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

Run 11	PFOS (µg/mL)			
QC (Theoretical Conc.)	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	PFOS (µg/mL)	
QC (Theoretical Conc.)	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

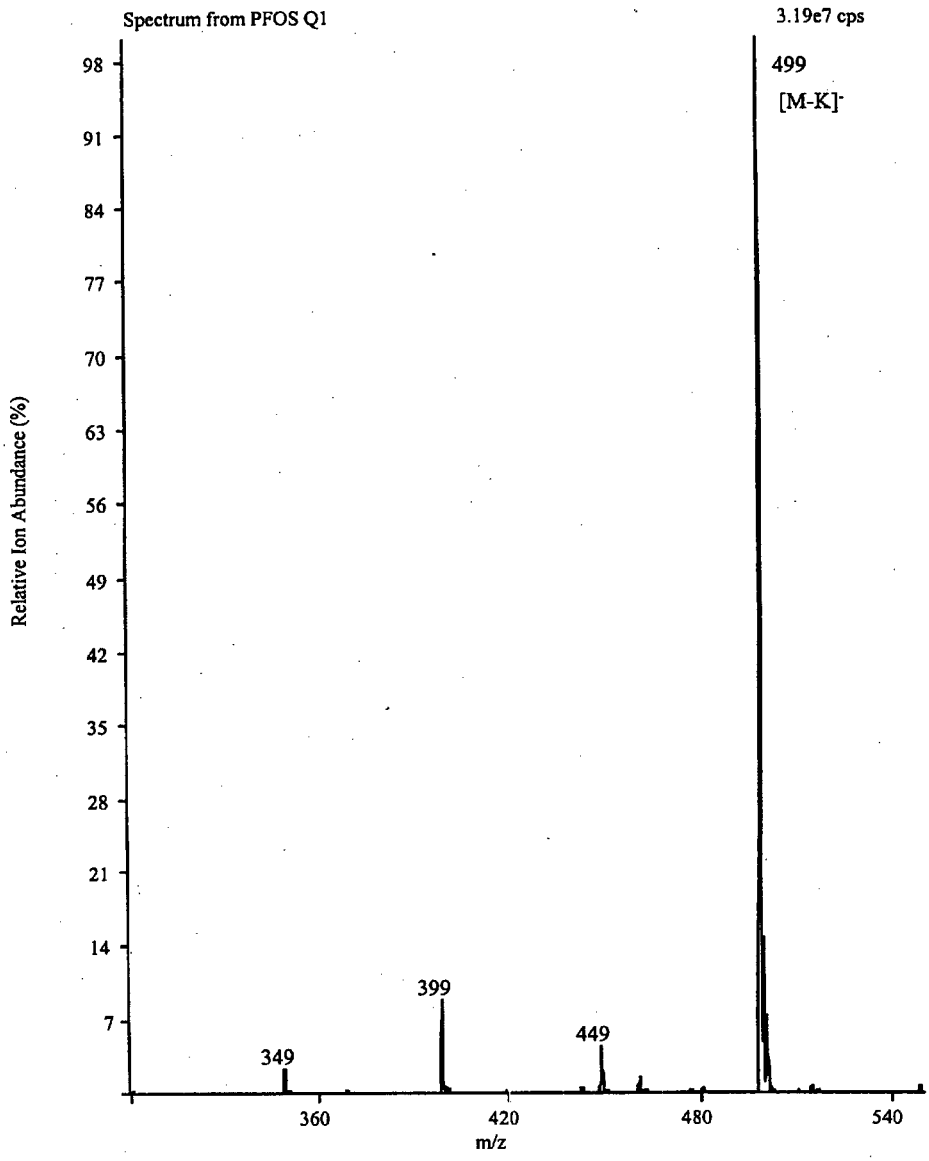


Figure 2: Full-Scan Single MS Mass Spectrum of Tetra-H-PFOS

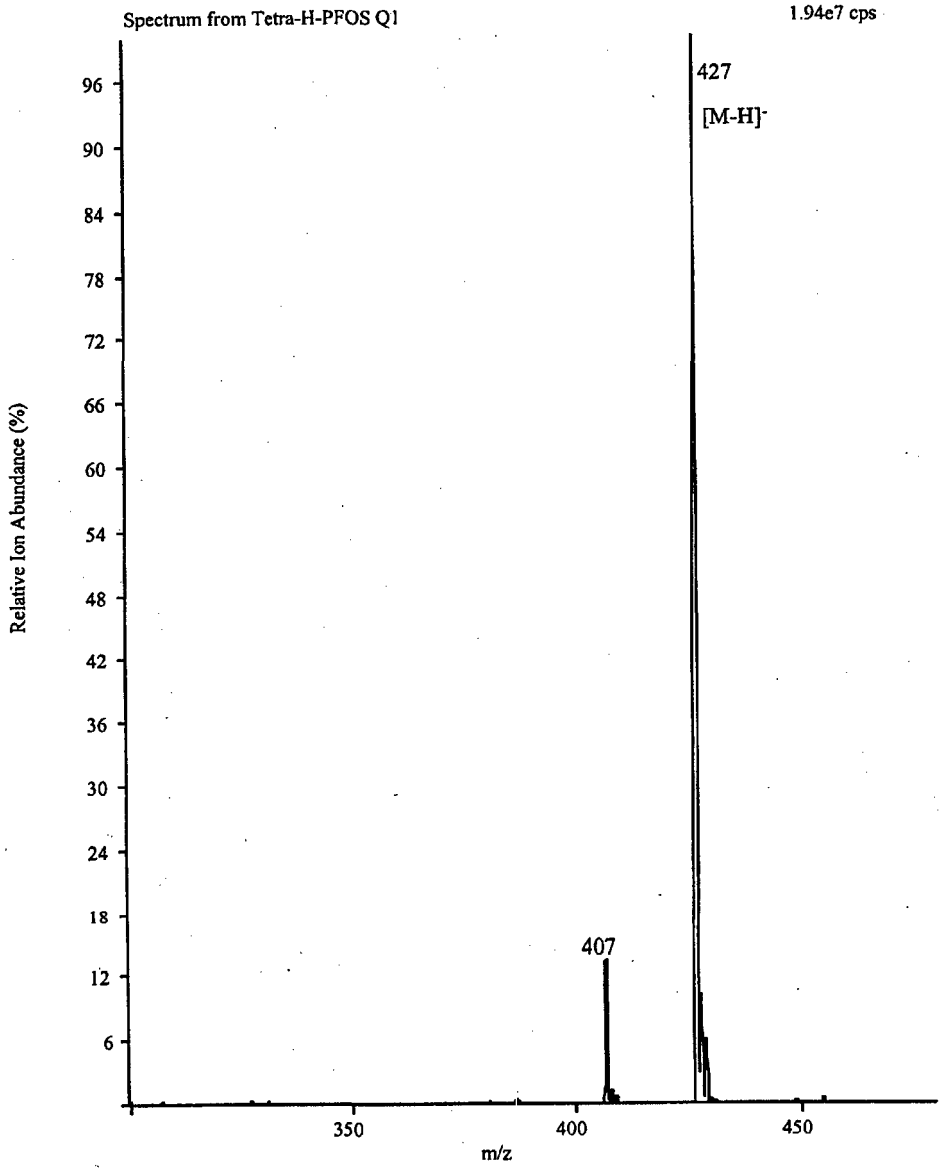
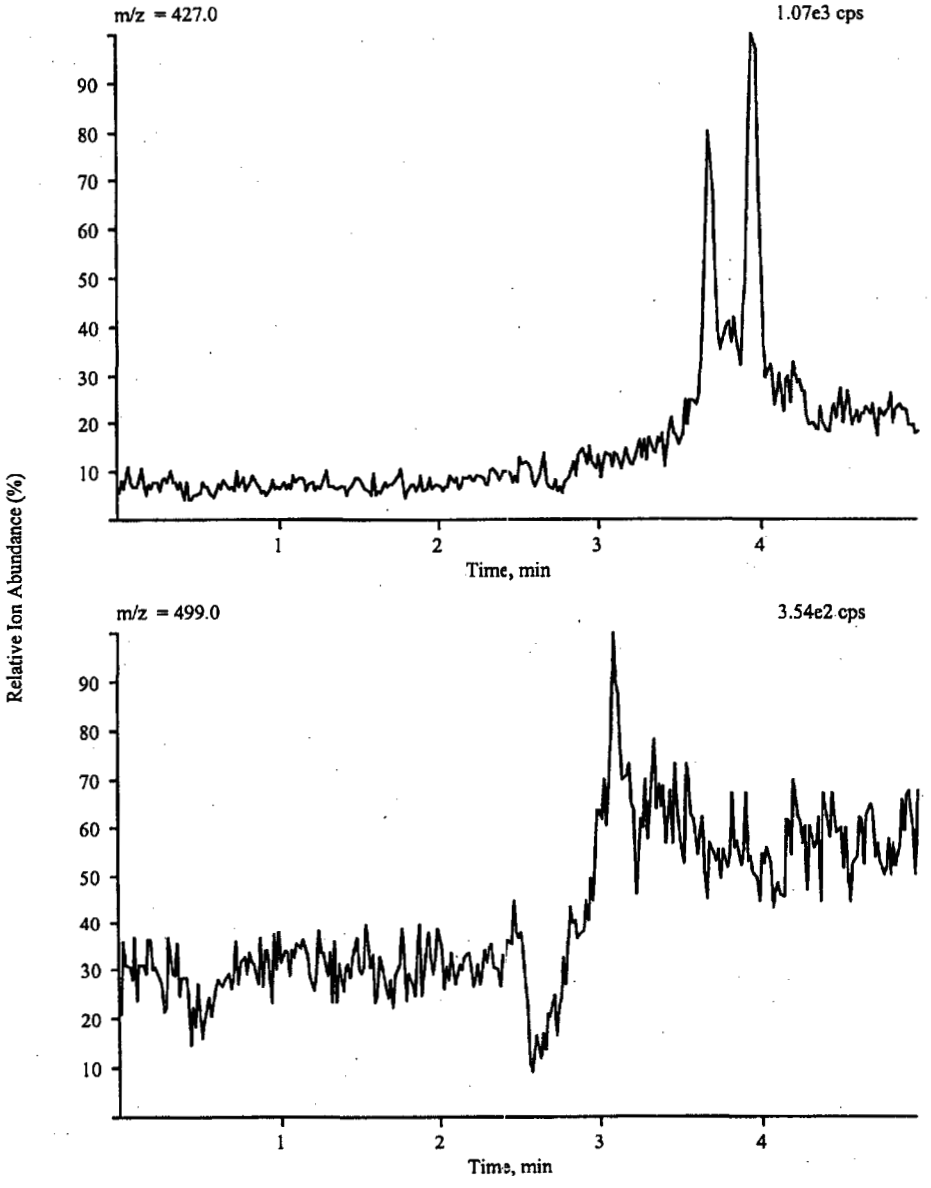


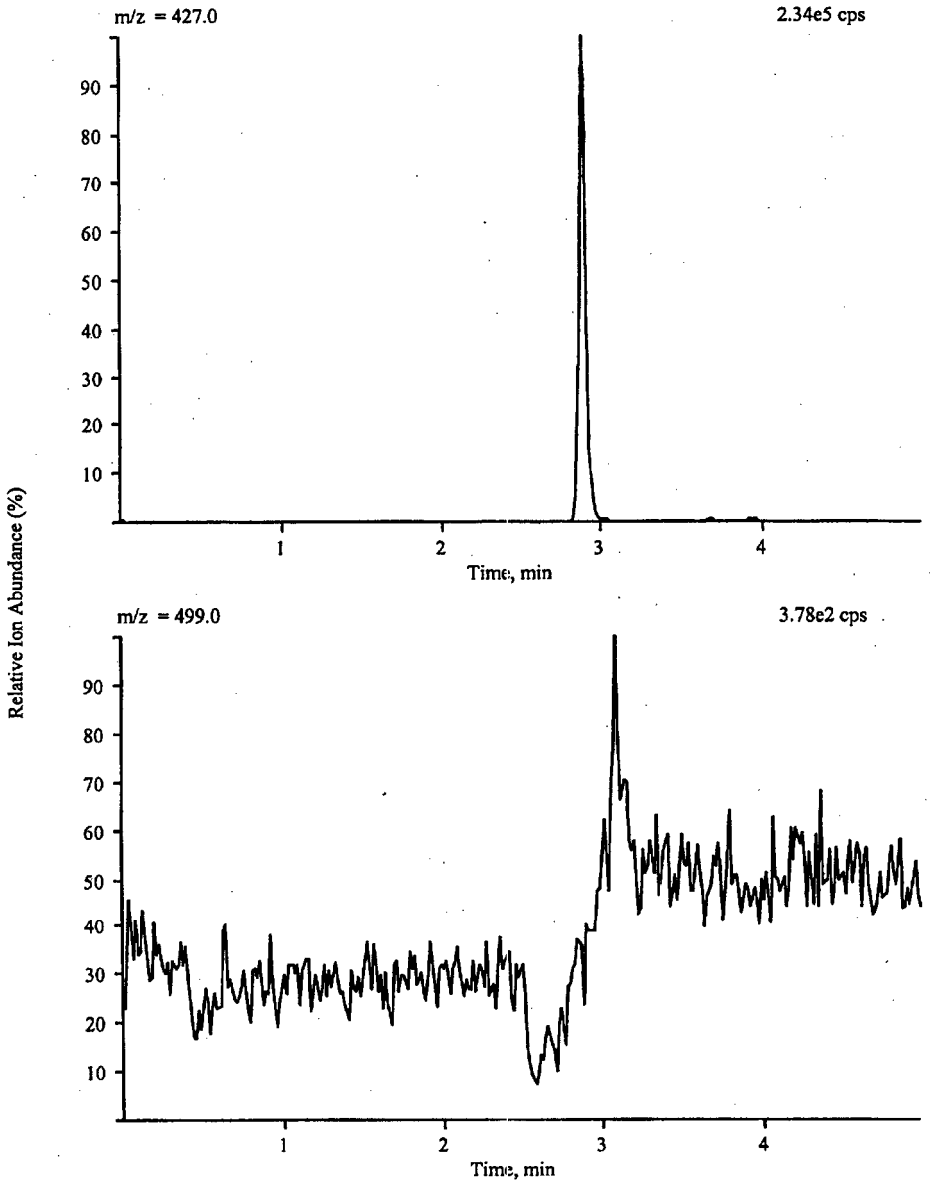
Figure 3: Mass Chromatograms of PFOS and its Internal Standard in Control Blank Rat Serum Extract



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

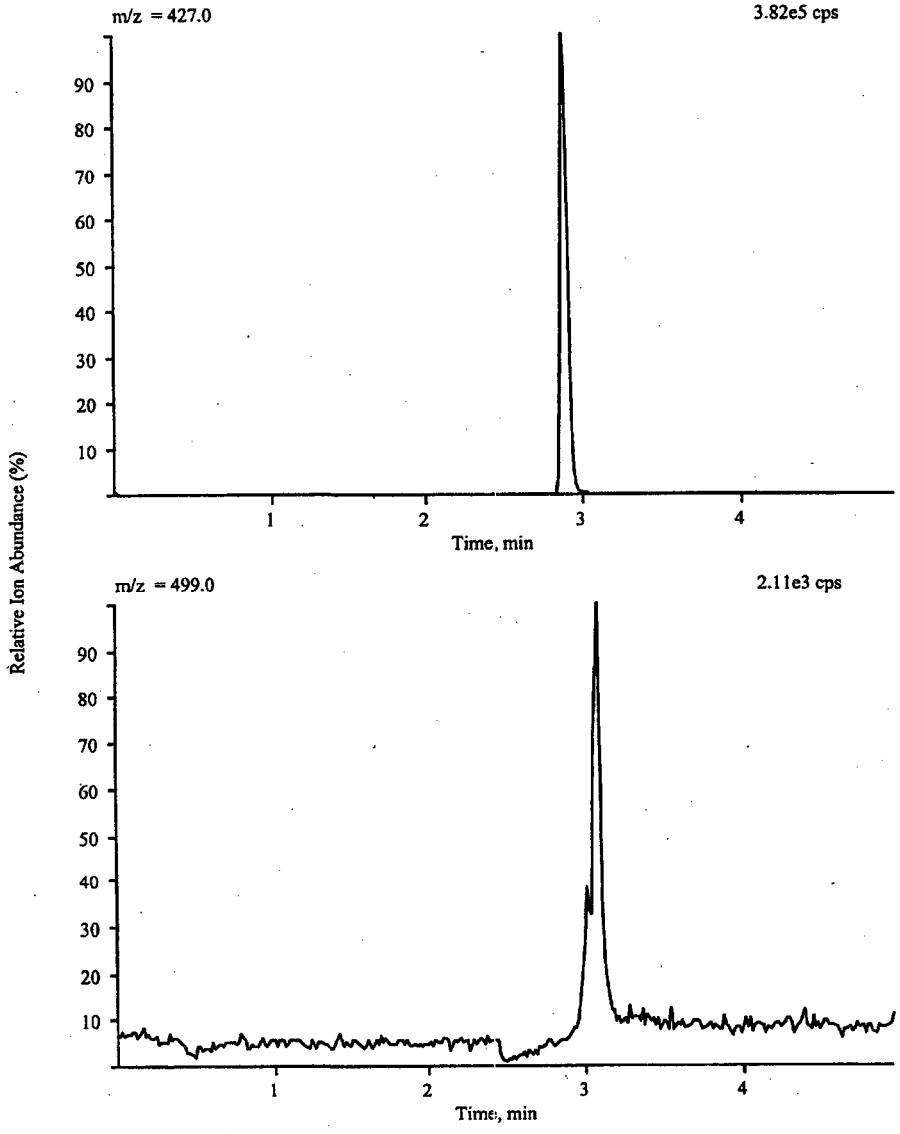
Figure 4: Mass Chromatograms of PFOS in a Rat Serum Extract Sample Containing Internal Standard Only (Zero Sample)



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

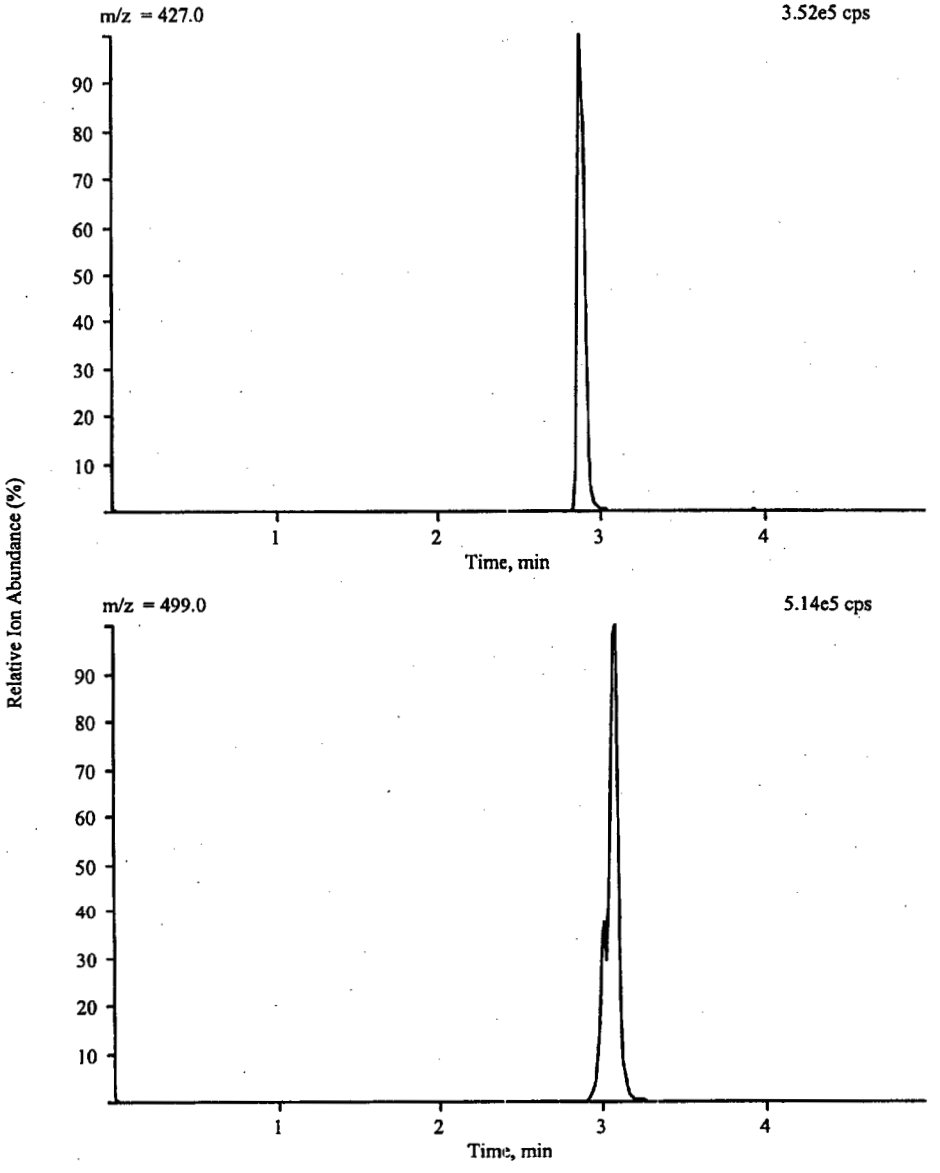
Figure 5: Mass Chromatograms of Calibration Standard 1 in Rat Serum Extract Containing PFOS (0.05 µg/mL) and the Internal Standard



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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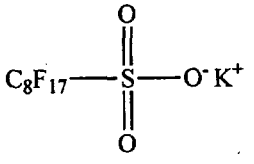
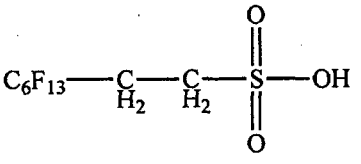
A.1.0 CHEMICAL STRUCTURE(S)



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.

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BIOANALYTICAL REPORT

Title:	Quantitative Determination of Perfluorooctanesulfonate (PFOS) in Rat Serum from Study #FACT-TOX-110 Using Turbo Ion Spray LC/MS
Date:	21 September 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.:	99ADJA02.MI.DOC
Study Protocol:	FACT-TOX-110
Number of Pages:	24

Summary and Conclusions

The concentration of perfluorooctanesulfonate (PFOS) was determined in rat serum samples collected during the study #FACT-TOX-110 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 six 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 ≤6.90%. The precision of this assay (RSD), as determined from the calibration standards for PFOS was ≤6.18%. The relative error (RE) of the assay, as determined from the analysis of the quality control samples ranged from 1.52 to 12.4%. The RE of the assay, as determined from the analysis of calibration standards ranged from -4.62 to 10.3%.

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ADVANCED BIOANALYTICAL SERVICES

SIGNATURE PAGE

Title: Quantitative Determination of Perfluorooctanesulfonate (PFOS) in Rat Serum from Study #FACT-TOX-110 Using Turbo Ion Spray LC/MS

ABS Report No.: 99ADJA02.MI.DOC

Reported by: _____ 21 SEP 99
David J. Anderson, M.S. — Date
Research Scientist

Reviewed by: Melissa Mapes 21 SEP 99
Melissa Mapes, A.S. — Date
Associate Auditor

Authorized for Release by: _____ 21. SEP. 99
John R. Perkins, Ph.D. — Date
Assistant Scientific Director

QAU STATEMENT

Periodic inspections of the bioanalytical portion of study #FACT-TOX-110 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:

25 June 1999; 6, 7, 8, 21 July 1999; 31 August 1999.

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

25 June 1999; 6, 7, 8, 21 July 1999; 31 August 1999.

Results of the inspections were reported to the Study Director on 2 September 1999.

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

Melissa Mapes 21SEP99

Melissa Mapes, A.S. Date
Associate Auditor

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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-110, "Oral (Gavage) Pharmacokinetic 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 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-110 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 2.37 to 6.90% for PFOS. There was no marked inaccuracy in the results from these quality control samples; the relative error (RE) ranged from 1.52 to 12.4%.

The performance of daily calibration curves is documented in Table 3. The inter-assay precision (RSD) of the standards ranged from 1.70 to 6.18% for PFOS. There was no marked inaccuracy in the results from these standards; the relative error (RE) ranged between -4.62 to 10.3%.

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 0.9955 to 0.9965 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-110

PFOS concentrations in rat serum from Study #FACT-TOX-110 are presented in Tables 5 through 9. All data were rounded to no less than three significant figures before reporting in Tables 5 through 9. Study samples requiring repeat analysis for Study #FACT-TOX-110 are presented in Table 10.

4. SUMMARY AND CONCLUSIONS

The concentration of PFOS was determined in rat serum samples collected during the study #FACT-TOX-110.

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 ≤6.90% for PFOS.

5. DATA RETRIEVAL

The calculated concentration data from the analysis of rat serum samples for Study #FACT-TOX-110 are maintained on file in the archives of the Advanced BioAnalytical Services, Inc., Ithaca, NY in ABS Notebook 2324.

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-110

Species/Matrix:	Rat/Serum
Sample Collection and Storage Information:	
Anticoagulant/Stabilizer:	None
Reported Sample Collection Dates:	27 December 1998 to 22 January 1999
Dates Received at ABS:	16 June 1999
Storage Temperature at ABS:	–20 °C
Assay Information:	
Assay Period:	29 June 1999 to 8 July 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-110

	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)	QC4 100 (1 in 30 Dilution)
1	0.178	6.18	15.9	NA	NA	NA
	0.183	6.40	17.3	NA	NA	NA
	0.177	6.32	17.3	NA	NA	NA
	0.187	6.32	17.3	NA	NA	NA
3	0.210	6.24	17.9	NA	NA	NA
	0.215	5.79	17.2	NA	NA	NA
	0.211	5.99	17.8	NA	NA	NA
	0.211	5.87	17.9	NA	NA	NA
4	0.225	6.14	18.1	116*	NA	NA
	0.221	6.09	18.4	110	NA	NA
	0.222	6.11	18.2	110	NA	NA
	0.220	6.19	18.2	113	NA	NA
5	0.220	6.14	19.0	NA	116*	NA
	0.218	6.31	18.1	NA	112	NA
	0.218	6.27	18.5	NA	107	NA
	0.214	6.42	18.7	NA	114	NA
6	0.225	6.22	19.1	NA	108	NA
	0.222	6.15	19.5	NA	111	NA
	0.218	6.39	19.8	NA	111	NA
	0.219	6.27	19.3	NA	111	NA
7	0.221	6.11	18.6	NA	NA	106
	0.212	6.15	19.2	NA	NA	104
	0.215	6.17	18.5	NA	NA	100
	0.218	6.80	18.8	NA	NA	100
Mean	0.212	6.21	18.3	112	111	103
RSD (%)	6.90	3.17	4.84	2.37	2.55	2.73
RE	5.80	3.50	1.52	12.4	11.2	2.54

RSD = (SD/Mean) x 100%
RE = [(Mean-Nominal)/Nominal] x 100%
NA: Not applicable
*Individual replicate exceeds 15% acceptance criterion.

