]<(0.05)
18926P	<LLQ[0.0229]<(0.05)
18927	<LLQ[0.0305]<(0.05)
18927P	<LLQ[0.0318]<(0.05)

Table 1. Concentrations of PFOS in Rat Serum Samples from Study FACT Tox-108 (continued)
<LLQ: Less than the Lower Limit of Quantitation (0.05µg/mL)

RAT NUMBER (RAT NUMBER WITH A "P" INDICATES A PUP SAMPLE)	PFOS CONCENTRATION (µg/mL)
18929	2.40
18931	<LLQ[0.0382]<(0.05)
18931P	<LLQ[0.0196]<(0.05)
18932	<LLQ[0.0399]<(0.05)
18934	1.25
18934P	34.1
18935	<LLQ[0.0316]<(0.05)
18935P	<LLQ[0.0206]<(0.05)
18936	<LLQ[0.0302]<(0.05)
18936P	<LLQ[0.0230]<(0.05)
18937	1.80
18938	<LLQ[0.0359]<(0.05)
18939	<LLQ[0.0311]<(0.05)
18939P	<LLQ[0.0308]<(0.05)
18940	<LLQ[0.0330]<(0.05)
18940P	<LLQ[0.0231]<(0.05)
18941	5.34
18942	1.12
18943	65.1
18943P	82.2
18946	157
18947	86.9
18948	96.1
18950	66.4
18951	72.4
18951P	59.2
18952	90.8
18952P	48.3
18953	67.0
18953P	53.8
18956	97.5
18956P	89.3
18957	69.1
18957P	93.2
18958	68.3
18958P	56.2

Table 1. Concentrations of PFOS in Rat Serum Samples from Study FACT Tox-108 (continued)

<LLQ: Less than the Lower Limit of Quantitation (0.05µg/mL)

RAT NUMBER (RAT NUMBER WITH A "P" INDICATES A PUP SAMPLE)	PFOS CONCENTRATION (µg/mL)
18959	59.2
18960	78.0
18960P	93.6
18961	69.1
18961P	47.6
18963	83.6
18964	85.6
18964P	92.9
18965	75.9
18967	74.2
18968	80.9
18969	78.6
18970	99.3
18970P	96.9
18971	218
18972	66.9
18972P	58.2
18973	81.9
18973P	79.5
18974	172
18976	77.9
18977	124

Table 2. Repeat Analysis of Rat Serum Samples from Study FACT Tox-108

<ULQ: Greater than the Upper Limit of Quantitation (20.0µg/mL)

Rat Number (Rat Number with a "P" indicates a Pup Sample)	Treatment	Time	Original Concentration (µg/mL)	Original Curve Number	Reason for Reassay	Reassay Conc. (µg/mL)	Reassay Curve Number	Reported Conc. (ug/mL)	Reason for Reported Conc.
18903P	0	Day 14	>ULQ-28.5284>(20)	3	1	35.7	5	35.7	1
18907P	0	Day 14	>ULQ-30.8172>(20)	3	1	33.5	5	33.5	1
18924P	0	Day 14	>ULQ-27.5496>(20)	3	1	30.7	5	30.7	1
18934P	0	Day 14	>ULQ-36.8737>(20)	3	1	34.1	5	34.1	1
18971	1	Day 14	>ULQ-219.5172>(200)	1	2	218	3	218	2

Reasons for Reassay:
1) Run 3 concentration exceeded calibration range
2) Run 1 concentration exceeded calibration range

Reasons for Reported Conc:
1) Run 5 concentration within calibration range
2) Run 3 concentration within calibration range

Table 3. Pharmacokinetic Subgroup—Concentrations of PFOS in Dam and Litter Serum Samples from Study FACT Tox-108

<LLQ: Less than the Lower Limit of Quantitation (0.05µg/mL)