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Table 3: Inter-Assay Precision and Accuracy for PFOS Calibration Standards in Rat Serum for Study #FACT-TOX-110

	PFOS Concentration (µg/mL)									
Run No.	STD1	STD2	STD3	STD4	STD5	STD6	STD7	STD8	STD9	STD10
	0.05	0.1	0.25	0.5	1	2	4	8	16	20
1	0.0473	0.0981	0.246	0.466	0.888	2.06	4.41	7.78	16.5	19.8
	0.0552	0.0985	0.261	0.507	0.997	2.11	4.48	7.80	15.5	20.0
3	0.0467	0.0985	0.237	0.488	1.00	2.08	4.26	8.00	15.1	20.0
	0.0545	0.108	0.251	0.448	0.924	2.21	4.49	8.07	15.7	20.5
4	0.0521	0.103	0.231	0.498	0.975	2.16	4.22	7.83	15.9	20.6
	0.0474	0.108	0.241	0.500	0.928	2.07	4.52	7.62	15.7	19.7
5	0.0499	0.0983	0.234	0.450	0.944	2.22	4.44	7.90	15.8	20.0
	0.0526	0.100	0.250	0.489	0.989	2.20	4.38	7.86	15.4	20.2
6	0.0464	0.102	0.252	0.473	0.953	2.17	4.42	7.90	16.0	20.6
	0.0530	0.108	0.248	0.471	0.938	2.09	4.30	7.65	15.5	19.7
7	0.0501	0.100	0.266	0.476	0.958	2.15	4.54	7.91	16.1	20.2
	0.0528	0.0905	0.256	0.478	0.948	2.00	4.47	7.64	15.8	19.7
Mean	0.0507	0.101	0.248	0.479	0.954	2.13	4.41	7.83	15.8	20.1
RSD (%)	6.18	5.05	4.35	3.89	3.52	3.24	2.32	1.80	2.28	1.70
RE	1.33	1.05	-0.887	-4.27	-4.62	6.31	10.3	-2.14	-1.51	0.467

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-110

Run No.	Quadratic Coefficient (A)	Linear Coefficient (B)	Intercept (C)	Coefficient of Determination (r^2)
1	-0.0009199	0.1354	0.001580	0.9960
3	-0.0005189	0.1328	0.002324	0.9955
4	-0.0005052	0.1334	0.0004498	0.9965
5	-0.0003195	0.1137	0.0009957	0.9958
6	-0.0004256	0.1119	0.0001798	0.9963
7	-0.0003264	0.1117	0.0001116	0.9959
Mean	-0.0005026	0.1232	0.0009401	0.9960

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

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Table 5: Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-110 Following the 0 mg/kg Dose

Sampling Time (day)	PFOS Concentration (µg/mL) Rat Number*									
	19501	19502	19503	19504	19504F	19505	19505F	19506	19506F	19507
0	0.112	0.0701	0.0607	0.0514	NS	0.0550	NS	0.309	NS	0.0545
7	0.397	0.0535	NS	0.0926	NS	0.0514	NS	0.219	NS	0.0586
15	0.0803	0.0690	NS	<LLQ[0.0432]<(0.05)	NS	0.0609	NS	0.199	NS	0.0648
21	NS	NS	NS	<LLQ[0.0258]<(0.05)	0.0845	<LLQ[0.0353]<(0.05)	0.0880	0.0820	0.267	<LLQ[0.0247]<(0.05)

<LLQ = Less than the Lower Limit of Quantitation (0.05 µg/mL).
*Rat Number with "F" indicates fetal sample.
NS: No sample.

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Table 5: (Continued) Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-110 Following the 0 mg/kg Dose

Sampling Time (day)	PFOS Concentration (µg/mL) Rat Number*							
	19507F	19508	19509	19509F	19510	19511	19512	19512F
0	NS	0.0548	<LLQ[0.0483]<(0.05)	NS	0.0664	0.0619	<LLQ[0.0449]<(0.05)	NS
7	NS	<LLQ[0.0395]<(0.05)	<LLQ[0.0481]<(0.05)	NS	0.0636	0.250	0.197	NS
15	NS	<LLQ[0.0454]<(0.05)	<LLQ[0.0458]<(0.05)	NS	0.0614	0.176	0.238	NS
21	0.0842	NS	<LLQ[0.0269]<(0.05)	0.102	NS	NS	0.0833	0.219

<LLQ = Less than the Lower Limit of Quantitation (0.05 µg/mL).
*Rat Number with "F" indicates fetal sample.
NS: No sample.

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Table 5: (Continued) Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-110 Following the 0 mg/kg Dose

Sampling Time (day)	PFOS Concentration (µg/mL) Rat Number*				
	19513	19514	19515	19516	19516F
0	0.0527	<LLQ[0.0500]<(0.05)	<LLQ[0.0443]<(0.05)	0.0559	NS
7	<LLQ[0.0345]<(0.05)	0.0784	<LLQ[0.0488]<(0.05)	<LLQ[0.0447]<(0.05)	NS
15	<LLQ[0.0462]<(0.05)	0.0648	0.0581	<LLQ[0.0328]<(0.05)	NS
21	NS	NS	NS	<LLQ[0.0206]<(0.05)	0.165

<LLQ = Less than the Lower Limit of Quantitation (0.05 µg/mL).
*Rat Number with "F" indicates fetal sample.
NS: No sample.

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Table 6: Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-110 Following the 0.1 mg/kg Dose

Sampling Time (day)	PFOS Concentration (µg/mL) Rat Number*											
	19517	19518	19518F	19519	19519F	19520	19521	19521F	19522	19523	19523F	19524
0	9.07	9.87	NS	9.37	NS	9.73	11.1	NS	9.32	12.8	NS	10.2
7	8.63	10.3	NS	6.83	NS	NS	8.82	NS	8.19	9.65	NS	10.4
15	11.5	10.7	NS	9.18	NS	NS	14.1	NS	9.57	10.9	NS	9.20
21	NS	6.84	12.1	3.93	8.52	NS	4.93	11.2	NS	5.35	10.2	4.11

*Rat Number with "F" indicates fetal sample.
NS: No sample.

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Table 6: (Continued) Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-110 Following the 0.1 mg/kg Dose

Sampling Time (day)	PFOS Concentration (µg/mL) Rat Number*										
	19524F	19525	19526	19527	19528	19528F	19529	19529F	19530	19531	19532
0	NS	12.0	9.48	12.0	10.0	NS	11.6	NS	8.48	9.10	10.6
7	NS	9.62	11.2	8.78	7.80	NS	10.3	NS	7.65	7.57	10.1
15	NS	10.0	11.7	8.78	8.04	NS	7.31	NS	11.8	10.4	9.66
21	10.3	NS	NS	NS	6.19	10.8	3.04	10.4	NS	NS	NS

*Rat Number with "F" indicates fetal sample.
NS: No sample.

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Table 7: Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-110 Following the 0.4 mg/kg Dose

Sampling Time (day)	PFOS Concentration (µg/mL) Rat Number*											
	19533	19533F	19534	19534F	19535	19536	19536F	19537	19538	19539	19540	19541
0	53.9	NS	46.9	NS	50.1	38.6	NS	56.6	46.5	45.2	42.9	44.4
7	44.3	NS	50.0	NS	56.6	36.6	NS	58.4	48.1	44.6	45.8	NS
15	47.3	NS	56.1	NS	48.2	43.3	NS	50.7	53.5	37.2	48.6	NS
21	20.4	42.5	20.5	41.4	NS	16.2	32.2	NS	NS	NS	NS	NS

*Rat Number with "F" indicates fetal sample.
NS: No sample.

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Table 7: (Continued) Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-110 Following the 0.4 mg/kg Dose

Sampling Time (day)	PFOS Concentration (µg/mL) Rat Number*								
	19542	19543	19543F	19544	19545	19546	19546F	19547	19548
0	45.3	46.3	NS	45.8	47.9	43.5	NS	42.2	57.6
7	40.6	46.1	NS	42.8	NS	46.5	NS	42.2	60.0
15	44.0	43.9	NS	49.1	NS	47.3	NS	43.3	58.0
21	NS	23.0	34.1	NS	NS	35.8	48.4	NS	65.7

*Rat Number with "F" indicates fetal sample.
NS: No sample.

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Table 8: Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-110 Following the 1.6 mg/kg Dose

Sampling Time (day)	PFOS Concentration (μg/mL) Rat Number											
	19549	19550	19551	19552	19553	19554	19555	19556	19557	19558	19559	19560
0	166	200	184	165	182	203	166	199	198	196	171	200
7	NS	216	NS	NS	160	189	195	186	NS	171	160	171
15	NS	160	NS	NS	135	176	225	202	NS	164	192	215
21	NS	NS	NS	NS	NS	NS	NS	251	NS	NS	237	79.4

NS: No sample.

Table 8: (Continued) Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-110 Following the 1.6 mg/kg Dose

Sampling Time (day)	PFOS Concentration (μg/mL) Rat Number*					
	19560F	19561	19562	19562F	19563	19564
0	NS	193	185	NS	163	185
7	NS	181	179	NS	168	169
15	NS	219	142	NS	183	159
21	131	NS	63.7	102	NS	NS

*Rat Number with "F" indicates fetal sample.
NS: No sample.

Table 9: Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-110 Following the 3.2 mg/kg Dose

Sampling Time (day)	PFOS Concentration (µg/mL) Rat Number*											
	19565	19566	19567	19568	19568F	19569	19569F	19570	19571	19571F	19572	19572F
0	370	337	366	339	NS	334	NS	351	348	NS	357	NS
7	392	280	327	340	NS	323	NS	337	371	NS	305	NS
15	345	311	294	336	NS	314	NS	269	308	NS	317	NS
21	NS	NS	NS	241	228	142	176	NS	165	166	123	166

*Rat Number with "F" indicates fetal sample.
NS: No sample.

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Table 9: (Continued) Concentrations of PFOS in Rat Serum Samples from Study #FACT-TOX-110 Following the 3.2 mg/kg Dose

Sampling Time (day)	PFOS Concentration (µg/mL) Rat Number*									
	19573	19574	19575	19576	19577	19577F	19578	19579	19579F	19580
0	364	365	413	383	417	NS	373	387	NS	378
7	343	393	387	NS	395	NS	NS	399	NS	357
15	317	299	284	NS	361	NS	NS	386	NS	323
21	NS	NS	NS	NS	221	226	NS	186	181	NS

*Rat Number with "F" indicates fetal sample.
NS: No sample.

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Table 10: Repeat Analysis of Rat Serum Samples from Study #FACT-TOX-110

Rat Number	Treatment	Time	Original Conc. µg/ml	Original Curve Number	Reason for Reassay	Reassay Conc. µg/ml	Reassay Curve Number	Reported Conc. µg/ml	Reason for Reported Conc.
19565	5	Day 7	>ULQ-428.6743>(400)	6	1	392	7	392	1
19565	5	Day 15	>ULQ-440.2439>(400)	6	1	345	7	345	1
19567	5	Day 0	>ULQ-402.0010>(400)	6	1	366	7	366	1
19574	5	Day 7	>ULQ-404.8033>(400)	6	1	393	7	393	1
19575	5	Day 0	>ULQ-436.6951>(400)	6	1	413	7	413	1
19577	5	Day 0	>ULQ-407.5918>(400)	6	1	417	7	417	1
19579	5	Day 0	>ULQ-403.4253>(400)	6	1	387	7	387	1

>ULQ: Greater than the Upper Limit of Quantitation.
REASONS FOR REASSAY:
1). Run 6 concentration exceeded calibration range.

REASONS FOR REPORTED CONC:
1). Run 7 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-110 Using Turbo Ion Spray LC/MS**
Date:	**13 October 1999**
Addendum Report:	**99ADJA02.MI.ADD.DOC**
Original Report:	**99ADJA02.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-110 Using Turbo Ion Spray LC/MS

ABS Report No.: 99ADJA02.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.

RE-ISSUED ANALYTICAL REPORT

STUDY TITLE

Oral (Gavage) Pharmacokinetic Study of PFOS in Rats

DATA REQUIREMENTS

Analytical Method Requirements

STUDY DIRECTOR

Marvin T. Case, 3M

PRINCIPAL INVESTIGATOR

Enaksha Wickremesinhe, Centre

RE-ISSUED ANALYTICAL REPORT COMPLETION DATE

October 19, 2000

PERFORMING LABORATORY / TESTING FACILITY

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

STUDY SPONSOR

3M Toxicology Services – Medical Department
3M Center, Building 220-2E-02
St. Paul, MN 55144-1000

PROJECT IDENTIFICATION

Sponsor Protocol Number: FACT-TOX-110
Centre Study Number: 023-016

Total Pages: 103

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Centre Study Number 023-016, entitled "Oral (Gavage) Pharmacokinetic Study of PFOS in Rats," conducted for 3M Environmental Toxicology Services – Medical Department, was performed in compliance with US FDA Good Laboratory Practice Standards (21 CFR 58) by Centre Analytical Laboratories, Inc. with the following exceptions:

1. The reference substances (analytical standards) used in this study (PFOS and THPFOS) have not been characterized under 21 CFR 58.105

2. The automated data collection systems used in this study are not fully compliant with 21 CFR 58.130(e).

Richard A. Grazzini
FOR ENAKSHA WICKREMESINHE — 19-OCT-00

Enaksha Wickremesinhe, Ph.D* — Date
Principal Investigator
Centre Analytical Laboratories, Inc.

*No longer employed at Centre. However, facility management, Rick Grazzini will be signing in the principal investigator's stead.

Marvin T Case — 23 October 2000

Marvin T Case, Ph.D. — Date
Study Director
3M Toxicology Services

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

QUALITY ASSURANCE STATEMENT

Centre Analytical Laboratories' Quality Assurance Unit reviewed Centre Study Number 023-016, entitled, "Oral (Gavage) Pharmacokinetic Study of PFOS in Rats". All phases were reviewed for conduct according to Centre Analytical Laboratories' Standard Operating Procedures, the Study Protocol, and all applicable Good Laboratory Practice Standards. All findings were reported to the Study Director and to management.

Phase	Date Inspected	Date Reported to Centre Management	Date Reported to Study Director and Sponsor Management
1. Protocol Review	5/17/00	6/1/00	7/14/00
2. Extraction	6/27/00	7/11/00	7/14/00
3. Raw Data Review	7/10,13,14,20/00	7/29/00	8/25/00
4. Draft Report Review	8/3-4/00	8/15/00	8/25/00
5. Final Report Review	9/8/00	9/11/00	9/11/00

Naomi Lovallo
Quality Assurance Auditor

10/19/00
Date

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

CERTIFICATION OF AUTHENTICITY

This report, for Centre Study Number 023-016, is a true and complete representation of the raw data for the study.

Submitted by: Centre Analytical Laboratories, Inc.
3048 Research Drive
State College, PA 16801
(814) 231-8032

Principal Investigator, Centre:

Richard A. Grazzini
FOR ENAKSHA WICKREMESINHE — 19-Oct-00

Enaksha Wickremesinhe, Ph.D.* — Date
Group/Team Leader
Centre Analytical Laboratories, Inc.

*No longer employed at Centre. However, facility management, Rick Grazzini will be signing in the principal investigator's stead.

Centre Analytical Laboratories, Inc. Facility Management:

John M Flaherty — 10/19/00

John Flaherty — Date
Laboratory Manager
Centre Analytical Laboratories, Inc.

Study Director, 3M:

Marvin T Case — 23 Oct 2000

Marvin T. Case, Ph.D. — Date
3M Toxicology Services

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

STUDY IDENTIFICATION

Oral (Gavage) Pharmacokinetic Study of PFOS in Rats

SPONSOR PROTOCOL NUMBER: FACT-TOX-110

TYPE OF STUDY:	Oral (Gavage) Pharmacokinetic
TEST SYSTEM:	Rat Feces
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
STUDY DIRECTOR:	From 6/10/99 to 2/10/00: Kris Hansen, Ph.D. 3M Environmental Technology and Safety Services Phone: (651) 778-6018 From 2/10/00 to Present: Marvin T. Case, D.V.M., Ph.D. 3M Toxicology Services Phone: (651) 733-5180
TESTING FACILITY:	Centre Analytical Laboratories, Inc. 3048 Research Drive State College, PA 16801
PRINCIPAL INVESTIGATOR	Enaksha Wickremesinhe, Ph.D. Centre Analytical Laboratories, Inc. Phone: (814) 231-8032
ANALYTICAL PHASE TIMETABLE:	Study Initiation Date: 06/10/99 Analytical Start Date: 06/09/00 Analytical Termination Date: 07/01/00

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

PROJECT PERSONNEL

The Study Director for this project was Marvin T. Case at 3M Toxicology Services and the Principal Investigator for this project at Centre Analytical Laboratories, Inc. was Enaksha Wickremesinhe. The following personnel from Centre Analytical Laboratories, Inc., were associated with various analytical phases of the study:

Name	Title
Enaksha Wickremesinhe	Group/Team Leader
Emily Stauffer	Scientist
Karen Smith	Scientist
Tiffany Proctor	Technician
Rickey Keller	Sample Custodian
Lawrence Ord	Sample Custodian

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LIST OF APPENDICES

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

1.0 SUMMARY

The purpose of this study was to analyze residues of perfluorooctanesulfonate (PFOS) in rat feces as specified in 3M Protocol FACT-TOX-110. The analytical method used for this study was entitled, "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS, revision 2" (Centre method number: 00M-023-003, revision 2).

The limit of quantification for PFOS in rat feces was 10 ppb.

Residues (corrected for purity) ranging from non-detected levels to 59386.8 ppb were found in the rat feces samples.

Fortification recoveries ranged from 71-128% with an average of 98% and relative standard deviation of 15%.

2.0 OBJECTIVE

The objective of this study was to determine levels of perfluorooctanesulfonate (PFOS) in specimens of feces of rats using the analytical method entitled "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS, revision 2."

3.0 INTRODUCTION

This report details the results of the residues of PFOS detected in rat feces, using the analytical method entitled, "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (revision 2)." Complete details of the analytical methodology can be found in **Appendix B.**

The study was initiated on June 10, 2000, when the study director signed the protocol FACT-TOX-110 Amendment #4. The complete protocol and amendments can be found in **Appendix A.** The analytical start date was June 9, 2000, and the analytical termination date was July 1, 2000.

4.0 TEST SYSTEM

The 250 rat feces samples analyzed in this study were received frozen on dry ice from 3M Environmental Laboratory St. Paul, MN on May 26, 2000 and stored frozen upon receipt. The samples were then logged in on May 31, 2000 by Centre personnel and transferred to different frozen storage locale.

The control rat feces used for standards and blanks was purchased from Lampire Biological Laboratories, Inc., Pipersville, PA and received at Centre frozen on dry ice on

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May 4, 2000 and June 22, 2000, logged in by Centre personnel and placed in frozen storage (<-10°C).

Sample login and chain of custody information can be found in the raw data package associated with this study. Storage records will be kept at Centre Analytical Laboratories, Inc. and a true copy of the storage records can be found in the raw data package associated with this study.

5.0 REFERENCE MATERIAL

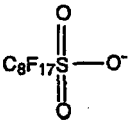
The analytical standard PFOS (as the potassium salt) was received at Centre on November 12, 1999 and the surrogate standard THPFOS was received on December 3, 1999 from 3M Environmental Technology and Services. Characterization of the reference material PFOS was performed on August 31, 2000 at Centre and documentation can be found in raw data package associated with this report.

The available information for the reference materials is listed below. The reference materials were stored at room temperature.

Compound	Centre Control No.	Batch No.	Purity (%)	Expiration Date
PFOS	99-023-002	217	86.9	08/31/01
THPFOS	99-023-011	53406	NA	01/01/10

Molecular structures of PFOS and THPFOS are given below.

PFOS
Chemical Name: Perfluorooctane sulfonate
Molecular weight: 499 ($C_8F_{17}SO_3^-$)



Note: The neutral molecule and standard form from which PFOS (anion) is derived, is potassium perfluorooctane sulfonate [$C_8F_{17}SO_3K$], molecular weight 538.

THPFOS
Chemical Name: 4-H, perfluorooctane sulfonic acid
Molecular weight: 428

1-H, 1-H, 2-H, 2-H, $C_8F_{13}SO_3H$

6.0 EXPERIMENTAL DESIGN

Samples were extracted according to group number and sampling day. Each set of samples contained two matrix blanks, two matrix blanks spiked with the surrogate standard (zero blank), two matrix spikes, and 12 – 16 samples.

The extracts were analyzed by LC/MS/MS. Samples with residues outside the linear range of the calibration curve were diluted and re-analyzed.

7.0 DESCRIPTION OF ANALYTICAL METHOD

Analytical method entitled "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2)" was used for this study.

7.1 Extraction Procedure

One gram ± 0.05 of sample was weighed into 20 mL polypropylene scintillation vials and fortified (if necessary) using disposable micropipettes. Ten mL of acetonitrile was added to the vial, capped tightly, and placed on a wrist-action shaker for ~ 30 min. The samples were filtered through a glass acrodisc filter. The filtered extract was passed through a conditioned carbon SPE column and collected. The columns were then eluted with ~10 mL acetonitrile followed by ~20 mL 90:10 acetonitrile:2% ascorbic acid in methanol. The combined extracts were evaporated down to almost dryness with a rotary evaporator and then re-constituted with methanol, making final volume 2 mL. The samples were analyzed using electrospray LC/MS/MS.

7.2 Preparation of Standards and Fortification Solutions

Standard solutions were prepared on May 3, 2000 as specified in Centre Analytical Laboratories' analytical method entitled, "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2)." An individual stock standard solution of PFOS was prepared at a concentration of 100 µg/mL by dissolving 10 mg of the standard (corrected for salt purity only) in methanol. From this solution, a 10 µg/mL fortification standard solution was prepared by taking 10 mL of the stock and bringing the volume up to 100 mL with methanol. Also, a stock standard of THPFOS was prepared at 100 µg/mL by dissolving 10 mg of the standard in methanol. An individual 10 µg/mL solution of THPFOS was prepared in the same fashion described above.

A 2.5 µg/mL fortification standard of PFOS was prepared by taking 25 mL of the 10 µg/mL solution of PFOS and bringing the volume up to 100 mL with methanol. An individual fortification solution of THPFOS was prepared in the same manner. A 0.5 µg/mL PFOS standard was prepared by taking 20 mL of the 2.5 µg/mL PFOS

standard and bringing to 100 mL with methanol. To make the 0.1 µg/mL PFOS fortification standard, 20 mL of the 0.5 µg/mL of PFOS was brought to 100 mL with methanol.

Calibration standards were processed through the extraction procedure, identical to the samples. The fortification of the standards before extraction was done according to the following table:

Conc. of PFOS Fortification Solution (µg/ml)	Fortification Volume (µL)	Weight of Control Sample (g) ± 0.05	Fort. Level of Extracted Calibration Standard (ppb)	Vol. of Surrogate Standard* added (µL)
0.1	100	1.0	10	200
0.1	200	1.0	20	200
0.5	100	1.0	50	200
0.5	200	1.0	100	200
2.5	100	1.0	250	200
2.5	200	1.0	500	200

* 2.5 µg/ml THPFOS fortification solution.

The stock standard solution and all fortification and calibration standard solutions were stored in a refrigerator (4° ± 2°C) when not in use. Documentation of standard preparation and extraction can be found in the raw data associated with this report.

7.3 Chromatography

Quantification of PFOS and THPFOS was accomplished by LC/MS/MS analysis using electrospray LC/MS/MS. The retention times of PFOS and THPFOS were ~ 5.2 min. and ~ 5.0 min., respectively, with no significant interfering peaks in the control matrices corresponding to either of the analyte retention times.

7.4 Instrument Sensitivity

The smallest standard amount injected during the chromatographic run was equivalent to 5 ng/mL of PFOS and 250 ng/mL of THPFOS in the rat feces matrix.

7.5 Description of Instrument and Operating Conditions

A Micromass Quattro Ultima LC/MS/MS coupled to a Hewlet Packard HPLC system was used. Data acquisition and processing were performed using Masslynx 3.4 software. Detailed operating conditions are listed below:

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Instrument: Micromass Quattro Ultima

ELECTROSPRAY ION SOURCE:

Capillary: 3.0 kV	Hexapole 2: 0.3 V
Hexapole 1: 0.1 V	Source Block Temp.: 100°C
Aperture 1: 0.2 V	Desolvation Temp.: 350°C

ANALYZER:

LM Res 1: 10.5 V	LM Res 2: 13.0 V
HM Res 1: 10.5 V	HM Res 2: 13.0 V
IEnergy 1: 1.0 V	IEnergy 2: 2.0 V
Entrance: -2 V	Multiplier: 650 V
Exit: 2 V	

GAS FLOWS AND PRESSURE:

Desolvation N_2 Flow Rate: ~650 L/hr
Nebuliser N_2 Flow Rate: ~150 L/hr
Gas Cell Pressure: ~0.003 mbar

Computer: COMPAQ Professional Workstation AP200

Software: Microsoft Windows NT: Version 4 Build 1381: Service Pack 5
Micromass Limited: Masslynx 3.4 Build 004

HPLC Equipment: Hewlett Packard (HP) Series 1100

HP Binary Pump	HP Vacuum Degasser
HP Autosampler	HP Column Oven

HPLC Column: Genesis C-8, 5 cm x 2.1 mm i.d. x 4 µ
Column Temperature: 35°C
Mobile Phase (A) : 2 mM Ammonium Acetate in Type I Water
Mobile Phase (B) : Methanol

Gradient:

Time (min)	% A	% B	Flow Rate (mL/min)
0.0	60.0	40.0	0.3
0.4	60.0	40.0	0.3
1.0	10.0	90.0	0.3
7.0	10.0	90.0	0.3
7.5	0.0	100.0	0.3
10.5	0.0	100.0	0.4
11.0	60.0	40.0	0.4
14.5	60.0	40.0	0.4
15.0	60.0	40.0	0.3

Injected Volume: 10 µL
Ions monitored :

Analyte	Transition Monitored	Dwell (secs)	Coll Energy (eV)	Cone (V)
PFOS	499 → 99	0.2	43	76
THPFOS	427 → 80	0.2	35	34

7.6 Quantitation and Example Calculation

Ten microliters of sample or extracted calibration standard was injected into the LC/MS/MS. The peak area was measured and the standard curve was generated (using 1/x weighted linear regression) by Masslynx software using six concentrations of standards. The surrogate standard, THPFOS, was only used to monitor the efficiency of the extraction procedure and was not used for quantitation of PFOS. The residue concentration in rat feces was determined using the following equations:

Equations 1 and 2 were used to calculate the amount of analyte found (in ppb, based on peak area) using the standard curve generated by the Masslynx software program.

Equation 1:

$$\text{Analyte found (ng/mL)} = \frac{\text{(Peak area - intercept)}}{\text{slope}}$$

Equation 2:

$$\text{Analyte found (ppb)} = \frac{\text{(analyte found (ng/mL)} \times \text{final vol. (mL)} \times \text{DF)}}{\text{sample wt. (g)}}$$

where DF = dilution factor.

For samples fortified with known amounts of analytes prior to extraction, use Equation 3 to calculate the percent recovery.

Equation 3:

$$\text{Recovery (\%)} = \frac{[\text{analyte found (ng/mL)} \times \text{final vol. (mL)} \times \text{DF}]}{\text{analyte added (ng)}} \times 100$$

Equation 4:

This calculation was only performed on actual samples and not QC recoveries.

Corrected analyte found (ppb) = analyte found (ppb) × % purity

% purity for PFOS lot 217 = 86.9% (0.869)

An example of a calculation using an actual sample analyzed with extracted standards follows:
Rat feces sample Centre ID 0004127 Spk A (Set: 062700A), fortified at 100 ng with PFOS.
Where:

peak area	=	32320
intercept	=	2653.87
slope	=	595.935
dilution factor	=	1

ng added (fort level) = 100
final vol. = 2 mL
sample wt. = 1.01 g

From equation 1:

Analyte found (ng/mL) = $\frac{[32320 - 2653.87]}{595.935}$

= 49.8 ng/mL

From equation 2:

Analyte found (ppb) = $\frac{[49.8 \times 2\ mL \times 1]}{1.01\ g}$

= 98.6 ppb

From equation 3:

% Recovery = $\frac{(49.8\ ng/mL \times 2\ mL \times 1)}{100\ ng} \times 100$

= 100%

Note: This example calculation was done using rounded numbers, and therefore may be slightly different from the values shown in the RAW DATA.

The amount of surrogate standard THPFOS found was calculated using the following equation:

Analyte found (ng/mL): $\frac{\text{peak area}}{\text{mean response factor}}$

Other statistical methods used in analyzing this data were:

$$\textit{Standard Deviation} = \sqrt{\frac{\sum_{i=1}^{n}(x_i - \bar{x})^2}{n-1}} \qquad \textit{Mean} = \bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

Relative Standard Deviation (RSD or Coefficient of Variation (CV)) =

$\frac{\text{Standard Deviation}}{\text{Mean}} \times 100\%$

8.0 RESULTS AND DISCUSSION

Although, the majority of the control samples did not contain significant interferences, a couple of samples did contain residues that were comparable to control group levels. A summary of residues found in all of the matrix blanks and matrix zero blanks is detailed in **Table I.**

Residues (corrected for purity) ranging from non-detected levels to 59386.8 ppb were found in the rat feces samples. The residues found in all of the samples plus the averages and standard deviations for each group at each interval are detailed in **Tables II-XXI.**

For this report the residues found in Tables I-XXI were corrected for the purity of the PFOS standard lot 217 based on the certificate of analysis finalized on September 7, 2000.

Fortification recoveries ranged from 71-128% with an average of 98% and relative standard deviation of 15% (n = 34). A summary of all of the fortification recoveries can be found in **Table XXII.**

Typical calibration curves and chromatograms representing standards, controls, fortifications, and samples are depicted in **Figures 1-7.**

9.0 CIRCUMSTANCES THAT MAY HAVE AFFECTED THE DATA

Electronic records are not fully compliant with 21 CFR 11, "Electronic Records: Electronic Signature." However, fully approved SOP's were in place and all chromatography instrumentation used in this study was fully validated (IQ, PQ, OQ), calibrated and operational. All original data were printed as hard copies and fully audited by quality assurance. Verified exact copies and the electronic data have been stored in the archives at Centre Analytical Laboratories, Inc. Original raw data have been returned to the sponsor.

10.0 RETENTION OF DATA AND SAMPLES

When the final report is complete, all original paper data generated by Centre Analytical Laboratories, Inc. will be shipped to the sponsor. This does not include facility-specific raw data such as instrument logs, however exact copies of temperature logs will be submitted. Exact copies of all raw data, as well as a signed copy of the final analytical report and all original facility-specific raw data, will be retained in the Centre Analytical Laboratories, Inc. archives for the period of time specified in 21 CFR Part 58. Retained samples of reference substances are archived by the sponsor.

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

11.0 TABLES

Centre Study No.: 023-016
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Table I. Summary of PFOS residues in Matrix Blanks and Matrix Zero Blanks

ND = Not Detected and NQ = Not Quantifiable

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
na	0004277 Blank A	061200AR	6/12/00	ND	ND
na	0004277 Blank B	061200AR	6/12/00	ND	ND
na	0004277 Zero Blank C	061200AR	6/12/00	ND	ND
na	0004277 Zero Blank D	061200AR	6/12/00	ND	ND
na	0004277 Blank A	061400A	6/14/00	NQ	NQ
na	0004277 Blank B	061400A	6/14/00	NQ	NQ
na	0004277 Zero Blank C	061400A	6/14/00	NQ	NQ
na	0004277 Zero Blank D	061400A	6/14/00	ND	ND
na	0004277 Blank A	061500AD	6/15/00	NQ	NQ
na	0004277 Blank B	061500AD	6/15/00	NQ	NQ
na	0004277 Zero Blank C	061500AD	6/15/00	1.6	1.4
na	0004277 Zero Blank D	061500AD	6/15/00	1.3	1.1
na	0004277 Blank E	061600A	6/16/00	2.0	1.7
na	0004277 Blank F	061600A	6/16/00	NQ	NQ
na	0004277 Zero Blank G	061600A	6/16/00	NQ	NQ
na	0004277 Zero Blank H	061600A	6/16/00	NQ	NQ
na	0004277 Blank A	061900AD	6/19/00	NQ	NQ
na	0004277 Blank B	061900AD	6/19/00	NQ	NQ
na	0004277 Zero Blank C	061900AD	6/19/00	NQ	NQ
na	0004277 Zero Blank D	061900AD	6/19/00	NQ	NQ
na	0003466 Blank A	062000A	6/20/00	NQ	NQ
na	0003466 Blank B	062000A	6/20/00	NQ	NQ
na	0003466 Zero Blank C	062000A	6/20/00	NQ	NQ
na	0003466 Zero Blank D	062000A	6/20/00	NQ	NQ
na	0004277 Blank A	062100A	6/21/00	NQ	NQ
na	0004277 Blank B	062100A	6/21/00	NQ	NQ
na	0004277 Zero Blank C	062100A	6/21/00	NQ	NQ
na	0004277 Zero Blank D	062100A	6/21/00	NQ	NQ
na	0004277 Blank A	062200A	6/22/00	6.5	5.6
na	0004277 Blank B	062200A	6/22/00	ND	ND
na	0004277 Zero Blank C	062200A	6/22/00	ND	ND
na	0004277 Zero Blank D	062200A	6/22/00	ND	ND
na	0004277 Blank E	062200B	6/22/00	ND	ND
na	0004277 Blank F	062200B	6/22/00	ND	ND
na	0004277 Zero Blank G	062200B	6/22/00	ND	ND

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Table I (cont.) Summary of PFOS residues in Matrix Blanks and Matrix Zero Blanks ND = Not Detected and NQ = Not Quantifiable

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
na	0004277 Zero Blank H	062200B	6/22/00	ND	ND
na	0004277 Blank A	062300A	6/23/00	ND	ND
na	0004277 Blank B	062300A	6/23/00	ND	ND
na	0004277 Zero Blank C	062300A	6/23/00	ND	ND
na	0004277 Zero Blank D	062300A	6/23/00	ND	ND
na	0004277 Blank A	062300B	6/23/00	NQ	NQ
na	0004277 Blank B	062300B	6/23/00	NQ	NQ
na	0004277 Zero Blank C	062300B	6/23/00	NQ	NQ
na	0004277 Zero Blank D	062300B	6/23/00	NQ	NQ
na	0005738 Blank A	062600A	6/26/00	0.5	0.4
na	0005738 Blank B	062600A	6/26/00	12.6	10.9
na	0005738 Zero Blank C	062600A	6/26/00	ND	ND
na	0005738 Zero Blank D	062600A	6/26/00	ND	ND
na	0005738 Blank E	062600B	6/26/00	ND	ND
na	0005738 Blank F	062600B	6/26/00	ND	ND
na	0005738 Zero Blank G	062600B	6/26/00	ND	ND
na	0005738 Zero Blank H	062600B	6/26/00	ND	ND
na	0005738 Blank A	062700A	6/27/00	ND	ND
na	0005738 Blank B	062700A	6/27/00	ND	ND
na	0005738 Zero Blank C	062700A	6/27/00	ND	ND
na	0005738 Zero Blank D	062700A	6/27/00	ND	ND
na	0005738 Blank A	062800A	6/28/00	ND	ND
na	0005738 Blank B	062800A	6/28/00	ND	ND
na	0005738 Zero Blank C	062800A	6/28/00	ND	ND
na	0005738 Zero Blank D	062800A	6/28/00	ND	ND
na	0005738 Blank A	062900A	6/29/00	30.1	26.2
na	0005738 Blank B	062900A	6/29/00	ND	ND
na	0005738 Zero Blank C	062900A	6/29/00	ND	ND
na	0005738 Zero Blank D	062900A	6/29/00	ND	ND
na	0005738 Blank A	063000A	6/30/00	ND	ND
na	0005738 Blank B	063000A	6/30/00	ND	ND
na	0005738 Zero Blank C	063000A	6/30/00	ND	ND
na	0005738 Zero Blank D	063000A	6/30/00	ND	ND
			AVERAGE:	**0.8**	**0.7**
			STANDARD DEVIATION:	**4**	**3**

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Table II. Summary of PFOS residues in G1-Control-GD0

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19501F,F0,G1-Control-GD0	0003977	061200AR	6/12/00	0.0	0.0
19502F,F0,G1-Control-GD0	0003978	061200AR	6/12/00	0.0	0.0
19503F,F0,G1-Control-GD0	0003979	061200AR	6/12/00	5.9	5.1
19504F,F0,G1-Control-GD0	0003980	061200AR	6/12/00	0.0	0.0
19505F,F0,G1-Control-GD0	0003981	061200AR	6/12/00	0.0	0.0
19506F,F0,G1-Control-GD0	0003982	061200AR	6/12/00	30.1	26.2
19507F,F0,G1-Control-GD0	0003983	061200AR	6/12/00	0.0	0.0
19508F,F0,G1-Control-GD0	0003984	061200AR	6/12/00	5.9	5.1
19509F,F0,G1-Control-GD0	0003985	061200AR	6/12/00	40.0	34.8
19510F,F0,G1-Control-GD0	0003986	061200AR	6/12/00	0.0	0.0
19511F,F0,G1-Control-GD0	0003987	061200AR	6/12/00	0.0	0.0
19512F,F0,G1-Control-GD0	0003988	061200AR	6/12/00	0.0	0.0
19513F,F0,G1-Control-GD0	0003989	061200AR	6/12/00	0.0	0.0
19514F,F0,G1-Control-GD0	0003990	061200AR	6/12/00	0.0	0.0
19515F,F0,G1-Control-GD0	0003991	061200AR	6/12/00	0.0	0.0
19516F,F0,G1-Control-GD0	0003992	061200AR	6/12/00	60.9	52.9
			AVERAGE:	**8.9**	**7.8**
			STANDARD DEVIATION:	**18**	**16**

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Table III. Summary of PFOS residues in G2-0.1MKD on GD0

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19517F,F0,G2-0.1MKD-GD0	0003993	061400AD	6/14/00	700.8	609.0
19518F,F0,G2-0.1MKD-GD0	0003994	061400A	6/14/00	455.7	396.0
19519F,F0,G2-0.1MKD-GD0	0003995	061400A	6/14/00	389.7	338.6
19520F,F0,G2-0.1MKD-GD0	0003996	061400AD	6/14/00	675.5	587.0
19521F,F0,G2-0.1MKD-GD0	0003997	061400A	6/14/00	494.8	430.0
19522F,F0,G2-0.1MKD-GD0	0003998	061400A	6/14/00	381.0	331.1
19523F,F0,G2-0.1MKD-GD0	0003999	061400A	6/14/00	458.2	398.2
19524F,F0,G2-0.1MKD-GD0	0004000	061400AD	6/14/00	832.8	723.7
19525F,F0,G2-0.1MKD-GD0	0004001	061400A	6/14/00	473.0	411.0
19526F,F0,G2-0.1MKD-GD0	0004002	061400A	6/14/00	446.1	387.7
19527F,F0,G2-0.1MKD-GD0	0004003	061400A	6/14/00	497.4	432.2
19528F,F0,G2-0.1MKD-GD0	0004004	061400AD	6/14/00	700.7	608.9
19529F,F0,G2-0.1MKD-GD0	0004005	061400AD	6/14/00	796.9	692.5
19530F,F0,G2-0.1MKD-GD0	0004006	061400A	6/14/00	393.7	342.1
19531F,F0,G2-0.1MKD-GD0	0004007	061400AD	6/14/00	734.7	638.5
19532F,F0,G2-0.1MKD-GD0	0004008	061400AD	6/14/00	748.2	650.2
			AVERAGE:	**573.7**	**498.5**
			STANDARD DEVIATION:	**160**	**139**

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Table IV. Summary of PFOS residues in G3-0.4MKD on GD0

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19533F,F0,G3-0.4MKD-GD0	0004009	061500AD	6/15/00	2598.1	2257.7
19534F,F0,G3-0.4MKD-GD0	0004010	061500AD	6/15/00	3750.7	3259.4
19535F,F0,G3-0.4MKD-GD0	0004011	061500AD	6/15/00	2801.3	2434.3
19536F,F0,G3-0.4MKD-GD0	0004012	061500AD	6/15/00	3174.3	2758.5
19537F,F0,G3-0.4MKD-GD0	0004013	061500AD	6/15/00	2777.9	2414.0
19538F,F0,G3-0.4MKD-GD0	0004014	061500AD	6/15/00	3083.8	2679.8
19539F,F0,G3-0.4MKD-GD0	0004015	061500AD	6/15/00	3680.4	3198.3
19540F,F0,G3-0.4MKD-GD0	0004016	061500AD	6/15/00	2044.0	1776.2
19541F,F0,G3-0.4MKD-GD0	0004017	061500AD	6/15/00	2104.0	1828.4
19542F,F0,G3-0.4MKD-GD0	0004018	061500AD	6/15/00	3290.2	2859.2
19543F,F0,G3-0.4MKD-GD0	0004019	061500AD	6/15/00	2113.4	1836.5
19544F,F0,G3-0.4MKD-GD0	0004020	061500AD	6/15/00	3430.8	2981.4
19545F,F0,G3-0.4MKD-GD0	0004021	061600AD	6/16/00	2755.3	2394.4
19546F,F0,G3-0.4MKD-GD0	0004022	061500AD	6/15/00	2334.2	2028.4
19547F,F0,G3-0.4MKD-GD0	0004023	061500AD	6/15/00	2215.6	1925.4
19548F,F0,G3-0.4MKD-GD0	0004024	061500AD	6/15/00	2334.8	2028.9
			AVERAGE:	**2780.6**	**2416.3**
			STANDARD DEVIATION:	**570**	**496**

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Table V. Summary of PFOS residues in G4-1.6MKD on GD0

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19549F,F0,G4-1.6MKD-GD0	0004025	061600AD	6/16/00	14122.8	12272.7
19550F,F0,G4-1.6MKD-GD0	0004026	061600AD	6/16/00	16858.7	14650.2
19551F,F0,G4-1.6MKD-GD0	0004027	061600AD	6/16/00	10394.9	9033.2
19552F,F0,G4-1.6MKD-GD0	0004028	061600AD	6/16/00	13143.8	11422.0
19553F,F0,G4-1.6MKD-GD0	0004029	061600AD	6/16/00	13712.0	11915.7
19554F,F0,G4-1.6MKD-GD0	0004030	061600AD	6/16/00	15507.9	13476.4
19555F,F0,G4-1.6MKD-GD0	0004031	061600AD	6/16/00	10328.8	8975.7
19556F,F0,G4-1.6MKD-GD0	0004032	061600AD	6/16/00	10253.1	8909.9
19557F,F0,G4-1.6MKD-GD0	0004033	061600AD	6/16/00	8961.3	7787.4
19558F,F0,G4-1.6MKD-GD0	0004034	061600AD	6/16/00	6845.2	5948.5
19559F,F0,G4-1.6MKD-GD0	0004035	061600AD	6/16/00	7677.3	6671.6
19560F,F0,G4-1.6MKD-GD0	0004036	061600AD	6/16/00	16518.0	14354.1
19561F,F0,G4-1.6MKD-GD0	0004037	061600AD	6/16/00	12121.6	10533.7
19562F,F0,G4-1.6MKD-GD0	0004038	061600AD	6/16/00	9585.4	8329.7
19563F,F0,G4-1.6MKD-GD0	0004039	061600AD	6/16/00	7465.7	6487.7
19564F,F0,G4-1.6MKD-GD0	0004040	061600AD	6/16/00	16957.0	14735.6
			AVERAGE:	**11903.3**	**10344.0**
			STANDARD DEVIATION:	**3453**	**3000**

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Table VI. Summary of PFOS residues in G5-3.2MKD on GD0

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19565F,F0,G5-3.2MKD-GD0	0004041	061900AD	6/19/00	23450.7	20378.7
19566F,F0,G5-3.2MKD-GD0	0004042	061900AD	6/19/00	29548.0	25677.2
19567F,F0,G5-3.2MKD-GD0	0004043	061900AD	6/19/00	30310.7	26340.0
19568F,F0,G5-3.2MKD-GD0	0004044	061900AD	6/19/00	24160.4	20995.4
19569F,F0,G5-3.2MKD-GD0	0004045	061900AD	6/19/00	27052.1	23508.3
19570F,F0,G5-3.2MKD-GD0	0004046	061900AD	6/19/00	19671.6	17094.6
19571F,F0,G5-3.2MKD-GD0	0004047	061900AD	6/19/00	29872.2	25958.9
19572F,F0,G5-3.2MKD-GD0	0004048	061900AD	6/19/00	25395.0	22068.3
19573F,F0,G5-3.2MKD-GD0	0004049	061900AD	6/19/00	23006.0	19992.2
19574F,F0,G5-3.2MKD-GD0	0004050	061900AD	6/19/00	29300.3	25462.0
19575F,F0,G5-3.2MKD-GD0	0004051	061900AD	6/19/00	23037.0	20019.2
19576F,F0,G5-3.2MKD-GD0	0004052	061900AD	6/19/00	36326.7	31567.9
19577F,F0,G5-3.2MKD-GD0	0004053	061900AD	6/19/00	34238.9	29753.6
19578F,F0,G5-3.2MKD-GD0	0004054	061900AD	6/19/00	34808.0	30248.2
19579F,F0,G5-3.2MKD-GD0	0004055	061900AD	6/19/00	25356.7	22035.0
19580F,F0,G5-3.2MKD-GD0	0004056	061900AD	6/19/00	24851.5	21596.0
			AVERAGE:	**27524.1**	**23918.5**
			STANDARD DEVIATION:	**4781**	**4155**

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Table VII. Summary of PFOS residues in G1-Control on GD7

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19501F,F0,G1-Control-GD7	0004057	062000A	6/20/00	ND	ND
19502F,F0,G1-Control-GD7	0004058	062000A	6/20/00	NQ	NQ
19504F,F0,G1-Control-GD7	0004059	062000A	6/20/00	NQ	NQ
19505F,F0,G1-Control-GD7	0004060	062000A	6/20/00	ND	ND
19506F,F0,G1-Control-GD7	0004061	062000A	6/20/00	17.8	15.5
19507F,F0,G1-Control-GD7	0004062	062000A	6/20/00	ND	ND
19508F,F0,G1-Control-GD7	0004063	062000A	6/20/00	0.3	0.3
19509F,F0,G1-Control-GD7	0004064	062000A	6/20/00	ND	ND
19510F,F0,G1-Control-GD7	0004065	062000A	6/20/00	ND	ND
19511F,F0,G1-Control-GD7	0004066	062000AR	6/20/00	ND	ND
19512F,F0,G1-Control-GD7	0004067	062000AR	6/20/00	ND	ND
19513F,F0,G1-Control-GD7	0004068	062000A	6/20/00	ND	ND
19514F,F0,G1-Control-GD7	0004069	062000A	6/20/00	ND	ND
19515F,F0,G1-Control-GD7	0004070	062000AR	6/20/00	ND	ND
19516F,F0,G1-Control-GD7	0004071	062000AR	6/20/00	NQ	NQ
			AVERAGE:	**1.2**	**1.0**
			STANDARD DEVIATION:	**5**	**4**

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Table VIII. Summary of PFOS residues in G2-0.1MKD on GD7

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19517F,F0,G2-0.1MKD-GD7	0004072	062100A	6/21/00	409.4	355.8
19518F,F0,G2-0.1MKD-GD7	0004073	062100AD	6/21/00	561.4	487.9
19519F,F0,G2-0.1MKD-GD7	0004074	062100A	6/21/00	344.6	299.5
19521F,F0,G2-0.1MKD-GD7	0004075	062100A	6/21/00	464.6	403.7
19522F,F0,G2-0.1MKD-GD7	0004076	062100AD	6/21/00	778.0	676.1
19523F,F0,G2-0.1MKD-GD7	0004077	062100AD	6/21/00	624.6	542.8
19524F,F0,G2-0.1MKD-GD7	0004078	062100AD	6/21/00	575.5	500.1
19525F,F0,G2-0.1MKD-GD7	0004079	062100AD	6/21/00	594.6	516.7
19526F,F0,G2-0.1MKD-GD7	0004080	062100A	6/21/00	469.2	407.7
19527F,F0,G2-0.1MKD-GD7	0004081	062100AD	6/21/00	741.9	644.7
19528F,F0,G2-0.1MKD-GD7	0004082	062100AD	6/21/00	531.8	462.1
19529F,F0,G2-0.1MKD-GD7	0004083	062100AD	6/21/00	744.4	646.9
19530F,F0,G2-0.1MKD-GD7	0004084	062100A	6/21/00	424.3	368.7
19531F,F0,G2-0.1MKD-GD7	0004085	062100AD	6/21/00	623.9	542.2
19532F,F0,G2-0.1MKD-GD7	0004086	062100AD	6/21/00	573.9	498.7
			AVERAGE:	**564.1**	**490.2**
			STANDARD DEVIATION:	**128**	**111**

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Table IX. Summary of PFOS residues in G3-0.4MKD on GD7

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19533F,F0,G3-0.4MKD-GD7	0004087	062200AD	6/22/00	2548.5	2214.6
19534F,F0,G3-0.4MKD-GD7	0004088	062200AD	6/22/00	2693.6	2340.7
19535F,F0,G3-0.4MKD-GD7	0004089	062200AD	6/22/00	1824.8	1585.8
19536F,F0,G3-0.4MKD-GD7	0004090	062200AD	6/22/00	3261.1	2833.9
19537F,F0,G3-0.4MKD-GD7	0004091	062200AD	6/22/00	2611.1	2269.0
19538F,F0,G3-0.4MKD-GD7	0004092	062200AD	6/22/00	2934.3	2549.9
19539F,F0,G3-0.4MKD-GD7	0004093	062200AD	6/22/00	2093.4	1819.2
19540F,F0,G3-0.4MKD-GD7	0004094	062200AD	6/22/00	2267.8	1970.7
19542F,F0,G3-0.4MKD-GD7	0004095	062200AD	6/22/00	3003.0	2609.6
19543F,F0,G3-0.4MKD-GD7	0004096	062200AD	6/22/00	2797.7	2431.2
19544F,F0,G3-0.4MKD-GD7	0004097	062200AD	6/22/00	2284.0	1984.8
19546F,F0,G3-0.4MKD-GD7	0004098	062200AD	6/22/00	1523.1	1323.6
19547F,F0,G3-0.4MKD-GD7	0004099	062200AD	6/22/00	2055.6	1786.3
19548F,F0,G3-0.4MKD-GD7	0004100	062200AD	6/22/00	2847.6	2474.6
			AVERAGE:	**2481.8**	**2156.7**
			STANDARD DEVIATION:	**492**	**428**

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Table X. Summary of PFOS residues in G4-1.6MKD on GD7