Dosage Group	Dam Number	PFOS Concentration (µg/mL)	Litter Number	PFOS Concentration (µg/mL)
I	18910	<LLQ[0.0316]<(0.05)	18910P	<LLQ[0.0382]<(0.05)
I	18911	0.0798	18911P	0.123
I	18913	<LLQ[0.0341]<(0.05)	18913P	<LLQ[0.0398]<(0.05)
I	18915	<LLQ[0.0221]<(0.05)	18915P	<LLQ[0.0242]<(0.05)
I	18926	<LLQ[0.0323]<(0.05)	18926P	<LLQ[0.0229]<(0.05)
I	18927	<LLQ[0.0305]<(0.05)	18927P	<LLQ[0.0318]<(0.05)
I	18939	<LLQ[0.0311]<(0.05)	18939P	<LLQ[0.0308]<(0.05)
I	18940	<LLQ[0.0330]<(0.05)	18940P	<LLQ[0.0231]<(0.05)
II	18956	97.5	18956P	89.3
II	18971	218	18971P	NA

NA–Sample not assayed. Insufficient sample volume for analysis.

Table 4. PFOS Concentrations in Dam and Cross-Fostered Litter Serum Samples by Dosage Group

<LLQ: Less than the Lower Limit of Quantitation (0.05µg/mL) —: Sample not received

Dosage Group	Dam Number	PFOS Concentration (µg/mL)	Subset Number	Cross Foster Litter Number	PFOS Concentration (µg/mL)
I Untreated Dams			A Treated Litters		
I	18903	4.80	A	18976P	—
I	18907	1.35	A	18963P	—
I	18908	2.41	A	18953P	53.8
I	18909	1.06	A	18961P	47.6
I	18918	<LLQ[0.0323]<(0.05)	A	18946P	—
I	18920	1.96	A	18952P	48.3
I	18924	2.72	A	18972P	58.2
I	18929	2.40	A	18950P	—
I	18934	1.25	A	18959P	—
I	18936	<LLQ[0.0302]<(0.05)	A	18977P	—
I	18937	1.80	A	18958P	56.2
I	18941	5.34	A	18951P	59.2
I	18942	1.12	A	18965P	—
I Untreated Dams			B Untreated Litters		
I	18901	0.0507	B	18931P	<LLQ[0.0196]<(0.05)
I	18904	<LLQ[0.0462]<(0.05)	B	18932P	—
I	18906	<LLQ[0.0329]<(0.05)	B	18938P	—
I	18912	<LLQ[0.0376]<(0.05)	B	18935P	<LLQ[0.0206]<(0.05)
I	18917	<LLQ[0.0355]<(0.05)	B	18922P	<LLQ[0.0367]<(0.05)
I	18919	<LLQ[0.0340]<(0.05)	B	18923P	<LLQ[0.0331]<(0.05)
I	18922	<LLQ[0.0412]<(0.05)	B	18917P	<LLQ[0.0221]<(0.05)
I	18923	<LLQ[0.0291]<(0.05)	B	18919P	—
I	18931	<LLQ[0.0382]<(0.05)	B	18901P	<LLQ[0.0323]<(0.05)
I	18932	<LLQ[0.0399]<(0.05)	B	18904P	—
I	18935	<LLQ[0.0316]<(0.05)	B	18912P	—
I	18938	<LLQ[0.0359]<(0.05)	B	18906P	—
II Treated Dams			C Treated Litters		
II	18943	65.1	C	18968P	—
II	18947	86.9	C	18973P	79.5
II	18948	96.1	C	18970P	96.9
II	18957	69.1	C	18969P	—
II	18960	78.0	C	18974P	—
II	18964	85.6	C	18967P	—
II	18967	74.2	C	18964P	92.9
II	18968	80.9	C	18943P	82.2
II	18969	78.6	C	18957P	93.2
II	18970	99.3	C	18948P	—
II	18973	81.9	C	18947P	—
II	18974	172	C	18960P	93.6
II Treated Dams			D Untreated Litters		
II	18946	157	D	18918P	<LLQ[0.0247]<(0.05)
II	18950	66.4	D	18929P	—
II	18951	72.4	D	18941P	—
II	18952	90.8	D	18920P	—
II	18953	67.0	D	18908P	—
II	18958	68.3	D	18937P	—
II	18959	59.2	D	18934P	34.1
II	18961	69.1	D	18909P	—
II	18963	83.6	D	18907P	33.5
II	18965	75.9	D	18942P	—
II	18972	66.9	D	18924P	30.7
II	18976	77.9	D	18903P	35.7
II	18977	124	D	18936P	<LLQ[0.0230]<(0.05)