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19550F,F0,G4-1.6MKD-GD7	0004101	062200BD	6/22/00	14977.5	13015.4
19553F,F0,G4-1.6MKD-GD7	0004102	062200BD	6/22/00	12262.8	10656.4
19554F,F0,G4-1.6MKD-GD7	0004103	062200BD	6/22/00	11777.1	10234.3
19555F,F0,G4-1.6MKD-GD7	0004104	062200BD	6/22/00	9607.3	8348.7
19556F,F0,G4-1.6MKD-GD7	0004105	062200BD	6/22/00	10222.3	8883.2
19558F,F0,G4-1.6MKD-GD7	0004106	062200BD	6/22/00	5886.5	5115.4
19559F,F0,G4-1.6MKD-GD7	0004107	062200BD	6/22/00	6894.8	5991.6
19560F,F0,G4-1.6MKD-GD7	0004108	062200BD	6/22/00	7024.2	6104.0
19561F,F0,G4-1.6MKD-GD7	0004109	062200BD	6/22/00	8877.7	7714.7
19562F,F0,G4-1.6MKD-GD7	0004110	062200BD	6/22/00	10845.1	9424.4
19563F,F0,G4-1.6MKD-GD7	0004111	062200BD	6/22/00	14910.3	12957.1
19564F,F0,G4-1.6MKD-GD7	0004112	062200BD	6/22/00	13656.0	11867.1
			AVERAGE:	**10578.5**	**9192.7**
			STANDARD DEVIATION:	**3076**	**2673**

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Table XI. Summary of PFOS residues in G5-3.2MKD on GD7

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19565F,F0,G5-3.2MKD-GD7	0004113	062300AD	6/23/00	51657.5	44890.4
19566F,F0,G5-3.2MKD-GD7	0004114	062300AD	6/23/00	26453.8	22988.4
19567F,F0,G5-3.2MKD-GD7	0004115	062300AD	6/23/00	35913.2	31208.6
19568F,F0,G5-3.2MKD-GD7	0004116	062300AD	6/23/00	23007.4	19993.4
19569F,F0,G5-3.2MKD-GD7	0004117	062300AD	6/23/00	42878.2	37261.2
19570F,F0,G5-3.2MKD-GD7	0004118	062300AD	6/23/00	37368.9	32473.6
19571F,F0,G5-3.2MKD-GD7	0004119	062300AD	6/23/00	42995.9	37363.4
19572F,F0,G5-3.2MKD-GD7	0004120	062300AD	6/23/00	38826.6	33740.3
19573F,F0,G5-3.2MKD-GD7	0004121	062300AD	6/23/00	26685.9	23190.0
19574F,F0,G5-3.2MKD-GD7	0004122	062300AD	6/23/00	36333.3	31573.6
19575F,F0,G5-3.2MKD-GD7	0004123	062300AD	6/23/00	35455.7	30811.0
19577F,F0,G5-3.2MKD-GD7	0004124	062300AD	6/23/00	68339.2	59386.8
19579F,F0,G5-3.2MKD-GD7	0004125	062300AD	6/23/00	35856.5	31159.3
19580F,F0,G5-3.2MKD-GD7	0004126	062300AD	6/23/00	30065.5	26126.9
			AVERAGE:	**37988.4**	**33011.9**
			STANDARD DEVIATION:	**11516**	**10008**

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Table XII. Summary of PFOS residues in G1-Control on GD15

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19501F,F0,G1-Control-GD15	0004127	062300B	6/23/00	ND	0.0
19502F,F0,G1-Control-GD15	0004128	062300B	6/23/00	ND	0.0
19504F,F0,G1-Control-GD15	0004129	062300B	6/23/00	ND	0.0
19505F,F0,G1-Control-GD15	0004130	062300B	6/23/00	ND	0.0
19506F,F0,G1-Control-GD15	0004131	062300B	6/23/00	44.5	38.7
19507F,F0,G1-Control-GD15	0004132	062300B	6/23/00	ND	0.0
19508F,F0,G1-Control-GD15	0004133	062300B	6/23/00	ND	0.0
19509F,F0,G1-Control-GD15	0004134	062300B	6/23/00	24.2	21.0
19510F,F0,G1-Control-GD15	0004135	062300B	6/23/00	7.9	6.9
19511F,F0,G1-Control-GD15	0004136	062300B	6/23/00	ND	0.0
19512F,F0,G1-Control-GD15	0004137	062300B	6/23/00	57.9	50.3
19513F,F0,G1-Control-GD15	0004138	062300B	6/23/00	ND	0.0
19514F,F0,G1-Control-GD15	0004139	062300B	6/23/00	ND	0.0
19515F,F0,G1-Control-GD15	0004140	062300B	6/23/00	ND	0.0
19516F,F0,G1-Control-GD15	0004141	062300B	6/23/00	21.6	18.6
			AVERAGE:	**10.4**	**9.0**
			STANDARD DEVIATION:	**19**	**16**

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Table XIII. Summary of PFOS residues in G2-0.1MKD on GD15

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19517F,F0,G2-0.1MKD-GD15	0004142	062600AD	6/26/00	758.1	658.8
19518F,F0,G2-0.1MKD-GD15	0004143	062600AD	6/26/00	671.8	583.8
19519F,F0,G2-0.1MKD-GD15	0004144	062600AD	6/26/00	782.2	679.7
19521F,F0,G2-0.1MKD-GD15	0004145	062600AD	6/26/00	632.3	549.5
19522F,F0,G2-0.1MKD-GD15	0004146	062600AD	6/26/00	817.4	710.3
19523F,F0,G2-0.1MKD-GD15	0004147	062600AD	6/26/00	641.7	557.6
19524F,F0,G2-0.1MKD-GD15	0004148	062600AD	6/26/00	834.2	724.9
19525F,F0,G2-0.1MKD-GD15	0004149	062600AD	6/26/00	1080.8	939.2
19526F,F0,G2-0.1MKD-GD15	0004150	062600AD	6/26/00	777.9	676.0
19527F,F0,G2-0.1MKD-GD15	0004151	062600AD	6/26/00	798.5	693.9
19528F,F0,G2-0.1MKD-GD15	0004152	062600AD	6/26/00	844.8	734.1
19529F,F0,G2-0.1MKD-GD15	0004153	062600AD	6/26/00	723.6	628.8
19530F,F0,G2-0.1MKD-GD15	0004154	062600AD	6/26/00	724.4	629.5
19531F,F0,G2-0.1MKD-GD15	0004155	062600AD	6/26/00	777.3	675.5
19532F,F0,G2-0.1MKD-GD15	0004156	062600AD	6/26/00	568.2	493.8
			AVERAGE:	**762.2**	**662.4**
			STANDARD DEVIATION:	**119**	**104**

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Table XIV. Summary of PFOS residues in G3-0.4MKD on GD15

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19533F,F0,G3-0.4MKD-GD15	0004157	062600BD	6/26/00	2759.7	2398.2
19534F,F0,G3-0.4MKD-GD15	0004158	062600BD	6/26/00	4817.3	4186.2
19535F,F0,G3-0.4MKD-GD15	0004159	062600BD	6/26/00	3627.9	3152.6
19536F,F0,G3-0.4MKD-GD15	0004160	062600BD	6/26/00	3186.8	2769.3
19537F,F0,G3-0.4MKD-GD15	0004161	062600BD	6/26/00	3397.0	2952.0
19538F,F0,G3-0.4MKD-GD15	0004162	062600BD	6/26/00	3143.5	2731.7
19539F,F0,G3-0.4MKD-GD15	0004163	062600BD	6/26/00	3094.1	2688.8
19540F,F0,G3-0.4MKD-GD15	0004164	062600BD	6/26/00	4151.1	3607.3
19542F,F0,G3-0.4MKD-GD15	0004165	062600BD	6/26/00	1881.6	1635.1
19543F,F0,G3-0.4MKD-GD15	0004166	062600BD	6/26/00	3420.1	2972.1
19544F,F0,G3-0.4MKD-GD15	0004167	062600BD	6/26/00	3372.6	2930.8
19546F,F0,G3-0.4MKD-GD15	0004168	062600BD	6/26/00	2625.1	2281.2
19547F,F0,G3-0.4MKD-GD15	0004169	062600BD	6/26/00	3863.8	3357.6
19548F,F0,G3-0.4MKD-GD15	0004170	062600BD	6/26/00	3910.6	3398.3
			- AVERAGE:	**3375.1**	**2932.9**
			STANDARD DEVIATION:	**713**	**620**

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Table XV. Summary of PFOS residues in G4-1.6MKD on GD15

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19550F,F0,G4-1.6MKD-GD15	0004171	062700AD	6/27/00	13868.2	12051.5
19553F,F0,G4-1.6MKD-GD15	0004172	062700AD	6/27/00	14838.7	12894.8
19554F,F0,G4-1.6MKD-GD15	0004173	062700AD	6/27/00	14172.4	12315.8
19555F,F0,G4-1.6MKD-GD15	0004174	062700AD	6/27/00	16258.1	14128.3
19556F,F0,G4-1.6MKD-GD15	0004175	062700AD	6/27/00	9066.1	7878.4
19558F,F0,G4-1.6MKD-GD15	0004176	062700AD	6/27/00	7174.9	6235.0
19559F,F0,G4-1.6MKD-GD15	0004177	062700AD	6/27/00	7477.4	6497.9
19560F,F0,G4-1.6MKD-GD15	0004178	062700AD	6/27/00	14880.9	12931.5
19561F,F0,G4-1.6MKD-GD15	0004179	062700AD	6/27/00	17442.7	15157.7
19562F,F0,G4-1.6MKD-GD15	0004180	062700AD	6/27/00	11417.2	9921.5
19563F,F0,G4-1.6MKD-GD15	0004181	062700AD	6/27/00	8949.0	7776.7
19564F,F0,G4-1.6MKD-GD15	0004182	062700AD	6/27/00	17342.4	15070.5
			AVERAGE:	**12740.7**	**11071.6**
			STANDARD DEVIATION:	**3769**	**3275**

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Table XVI. Summary of PFOS residues in G5-3.2MKD on GD15

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19565F,F0,G5-3.2MKD-GD15	0004183	062800AD	6/28/00	35175.8	30567.8
19566F,F0,G5-3.2MKD-GD15	0004184	062800AD	6/28/00	15192.6	13202.4
19567F,F0,G5-3.2MKD-GD15	0004185	062800AD	6/28/00	20391.7	17720.4
19568F,F0,G5-3.2MKD-GD15	0004186	062800AD	6/28/00	46897.0	40753.5
19569F,F0,G5-3.2MKD-GD15	0004187	062800AD	6/28/00	36307.7	31551.4
19570F,F0,G5-3.2MKD-GD15	0004188	062800AD	6/28/00	31052.5	26984.6
19571F,F0,G5-3.2MKD-GD15	0004189	062800AD	6/28/00	28440.6	24714.9
19572F,F0,G5-3.2MKD-GD15	0004190	062800AD	6/28/00	20549.6	17857.6
19573F,F0,G5-3.2MKD-GD15	0004191	062800AD	6/28/00	39763.7	34554.7
19574F,F0,G5-3.2MKD-GD15	0004192	062800AD	6/28/00	47267.6	41075.5
19575F,F0,G5-3.2MKD-GD15	0004193	062800AD	6/28/00	40708.5	35375.7
19577F,F0,G5-3.2MKD-GD15	0004194	062800AD	6/28/00	47288.1	41093.4
19579F,F0,G5-3.2MKD-GD15	0004195	062800AD	6/28/00	33436.6	29056.4
19580F,F0,G5-3.2MKD-GD15	0004196	062800AD	6/28/00	32709.0	28424.1
			AVERAGE:	**33941.5**	**29495.2**
			STANDARD DEVIATION:	**10248**	**8905**

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Table XVII. Summary of PFOS residues in G1-Control on GD21

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19504F,F0,G1-Control-GD21	0004197	062900A	6/29/00	ND	ND
19505F,F0,G1-Control-GD21	0004198	062900A	6/29/00	ND	ND
19506F,F0,G1-Control-GD21	0004199	062900A	6/29/00	12.3	10.7
19507F,F0,G1-Control-GD21	0004200	062900A	6/29/00	ND	ND
19509F,F0,G1-Control-GD21	0004201	062900A	6/29/00	84.3	73.3
19512F,F0,G1-Control-GD21	0004202	062900A	6/29/00	21.5	18.7
19516F,F0,G1-Control-GD21	0004203	062900A	6/29/00	2.0	1.7
			AVERAGE:	**17.2**	**14.9**
			STANDARD DEVIATION:	**31**	**27**

Table XVIII. Summary of PFOS residues in G2-0.1MKD on GD21

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19518F,F0,G2-0.1MKD-GD21	0004204	0629-3000AD	6/29/00	735.4	639.1
19519F,F0,G2-0.1MKD-GD21	0004205	062900A	6/29/00	473.0	411.0
19521F,F0,G2-0.1MKD-GD21	0004206	062900A	6/29/00	403.4	350.6
19523F,F0,G2-0.1MKD-GD21	0004207	062900A	6/29/00	402.1	349.4
19524F,F0,G2-0.1MKD-GD21	0004208	062900A	6/29/00	493.9	429.2
19528F,F0,G2-0.1MKD-GD21	0004209	062900A	6/29/00	456.1	396.4
19529F,F0,G2-0.1MKD-GD21	0004210	062900A	6/29/00	391.4	340.1
			AVERAGE:	**479.3**	**416.5**
			STANDARD DEVIATION:	**120**	**104**

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Table XIX. Summary of PFOS residues in G3-0.4MKD on GD21

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19533F,F0,G3-0.4MKD-GD21	0004211	0629-3000AD	6/30/00	1684.4	1463.7
19534F,F0,G3-0.4MKD-GD21	0004212	0629-3000AD	6/30/00	3102.9	2696.4
19536F,F0,G3-0.4MKD-GD21	0004213	0629-3000AD	6/30/00	1848.1	1606.0
19543F,F0,G3-0.4MKD-GD21	0004214	0629-3000AD	6/30/00	2233.1	1940.6
19546F,F0,G3-0.4MKD-GD21	0004215	0629-3000AD	6/30/00	2229.2	1937.2
19548F,F0,G3-0.4MKD-GD21	0004216	0629-3000AD	6/30/00	5417.3	4707.6
			AVERAGE:	**2752.5**	**2391.9**
			STANDARD DEVIATION:	**1395**	**1212**

Table XX. Summary of PFOS residues in G4-1.6MKD on GD21

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19556F,F0,G4-1.6MKD-GD21	0004217	0629-3000AD	6/30/00	18272.7	15879.0
19559F,F0,G4-1.6MKD-GD21	0004218	0629-3000AD	6/30/00	10574.8	9189.5
19560F,F0,G4-1.6MKD-GD21	0004219	0629-3000AD	6/30/00	11190.1	9724.2
19562F,F0,G4-1.6MKD-GD21	0004220	0629-3000AD	6/30/00	5681.8	4937.5
			AVERAGE:	**11429.9**	**9932.5**
			STANDARD DEVIATION:	***5185***	***4506***

Table XXI. Summary of PFOS residues in G5-3.2MKD on GD21

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (ng/g)	Corrected Residue Found (ng/g)
19568F,F0,G5-3.2MKD-GD21	0004221	0629-3000AD	6/30/00	25084.0	21798.0
19569F,F0,G5-3.2MKD-GD21	0004222	0629-3000AD	6/30/00	21667.4	18829.0
19571F,F0,G5-3.2MKD-GD21	0004223	0629-3000AD	6/30/00	21465.5	18653.5
19572F,F0,G5-3.2MKD-GD21	0004224	0629-3000AD	6/30/00	15006.4	13040.6
19577F,F0,G5-3.2MKD-GD21	0004225	0629-3000AD	6/30/00	27066.6	23520.9
19579F,F0,G5-3.2MKD-GD21	0004226	0629-3000AD	6/30/00	28290.3	24584.3
			AVERAGE:	**23096.7**	**20071.0**
			STANDARD DEVIATION:	**4834**	**4201**

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Table XXII. Summary of PFOS recoveries in Fortified Samples

Sponsor ID	Centre ID	Set Number	Date Extracted	Amt. Added (ng)	% Recovery
501F,F0,G1-Control-GD0	0003977 Spk A	061200AR	6/12/00	50	126
504F,F0,G1-Control-GD0	0003980 Spk B	061200AR	6/12/00	250	116
505F,F0,G1-Control-GD0	0003981 Spk A	061400A	6/14/00	50	98
07F,F0,G1-Control-GD0	0003983 Spk B	061400A	6/14/00	250	97
11F,F0,G1-Control-GD0	0003987 Spk A	061500AD	6/15/00	50	128
2F,F0,G1-Control-GD0	0003988 Spk B	061500AD	6/15/00	250	110
3F,F0,G1-Control-GD0	0003989 Spk A	061600A	6/16/00	50	80
5F,F0,G1-Control-GD0	0003991 Spk B	061600A	6/16/00	1000	89
)F,F0,G1-Control-GD0	0003986 Spk A	061900AD	6/19/00	50	107
1 F,F0,G1-Control-GD0	0003987 Spk B	061900AD	6/19/00	5000	78
1 F,F0,G1-Control-GD0	0003986 Spk C	062000A	6/20/00	50	128
1 F,F0,G1-Control-GD0	0003987 Spk D	062000A	6/20/00	20000	96
1 F,F0,G1-Control-GD0	0003989 Spk A	062100AD	6/21/00	1000	104
19 F,F0,G1-Control-GD0	0003991 Spk B	062100A	6/21/00	20000	80
19 F,F0,G1-Control-GD7	0004057 Spk A	062200A	6/22/00	1000	97
19 F0,G1-Control-GD7	0004060 Spk B	062200A	6/22/00	20000	79
1950 ,G1-Control-GD7	0004062 Spk A	062200B	6/22/00	1000	99
19509F,F0,G1-Control-GD7	0004064 Spk B	062200B	6/22/00	20000	86
19510F,F0,G1-Control-GD7	0004065 Spk A	062300A	6/23/00	1000	93
19511F,F0,G1-Control-GD7	0004066 Spk B	062300A	6/23/00	20000	71
19512F,F0,G1-Control-GD7	0004067 Spk A	062300B	6/23/00	1000	85
19513F,F0,G1-Control-GD7	0004068 Spk B	062300B	6/23/00	20000	81
19514F,F0,G1-Control-GD7	0004069 Spk A	062600A	6/26/00	50	111
19516F,F0,G1-Control-GD7	0004071 Spk B	062600A	6/26/00	1000	94
19501F,F0,G1-Control-GD7	0004057 Spk C	062600B	6/26/00	50	82
19505F,F0,G1-Control-GD7	0004060 Spk D	062600B	6/26/00	2000	100
19501F,F0,G1-Control-GD15	0004127 Spk A	062700A	6/27/00	100	100
19502F,F0,G1-Control-GD15	0004128 Spk B	062700A	6/27/00	20000	100
19504F,F0,G1-Control-GD15	0004129 Spk A	062800A	6/28/00	500	99
19505F,F0,G1-Control-GD15	0004130 Spk B	062800A	6/28/00	100000	89
19507F,F0,G1-Control-GD15	0004132 Spk A	062900A	6/29/00	50	119
19508F,F0,G1-Control-GD15	0004133 Spk B	062900A	6/29/00	2000	95
19513F,F0,G1-Control-GD15	0004138 Spk A	063000A	6/30/00	500	113
19514F,F0,G1-Control-GD15	0004139 Spk B	063000A	6/30/00	20000	85
				AVERAGE:	**98**
				STANDARD DEVIATION:	**15**
				RELATIVE STANDARD DEVIATION:	**15**

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12.0 FIGURES

Figure 1. Typical Calibration Curve for PFOS

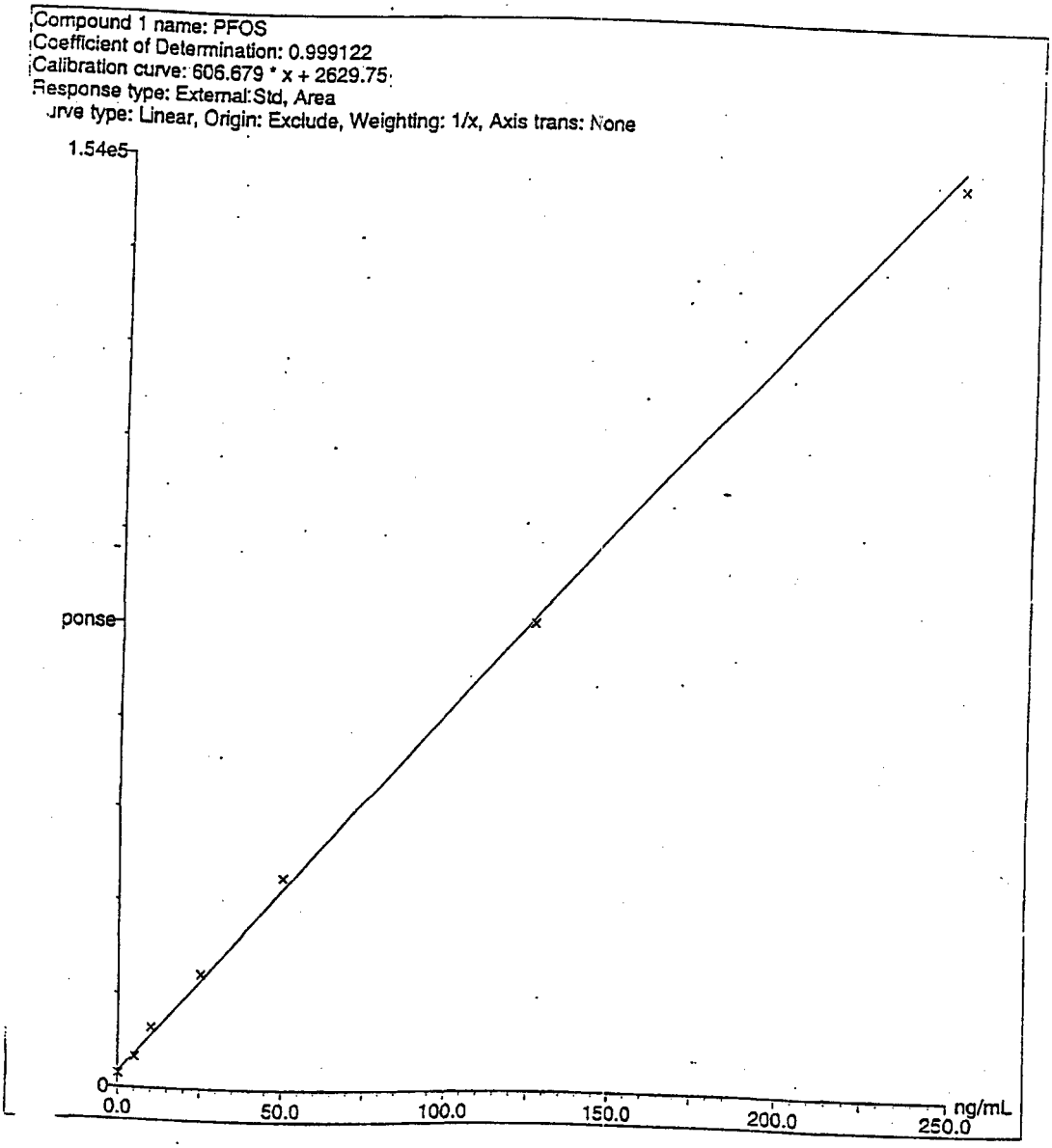

Figure 2. Typical Mean Response Factor for THPFOS

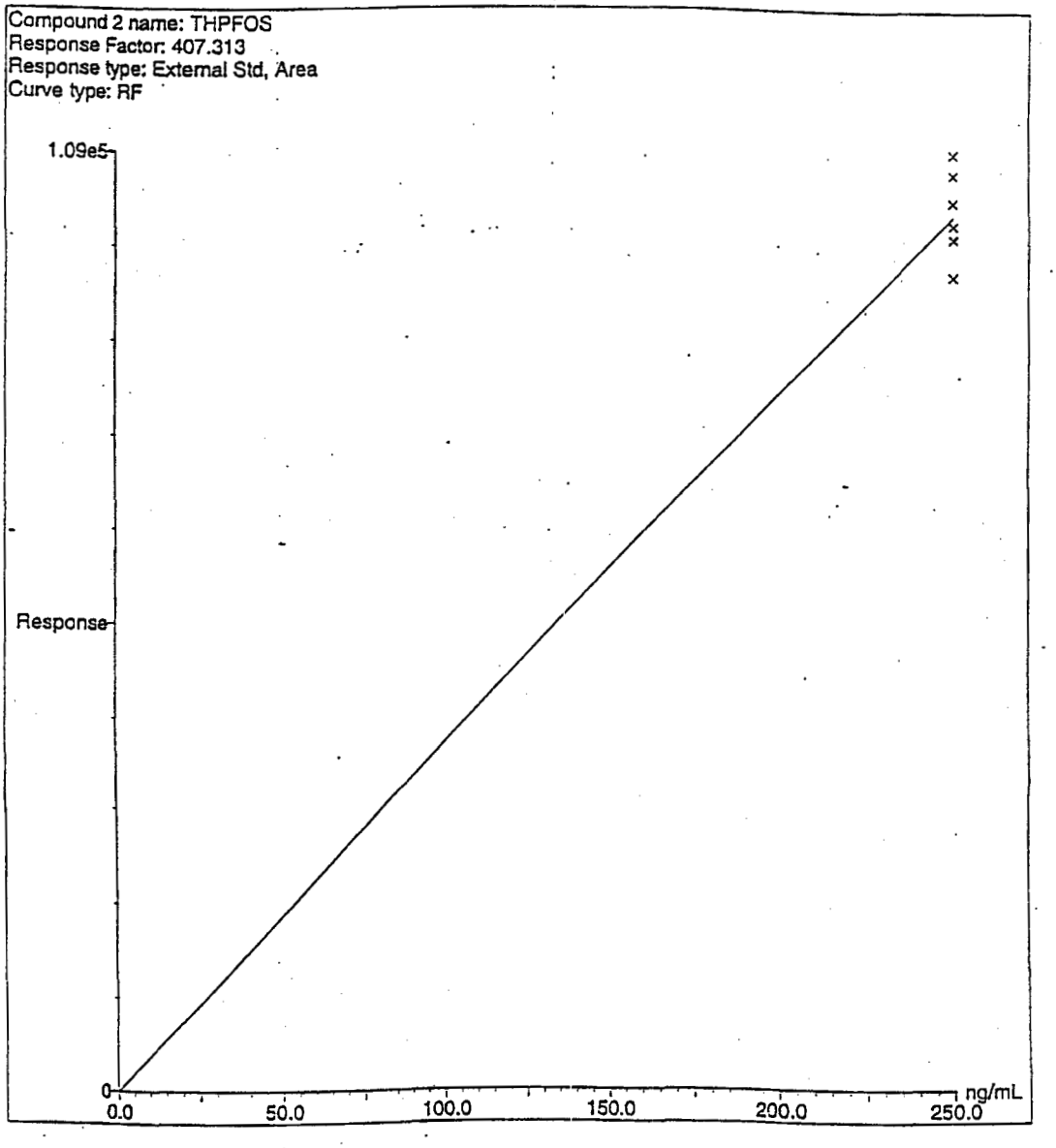

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Figure 3. Chromatogram Representing a 5 ng/mL extracted standard for PFOS and 250 ng/mL fortification of THPFOS