ATTACHMENT F
ANALYTICAL REPORTS FROM CONTRIBUTING LABORATORIES

ADVANCED
BIOANALYTICAL
SERVICES, INC.

BIOANALYTICAL REPORT

Title:	Quantitative Determination of Perfluorooctanesulfonate (PFOS) in Rat Serum from Study #FACT-TOX-108 Using Turbo Ion Spray LC/MS
Date:	27 August 1999
Authors:	David J. Anderson, M.S. Amie J. Prince, B.S. Holly D. Ross, M.S.
Prepared For:	3M Environmental Technology and Safety Services St. Paul, MN 55133-3331
ABS Report No.:	99ADJA01.MI.DOC
Study Protocol:	FACT-TOX-108
Number of Pages:	16

Summary and Conclusions

The concentration of perfluorooctanesulfonate (PFOS) was determined in rat serum samples collected during the study #FACT-TOX-108 using a sensitive, specific, accurate, and reproducible analytical method developed by Advanced BioAnalytical Services, Inc., Ithaca, NY.

Rat serum samples (50 µL) were extracted by a liquid-liquid extraction procedure to isolate PFOS and the internal standard, 1H,1H,2H,2H-perfluorooctane sulfonic acid (Tetra-H-PFOS). Following evaporation and reconstitution, sample extracts were analyzed by turbo ion spray liquid chromatography/mass spectrometry (LC/MS) in the negative ion mode.

All samples were successfully analyzed within four runs. The lower limit of quantitation was 0.05 µg/mL for PFOS. The precision of this assay (RSD) as determined from the analysis of quality control samples for PFOS was ≤4.01%. The precision of this assay (RSD), as determined from the calibration standards for PFOS was ≤5.83%. The relative error (RE) of the assay, as determined from the analysis of the quality control samples ranged from -7.32 to 10.1%. The RE of the assay, as determined from the analysis of calibration standards ranged from -5.86 to 11.0%.

ADVANCED BIOANALYTICAL SERVICES

SIGNATURE PAGE

Title: Quantitative Detennination of Perfluorooctanesulfonate (PFOS) in Rat Serum from Study #FACT-TOX-108 Using Turbo Ion Spray LC/MS

ABS Report No.: 99ADJA01.MI.DOC

Reported by: _____ 27 August 1999
David J. Anderson, M.S. Date
Research Scientist

Reviewed by: _____ 27 Aug 99
Kathleen Cormack, B.S. Date
Associate Auditor

Authorized for Release by: _____ 27. AUG. 99
John R. Perkins, Ph.D. Date
Assistant Scientific Director

QAU STATEMENT

Periodic inspections of the bioanalytical portion of study #FACT-TOX-108 were conducted by the Quality Assurance Unit of Advanced BioAnalytical Services (ABS) for compliance with EPA GLP regulations (40 CFR Part 792).

The study was inspected on the following dates:

22 June 1999; 1, 2, 7, July 1999; 12 August 1999.

Results of the inspection were reported to ABS Management and Project Team Leader on:

22 June 1999; 1, 2, 7, July 1999; 12 August 1999.

Results of the inspections were reported to the Study Director on 12 August 1999.

Based on the inspections and the data reviewed, this report is a complete and accurate representation of the data.