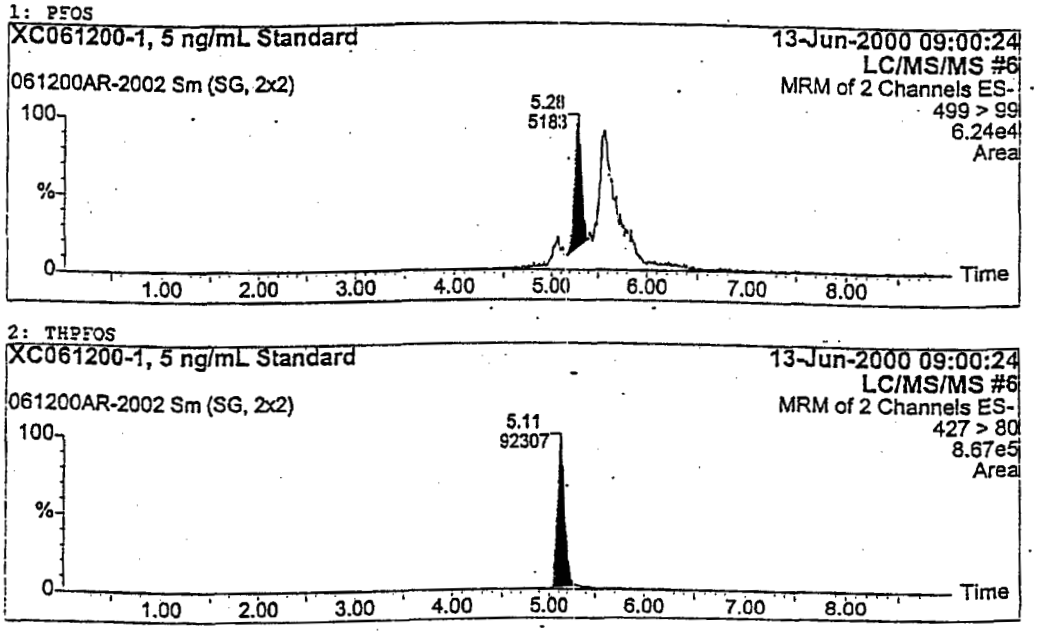

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Figure 4. Chromatogram Representing a 125 ng/mL extracted standard for PFOS and 250 ng/mL fortification of THPFOS

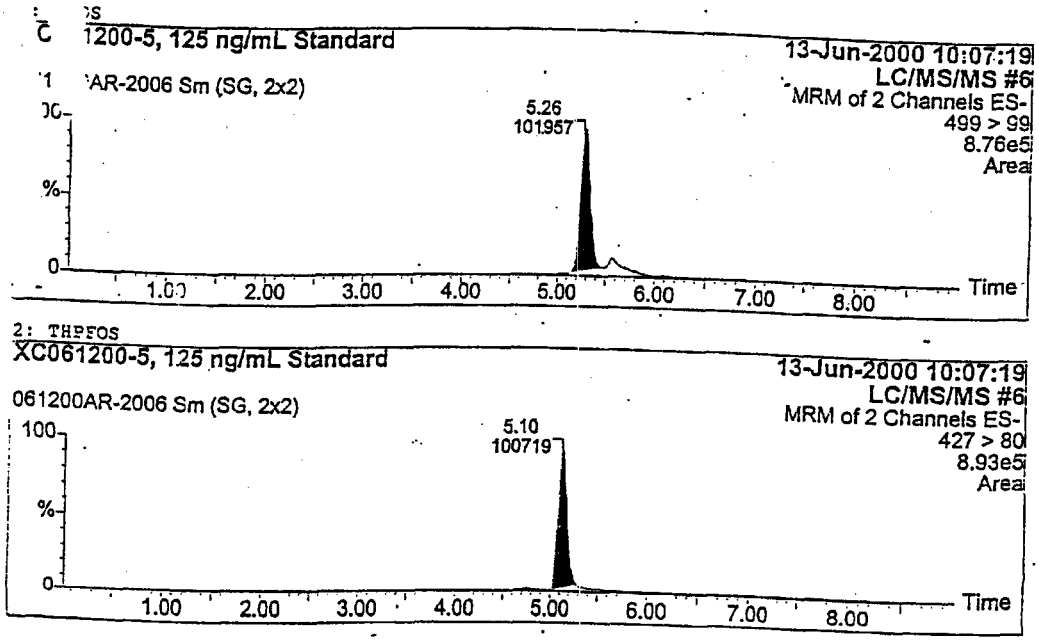

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Figure 5. Chromatogram Representing Control Rat Feces for PFOS and THPFOS (Centre ID: 0004277 Blank B, Set: 062300A)

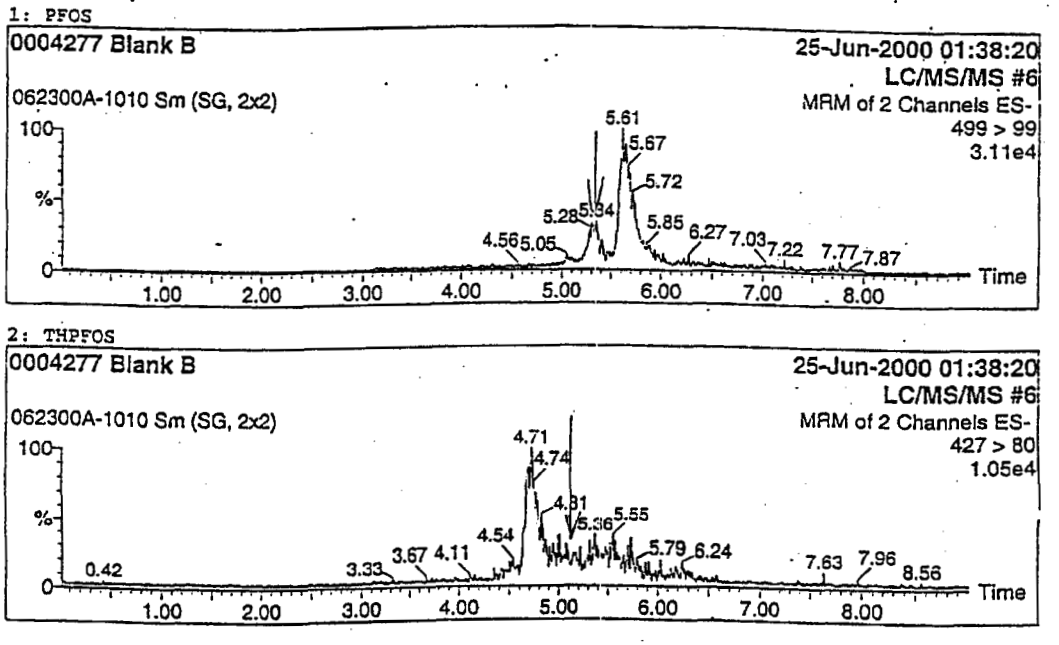
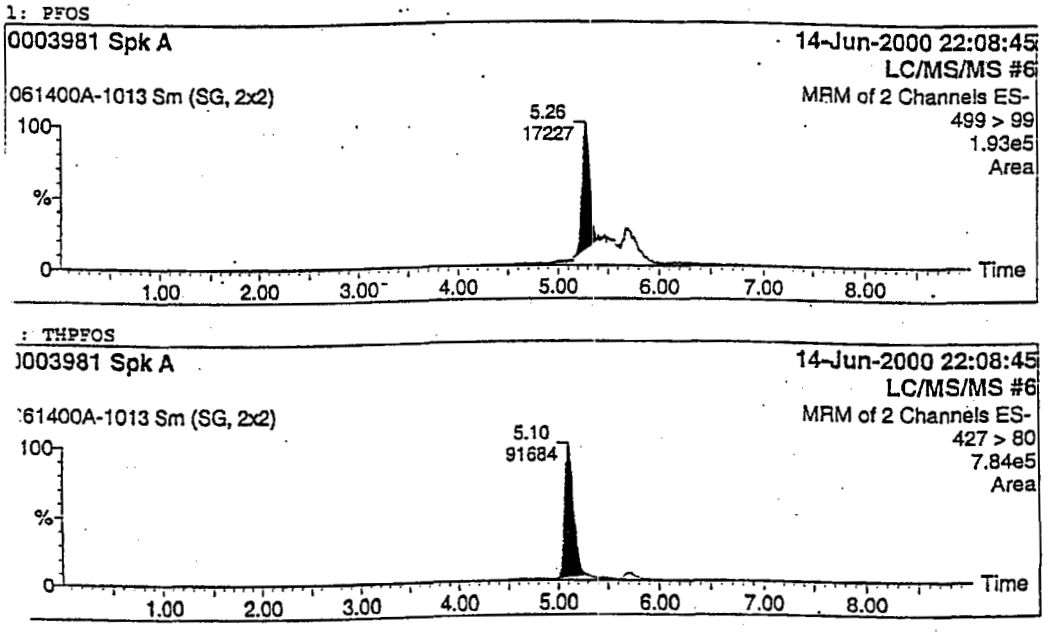


Figure 6. Chromatogram Representing Control Rat Feces Fortified with 50 ng of PFOS and 500 ng of THPFOS (Centre ID: 0003981 Spk A, Set: 061400A)



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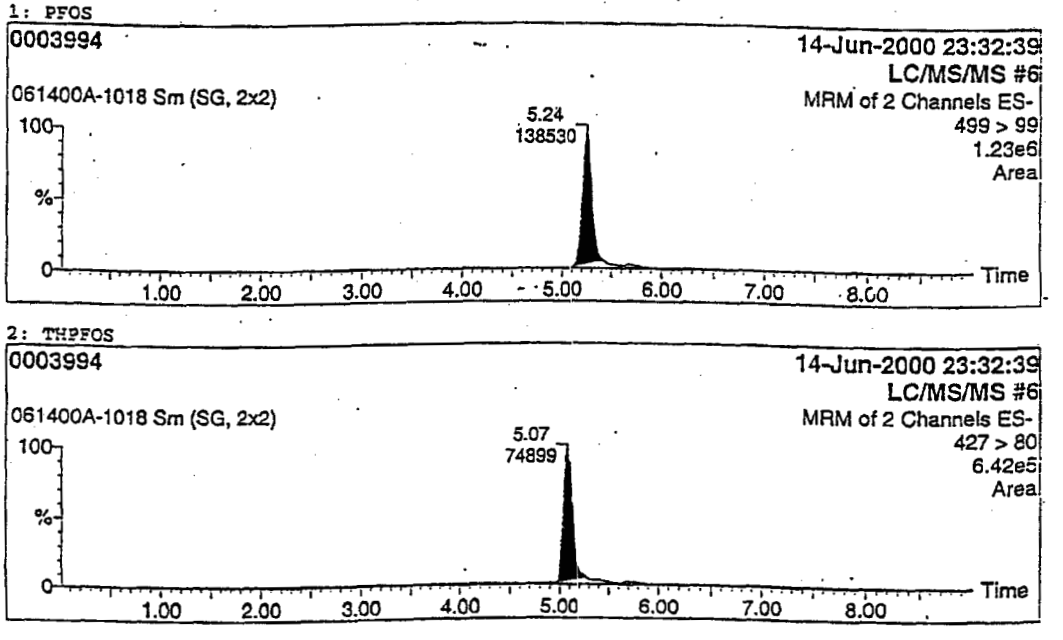
Sponsor Protocol No: FACT-TOX-110

Figure 7. Chromatogram of Rat Feces Sample from G2 on GD0 (Centre ID: 0003994, Set: 061400A)



13.0 APPENDICES

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

APPENDIX A

Study Protocol
FACT-TOX-110
(Centre Study No. 023-016)
and
Amendments and Deviations

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

3M Environmental Technology and Services

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

Protocol #FACT-TOX-110

3M

Study Title

Oral (Gavage) Pharmacokinetic 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-110
U2849

Exact Copy of Original
LAC 05/26/00
Initial Date

Centre Study # 023-016
EW 5-11-00

Study Identification

Oral (Gavage) Pharmacokinetic 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

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

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 8, 1999
Study Completion Date	August 8, 2000

1. STUDY

Oral (gavage) pharmacokinetic 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 #418-013. 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 #418-013.

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

Rats were used as the test system and were maintained and dosed as described in the Argus Research protocol #418-013. The female rats will be given the test material or control once daily beginning 42 days prior to cohabitation and continue through day 14 or 20 of presumed gestation. See table 1 for more dosage information.

Table 1
Dosage Levels, Concentration, and Volumes

Dosage Group	Number of female rats	Dosage mg/kg/day	Concentration mg/kg/day	Dosage volume mL/kg
1	16	0	0	5
2	16	0.1	0.02	5
3	16	0.4	0.08	5
4	16	1.6	0.32	5
5	16	3.2	0.64	5

9. SPECIMEN AND SAMPLE RECEIPT

The 3M Environmental Laboratory will receive homogeneity samples for dose analysis and specimens of the following body tissues and fluids from the indicated points in the study. See table 2 for specimen information. All specimens will be packed on dry ice for shipping.

Table 2
Specimen Information

Body tissue/fluid	Collected	Expected # of specimens
Serum – Dam and Fetus animals	Dam-Predose, Days 7, 15, and 21 Fetus-At termination of the study	320 Dam and 320 Fetus (pooled)
Urine and feces – Dam animals	Predose, Days 7, 15, and 21	320 urine and 320 feces
Liver – Dam and Fetus animals	Dam and Fetus-At termination of the study	80 Dam and 80 Fetus (pooled)
Milk Secreting Glands – Dam animals	At the termination of the study	80
Amniotic Fluid – Dam animals	Day 15 and Day 21	80

Table 2 Cont. Specimen Information		
Body tissue/fluid	Collected	Expected # of specimens
Embryo's with Placentae – Dam animals	Day 15 and Day 21	80
Carcass – Fetus animal	At the termination of the study	160
Placentae – Fetus animal	Day 15 and Day 21	80
Liver/lung – Fetus animal	At the termination of the study	80

Total number of expected specimens: 2000
Total number of test animals: 64
Total number of control animals: 16

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 Approved protocol and amendments

16.1. Study correspondence

16.1. Shipping records

16.1. Raw data

16.1. 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 archive 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 Hansen — 6/16/99
Kristen J. Hansen, Ph.D., 3M Environmental Laboratory Study Director — Date

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Study Title
Oral (Gavage) Pharmacokinetic 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-TOX110
LIRN U2849

Exact Copy of Original
tAC 04/26/00
Initial Date

3M Environmental Laboratory

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Protocol FACT-TOX110
Amendment No. 1

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

1. *PROTOCOL READS:* Section 2.0 states this study is designed to determine potassium perfluorooctane sulfonic acid (PFOS) in specimens of liver and serum of rats.

AMEND TO READ: This study is designed to determine PFOS in specimens of rat liver, serum, and urine. All Day -1 and Gestation Day 21 rat urine specimens will be extracted and analyzed by the 3M Environmental Laboratory.

REASON: The urine extraction and analytical methods were not validated and approved prior to protocol approval.

2. *PROTOCOL READS:* Section 6.0 lists rat or rabbit liver and serum.

AMEND TO READ: Rat or rabbit urine from 3M Toxicology with a physical description of rat or rabbit urine.

REASON: The rat urine matrix was added after the protocol was approved.

3. *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-8-96.0 "Extraction of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds from Urine for Analysis Using HPLC-Electrospray/Mass Spectrometry/Mass Spectrometry"
ETS-8-7.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Liver Extracts Using HPLC-Electrospray/Mass Spectrometry"
ETS-8-97.0 "Analysis of Potassium Perfluorooctanesulfonate or Other Fluorochemical Compounds in Urine Extracts Using HPLC-Electrospray/Mass Spectrometry/Mass Spectrometry"

3M Environmental Laboratory

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Protocol FACT-TOX110
Amendment No. 1

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.
Methods ETS-8-96.0 and ETS-8-97.0 were not validated and approved until after the protocol was approved.

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

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

The extraction and analytical method to follow at Battelle Memorial Institute is:

"Method for Analysis of Perfluorooctane Sulfonate (PFOS) in Rat ~~Sera~~ Liver by LC/MS/MS, Version 1.0"

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

5. *PROTOCOL READS:* Section 12.2.1 b) Liver method detection limit is 15 ppb.

AMEND TO READ: 12.2.1 b) Liver method detection limit is 8.50 ppb (ng/g).
12.2.1 c) Urine method detection limit is 1.5 ppb (ng/g).

REASON: The validation supporting methods ETS-8-6.0 and ETS-8-7.0 included a lower method detection limit for PFOS in liver. A validation in urine was performed, after protocol approval, to support this method detection limit.

D Reuc 9/27/99

KJ 9/27/99

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Protocol FACT-TOX110
Amendment No. 1

Amendment Approval

Marvin Case

Marvin Case, D.V.M., Ph.D., Sponsor Representative — 17 August 1999 — Date

Kris J. Hansen

Kris J. Hansen, Ph.D., Study Director — 8/18/99 — Date

3M Environmental Laboratory

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Study Title
Oral (Gavage) Pharmacokinetic Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 2

Amendment Date:
September 28, 1999

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

Laboratory Project Identification
ET&SS FACT-TOX110
LIRN U2849

Exact Copy of Original
LAC 04/26/00
Initial Date

3M Environmental Laboratory

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Protocol FACT-TOX110
Amendment No. 2

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

1. *PROTOCOL READS:*
Section 2 states that all liver samples will be extracted and analyzed at Battelle Memorial Institute.

AMEND TO READ:
All dam liver specimens (female mother) and 27 fetus (pooled) liver specimens will be analyzed at Battelle Memorial Institute.

REASON:
The fetal liver/lung is not a target matrix and will not be extracted or analyzed.

2. *PROTOCOL READS:*
Section 9 states that 80 dam liver specimens (female mother) and 80 fetus (pooled) liver specimens will be sent to the Environmental Laboratory.

AMEND TO READ:
Seventy-three dam liver specimens (female mother), 27 fetus (pooled) liver specimens, and 81 fetal liver/lung specimens (27 liver/lung specimens sampled in triplicate) were sent to the Environmental Laboratory.

REASON:
The number of animals changed during the course of the study.

3. *PROTOCOL READS:*
Section 12.2.1 a) serum method detection limit is 1.75 ppb b) Liver method detection limit is 15 ppb.

AMEND TO READ:
The method detection limits for all compounds and matrices will be taken from the methods used for extraction and analysis.

REASON:
The method detection limits listed are specific to the 3M Environmental Laboratory. Statement was added to allow for sub-contracted analyses and/or revised methods.

3M Environmental Laboratory

4. ***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.

5. ***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 documentation when shipping specimens for analyses performed by a sub-contract laboratory.

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Protocol FACT-TOX110
Amendment No. 2

Amendment Approval

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

Kristen Hansen
Kristen J. Hansen, Ph.D., Study Director — 5 Oct 1999 — Date

3M Environmental Laboratory

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Study Title

Analytical Laboratory Report on the Determination of Perfluorooctanesulfonate (PFOS) Presence and Concentration in Serum, Liver, and Urine from the Gavage Study of T-6295.12

PROTOCOL AMENDMENT NO. 3

Amendment Date:
20 January 2000

Performing Laboratories

Urine Analyses	Liver Analyses	Serum Analyses
3M Environmental Technology and Safety Services Fluorine Analytical Chemistry Team Building 2-3E-09 935 Bush Avenue St. Paul, MN 55106	Battelle Memorial Institute 505 King Avenue Columbus, OH 43201-2693	Advanced Bioanalytical Services, Inc. 15 Catherwood Road Ithaca, NY 14850

Laboratory Project Identification
ET&SS LRN-U2849
FACT TOX-110
Argus Study: 418-013
3M Medical Department Study: T-6295.12

Exact Copy of Original
UAC 04/26/00
Initial Date

3M Environmental Laboratory

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Protocol LRN-U2849
Amendment Number 3

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

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

2. *PROTOCOL READS:*
The sponsor for the present study was identified as Marvin T. Case, D.V.M., Ph.D.
AMEND TO READ:
The role of sponsor for the present study was reassigned to John L. Butenhoff, Ph.D., as of 20 January 2000.
REASON:
To ensure that the study director does not also carry the duties of study sponsor, the sponsor role was reassigned. In this manner, personnel responsibilities and workload are more evenly balanced.

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Protocol LRN-U2849
Amendment Number 3

Amendment Approval

John L. Butenhoff — February 10, 2000
John L. Butenhoff, Ph.D., Sponsor Representative — Date

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

Marvin T. Case — 10 February 2000
Marvin T. Case, D.V.M., Ph.D., Incoming Study Director — Date

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Study Title
Oral (Gavage) Pharmacokinetic Study of PFOS in Rats

PROTOCOL AMENDMENT NO. 4

Amendment Date:
20 April 2000

Performing Laboratories

URINE ANALYSES:
3M Environmental Technology
and Safety Services
Fluorine Analytical Chemistry Team
Building 2-3E-09, 935 Bush Avenue
St. Paul, MN 55106

FECES ANALYSES:
Centre Analytical Laboratories, Inc.
3048 Research Drive
State College, PA 16801

LIVER ANALYSES:
Battelle Memorial Institute
505 King Avenue
Columbus, OH 43201-2693

SERUM ANALYSES:
Advanced Bioanalytical Services, Inc.
15 Catherwood Road
Ithaca, NY 14850

Laboratory Project Identification
ET&SS LRN-U2849
FACT TOX-110
Argus Study: 418-013
3M Medical Department Study: T-6295.12

Exact Copy of Original
LAC 04/26/00
Initial Date

3M Environmental Laboratory

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Protocol LRN-U2849
Amendment Number 4

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

1. *PROTOCOL READS:*
The amended section 2.0 text states that this study is designed to determine PFOS in specimens of rat liver, serum, and urine.
AMEND TO READ:
This study is designed to determine PFOS in specimens of rat liver, serum, feces, and urine.
REASON:
The analysis of fecal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the protocol. Feces extraction and analytical methods were not validated and approved prior to protocol approval.

2. *PROTOCOL READS:*
The amended section 6.0 lists rat or rabbit liver, serum, and urine.
AMEND TO READ:
Add: rat or rabbit feces with a physical description of rat or rabbit feces.
REASON:
Analysis of fecal tissue for the target chemical and/or its analytes was added to the scope of the study following the issuance of the original protocol.

3. *PROTOCOL READS:*
Section 13.1 lists all of the laboratories that will be conducting analyses for this study.
AMEND TO READ:
Add: Centre Analytical Laboratories, Inc., 3048 Research Drive, State College, PA 16801
REASON:
Feces analyses were added to the scope of this study. The sub-contract laboratory performing analyses was not in the original protocol.

4. *PROTOCOL READS:*
Sections 10.4 and 11.4 state that if the analyses are sub-contracted to other laboratories an amendment will be written to include these methods.
AMEND TO READ:
The feces extraction and analytical method used by Centre Analytical Laboratories will be; 00M-023-003 (Revision 2), "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS."
REASON:
The sub-contract laboratory performing feces analyses was added to the scope of this study; this method was not validated and approved prior to protocol approval.

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Protocol LRN-U2849
Amendment Number 4

Amendment Approval

John L. Butenhoff — April 19, 2000

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

Marvin Case — 19 April 2000

Marvin T. Case, D.V.M., Ph.D., Study Director — Date

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

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

PROTOCOL DEVIATION

Deviation Number: 1

Date of Occurrence: entire study

Centre Study Number: 023-016 Centre Protocol Number: FACT-TOX-110

DESCRIPTION OF DEVIATION

1. § 12.3 Page 7: Did not perform duplicate extractions on any of the samples for this study.

ACTIONS TAKEN

i.e., amendment issued, SOP revision, etc...

1. Protocol deviation issued.

Recorded By/Date: Kristy R Stauffer 7/28/00

IMPACT ON THE STUDY

1. No negative impact.

RATIONALE

1. Although none of the study samples were duplicated, at least one of the matrix fortifications (QC's) in each set was prepared to match one of the extracted calibration standards and a precision of < 30% was observed.

Principal Investigator Signature — 7/28/00 Date

Sponsor Representative Signature — 8/3/00 Date

CAL QAU Review WLL 7/28/00

February 12, 1998/2

Study Director — Date

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

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

PROTOCOL DEVIATION

Deviation Number: 2

Date of Occurrence: (1) entire study

Centre Study Number: 023-016 Centre Protocol Number: FACT-TOX-110

DESCRIPTION OF DEVIATION

1. § Amendment 2 Item #3: A method detection limit was not established for rat feces during the method validation. 8/4/00

ACTIONS TAKEN

i.e., amendment issued, SOP revision, etc...

1. Protocol deviation issued.

Recorded By/Date: Emily R. Stauffer 8/4/00

IMPACT ON THE STUDY

1. No negative impact.

RATIONALE

1. Any sample that contained residue with ~ 3:1 signal:noise was integrated and any number that was greater than 0 was reported. The limit of quantitation was established at 10 ppb during the method validation.