Kathleen Cormack 27Aug99

Kathleen Cormack, B.S. Date
Associate Auditor

TABLE OF CONTENTS

LIST OF TABLES

1. INTRODUCTION

1.1. Study Description and Objective

The objective of this study was to determine the concentrations of perfluorooctanesulfonate (PFOS) in rat serum samples collected during the study #FACT-TOX-108, "Oral (Gavage) Cross-Fostering Study of PFOS in Rats." Rat serum samples were analyzed using a liquid-liquid extraction and turbo ion spray liquid chromatography/mass spectrometry (LC/MS) assay.

2. METHODS

2.1. Analytical Procedure(s)

PFOS concentrations were determined in rat serum according to the validated LC/MS method (1). Serum samples (50 µL) were extracted by a liquid-liquid extraction procedure using ethyl acetate to isolate PFOS and the internal standard (IS), 1H,1H,2H,2H-perfluorooctane sulfonic acid (Tetra-H-PFOS) from rat serum. Following evaporation to dryness and subsequent reconstitution, sample extracts were separated by reversed-phase chromatography on a 2.1 x 50 mm (5 µm) Betasil™ C_{18} column (Keystone Scientific, Inc., Bellefonte, PA) with an initial mobile phase of 55% Eluent A (10:90 methanol:2 mM ammonium acetate) and 45% Eluent B (90:10 methanol:2 mM ammonium acetate). PFOS concentrations were determined by turbo ion spray liquid chromatography/mass spectrometry (LC/MS) in selected ion monitoring (SIM) mode. The negative ions monitored were:

$m/z = 499.0$ for PFOS $[M-K]^-$
$m/z = 427.0$ for Tetra-H-PFOS $[M-H]^-$

Study sample concentrations were determined from a weighted ($1/y^2$), quadratic regression of peak area ratios (peak area of PFOS/peak area of Tetra-H-PFOS) versus nominal concentration of ten calibration standards. The nominal concentrations of the calibration standards were 0.05, 0.1, 0.25, 0.5, 1, 2, 4, 8, 16, and 20 µg/mL PFOS.

The lower limit of quantitation (LLQ) for this assay was determined to be 0.05 µg/mL PFOS. Quality control (QC) serum samples at three different concentrations (0.2, 6, and 18 µg/mL PFOS) were analyzed with each assay batch in replicates of four. Dilution QC samples (QC4, 100 µg/mL) were prepared at each dilution that was used during a particular assay and were analyzed with each assay batch in replicates of four as needed.

The acceptance criteria for calibration standards stipulated that the back-calculated concentrations of at least three-fourths of the individual calibration standard replicates must not deviate more than ±15% from their nominal concentration (except at the LLQ). At least one duplicate at the LLQ must exhibit a deviation within ±20% of the nominal

concentration . The coefficient of determination (r^2) must be ≥0.98. The acceptance criteria followed are in accordance with ABS Standard Operating Procedures.

The acceptance criteria for the quality control samples stipulated that at least two-thirds of the individual QC sample replicates must not deviate more than ±15% from their nominal concentrations. At least one replicate at each QC concentration must exhibit a deviation within ±15%.

A summary of the sample and assay information for study #FACT-TOX-108 is given in Table 1.

2.2. Assay Site

All samples were analyzed at Advanced BioAnalytical Services, Inc., Ithaca, NY.

2.3. Data Processing

The data were collected using turbo ion spray LC/MS selected ion monitoring (SIM) in the negative ion mode. Peak areas were integrated by the PE SCIEX program MacQuan, version 1.4, residing on a Macintosh computer. Following peak area integration, the results tables from MacQuan were saved as text files and uploaded to the ABS file server where a weighted ($1/y^2$) quadratic regression was performed using the software package Watson (v 5.3.1.01 PSS, Inc., Wayne, PA 19087). Calculations were performed on unrounded numbers. All calibration standard and QC results were rounded to no less than three significant figures before reporting.