Principal Investigator Signature — Date: 8/8/00

Sponsor Representative Signature — Date: 21 Aug 2000

CAL QAU Review MLL 8/4/00

February 12, 1998/2

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

APPENDIX B

Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2) Method #00M-023-003, revision 2 and Deviations and Modifications

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

TITLE

Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS
(Revision 2)

AUTHORS

Enaksha Wickremesinhe, Shaozhi Zheng, and John Flaherty

DATE ISSUED

June 02, 2000

SPONSOR

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CENTRE STUDY NUMBER

023-003

CENTRE METHOD NUMBER

00M-023-003, revision 2

TOTAL NUMBER OF PAGES

20

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Centre Method No. : 00M-023-003, revision 2

MANAGEMENT APPROVAL

The work cited here was performed in conformance with applicable standard operating procedures and general GLP's.

6-02-00

Enaksha Wickremesinhe, Ph.D. Date
Principal Investigator
Centre Analytical Laboratories, Inc

6-02-00

Shaozhi Zheng Date
Principal Investigator
Centre Analytical Laboratories, Inc.

6/9/00

John Flaherty Date
Laboratory Manager
Centre Analytical Laboratories, Inc.

2-JUNE-00
2-JUNE-00

Richard A. Grazzini, Ph.D. Date
President
Centre Analytical Laboratories, Inc.

06/06/00

Kris Hansen, Ph.D. Date
Group Leader
3M Environmental Laboratory

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Centre Method No. : 00M-023-003, revision 2

TABLE OF CONTENTS

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Centre Method No. : 00M-023-003, revision 2

1. SUMMARY

This document details a method of analysis for residues of perfluorooctane sulfonate (PFOS), perfluorooctane sulfonylamide (PFOSA), perfluorooctane sulfonylamidoethyl-acetate (PFOSAA), perfluorooctanesulfonylethylamide (PFOSEA), perfluorooctanoate (POAA), 2(N-ethylperfluorooctanesulfonamido)-ethyl alcohol (Et-FOSE-OH), M570, and M556 in monkey feces. The chemical formulas of the analytes are given in Section 2 of this method.

This method is being re-issued as method 00M-023-003 revision 2. It is being revised to add the rat feces as a matrix, to document the use of 20-mL vials instead of 15 mL tubes for sample extraction, to add the option of homogenizing feces samples before weighing, to clarify the preparation of QC recovery samples and to correct some typographical errors in method 00M-023-003 revision 1. Rat feces is being added to the method, however, it was validated for the analysis of PFOS only. Therefore, this method should be used for the analysis of PFOS only, in rat feces.

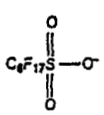
Residues of these fluorochemicals are extracted from feces with acetonitrile (ACN). The ACN extract is filtered and passed through a carbon solid-phase extraction (SPE) column. Approximately 3-4 drops of 1-octanol are added to the extract and evaporated to near dryness using rotary evaporation. Each extract is then reconstituted with methanol. Quantification of PFOS, PFOSA, PFOSAA, PFOSEA, Et-FOSE-OH, M556, M570, and POAA is accomplished by liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis using selected reaction monitoring (SRM).

The proposed limit of quantitation (LOQ) for this method is 10 ppb each for PFOS, PFOSA, PFOSAA, PFOSEA, Et-FOSE-OH, M556, M570, and POAA. This is based on studies conducted during the development of this method using control monkey feces.

2. FLUOROCHEMICAL STANDARDS

Molecular structures of PFOS, PFOSA, PFOSAA, PFOSEA, Et-FOSE-OH, M556, M570, and POAA are given below.

PFOS
Molecular weight: 499 ($C_8F_{17}SO_3^-$)



Chemical Name = Perfluorooctane sulfonate

Note: The neutral molecule and standard form that the PFOS (anion) is derived from is potassium perfluorooctane sulfonate [$C_8F_{17}SO_3K$], molecular weight 538.

Centre Study No.: 023-016
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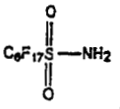
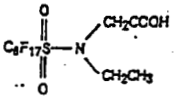
Centre Method No.: 00M-023-003, revision 2

PFOSA
Molecular weight: 499



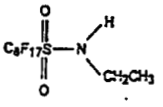
Chemical Name = Perfluorooctane sulfonylamide

PFOSAA
Molecular weight: 585



Chemical Name = perfluorooctane sulfonylamidoethylacetate

PFOSEA
Molecular weight: 527



Chemical Name = Perfluorooctanesulfonylethylamide

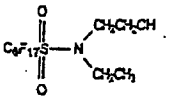
POAA
Molecular weight: 413

$C_7F_{15}CCO$-

Chemical Name = Perfluorooctanoate

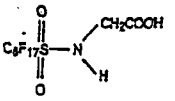
Note: The neutral molecule and standard form that the POAA (anion) is derived from is ammonium perfluoroctanoate [$C_7F_{15}COONH_4$], molecular weight 431.

Et-FOSE-OH
Molecular weight: 571



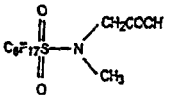
Chemical Name = 2(N-ethylperfluorooctanesulfonamido)-ethyl alcohol

M 556
Molecular weight: 557



Chemical Name = M556

M 570
Molecular weight: 571



Chemical Name = M570

THPFOS
Molecular weight: 428

1-H, 1-H, 2-H, 2-H, $C_8F_{13}SO_3H$

Chemical Name = 4-H, perfluorooctane sulfonic acid

3. CHEMICALS AND SUPPLIES

3.1. CHEMICALS

Chemical	Grade	Source	Catalog No.
Acetonitrile (ACN)	HPLC	(VWR) J. T. Baker	JT9017-3
Carbon 120/400 mesh	-	Supelco	57210-U
Methanol (MeOH)	HPLC	(VWR) J. T. Baker	JT9093-2
1-Octanol	Reagent	Aldrich Chemical	111-87-5
Ammonium Acetate	Reagent	Sigma-Aldrich	A-7330
L-Ascorbic acid	Reagent	Sigma-Aldrich	L-5960
Toluene	Reagent	(VWR) J. T. Baker	JT9460-3
Dimetyldichlorosilane	Reagent	Supelco	3-3009
Water	Type I	in house	-

(Type I water = electrical resistivity, minimum of 16.67 MΩ/cm at 25°C, from a Labconco waterpro workstation)

3.2. FLUOROCHEMICAL STANDARDS

Standard	Source
PFOS	3M
PFOSA	3M
PFOSAA	3M
PFOSEA	3M
Et-FOSE-OH	3M
M556	3M
M570	3M
POAA	3M
THPFOS	3M

3.3. EQUIPMENT AND SUPPLIES

Equipment	Source
Balance, analytical, (display at least 0.0001 g)	Mettler
Balance, top loading, (display at least 0.01 g)	Mettler
Rotary evaporator	Buchi
Rotary evaporator trap	Buchi
Pear-shaped flasks (125 mL)	Pyrex
20-mL SPE tubes (cat. no. N057177) with frits	Supelco
Vacuum pump	Buchi
Visiprep vacuum manifold	Supelco

Wrist-action shaker	Burrell
20-mL polyethylene scintillation vials with lids	Wheaton (VWR)
20-mL syringe	VWR
25-mm diameter glass fibre acrodisc (cat # 4523)	Gelman
Disposable pipets, test tubes etc.	VWR
Disposable micropipets, 100 – 200 µL.	VWR
125-mL LDPE narrow-mouth bottles	Nalgene
2-mL clear HPLC vial kit (cat # 5181-3400)	HP
Standard lab equipment (class A pipets and volumetric flask, graduated cylinders, etc.)	various
LC/MS/MS and HPLC systems	As described in Section 4.5.1

Notes:

1. In order to avoid contamination, the use of disposable containers/tubes/pipets etc. is highly recommended.
2. Teflon or teflon-lined containers or equipment, including teflon-lined HPLC vial caps should not be used.
3. Pear-shaped flasks are silanized before use.
4. It may be necessary to check the solvents (methanol) for the presence of contaminants (especially POAA) by LC/MS/MS before use. Certain lot numbers have been found to be unsuitable for use.
5. Disposable micropipets or pipets should be used to aliquot standard solutions, when preparing standards and samples for extraction.
6. Equivalent materials may be substituted for those specified in this method. However, the use of carbon from Supelco is strongly recommended.

3.4. SOLUTIONS

1. 50 mM Ammonium Acetate: Dissolve 3.85 g of ammonium acetate in 1 L of type I water.
2. 2 mM Ammonium Acetate: Dilute 40 mL of the 100 mM ammonium acetate solution in a liter of type I water, for mobile phase A.
3. Saturated Ascorbic Acid in Methanol: Dissolve ~ 10 g ascorbic acid in 100 mL methanol.
4. The 2% Ascorbic Acid in Methanol: Dissolve 2 g ascorbic acid in 100 mL methanol.

Note: The volumes shown are provided for guidance; alternative volumes may be prepared.

3.5. Preparation of Stock, Fortification, and Calibration Solutions

Analytical standards are used for two purposes: (1) to fortify control matrix samples designated to be extracted and processed as calibration standards that will be then used to calibrate the response of the detector used in the analysis; and (2) to fortify other control matrix samples to determine analytical recovery.

The absolute volumes of the standards may be varied by the analyst as long as the correct proportions of solute to solvent are maintained. The solutions cited below are given as an example; alternative concentrations may be prepared if needed.

3.5.1. Stock solutions

To prepare stock solutions of 100 µg/mL each PFOS, PFOSA, PFOSAA, PFOSEA, Et-FOSE-OH, M556, M570, POAA, and the surrogate standard THPFOS, weigh out 10 mg of analytical standard (corrected for purity and percent salt, if necessary) and dilute to 100 mL with methanol in a 100-mL volumetric flask. Prepare a separate solution for each analyte. These stock solutions (in 125-mL LDPE bottles) are to be stored in a refrigerator at 2°C to 6°C and are stable for a maximum period of 6 months from the date of preparation.

3.5.2. Fortification Solutions

a. 10 µg/mL Mixed Fortification Solution – Pipet 10.0 mL each of PFOS, PFOSA, PFOSAA, PFOSEA, Et-FOSE-OH, M556, M570, and POAA stock solution to a 100 mL volumetric flask. Bring up to volume with methanol.

b. 2.5 µg/mL Mixed Fortification Solution – Pipet 25.0 mL of the 10 µg/mL mixed fortification solution to a 100-mL volumetric flask and bring up to volume with methanol.

c. 0.5 µg/mL Mixed Fortification Solution – Pipet 20.0 mL of the 2.5 µg/mL mixed fortification solution to a 100-mL volumetric flask and bring up to volume with methanol.

d. 0.1 µg/mL Mixed Fortification Solution – Pipet 20.0 mL of the 0.5 µg/mL mixed fortification solution to a 100-mL volumetric flask and bring up to volume with methanol.

Follow Steps a and b above to make a 2.5 µg/mL solution of the surrogate standard (THPFOS) from the stock solution.

Store all fortification standard solutions (in 125-mL LDPE bottles) in a refrigerator at 2°C to 6°C for a maximum period of 6 months from the date of preparation.

3.5.3. Calibration Standards

Prepare LC/MS/MS calibration standards by fortifying weighed out aliquots of the same control feces sample (or a combined "bulk" control feces sample) before they are processed through the extraction procedure.

The following is a typical example; additional concentrations may be prepared as needed. It is recommended to prepare the fortification solutions so that the volume of fortification is between 50 and 200 µL and to use disposable micropipets for aliquoting fortification volumes.

Conc. of Mixed Fortification Solution (µg/ml)	Fortification Volume (µL)	Weight of Control Sample (g)	Conc. of Extracted Calibration Standard (ppb)	Vol. of Surrogate Standard* added (µL)
NA	NA	1.0	NA	200
0.1	100	1.0	10	200
0.1	200	1.0	20	200
0.5	100	1.0	50	200
0.5	200	1.0	100	200
2.5	100	1.0	250	200
2.5	200	1.0	500	200
10	100	1.0	1000	200

* 2.5 µg/ml THPFOS fortification solution.

Calibration standard preparations may be stored in a refrigerator at 2°C to 6°C, if needed, and used over a period of time as long as its stability has been verified.

4. METHOD

4.1. FLOW DIAGRAM

The flow diagram of the method is given below, followed by a detailed description of each step.

Method Flow Diagram

Weigh 1.0 g of sample
(fortify samples designated as calibration standards and QC recoveries)
↓
Extract with ACN and filter
↓
Carbon Solid-Phase Extraction
↓
Concentrate to almost dryness
↓
Adjust final volume (2.0 mL)
↓
LC/MS/MS

4.2. SAMPLE PROCESSING

All samples are received frozen and will be kept frozen (below -10°C) until time of extraction. The rat feces do not need any processing, however, the monkey feces may need to be homogenized in order to be able to weigh out the specified amounts.

4.3. SAMPLE PREPARATION

Prepare the following blank and calibration samples (Sections 4.3.1 to 4.3.4) from same control feces sample (or a combined "bulk" control feces sample).

4.3.1. Matrix Blanks

Prepare two blank samples without any fortifications, per set of samples analyzed. These will be used as the matrix blank.

4.3.2. Matrix Zero Blanks

Prepare two matrix blank samples fortified with the surrogate, per set of samples analyzed. These will be used as the matrix zero blanks.

4.3.3. Calibration Standards

Prepare calibration standards as described in Section 3.5.3.

4.3.4. Continuing Calibration Verification Standards

Two of the extracted calibration standards, a low-mid and a mid-high level standard, will be used as continuing calibration verification (CCV) standards.

4.3.5. QC Recovery Samples

Prepare QC recovery samples to represent every control matrix included in the study. Prepare at least one QC recovery sample in the low-range, and one in the high-range, to approximately bracket the expected range of analyte in the samples. For sets containing more than 20 samples, prepare an additional QC recovery sample for every group of 10 additional samples (for example, if one set has 24 samples, then 3 levels of QC recovery samples will be prepared). Prepare these additional QC recovery samples to bracket the range of analyte found in the samples, as appropriate.

Note: An example of a sample extraction worksheet is attached, as Attachment 1.

4.4. EXTRACTION

Note: A. Preparation/conditioning of SPE columns is described in Section 4.4.1.
B. Silanization of pear-shaped flasks is described in Section 4.4.2.
C. Evaluation/standardization of SPE columns (when a different supplier is used etc.) is described in Section 4.4.3.

a. Weigh approximately 1.0 g (± 0.05 g) of sample in to 20 mL polyethylene scintillation vial (Note: designated samples need to be fortified with appropriate aliquots of fortification standards).

b. Add 10 mL of ACN (note: break-up any clumps using a spatula or glass rod), shake on a wrist-action shaker for 30 minutes.

c. Let the vials sit for ~ 5 minutes, carefully decant, directly on to a 20 mL disposable syringe barrel connected to a glass acrodisc (Gelman) filter.

d. Transfer the filtrate to a conditioned SPE column (see Section 4.4.1). Collect the elute in a 125-mL "silanized"(see Section 4.4.2) pear-shaped flask.

Note: The 20 mL SPE column fits well inside the mouth (sleeve) of the pear-shaped flask. Make sure that the pear-shaped flask is well supported and will not topple. No vacuum is needed for this step.

e. Add 10 mL of ACN and then 20 ml of 90:10 ACN : 2% ascorbic acid in MeOH to the SPE column and collect the eluate in to the same pear shaped flask, combining the eluates.

f. Add 3-4 drop of 1-octanol to the pear shaped flask and rotary evaporate to near dryness (a minute amount of 1-octanol will remain) at a reduced pressure of ~ 40°C.

g. Reconstitute by adding 2.0 mL methanol (final volume = 2 mL) and swirl/mix to dissolve. Transfer the extract to HPLC vials using disposable pipets.

4.4.1 SPE Column Preparation

Pack 20-mL SPE tubes with 2 g carbon. Condition columns with 10 mL of saturated ascorbic acid in MeOH followed by 10 mL ACN. Discard all washes. Do not allow the column to dry.

Note: The SPE columns may be packed and conditioned at any point. Use the vacuum manifold to condition the columns. Do not let the SPE columns to run dry at any time.

4.4.2 Silanization of Pear-Shaped Flasks

Silanize the pear-shaped flasks before use, by rinsing with a 30% dimethyl-dichlorosilane in toluene solution followed by a toluene rinse and finally a methanol rinse.

4.4.3 Standardization of SPE Columns

When using carbon from a different supplier, SPE columns should be standardized in the following manner, prior to analyzing samples:

a. Using a mixed standard with a concentration between 100 and 500 ng/mL (each of all eight analytes) follow the elution schemes as outlined in steps 2 and 3. Collect all eluting fractions.

b. Collect a post-elution fraction, after step 3, eluting with an additional 20 mL of 90:10 (ACN : 2% ascorbic acid in MeOH).
c. Adjust the final volume of all the fractions to 10 mL with methanol.
d. Analyze all the fractions by LC/MS/MS.
e. If the target fraction contains a minimum of 85% of the respective analytes, it may be considered acceptable.
f. If the "post-elution" fraction contains significant amount of analytes, the target elution volume may be increased.

4.5. ANALYSIS BY LC/MS/MS

4.5.1. LC/MS/MS System and Operating Conditions (Electrospray)

Mass Spec:	Micromass Quattro Ultima (Micromass)
Interface:	Electrospray (Micromass)
	Harvard infusion pump
Computer:	COMPAQ Professional Workstation AP200
Software:	Windows NT, MassLynx 3.3
HPLC:	Hewlett Packard (HP) Series 1100
	HP Quat Pump
	HP Vacuum Degasser
	HP Autosampler
	HP Column Oven

Note: A 4 x 10 mm hypercarb drop-in guard cartridge (Keystone, part # 844017-400) is attached on-line after the purge valve and before the sample injector port to trap any residue contaminants that may be in the mobile phase and/or HPLC system.

HPLC Column: Genesis C_4 (Jones Chromatography), 2.1 mm x 50 mm, 4µ
Column Temp.: 35° C
Injection Vol.: 10 µL
Mobile Phase (A): 2 mM Ammonium Acetate in Type I water
Mobile Phase (B): Methanol

Time	% A	% B	Flow Rate (mL/min)
0	60	40	0.300
0.4	60	40	0.300
1.0	10	90	0.300
7.0	10	90	0.300
7.5	0	100	0.300
9.0	0	100	0.400
9.5	60	40	0.400
13.5	60	40	0.400
14.0	60	40	0.300

It may be necessary to adjust the HPLC gradient in order to optimize instrument performance. Columns with different dimensions (e.g. 2.1 mm x 30 mm) and also columns from different manufacturers (Keystone Betasil C_{18} etc.) can be used provided equivalent chromatography is obtained.

Ions monitored:

Analyte	Mode	Parent Ion	Product Ion	Approximate Retention Time (min)
PFOS	negative	499	99	5.3
PFOSA	negative	498	78	6.0
PFOSAA	negative	584	526	5.7
PFOSEA	negative	526	169	6.7
Et-FOSE-OH	negative	630	59	6.7
POAA	negative	413	369	5.1
M556	negative	556	498	5.4
M570	negative	570	419	5.6
THPFOS	negative	427	80	5.1

Note that the retention times may vary slightly, on a day-to-day basis, depending on the batch of mobile phase, etc.

4.5.2. Example Tune File Parameters

The following values are provided as an example. Actual values may vary from instrument to instrument. Also these values may be changed from time to time in order to optimize for greatest sensitivity.

The mass spectrometer is tuned using a solution of each analyte at ~0.5 µg/mL, prepared via dilution of the stock solution in methanol. The solution is infused (using a "T" connector) at 10 µL/min into a 0.2 mL/min stream of mobile phase consisting of 40% methanol and 60% 2 mM ammonium acetate. The analytes are initially tuned for the parent ion and then tuned for the product ion. The optimized parameters are saved as a "tune file". This tune file is then used during routine analysis.

Analyte	Dwell (s)	Collision Energy (eV)	Cone (V)
PFOS	0.2	43	76
PFOSA	0.2	28	34
PFOSAA	0.2	20	37
PFOSEA	0.2	28	49
Et-FOSE-OH	0.2	31	30
POAA	0.2	11	25
M556	0.2	28	50
M570	0.2	20	60
THPFOS	0.2	35	34

Source	Set
Capillary	2.56 kV
Hexapole 1	0.5 V
Aperture 1	0.2 V
Hexapole 2	0.8 V
Source Block Temp.	100°C
Desolvation Temp.	400°C

Analyzer	Set
LM Res 1	13.0 V
HM Res 1	13.0 V
IEnergy 1	0.7 V
Entrance	-2 V
Exit	1 V
LM Res 2	11.0 V
HM Res 2	11.0 V
IEnergy 2	1.0 V
Multiplier	650 V

Gas Flows	Set
Cone Gas	~150 L/hr
Desolvation	~700 L/hr

Pressures	Read back
Gas Cell	~3.0e-3 mbar

Note: An alternative LC/MS/MS system may be used once demonstrated to be equivalent.

4.5.3. Calibration Procedures

a. Inject an aliquot (10 µL) of each calibration standard into the LC/MS/MS system. Include standards corresponding to six or more concentration levels (ranging from the LOQ to the highest concentration level to be included in the analysis) in an analytical set.

b. Use a linear y = mx + b function for quantification. Linear standard curves are generated for each analyte by linear regression using 1/x weighting of peak area versus calibration standard concentration using the Windows NT, MassLynx 3.3 (or equivalent) software system. Any extracted standards that fall outside the 70 to 130%, based on the average response factor of the surrogate standard, must be excluded from the calibration curve.

c. The correlation coefficient (R) for calibration curves generated must be ≥0.98 (R^2≥0.96). If calibration results fall outside these limits, take appropriate steps to adjust instrument operation, and reanalyze the relevant sets of samples.

4.5.4. Sample Analysis

a. Each set of samples analyzed must include matrix blanks (without surrogate standard), martrix zero blanks (with the surrogate standard), a set of extracted standards, and QC recovery samples.

b. Inject an aliquot (10 µL) of each standard/sample/QC recovery/control into the LC/MS/MS system. Begin a set of samples with an entire set of calibrations. Include two continuing calibration verification (CCV) standards (one from the low-mid and one from the mid-high range) after every 5 to 10 samples, at the most.

c. Determine the concentration of each sample/QC recovery/control from the standard curve, based on the peak area of each analyte. The standard responses must bracket responses of the residue found in each sample set. Samples that fall outside the range of the standard curve must be diluted appropriately and re-analyzed.

d. Evaluate the ongoing acceptability of instrumental analysis based on the CCV results. Samples analyzed will be acceptable as long as both CCV's fall within ± 30% of their true value. Calculate the CCV concentration found using the linear calibration curve. If the CCV recovery is outside 70% to 130%, re-analyze samples analyzed after the last acceptable check.

e. Monitor the response of the surrogate standard (SS) for every sample, extracted standard, blank, and QC recovery sample. The response is calculated using the "average response factor" function using MassLynx 3.3 (or equivalent). Evaluate the acceptability of the sample extraction process based on the concentration of the SS found in all extracted standards, QC samples, blanks, and samples. The average response factor for all samples, blanks, extracted standards, and QC recovery samples must fall between 60% to 140% (± 40%). Those deviating by greater than 30% (outside 70% to 130% range) must be checked for possible errors and may need to be re-extracted. Any responses deviating by greater than 40% (falling outside the 60% to 140% range) will require re-extraction.

f. If analysis is delayed, samples must be stored refrigerated at approximately 2°C to 6°C until analysis, and analyzed preferably within a week.

4.6. Performance Criteria

The following two criteria should be met before the initial analysis of samples, especially when using different instrumentation set-ups than those cited in this method.

First Criterion - Run a standard solution on LC/MS/MS corresponding to the estimated LOQ (the 10 ppb extracted standard) and obtain a signal to noise ratio of at least 9:1 for all analytes. If this criterion cannot be met, optimize and change instrument operating parameters.

Second Criterion - Run a set of standards of six or more concentration levels, ranging from the proposed LOQ up to the highest concentration level to be included in the analysis. Generate a calibration curve for each analyte and obtain a linear regression with a correlation coefficient of at least 0.98 for each analyte.

4.7. Time Required for Analysis

A set of 14 samples can be taken through the extraction procedure in approximately six hours by one person. The LC/MS/MS analysis (12-14 standards and 14 samples) will take approximately seven hours.

5. CALCULATIONS

5.1. Analyte Found

Calculate the amount of analyte found (in ppb) using the standard curve generated by the Mass Lynx software program using Equation 1. Correction for sample weight (1 g) and final volume (2 mL) is not required since the samples and standards are extracted the same way.

Equation 1:

$$\text{Analyte found (ppb)} = \frac{(\text{peak area} - \text{intercept}) \times DF}{\text{slope}}$$

Where DF = dilution factor, if samples were diluted.

5.2. QC Recovery

For samples fortified with known amounts of analytes prior to extraction, calculate the percent recovery from Equation 2.

Equation 2:

% Recovery =

$$\frac{\text{analyte found (ppb)} - \text{analyte found in matrix blank (ppb)}}{\text{amount analyte added (ppb)}} \times 100$$

5.3. Mean Response Factor

The mean response factor (MRF) is calculated by the Mass Lynx software program by averaging the mean response for all calibration standards (response/amount added) and dividing by the amount added. The actual concentration from each injection is then calculated by dividing the individual response by the MRF. The recovery for each injection is the percent of the actual concentration to the amount added. This calculation is performed by the MassLynx 3.3 (or equivalent) software.

6. SAFETY

All samples must be treated as potential biohazards and appropriate universal precautions must be taken (follow standard operating procedures for the handling of biofluids). These include, but are not limited to, the use of double layers of latex or vinyl gloves, goggles, dust mask, and lab coat. All disposable materials must be treated as biohazards and handled accordingly.

Acetonitrile, methanol, dimethyldichlorosilane and toluene are highly flammable. Open and use under fume hood only.

All organic waste and solvent waste must be segregated and disposed of accordingly.

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

Centre Method No.: 00M-023-003, revision 2

ATTACHMENT 1.