3. RESULTS

3.1. Assay Performance

The performance of the assay for PFOS as determined from the analysis of daily quality control samples is documented in Table 2. The inter-assay precision (RSD) of quality control samples ranged from 1.57 to 4.01% for PFOS. There was no marked inaccuracy in the results from these quality control samples; the relative error (RE) ranged from -7.32 to 10.1%.

The performance of daily calibration curves is documented in Table 3. The inter-assay precision (RSD) of the standards ranged from 1.11 to 5.83% for PFOS. There was no marked inaccuracy in the results from these standards; the relative error (RE) ranged between -5.86 to 11.0%.

The slope, y-intercept, and coefficient of determination (r^2) for each analytical run are presented in Table 4. The r^2 values ranged from 0.9954 to 0.9982 for PFOS in rat serum.

All ABS personnel assigned to analyze samples for this project were required to successfully extract quality control (QC) samples and calibration standards. Results of the extraction were reviewed by a trained analyst against the pre-defined acceptance criteria for this assay. Results are maintained in the study records of ABS.

3.2. Analytical Results for Study #FACT-TOX-108

PFOS concentrations in rat serum from Study #FACT-TOX-108 are presented in Table 5. All data were rounded to no less than three significant figures before reporting in Table 5. Study samples requiring repeat analysis for Study #FACT-TOX-108 are presented in Table 6.

4. SUMMARY AND CONCLUSIONS

The concentration of PFOS was determined in rat serum samples collected during the study #FACT-TOX-108.

PFOS was isolated from serum by a liquid-liquid extraction procedure and determined by LC/MS.

The quality of the determinations was satisfactory throughout. The LLQ was 0.05 µg/mL PFOS. The precision of the assay, as determined from the analysis of quality control samples was ≤4.01% for PFOS.

5. DATA RETRIEVAL

The calculated concentration data from the analysis of rat serum samples for Study #FACT-TOX-108 are maintained on file in the archives of the Advanced BioAnalytical Services, Inc., Ithaca, NY in ABS Notebook 2319.

6. REFERENCES

1. Advanced BioAnalytical Services Validation Report 99VDJA01.MI.DOC. Method Validation for the Quantitation of Perfluorooctanesulfonate (PFOS) in Rat Serum by Turbo Ion Spray LC/MS. David J. Anderson, M.S. Amie J. Prince, B.S., and Holly D. Ross, M.S. 26 August 1999.

7. TABLES

Table 1: Summary of Sample and Assay Information for Protocol FACT-TOX-108

Species/Matrix:	Rat/Serum
Sample Collection and Storage Information:	
Anticoagulant/Stabilizer:	None
Reported Sample Collection Dates:	7 January 1999 to 29 January 1999
Dates Received at ABS:	16 June 1999
Storage Temperature at ABS:	–20 °C
Assay Information:	
Assay Period:	22 June 1999 to 28 June 1999
Analyte:	**Potassium perfluorooctanesulfonate (PFOS)**
Analytical Standard:	PFOS
Lot No:	171
Source:	3M
Internal Standard:	1H,1H,2H,2H-perfluorooctane sulfonic acid (Tetra-H-PFOS)
Lot No:	59909
Source:	3M
Calibration Range:	
Regression Method:	quadratic
Weighting Factor:	$1/y^2$
Lower Limit of Quantitation:	0.05 µg/mL
Upper Limit of Quantitation:	20 µg/mL

Table 2: Inter-Assay Precision and Accuracy for PFOS Quality Control Samples in Rat Serum for Study #FACT-TOX-108