Centre Analytical Laboratories, Inc

Page ___ of ___

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PREPARATION OF SAMPLES FOR EXTRACTION AND ANALYSIS

Protocol No.: NA Centre Study No.: 023-003 Matrix: Feces

Method No.: NA Analytes: PFOS, PFOSA, POAA, N-FOSE-OH, PFOSEA, PFOSAA, M 570, M 556, THPFOS

Sponsor Sample ID	Centre Sample ID	Sample Description	Sample Weight (g)	Surrogate Fort. Vol. (µL)	Fortification Standard ID	Fort. Conc. (pg/mL)	Fort. Vol. (µL)	Fort. Level (ppb)
		Matrix blank	1.0	-	-	-	-	-
		Matrix zero blank	1.0	200		-	-	-
		Std 1	1.0	200		0.1	100	10
		Std 2	1.0	200		0.1	200	20
		Std 3	1.0	200		0.5	100	50
		Std 4	1.0	200		0.5	200	100
		Std 5	1.0	200		2.5	100	250
		Std 6	1.0	200		2.5	200	500
		Std 7	1.0	200		10.0	100	1000
				Fortified with: _____ Capacity: _____ µL				
				Initials/Date: ____/____				

If a sample is not fortified, draw a line through the fortification section. Vertical arrows in a column indicate identical values.

Samples removed from freezer # _____ Time: _____ Initials/Date: ____/____

Samples were weighed on Balance # _____ Time: _____ Initials/Date: ____/____

Samples returned to freezer # _____ Time: _____ Initials/Date: ____/____

* Surrogate Std ID: _____ Initials/Date: ____/____

Samples extracted with ACN and filtered: Initials/Date: ____/____

Carbon SPE clean-up and rotovap: Initials/Date: ____/____

Final volume adjusted to 2 mL with methanol: Initials/Date: ____/____

Extracts placed in refrigerator # _____ Initials/Date: ____/____

Reagent	Lot #
Acetonitrile	
Glass Aerodisc	
Carbon 120/400	
Ascorbic Acid	
Methanol	
1-Octanol	
$C_2H_3Cl_3Si$	
Toluene	

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Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

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METHOD DEVIATION

Deviation Number: 1

Date of Occurrence: (1) 6/14/00, (2) 6/16/00, (3) 6/19/00, (4, 5, & 6), 6/22/00, (7) 6/27/00, (8) 6/28/00, (9) 6/29/00, (10) entire study

Centre Study Number: 023-016 Sponsor Protocol Number: FACT-TOX-110

DESCRIPTION OF DEVIATION

Deviations to method titled "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2)" (Centre Method 00M-023-003, revision 2)

1. Section 4.4.a : Only 0.23 g was weighed for Centre sample ID 0003997 because that was all of the sample available.
2. Section 4.4.a: Only 0.70 g was weighed for Centre sample 0004031 and only 0.43 g for 0004040 because that was all of the sample available.
3. Section 4.4.a : Only 0.31 g was weighed for Centre sample ID 0004052 because that was all of the sample available.
4. Section 4.5.4 (e): Accepted recovery of THPFOS of 43% for 0003989 Spk A in Set 062100AD.
5. Section 4.4.a : Only 0.70 g was weighed for Centre sample ID 0004097 because that was all of the sample available.
6. Section 4.4.a : THPFOS was inadvertently not spiked into samples 0004097, 0004098, 0004099, and 0004100 in Set 062200A. 4 @ EAS 7/21/00
7. Section 4.4.a : Only 0.57 g was weighed for Centre sample ID 0004173 because that was all of the sample available.
8. Section 4.4.a : Only 0.66 g and 0.33 g was weighed for Centre sample ID's 0004186 and 0004194, respectively, because that was all of the sample available.
9. Section 4.4.a : Only 0.30 g was weighed for Centre sample ID 0004132 Spk A because that was all of the sample available.
10. Section 4.4.b.: Occasionally, some samples that contained hard fecal matter did not completely disintegrate after shaking.

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

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METHOD DEVIATION

Deviation Number: 1

Date of Occurrence: (1) 6/14/00, (2) 6/16/00, (3) 6/19/00, (4, 5, & 6), 6/22/00, (7) 6/27/00, (8) 6/28/00, (9) 6/29/00, (10) entire study

Centre Study Number: 023-016 Sponsor Protocol Number: FACT-TOX-110

ACTIONS TAKEN
i.e., amendment issued, SOP revision, etc...

1-10. Method deviation issued.

Recorded By/Date: Emily L Stauffer 6/30/00

IMPACT ON THE STUDY

1-3, 5, 7-9 No negative impact
4. No negative impact because other Spk was acceptable and PFOS recovery was acceptable.
6. No negative impact because THPFOS is not used for quantitative purposes and the PFOS residues found were similar to the other samples in the set.
10. No negative impact because residues found were consistent.

Principal Investigator Signature 6/30/00 6/30/00 Date

Centre QAU Review 7/21/00

February 12, 1998/2

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

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Page 1 of 2 (2) 6/15 7/28/00

METHOD DEVIATION

Deviation Number: 2

Date of Occurrence: (1) 6/15-16/00, (2) 6/29/00, (3) 6/20-21/00

Centre Study Number: 023-016 Sponsor Protocol Number: FACT-TOX-110

DESCRIPTION OF DEVIATION

Deviations to method titled "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2)" (Centre Method 00M-023-003, revision 2)

1. Section 4.5.4.d: Accepted 60% for CCV recovery of PFOS in Set 061500A.
2. Section 4.5.4.c: Quantitated sample residue outside the range of the standard curve.
3. Section 4.5.4.d: Accepted 131% for CCV recovery of PFOS in Set 062000A.

ACTIONS TAKEN

i.e., amendment issued, SOP revision, etc...

1-2. Method deviation issued.

Recorded By/Date: Emily L Stauffer 7/28/00

IMPACT ON THE STUDY

1. No negative impact because entire set required dilution and re-injected with acceptable results.
2. No negative impact because sample response only ~2.5% higher than response of highest standard.
3. No negative impact because following CCV's were within acceptable range.

Principal Investigator Signature 7/28/00 Date

Centre QAU Review NCL 7/28/00

February 12, 1998/2

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

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METHOD DEVIATION

Deviation Number: 3

Date of Occurrence: (1) 6/29-30/00

Centre Study Number: 023-016 Sponsor Protocol Number: FACT-TOX-110

DESCRIPTION OF DEVIATION

Deviations to method titled "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2)" (Centre Method 00M-023-003, revision 2)

1. Section 4.3.5: Did not fortify any of the control samples from the last interval (GD21) of the study.

ACTIONS TAKEN
i.e., amendment issued, SOP revision, etc...

1. Method deviation issued.

Recorded By/Date: Emily R. Stauffer 8/9/00

IMPACT ON THE STUDY

1. No negative impact.

Principal Investigator Signature 8/30/00 Date

Centre QAU Review vcc 8/9/00

February 12, 1998/2

Centre Study No.: 023-016
Sponsor Protocol No: FACT-TOX-110

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METHOD MODIFICATION FORM

Modification No.: 1
Study Director: Marvin T. Case

Centre Study Number: 023-016 Sponsor Protocol Number: FACT-TOX-110

Method Title: Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2) Centre Method Number: 00M-023-003, revision 2

MODIFICATION:

Section 3.4 Solutions

2. The 100 mM ammonium acetate solution should be 50 mM ammounium acetate.

JUSTIFICATION:

To correct for a typographical error.

EFFECT ON STUDY:

No negative impact.

Originator: Lindy R Stauffer 7/7/00 Date

Principal Investigator: Nichole Winn 7/7/00 Date

Study director ~~Sponsor Management~~: Marvin Case 3 Aug 2000 Date

February 12, 1998/3

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METHOD MODIFICATION FORM

Modification No.: 2
Study Director: Marvin T. Case

Centre Study Number: 023-016 Sponsor Protocol Number: FACT-TOX-110

Method Title: Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2) Centre Method Number: 00M-023-003, revision 2

MODIFICATION:

1. Section 4.5.4 Sample Analysis (Item e.)
   Should read as follows:
   Monitor the response of the surrogate standard (SS) for every sample, extracted standard, blank, and QC recovery sample. The concentration is calculated using the "average response factor" function from MassLynx 3.3 software (or equivalent). Evaluate the acceptability of the sample extraction process based on the concentration of the SS found in all extracted standards, QC samples, blanks, and samples. The percent recovery for all samples, blanks, extracted standards, and QC recovery samples must fall between 60% and 140%. Those deviating by greater than 30% must be checked for possible errors and may need re-extracted. Any recoveries deviating by greater than 40% (falling outside the 60% to 140% range) will require re-extraction.

2. Section 5.1 Analyte Found
   Remove second sentence.

3. Section 5.1 Equations
   Modify Equation 1 to:

$$\text{Analyte found (ng/mL)} = \frac{\text{(Peak area - intercept)}}{\text{slope}}$$

   Change Equation 2 to:

$$\text{Analyte found (ppb)} = \frac{\text{(analyte found (ng/mL)} \times \text{final vol. (mL)} \times \text{DF)}}{\text{sample wt. (g)}}$$

   Add Equation 3:

$$\text{Recovery (\%)} = \frac{[\text{analyte found (ng/mL)} \times \text{final vol. (mL)} \times \text{DF}]}{\text{analyte added (ng)}} \times 100$$

4. Section 5.3 Mean Response Factor
   Modify first sentence to read:
   The mean response factor (MRF) is calculated by the MassLynx software program by averaging the response for all calibration standards and dividing by the amount added.

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METHOD MODIFICATION FORM

Modification No.: 2
Study Director: Marvin T. Case

Centre Study Number: 023-016 Sponsor Protocol Number: FACT-TOX-110

Method Title: Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (Revision 2) Centre Method Number: 00M-023-003, revision 2

JUSTIFICATION:

1. To clarify SS acceptable criteria.
2. Remove incorrect statement because calculations did include sample weight and final volume.
3. Modify calculations to correspond with those used in study.
4. Clarify calculation of mean response factor

EFFECT ON STUDY:

1-4. No negative impact.

Originator: M R Stauffer Date: 8/11/00

Principal Investigator: Date: 8/11/00

Sponsor Management: Marvin T Case Date: 22 Aug 2000

February 12, 1998/3

Centre Analytical Laboratories, Inc.
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21 Amended pages + amendment form

ANALYTICAL PHASE REPORT AMENDMENT

Amendment Number: 1

Effective Date: 9/20/00

Centre Study Number: 023-016 Sponsor Protocol Number: FACT-TOX-110

Report Title

Oral (Gavage) Pharmacokinetic Study of PFOS in Rats

Amended Section

Section 1.0 Summary: 59386.8 ppb was changed to 59.4 µg/g in the third paragraph (See attached page11).

Section 7.6 Quantitation and Example Calculation:
Equation 5: Residue Found (µg/g) = Residue Found (ng/g) * (1 µg / 1000 ng)

Equation 6: Corrected Residue Found (µg/g) = Residue Found (µg/g) * 0.869

These equations were only applied to the tables in the report.

Section 8.0 Results: 59386.8 was changed to 59.4 µg/g in the second paragraph (See attached page 18).

Section 11.0: Tables I – XXI (See attached pages 20 – 38)

Reason for Amendment

The units reported in the tables and text were changed from ng/g to µg/g, the number of significant figures represented in the tables and text was changed to three, the zeros reported in Tables II and XII were changed to ND's, and any value less than 0.0100 µg/g (LOQ) was reported as < 0.0100.

Impact on the Study

No negative impact

Principal Investigator Signature — Date 9/25/00

Study Director Signature — Date 9 Oct 2000

CAL QAU Review NCC 9/25/00

November 1995/0

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

1.0 SUMMARY

The purpose of this study was to analyze residues of perfluorooctanesulfonate (PFOS) in rat feces as specified in 3M Protocol FACT-TOX-110. The analytical method used for this study was entitled, "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS, revision 2" (Centre method number: 00M-023-003, revision 2).

The limit of quantification for PFOS in rat feces was 10 ppb (0.0100 µg/g).

Residues (corrected for purity) ranging from non-detected levels to 59.4 µg/g were found in the rat feces samples.

Fortification recoveries ranged from 71-128% with an average of 98% and relative standard deviation of 15%.

2.0 OBJECTIVE

The objective of this study was to determine levels of perfluorooctanesulfonate (PFOS) in specimens of feces of rats using the analytical method entitled "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS, revision 2."

3.0 INTRODUCTION

This report details the results of the residues of PFOS detected in rat feces, using the analytical method entitled, "Determination of Fluorochemical Residues in Monkey/Rat Feces by LC/MS/MS (revision 2)." Complete details of the analytical methodology can be found in **Appendix B.**

The study was initiated on June 10, 2000, when the study director signed the protocol FACT-TOX-110 Amendment #4. The complete protocol and amendments can be found in **Appendix A.** The analytical start date was June 9, 2000, and the analytical termination date was July 1, 2000.

4.0 TEST SYSTEM

The 250 rat feces samples analyzed in this study were received frozen on dry ice from 3M Environmental Laboratory St. Paul, MN on May 26, 2000 and stored frozen upon receipt. The samples were then logged in on May 31, 2000 by Centre personnel and transferred to different frozen storage locale.

The control rat feces used for standards and blanks was purchased from Lampire Biological Laboratories, Inc., Pipersville, PA and received at Centre frozen on dry ice on

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

8.0 RESULTS AND DISCUSSION

Although, the majority of the control samples did not contain significant interferences, a couple of samples did contain residues that were comparable to control group levels. A summary of residues found in all of the matrix blanks and matrix zero blanks is detailed in **Table I.**

Residues (corrected for purity) ranging from non-detected levels to 59.4 µg/g were found in the rat feces samples. The residues found in all of the samples plus the averages and standard deviations for each group at each interval are detailed in **Tables II-XXI.**

For this report the residues found in Tables I-XXI were corrected for the purity of the PFOS standard lot 217 based on the certificate of analysis finalized on September 7, 2000.

Fortification recoveries ranged from 71-128% with an average of 98% and relative standard deviation of 15% (n = 34). A summary of all of the fortification recoveries can be found in **Table XXII.**

Typical calibration curves and chromatograms representing standards, controls, fortifications, and samples are depicted in **Figures 1-7.**

9.0 CIRCUMSTANCES THAT MAY HAVE AFFECTED THE DATA

Electronic records are not fully compliant with 21 CFR 11, "Electronic Records: Electronic Signature." However, fully approved SOP's were in place and all chromatography instrumentation used in this study was fully validated (IQ, PQ, OQ), calibrated and operational. All original data were printed as hard copies and fully audited by quality assurance. Verified exact copies and the electronic data have been stored in the archives at Centre Analytical Laboratories, Inc. Original raw data have been returned to the sponsor.

10.0 RETENTION OF DATA AND SAMPLES

When the final report is complete, all original paper data generated by Centre Analytical Laboratories, Inc. will be shipped to the sponsor. This does not include facility-specific raw data such as instrument logs, however exact copies of temperature logs will be submitted. Exact copies of all raw data, as well as a signed copy of the final analytical report and all original facility-specific raw data, will be retained in the Centre Analytical Laboratories, Inc. archives for the period of time specified in 21 CFR Part 58. Retained samples of reference substances are archived by the sponsor.

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table I. Summary of PFOS residues in Matrix Blanks and Matrix Zero Blanks

ND = Not Detected and NQ = Not Quantifiable

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
na	0004277 Blank A	061200AR	6/12/00	ND	ND
na	0004277 Blank B	061200AR	6/12/00	ND	ND
na	0004277 Zero Blank C	061200AR	6/12/00	ND	ND
na	0004277 Zero Blank D	061200AR	6/12/00	ND	ND
na	0004277 Blank A	061400A	6/14/00	NQ	NQ
na	0004277 Blank B	061400A	6/14/00	NQ	NQ
na	0004277 Zero Blank C	061400A	6/14/00	NQ	NQ
na	0004277 Zero Blank D	061400A	6/14/00	ND	ND
na	0004277 Blank A	061500AD	6/15/00	NQ	NQ
na	0004277 Blank B	061500AD	6/15/00	NQ	NQ
na	0004277 Zero Blank C	061500AD	6/15/00	< 0.0100	< 0.00869
na	0004277 Zero Blank D	061500AD	6/15/00	< 0.0100	< 0.00869
na	0004277 Blank E	061600A	6/16/00	< 0.0100	< 0.00869
na	0004277 Blank F	061600A	6/16/00	NQ	NQ
na	0004277 Zero Blank G	061600A	6/16/00	NQ	NQ
na	0004277 Zero Blank H	061600A	6/16/00	NQ	NQ
na	0004277 Blank A	061900AD	6/19/00	NQ	NQ
na	0004277 Blank B	061900AD	6/19/00	NQ	NQ
na	0004277 Zero Blank C	061900AD	6/19/00	NQ	NQ
na	0004277 Zero Blank D	061900AD	6/19/00	NQ	NQ
na	0003466 Blank A	062000A	6/20/00	NQ	NQ
na	0003466 Blank B	062000A	6/20/00	NQ	NQ
na	0003466 Zero Blank C	062000A	6/20/00	NQ	NQ
na	0003466 Zero Blank D	062000A	6/20/00	NQ	NQ
na	0004277 Blank A	062100A	6/21/00	NQ	NQ
na	0004277 Blank B	062100A	6/21/00	NQ	NQ
na	0004277 Zero Blank C	062100A	6/21/00	NQ	NQ
na	0004277 Zero Blank D	062100A	6/21/00	NQ	NQ
na	0004277 Blank A	062200A	6/22/00	< 0.0100	< 0.00869
na	0004277 Blank B	062200A	6/22/00	ND	ND
na	0004277 Zero Blank C	062200A	6/22/00	ND	ND
na	0004277 Zero Blank D	062200A	6/22/00	ND	ND
na	0004277 Blank E	062200B	6/22/00	ND	ND
na	0004277 Blank F	062200B	6/22/00	ND	ND
na	0004277 Zero Blank G	062200B	6/22/00	ND	ND

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table I (cont.) Summary of PFOS residues in Matrix Blanks and Matrix Zero Blanks ND = Not Detected and NQ = Not Quantifiable

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
na	0004277 Zero Blank H	062200B	6/22/00	ND	ND
na	0004277 Blank A	062300A	6/23/00	ND	ND
na	0004277 Blank B	062300A	6/23/00	ND	ND
na	0004277 Zero Blank C	062300A	6/23/00	ND	ND
na	0004277 Zero Blank D	062300A	6/23/00	ND	ND
na	0004277 Blank A	062300B	6/23/00	NQ	NQ
na	0004277 Blank B	062300B	6/23/00	NQ	NQ
na	0004277 Zero Blank C	062300B	6/23/00	NQ	NQ
na	0004277 Zero Blank D	062300B	6/23/00	NQ	NQ
na	0005738 Blank A	062600A	6/26/00	< 0.0100	< 0.00869
na	0005738 Blank B	062600A	6/26/00	0.0126	0.0109
na	0005738 Zero Blank C	062600A	6/26/00	ND	ND
na	0005738 Zero Blank D	062600A	6/26/00	ND	ND
na	0005738 Blank E	062600B	6/26/00	ND	ND
na	0005738 Blank F	062600B	6/26/00	ND	ND
na	0005738 Zero Blank G	062600B	6/26/00	ND	ND
na	0005738 Zero Blank H	062600B	6/26/00	ND	ND
na	0005738 Blank A	062700A	6/27/00	ND	ND
na	0005738 Blank B	062700A	6/27/00	ND	ND
na	0005738 Zero Blank C	062700A	6/27/00	ND	ND
na	0005738 Zero Blank D	062700A	6/27/00	ND	ND
na	0005738 Blank A	062800A	6/28/00	ND	ND
na	0005738 Blank B	062800A	6/28/00	ND	ND
na	0005738 Zero Blank C	062800A	6/28/00	ND	ND
na	0005738 Zero Blank D	062800A	6/28/00	ND	ND
na	0005738 Blank A	062900A	6/29/00	0.0301	0.0262
na	0005738 Blank B	062900A	6/29/00	ND	ND
na	0005738 Zero Blank C	062900A	6/29/00	ND	ND
na	0005738 Zero Blank D	062900A	6/29/00	ND	ND
na	0005738 Blank A	063000A	6/30/00	ND	ND
na	0005738 Blank B	063000A	6/30/00	ND	ND
na	0005738 Zero Blank C	063000A	6/30/00	ND	ND
na	0005738 Zero Blank D	063000A	6/30/00	ND	ND
			AVERAGE:	**< 0.0100**	**< 0.00869**
			STANDARD DEVIATION:	**NQ**	**NQ**

Note: The average and standard deviation were calculated by using 0.0100 as the value for those samples reported as < 0.0100, 0.00869 for those reported as < 0.00869, and zero for those reported as ND or NQ.

Amended Page 21 of 103

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Sponsor Protocol No.: FACT-TOX-110

Table II. Summary of PFOS residues in G1-Control-GD0

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19501F,F0,G1-Control-GD0	0003977	061200AR	6/12/00	ND	ND
19502F,F0,G1-Control-GD0	0003978	061200AR	6/12/00	ND	ND
19503F,F0,G1-Control-GD0	0003979	061200AR	6/12/00	< 0.0100	< 0.00869
19504F,F0,G1-Control-GD0	0003980	061200AR	6/12/00	ND	ND
19505F,F0,G1-Control-GD0	0003981	061200AR	6/12/00	ND	ND
19506F,F0,G1-Control-GD0	0003982	061200AR	6/12/00	0.0301	0.0262
19507F,F0,G1-Control-GD0	0003983	061200AR	6/12/00	ND	ND
19508F,F0,G1-Control-GD0	0003984	061200AR	6/12/00	< 0.0100	< 0.00869
19509F,F0,G1-Control-GD0	0003985	061200AR	6/12/00	0.0400	0.0348
19510F,F0,G1-Control-GD0	0003986	061200AR	6/12/00	ND	ND
19511F,F0,G1-Control-GD0	0003987	061200AR	6/12/00	ND	ND
19512F,F0,G1-Control-GD0	0003988	061200AR	6/12/00	ND	ND
19513F,F0,G1-Control-GD0	0003989	061200AR	6/12/00	ND	ND
19514F,F0,G1-Control-GD0	0003990	061200AR	6/12/00	ND	ND
19515F,F0,G1-Control-GD0	0003991	061200AR	6/12/00	ND	ND
19516F,F0,G1-Control-GD0	0003992	061200AR	6/12/00	0.0609	0.0529
			AVERAGE:	**< 0.0100**	**< 0.00869**
			STANDARD DEVIATION:	**NQ**	**NQ**

ND = Not Detected and NQ = Not Quantifiable

Note: The average and standard deviation were calculated by using 0.0100 as the value for those samples reported as < 0.0100, 0.00869 for those reported as < 0.00869, and zero for those reported as ND or NQ.