	PFOS Concentration (µg/mL)				
Run No.	QC1 0.2	QC2 6	QC3 18	QC4 100 (1 in 10 Dilution)	QC4 100 (1 in 20 Dilution)
1	0.187	6.19	17.5	110	NA
	0.192	6.22	17.3	109	NA
	0.191	6.22	17.5	110	NA
	0.182	6.14	17.3	108	NA
2	0.191	6.13	16.9	NA	NA
	0.191	5.91	16.8	NA	NA
	0.190	6.20	16.5	NA	NA
	0.192	6.19	16.7	NA	NA
3	0.172	6.02	17.0	114	107
	0.174	6.22	16.7	111	106
	0.175	5.94	17.0	110	100
	0.177	6.08	17.1	109	103
5	0.184	6.37	17.3	109	NA
	0.187	6.36	17.5	113	NA
	0.197	6.39	17.5	111	NA
	0.183	6.33	17.1	109	NA
Mean	0.185	6.18	17.1	110	104
RSD (%)	4.01	2.33	1.92	1.57	2.92
RE	-7.32	3.04	-5.00	10.1	3.97

RSD = (SD/Mean) x 100%
RE = [(Mean-Nominal)/Nominal] x 100%
NA: Not Applicable.

Table 3: Inter-Assay Precision and Accuracy for PFOS Calibration Standards in Rat Serum for Study #FACT-TOX-108

	PFOS Concentration (µg/mL)									
Run No.	STD1 0.05	STD2 0.1	STD3 0.25	STD4 0.5	STD5 1	STD6 2	STD7 4	STD8 8	STD9 16	STD10 20
1	0.0479	0.105	0.240	0.488	0.891	2.03	4.24	7.64	15.4	20.5
	0.0490	0.112	0.258	0.509	0.935	2.12	4.51	8.03	16.1	20.0
2	0.0508	0.102	0.245	0.488	0.973	2.07	4.37	7.97	15.4	19.8
	0.0503	0.0985	0.240	0.483	0.970	2.05	4.41	7.93	15.9	20.4
3	0.0474	0.105	0.237	0.476	0.944	2.12	4.52	7.91	15.6	20.1
	0.0513	0.109	0.245	0.479	0.934	2.07	4.54	7.84	15.8	20.0
5	0.0498	0.0936	0.231	0.483	0.952	2.19	4.45	7.79	15.8	19.9
	0.0553	0.0982	0.244	0.507	0.933	2.21	4.47	7.89	15.6	20.1
Mean	0.0502	0.103	0.242	0.489	0.941	2.11	4.44	7.88	15.7	20.1
RSD (%)	4.90	5.83	3.20	2.54	2.71	3.09	2.23	1.52	1.62	1.11
RE	0.450	2.82	-3.04	-2.21	-5.86	5.34	11.0	-1.55	-1.86	0.446

RSD = (SD/Mean) x 100%

RE = [(Mean-Nominal)/Nominal] x 100%

Table 4: Calibration Curve Statistics for the Determination of PFOS in Rat Serum for Study #FACT-TOX-108

Run No.	Quadratic Coefficient (A)	Linear Coefficient (B)	Intercept (C)	Coefficient of Determination (r^2)
1	-0.0007277	0.1225	0.001121	0.9959
2	-0.0005896	0.1392	0.0009484	0.9982
3	-0.0006758	0.1401	0.001454	0.9959
5	-0.0004928	0.1345	0.001190	0.9954
Mean	-0.0006215	0.1341	0.001178	0.9964

$y = Ax^2 + Bx + C$, weighted $1/y^2$

Table 5: Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-108

Rat Number*	PFOS Concentration (µg/mL)
18901	0.0507
18901P	<LLQ[0.0323]<(0.05)
18903	4.80
18903P	35.7
18904	<LLQ[0.0462]<(0.05)
18906	<LLQ[0.0329]<(0.05)
18907	1.35
18907P	33.5
18908	2.41
18909	1.06
18910	<LLQ[0.0316]<(0.05)
18910P	<LLQ[0.0382]<(0.05)
18911	0.0798
18911P	0.123
18912	<LLQ[0.0376]<(0.05)
18913	<LLQ[0.0341]<(0.05)
18913P	<LLQ[0.0398]<(0.05)
18915	<LLQ[0.0221]<(0.05)
18915P	<LLQ[0.0242]<(0.05)
18917	<LLQ[0.0355]<(0.05)
18917P	<LLQ[0.0221]<(0.05)
18918	<LLQ[0.0323]<(0.05)
18918P	<LLQ[0.0247]<(0.05)
18919	<LLQ[0.0340]<(0.05)
18920	1.96
18922	<LLQ[0.0412]<(0.05)
18922P	<LLQ[0.0367]<(0.05)
18923	<LLQ[0.0291]<(0.05)
18923P	<LLQ[0.0331]<(0.05)
18924	2.72
18924P	30.7
18926	<LLQ[0.0323]<(0.05)
18926P	<LLQ[0.0229]<(0.05)
18927	<LLQ[0.0305]<(0.05)
18927P	<LLQ[0.0318]<(0.05)
18929	2.40

*Rat Number with a "P" indicates a pup sample.
<LLQ: Less than the Lower Limit of Quantitation (0.05 µg/mL)

Table 5: **(Continued) Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-108**

Rat Number*	PFOS Concentration (µg/mL)
18931	<LLQ[0.0382]<(0.05)
18931P	<LLQ[0.0196]<(0.05)
18932	<LLQ[0.0399]<(0.05)
18934	1.25
18934P	34.1
18935	<LLQ[0.0316]<(0.05)
18935P	<LLQ[0.0206]<(0.05)
18936	<LLQ[0.0302]<(0.05)
18936P	<LLQ[0.0230]<(0.05)
18937	1.80
18938	<LLQ[0.0359]<(0.05)
18939	<LLQ[0.0311]<(0.05)
18939P	<LLQ[0.0308]<(0.05)
18940	<LLQ[0.0330]<(0.05)
18940P	<LLQ[0.0231]<(0.05)
18941	5.34
18942	1.12
18943	65.1
18943P	82.2
18946	157
18947	86.9
18948	96.1
18950	66.4
18951	72.4
18951P	59.2
18952	90.8
18952P	48.3
18953	67.0
18953P	53.8
18956	97.5
18956P	89.3
18957	69.1
18957P	93.2
18958	68.3
18958P	56.2

*Rat Number with a "P" indicates a pup sample.
<LLQ: Less than the Lower Limit of Quantitation (0.05 µg/mL)

Table 5: **(Continued) Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-108**

Rat Number*	PFOS Concentration (µg/mL)
18959	59.2
18960	78.0
18960P	93.6
18961	69.1
18961P	47.6
18963	83.6
18964	85.6
18964P	92.9
18965	75.9
18967	74.2
18968	80.9
18969	78.6
18970	99.3
18970P	96.9
18971	218
18972	66.9
18972P	58.2
18973	81.9
18973P	79.5
18974	172
18976	77.9
18977	124

*Rat Number with a "P" indicates a pup sample.

Table 6: Repeat Analysis of Rat Serum Samples from Study #FACT-TOX-108

Rat Number*	Treatment	Time	Original Conc. ug/ml	Original Curve Number	Reason for Reassay	Reassay Conc. ug/ml	Reassay Curve Number	Reported Conc. ug/ml	Reason for Reported Conc.
18903P	0	Day 14	>ULQ-28.5284>(20)	3	1	35.7	5	35.7	1
18907P	0	Day 14	>ULQ-30.8172>(20)	3	1	33.5	5	33.5	1
18924P	0	Day 14	>ULQ-27.5496>(20)	3	1	30.7	5	30.7	1
18934P	0	Day 14	>ULQ-36.8737>(20)	3	1	34.1	5	34.1	1
18971	1	Day 14	>ULQ-219.5172>(200)	1	2	218	3	218	2

>ULQ: Greater than the Upper Limit of Quantitation.
*Rat Number with a "P" indicates a pup sample.

REASONS FOR REASSAY:
1). Run 3 concentration exceeded calibration range.
2). Run 1 concentration exceeded calibration range.
REASONS FOR REPORTED CONC:
1). Run 5 concentration within calibration range.
2). Run 3 concentration within calibration range.

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ADDENDUM TO THE BIOANALYTICAL REPORT

Title:	**Addendum - Quantitative Determination of Perfluorooctanesulfonate (PFOS) in Rat Serum from Study #FACT-TOX-108 Using Turbo Ion Spray LC/MS**
Date:	**13 October 1999**
Addendum Report:	**99ADJA01.MI.ADD.DOC**
Original Report:	**99ADJA01.MI.DOC**
Authors:	**David J. Anderson, M.S.** **Amie J. Prince, B.S.** **Holly D. Ross, M.S.**
Prepared For:	**3M Environmental Technology and Safety Services** **St. Paul, MN 55133-3331**
Number of Pages:	**3**

15 Catherwood Road ■ Ithaca, New York 14850 ■ (607) 266-0665 ■ Fax (607) 266-0749

ADVANCED BIOANALYTICAL SERVICES

SIGNATURE PAGE

Title: Addendum - Quantitative Determination of Perfluorooctanesulfonate (PFOS) in Rat Serum from Study #FACT-TOX-108 Using Turbo Ion Spray LC/MS

ABS Report No.: 99ADJA01.MI.ADD.DOC

Reported by: _____ 13 OCT 99
David J. Anderson, M.S. Date
Research Scientist

Reviewed by: Kathleen Cormack 13 Oct 99
Kathleen Cormack, B.S.. Date
Associate Auditor

Authorized for Release by: _____ 13 OCT 99
John R. Perkins, Ph.D. Date
Assistant Scientific Director

Accepted by: _____ 14 Oct 99
Kris Hanson, Ph.D. Date
3M Study Director

ADDENDUM SUMMARY

ADDENDUM

Addition of Study Director signature on the Signature Page. The signature page of this addendum indicates acceptance of the original report.

REASON FOR ADDENDUM

Original GLP report requires the signature of the Study Director.

ATTACHMENT G
REPORT SIGNATURE PAGE

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

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

Dale L. Bacon, Laboratory Manager — 2/14/00 Date

Study Title

Analytical Laboratory Report on the Determination of the Presence and Concentration of Perfluorooctanesulfonate (PFOS) in the Serum of Sprague-Dawley® Rats Exposed to Potassium Perfluorooctanesulfonate via Gavage

REPORT AMENDMENT NO. 1

Amendment Date:

19 April 2000

Performing Laboratory

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

Laboratory Project Identification

ET&SS LRN-U2779
ET&SS FACT Tox-108

This amendment modifies the following portion(s) of the final report:

1. ***FINAL REPORT:***
Make the following addition to the final report text.
ADD:
After the final report was issued (signed and archived), it was discovered that the purity values stated for the analytical reference material and test substance were inaccurate. These values were based only on NMR analyses. Subsequent chemical characterization is occurring to determine the actual purity of theses substances (PFOS Lot 171 and 217). The final report will be amended to detail the analytical results using the purity values from the certificates of analysis when they are issued for PFOS Lot 171 and 217.
REASON:
To describe the changes that will be made to the final report when the certificate of analysis is issued for PFOS Lot 171 and 217.

2. ***FINAL REPORT:***
Make the following addition to the exceptions list in the Statement of Compliance section.
AMEND TO READ:
   - The purity and stability of the test substance and analytical reference material are unknown and was not determined prior to the initiation of this study.

REASON:
The Statement of Compliance section was incomplete.

3. ***FINAL REPORT:***
The purity of the test substance is 99.28% (Lot 217), and analytical reference material is 99.49% (Lot 171) as listed in Table 2.
AMEND TO READ:
The purity of the test substance and analytical reference material listed in Table 2 is unknown.
REASON:
The purity values stated for the test substance and analytical reference material listed in Table 2 are incorrect.

Amendment Approval

Marvin T Case — 19 April 2000

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

Kristen Hansen — 04/19/00

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