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table III. Summary of PFOS residues in G2-0.1MKD on GD0

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19517F,F0,G2-0.1MKD-GD0	0003993	061400AD	6/14/00	0.701	0.609
19518F,F0,G2-0.1MKD-GD0	0003994	061400A	6/14/00	0.456	0.396
19519F,F0,G2-0.1MKD-GD0	0003995	061400A	6/14/00	0.390	0.339
19520F,F0,G2-0.1MKD-GD0	0003996	061400AD	6/14/00	0.676	0.587
19521F,F0,G2-0.1MKD-GD0	0003997	061400A	6/14/00	0.495	0.430
19522F,F0,G2-0.1MKD-GD0	0003998	061400A	6/14/00	0.381	0.331
19523F,F0,G2-0.1MKD-GD0	0003999	061400A	6/14/00	0.458	0.398
19524F,F0,G2-0.1MKD-GD0	0004000	061400AD	6/14/00	0.833	0.724
19525F,F0,G2-0.1MKD-GD0	0004001	061400A	6/14/00	0.473	0.411
19526F,F0,G2-0.1MKD-GD0	0004002	061400A	6/14/00	0.446	0.388
19527F,F0,G2-0.1MKD-GD0	0004003	061400A	6/14/00	0.497	0.432
19528F,F0,G2-0.1MKD-GD0	0004004	061400AD	6/14/00	0.701	0.609
19529F,F0,G2-0.1MKD-GD0	0004005	061400AD	6/14/00	0.797	0.693
19530F,F0,G2-0.1MKD-GD0	0004006	061400A	6/14/00	0.394	0.342
19531F,F0,G2-0.1MKD-GD0	0004007	061400AD	6/14/00	0.735	0.638
19532F,F0,G2-0.1MKD-GD0	0004008	061400AD	6/14/00	0.748	0.650
			AVERAGE:	**0.574**	**0.499**
			STANDARD DEVIATION:	**0.160**	**0.139**

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table IV. Summary of PFOS residues in G3-0.4MKD on GD0

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19533F,F0,G3-0.4MKD-GD0	0004009	061500AD	6/15/00	2.60	2.26
19534F,F0,G3-0.4MKD-GD0	0004010	061500AD	6/15/00	3.75	3.26
19535F,F0,G3-0.4MKD-GD0	0004011	061500AD	6/15/00	2.80	2.43
19536F,F0,G3-0.4MKD-GD0	0004012	061500AD	6/15/00	3.17	2.76
19537F,F0,G3-0.4MKD-GD0	0004013	061500AD	6/15/00	2.78	2.41
19538F,F0,G3-0.4MKD-GD0	0004014	061500AD	6/15/00	3.08	2.68
19539F,F0,G3-0.4MKD-GD0	0004015	061500AD	6/15/00	3.68	3.20
19540F,F0,G3-0.4MKD-GD0	0004016	061500AD	6/15/00	2.04	1.78
19541F,F0,G3-0.4MKD-GD0	0004017	061500AD	6/15/00	2.10	1.83
19542F,F0,G3-0.4MKD-GD0	0004018	061500AD	6/15/00	3.29	2.86
19543F,F0,G3-0.4MKD-GD0	0004019	061500AD	6/15/00	2.11	1.84
19544F,F0,G3-0.4MKD-GD0	0004020	061500AD	6/15/00	3.43	2.98
19545F,F0,G3-0.4MKD-GD0	0004021	061600AD	6/16/00	2.76	2.39
19546F,F0,G3-0.4MKD-GD0	0004022	061500AD	6/15/00	2.33	2.03
19547F,F0,G3-0.4MKD-GD0	0004023	061500AD	6/15/00	2.22	1.93
19548F,F0,G3-0.4MKD-GD0	0004024	061500AD	6/15/00	2.33	2.03
			AVERAGE:	**2.78**	**2.42**
			STANDARD DEVIATION:	**0.570**	**0.496**

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table V. Summary of PFOS residues in G4-1.6MKD on GD0

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19549F,F0,G4-1.6MKD-GD0	0004025	061600AD	6/16/00	14.1	12.3
19550F,F0,G4-1.6MKD-GD0	0004026	061600AD	6/16/00	16.9	14.7
19551F,F0,G4-1.6MKD-GD0	0004027	061600AD	6/16/00	10.4	9.03
19552F,F0,G4-1.6MKD-GD0	0004028	061600AD	6/16/00	13.1	11.4
19553F,F0,G4-1.6MKD-GD0	0004029	061600AD	6/16/00	13.7	11.9
19554F,F0,G4-1.6MKD-GD0	0004030	061600AD	6/16/00	15.5	13.5
19555F,F0,G4-1.6MKD-GD0	0004031	061600AD	6/16/00	10.3	8.98
19556F,F0,G4-1.6MKD-GD0	0004032	061600AD	6/16/00	10.3	8.91
19557F,F0,G4-1.6MKD-GD0	0004033	061600AD	6/16/00	8.96	7.79
19558F,F0,G4-1.6MKD-GD0	0004034	061600AD	6/16/00	6.85	5.95
19559F,F0,G4-1.6MKD-GD0	0004035	061600AD	6/16/00	7.68	6.67
19560F,F0,G4-1.6MKD-GD0	0004036	061600AD	6/16/00	16.5	14.4
19561F,F0,G4-1.6MKD-GD0	0004037	061600AD	6/16/00	12.1	10.5
19562F,F0,G4-1.6MKD-GD0	0004038	061600AD	6/16/00	9.59	8.33
19563F,F0,G4-1.6MKD-GD0	0004039	061600AD	6/16/00	7.47	6.49
19564F,F0,G4-1.6MKD-GD0	0004040	061600AD	6/16/00	17.0	14.7
			AVERAGE:	**11.9**	**10.3**
			STANDARD DEVIATION:	**3.45**	**3.00**

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table VI. Summary of PFOS residues in G5-3.2MKD on GD0

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19565F,F0,G5-3.2MKD-GD0	0004041	061900AD	6/19/00	23.5	20.4
19566F,F0,G5-3.2MKD-GD0	0004042	061900AD	6/19/00	29.5	25.7
19567F,F0,G5-3.2MKD-GD0	0004043	061900AD	6/19/00	30.3	26.3
19568F,F0,G5-3.2MKD-GD0	0004044	061900AD	6/19/00	24.2	21.0
19569F,F0,G5-3.2MKD-GD0	0004045	061900AD	6/19/00	27.1	23.5
19570F,F0,G5-3.2MKD-GD0	0004046	061900AD	6/19/00	19.7	17.1
19571F,F0,G5-3.2MKD-GD0	0004047	061900AD	6/19/00	29.9	26.0
19572F,F0,G5-3.2MKD-GD0	0004048	061900AD	6/19/00	25.4	22.1
19573F,F0,G5-3.2MKD-GD0	0004049	061900AD	6/19/00	23.0	20.0
19574F,F0,G5-3.2MKD-GD0	0004050	061900AD	6/19/00	29.3	25.5
19575F,F0,G5-3.2MKD-GD0	0004051	061900AD	6/19/00	23.0	20.0
19576F,F0,G5-3.2MKD-GD0	0004052	061900AD	6/19/00	36.3	31.6
19577F,F0,G5-3.2MKD-GD0	0004053	061900AD	6/19/00	34.2	29.8
19578F,F0,G5-3.2MKD-GD0	0004054	061900AD	6/19/00	34.8	30.2
19579F,F0,G5-3.2MKD-GD0	0004055	061900AD	6/19/00	25.4	22.0
19580F,F0,G5-3.2MKD-GD0	0004056	061900AD	6/19/00	24.9	21.6
			AVERAGE:	**27.5**	**23.9**
			STANDARD DEVIATION:	**4.78**	**4.15**

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table VII. Summary of PFOS residues in G1-Control on GD7

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19501F,F0,G1-Control-GD7	0004057	062000A	6/20/00	ND	ND
19502F,F0,G1-Control-GD7	0004058	062000A	6/20/00	NQ	NQ
19504F,F0,G1-Control-GD7	0004059	062000A	6/20/00	NQ	NQ
19505F,F0,G1-Control-GD7	0004060	062000A	6/20/00	ND	ND
19506F,F0,G1-Control-GD7	0004061	062000A	6/20/00	0.0178	0.0155
19507F,F0,G1-Control-GD7	0004062	062000A	6/20/00	ND	ND
19508F,F0,G1-Control-GD7	0004063	062000A	6/20/00	< 0.0100	< 0.00869
19509F,F0,G1-Control-GD7	0004064	062000A	6/20/00	ND	ND
19510F,F0,G1-Control-GD7	0004065	062000A	6/20/00	ND	ND
19511F,F0,G1-Control-GD7	0004066	062000AR	6/20/00	ND	ND
19512F,F0,G1-Control-GD7	0004067	062000AR	6/20/00	ND	ND
19513F,F0,G1-Control-GD7	0004068	062000A	6/20/00	ND	ND
19514F,F0,G1-Control-GD7	0004069	062000A	6/20/00	ND	ND
19515F,F0,G1-Control-GD7	0004070	062000AR	6/20/00	ND	ND
19516F,F0,G1-Control-GD7	0004071	062000AR	6/20/00	NQ	NQ
			AVERAGE:	**< 0.0100**	**< 0.00869**
			STANDARD DEVIATION:	NQ	NQ

ND = Not Detected and NQ = Not Quantifiable

Note: The average and standard deviation were calculated by using 0.0100 as the value for those samples reported as < 0.0100, 0.00869 for those reported as < 0.00869, and zero for those reported as ND or NQ.

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table VIII. Summary of PFOS residues in G2-0.1MKD on GD7

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	*Corrected* Residue Found (µg/g)
19517F,F0,G2-0.1MKD-GD7	0004072	062100A	6/21/00	0.409	0.356
19518F,F0,G2-0.1MKD-GD7	0004073	062100AD	6/21/00	0.561	0.488
19519F,F0,G2-0.1MKD-GD7	0004074	062100A	6/21/00	0.345	0.299
19521F,F0,G2-0.1MKD-GD7	0004075	062100A	6/21/00	0.465	0.404
19522F,F0,G2-0.1MKD-GD7	0004076	062100AD	6/21/00	0.778	0.676
19523F,F0,G2-0.1MKD-GD7	0004077	062100AD	6/21/00	0.625	0.543
19524F,F0,G2-0.1MKD-GD7	0004078	062100AD	6/21/00	0.576	0.500
19525F,F0,G2-0.1MKD-GD7	0004079	062100AD	6/21/00	0.595	0.517
19526F,F0,G2-0.1MKD-GD7	0004080	062100A	6/21/00	0.469	0.408
19527F,F0,G2-0.1MKD-GD7	0004081	062100AD	6/21/00	0.742	0.645
19528F,F0,G2-0.1MKD-GD7	0004082	062100AD	6/21/00	0.532	0.462
19529F,F0,G2-0.1MKD-GD7	0004083	062100AD	6/21/00	0.744	0.647
19530F,F0,G2-0.1MKD-GD7	0004084	062100A	6/21/00	0.424	0.369
19531F,F0,G2-0.1MKD-GD7	0004085	062100AD	6/21/00	0.624	0.542
19532F,F0,G2-0.1MKD-GD7	0004086	062100AD	6/21/00	0.574	0.499
			AVERAGE:	**0.564**	**0.490**
			STANDARD DEVIATION:	**0.128**	**0.111**

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table IX. Summary of PFOS residues in G3-0.4MKD on GD7

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19533F,F0,G3-0.4MKD-GD7	0004087	062200AD	6/22/00	2.55	2.21
19534F,F0,G3-0.4MKD-GD7	0004088	062200AD	6/22/00	2.69	2.34
19535F,F0,G3-0.4MKD-GD7	0004089	062200AD	6/22/00	1.82	1.59
19536F,F0,G3-0.4MKD-GD7	0004090	062200AD	6/22/00	3.26	2.83
19537F,F0,G3-0.4MKD-GD7	0004091	062200AD	6/22/00	2.61	2.27
19538F,F0,G3-0.4MKD-GD7	0004092	062200AD	6/22/00	2.93	2.55
19539F,F0,G3-0.4MKD-GD7	0004093	062200AD	6/22/00	2.09	1.82
19540F,F0,G3-0.4MKD-GD7	0004094	062200AD	6/22/00	2.27	1.97
19542F,F0,G3-0.4MKD-GD7	0004095	062200AD	6/22/00	3.00	2.61
19543F,F0,G3-0.4MKD-GD7	0004096	062200AD	6/22/00	2.80	2.43
19544F,F0,G3-0.4MKD-GD7	0004097	062200AD	6/22/00	2.28	1.98
19546F,F0,G3-0.4MKD-GD7	0004098	062200AD	6/22/00	1.52	1.32
19547F,F0,G3-0.4MKD-GD7	0004099	062200AD	6/22/00	2.06	1.79
19548F,F0,G3-0.4MKD-GD7	0004100	062200AD	6/22/00	2.85	2.47
			AVERAGE:	**2.48**	**2.16**
			STANDARD DEVIATION:	**0.492**	**0.428**

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table X. Summary of PFOS residues in G4-1.6MKD on GD7

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19550F,F0,G4-1.6MKD-GD7	0004101	062200BD	6/22/00	15.0	13.0
19553F,F0,G4-1.6MKD-GD7	0004102	062200BD	6/22/00	12.3	10.7
19554F,F0,G4-1.6MKD-GD7	0004103	062200BD	6/22/00	11.8	10.2
19555F,F0,G4-1.6MKD-GD7	0004104	062200BD	6/22/00	9.61	8.35
19556F,F0,G4-1.6MKD-GD7	0004105	062200BD	6/22/00	10.2	8.88
19558F,F0,G4-1.6MKD-GD7	0004106	062200BD	6/22/00	5.89	5.12
19559F,F0,G4-1.6MKD-GD7	0004107	062200BD	6/22/00	6.89	5.99
19560F,F0,G4-1.6MKD-GD7	0004108	062200BD	6/22/00	7.02	6.10
19561F,F0,G4-1.6MKD-GD7	0004109	062200BD	6/22/00	8.88	7.71
19562F,F0,G4-1.6MKD-GD7	0004110	062200BD	6/22/00	10.8	9.42
19563F,F0,G4-1.6MKD-GD7	0004111	062200BD	6/22/00	14.9	13.0
19564F,F0,G4-1.6MKD-GD7	0004112	062200BD	6/22/00	13.7	11.9
			AVERAGE:	**10.6**	**9.19**
			STANDARD DEVIATION:	**3.08**	**2.67**

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table XI. Summary of PFOS residues in G5-3.2MKD on GD7

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19565F,F0,G5-3.2MKD-GD7	0004113	062300AD	6/23/00	51.7	44.9
19566F,F0,G5-3.2MKD-GD7	0004114	062300AD	6/23/00	26.5	23.0
19567F,F0,G5-3.2MKD-GD7	0004115	062300AD	6/23/00	35.9	31.2
19568F,F0,G5-3.2MKD-GD7	0004116	062300AD	6/23/00	23.0	20.0
19569F,F0,G5-3.2MKD-GD7	0004117	062300AD	6/23/00	42.9	37.3
19570F,F0,G5-3.2MKD-GD7	0004118	062300AD	6/23/00	37.4	32.5
19571F,F0,G5-3.2MKD-GD7	0004119	062300AD	6/23/00	43.0	37.4
19572F,F0,G5-3.2MKD-GD7	0004120	062300AD	6/23/00	38.8	33.7
19573F,F0,G5-3.2MKD-GD7	0004121	062300AD	6/23/00	26.7	23.2
19574F,F0,G5-3.2MKD-GD7	0004122	062300AD	6/23/00	36.3	31.6
19575F,F0,G5-3.2MKD-GD7	0004123	062300AD	6/23/00	35.5	30.8
19577F,F0,G5-3.2MKD-GD7	0004124	062300AD	6/23/00	68.3	59.4
19579F,F0,G5-3.2MKD-GD7	0004125	062300AD	6/23/00	35.9	31.2
19580F,F0,G5-3.2MKD-GD7	0004126	062300AD	6/23/00	30.1	26.1
			AVERAGE:	**38.0**	**33.0**
			STANDARD DEVIATION:	**11.5**	**10.0**

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table XII. Summary of PFOS residues in G1-Control on GD15

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19501F,F0,G1-Control-GD15	0004127	062300B	6/23/00	ND	ND
19502F,F0,G1-Control-GD15	0004128	062300B	6/23/00	ND	ND
19504F,F0,G1-Control-GD15	0004129	062300B	6/23/00	ND	ND
19505F,F0,G1-Control-GD15	0004130	062300B	6/23/00	ND	ND
19506F,F0,G1-Control-GD15	0004131	062300B	6/23/00	0.0445	0.0387
19507F,F0,G1-Control-GD15	0004132	062300B	6/23/00	ND	ND
19508F,F0,G1-Control-GD15	0004133	062300B	6/23/00	ND	ND
19509F,F0,G1-Control-GD15	0004134	062300B	6/23/00	0.0242	0.0210
19510F,F0,G1-Control-GD15	0004135	062300B	6/23/00	< 0.0100	< 0.00869
19511F,F0,G1-Control-GD15	0004136	062300B	6/23/00	ND	ND
19512F,F0,G1-Control-GD15	0004137	062300B	6/23/00	0.0579	0.0503
19513F,F0,G1-Control-GD15	0004138	062300B	6/23/00	ND	ND
19514F,F0,G1-Control-GD15	0004139	062300B	6/23/00	ND	ND
19515F,F0,G1-Control-GD15	0004140	062300B	6/23/00	ND	ND
19516F,F0,G1-Control-GD15	0004141	062300B	6/23/00	0.0216	0.0188
			AVERAGE:	**0.0105**	**0.00917**
			STANDARD DEVIATION:	**0.0185**	**0.0161**

ND = Not Detected and NQ = Not Quantifiable

Note: The average and standard deviation were calculated by using 0.0100 as the value for those samples reported as < 0.0100, 0.00869 for those reported as < 0.00869, and zero for those reported as ND or NQ.

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table XIII. Summary of PFOS residues in G2-0.1MKD on GD15

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19517F,F0,G2-0.1MKD-GD15	0004142	062600AD	6/26/00	0.758	0.659
19518F,F0,G2-0.1MKD-GD15	0004143	062600AD	6/26/00	0.672	0.584
19519F,F0,G2-0.1MKD-GD15	0004144	062600AD	6/26/00	0.782	0.680
19521F,F0,G2-0.1MKD-GD15	0004145	062600AD	6/26/00	0.632	0.549
19522F,F0,G2-0.1MKD-GD15	0004146	062600AD	6/26/00	0.817	0.710
19523F,F0,G2-0.1MKD-GD15	0004147	062600AD	6/26/00	0.642	0.558
19524F,F0,G2-0.1MKD-GD15	0004148	062600AD	6/26/00	0.834	0.725
19525F,F0,G2-0.1MKD-GD15	0004149	062600AD	6/26/00	1.08	0.939
19526F,F0,G2-0.1MKD-GD15	0004150	062600AD	6/26/00	0.778	0.676
19527F,F0,G2-0.1MKD-GD15	0004151	062600AD	6/26/00	0.799	0.694
19528F,F0,G2-0.1MKD-GD15	0004152	062600AD	6/26/00	0.845	0.734
19529F,F0,G2-0.1MKD-GD15	0004153	062600AD	6/26/00	0.724	0.629
19530F,F0,G2-0.1MKD-GD15	0004154	062600AD	6/26/00	0.724	0.630
19531F,F0,G2-0.1MKD-GD15	0004155	062600AD	6/26/00	0.777	0.675
19532F,F0,G2-0.1MKD-GD15	0004156	062600AD	6/26/00	0.568	0.494
			AVERAGE:	**0.762**	**0.662**
			STANDARD DEVIATION:	**0.119**	**0.104**

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table XIV. Summary of PFOS residues in G3-0.4MKD on GD15

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19533F,F0,G3-0.4MKD-GD15	0004157	062600BD	6/26/00	2.76	2.40
19534F,F0,G3-0.4MKD-GD15	0004158	062600BD	6/26/00	4.82	4.19
19535F,F0,G3-0.4MKD-GD15	0004159	062600BD	6/26/00	3.63	3.15
19536F,F0,G3-0.4MKD-GD15	0004160	062600BD	6/26/00	3.19	2.77
19537F,F0,G3-0.4MKD-GD15	0004161	062600BD	6/26/00	3.40	2.95
19538F,F0,G3-0.4MKD-GD15	0004162	062600BD	6/26/00	3.14	2.73
19539F,F0,G3-0.4MKD-GD15	0004163	062600BD	6/26/00	3.09	2.69
19540F,F0,G3-0.4MKD-GD15	0004164	062600BD	6/26/00	4.15	3.61
19542F,F0,G3-0.4MKD-GD15	0004165	062600BD	6/26/00	1.88	1.64
19543F,F0,G3-0.4MKD-GD15	0004166	062600BD	6/26/00	3.42	2.97
19544F,F0,G3-0.4MKD-GD15	0004167	062600BD	6/26/00	3.37	2.93
19546F,F0,G3-0.4MKD-GD15	0004168	062600BD	6/26/00	2.63	2.28
19547F,F0,G3-0.4MKD-GD15	0004169	062600BD	6/26/00	3.86	3.36
19548F,F0,G3-0.4MKD-GD15	0004170	062600BD	6/26/00	3.91	3.40
			AVERAGE:	**3.38**	**2.93**
			STANDARD DEVIATION:	**0.713**	**0.620**

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table XV. Summary of PFOS residues in G4-1.6MKD on GD15

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19550F,F0,G4-1.6MKD-GD15	0004171	062700AD	6/27/00	13.9	12.1
19553F,F0,G4-1.6MKD-GD15	0004172	062700AD	6/27/00	14.8	12.9
19554F,F0,G4-1.6MKD-GD15	0004173	062700AD	6/27/00	14.2	12.3
19555F,F0,G4-1.6MKD-GD15	0004174	062700AD	6/27/00	16.3	14.1
19556F,F0,G4-1.6MKD-GD15	0004175	062700AD	6/27/00	9.07	7.88
19558F,F0,G4-1.6MKD-GD15	0004176	062700AD	6/27/00	7.17	6.23
19559F,F0,G4-1.6MKD-GD15	0004177	062700AD	6/27/00	7.48	6.50
19560F,F0,G4-1.6MKD-GD15	0004178	062700AD	6/27/00	14.9	12.9
19561F,F0,G4-1.6MKD-GD15	0004179	062700AD	6/27/00	17.4	15.2
19562F,F0,G4-1.6MKD-GD15	0004180	062700AD	6/27/00	11.4	9.92
19563F,F0,G4-1.6MKD-GD15	0004181	062700AD	6/27/00	8.95	7.78
19564F,F0,G4-1.6MKD-GD15	0004182	062700AD	6/27/00	17.3	15.1
			AVERAGE:	**12.7**	**11.1**
			STANDARD DEVIATION:	**3.77**	**3.28**

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table XVI. Summary of PFOS residues in G5-3.2MKD on GD15

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19565F,F0,G5-3.2MKD-GD15	0004183	062800AD	6/28/00	35.2	30.6
19566F,F0,G5-3.2MKD-GD15	0004184	062800AD	6/28/00	15.2	13.2
19567F,F0,G5-3.2MKD-GD15	0004185	062800AD	6/28/00	20.4	17.7
19568F,F0,G5-3.2MKD-GD15	0004186	062800AD	6/28/00	46.9	40.8
19569F,F0,G5-3.2MKD-GD15	0004187	062800AD	6/28/00	36.3	31.6
19570F,F0,G5-3.2MKD-GD15	0004188	062800AD	6/28/00	31.1	27.0
19571F,F0,G5-3.2MKD-GD15	0004189	062800AD	6/28/00	28.4	24.7
19572F,F0,G5-3.2MKD-GD15	0004190	062800AD	6/28/00	20.5	17.9
19573F,F0,G5-3.2MKD-GD15	0004191	062800AD	6/28/00	39.8	34.6
19574F,F0,G5-3.2MKD-GD15	0004192	062800AD	6/28/00	47.3	41.1
19575F,F0,G5-3.2MKD-GD15	0004193	062800AD	6/28/00	40.7	35.4
19577F,F0,G5-3.2MKD-GD15	0004194	062800AD	6/28/00	47.3	41.1
19579F,F0,G5-3.2MKD-GD15	0004195	062800AD	6/28/00	33.4	29.1
19580F,F0,G5-3.2MKD-GD15	0004196	062800AD	6/28/00	32.7	28.4
			AVERAGE:	**33.9**	**29.5**
			STANDARD DEVIATION:	**10.2**	**8.91**

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table XVII. Summary of PFOS residues in G1-Control on GD21

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19504F,F0,G1-Control-GD21	0004197	062900A	6/29/00	ND	ND
19505F,F0,G1-Control-GD21	0004198	062900A	6/29/00	ND	ND
19506F,F0,G1-Control-GD21	0004199	062900A	6/29/00	0.0123	0.0107
19507F,F0,G1-Control-GD21	0004200	062900A	6/29/00	ND	ND
19509F,F0,G1-Control-GD21	0004201	062900A	6/29/00	0.0843	0.0733
19512F,F0,G1-Control-GD21	0004202	062900A	6/29/00	0.0215	0.0187
19516F,F0,G1-Control-GD21	0004203	062900A	6/29/00	< 0.0100	< 0.00869
			AVERAGE:	**0.0183**	**0.0159**
			STANDARD DEVIATION:	**0.0302**	**0.0263**

ND = Not Detected and NQ = Not Quantifiable

Note: The average and standard deviation were calculated by using 0.0100 as the value for those samples reported as < 0.0100, 0.00869 for those reported as < 0.00869, and zero for those reported as ND or NQ.

Table XVIII. Summary of PFOS residues in G2-0.1MKD on GD21

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19518F,F0,G2-0.1MKD-GD21	0004204	0629-3000AD	6/29/00	0.735	0.639
19519F,F0,G2-0.1MKD-GD21	0004205	062900A	6/29/00	0.473	0.411
19521F,F0,G2-0.1MKD-GD21	0004206	062900A	6/29/00	0.403	0.351
19523F,F0,G2-0.1MKD-GD21	0004207	062900A	6/29/00	0.402	0.349
19524F,F0,G2-0.1MKD-GD21	0004208	062900A	6/29/00	0.494	0.429
19528F,F0,G2-0.1MKD-GD21	0004209	062900A	6/29/00	0.456	0.396
19529F,F0,G2-0.1MKD-GD21	0004210	062900A	6/29/00	0.391	0.340
			AVERAGE:	**0.479**	**0.417**
			STANDARD DEVIATION:	**0.120**	**0.104**

Centre Study No.: 023-016
Sponsor Protocol No.: FACT-TOX-110

Table XIX. Summary of PFOS residues in G3-0.4MKD on GD21

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19533F,F0,G3-0.4MKD-GD21	0004211	0629-3000AD	6/30/00	1.68	1.46
19534F,F0,G3-0.4MKD-GD21	0004212	0629-3000AD	6/30/00	3.10	2.70
19536F,F0,G3-0.4MKD-GD21	0004213	0629-3000AD	6/30/00	1.85	1.61
19543F,F0,G3-0.4MKD-GD21	0004214	0629-3000AD	6/30/00	2.23	1.94
19546F,F0,G3-0.4MKD-GD21	0004215	0629-3000AD	6/30/00	2.23	1.94
19548F,F0,G3-0.4MKD-GD21	0004216	0629-3000AD	6/30/00	5.42	4.71
			AVERAGE:	**2.75**	**2.39**
			STANDARD DEVIATION:	**1.39**	**1.21**

Table XX. Summary of PFOS residues in G4-1.6MKD on GD21

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19556F,F0,G4-1.6MKD-GD21	0004217	0629-3000AD	6/30/00	18.3	15.9
19559F,F0,G4-1.6MKD-GD21	0004218	0629-3000AD	6/30/00	10.6	9.19
19560F,F0,G4-1.6MKD-GD21	0004219	0629-3000AD	6/30/00	11.2	9.72
19562F,F0,G4-1.6MKD-GD21	0004220	0629-3000AD	6/30/00	5.68	4.94
			AVERAGE:	**11.4**	**9.93**
			STANDARD DEVIATION:	**5.19**	**4.51**

Table XXI. Summary of PFOS residues in G5-3.2MKD on GD21

Sponsor ID	Centre ID	Set Number	Date Extracted	Residue Found (µg/g)	Corrected Residue Found (µg/g)
19568F,F0,G5-3.2MKD-GD21	0004221	0629-3000AD	6/30/00	25.1	21.8
19569F,F0,G5-3.2MKD-GD21	0004222	0629-3000AD	6/30/00	21.7	18.8
19571F,F0,G5-3.2MKD-GD21	0004223	0629-3000AD	6/30/00	21.5	18.7
19572F,F0,G5-3.2MKD-GD21	0004224	0629-3000AD	6/30/00	15.0	13.0
19577F,F0,G5-3.2MKD-GD21	0004225	0629-3000AD	6/30/00	27.1	23.5
19579F,F0,G5-3.2MKD-GD21	0004226	0629-3000AD	6/30/00	28.3	24.6
			AVERAGE:	**23.1**	**20.1**
			STANDARD DEVIATION:	**4.83**	**4.20**

Appendix H: Interim Certificate of Analyses

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

INTERIM CERTIFICATE OF ANALYSIS

Revision 1(9/7/00)

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

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

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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018B

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/01

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

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

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

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

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

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

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

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Centre Analytical Laboratories COA Reference #: 023-018B

LC/MS Purity Profile:

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

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

Prepared By: _____ 9/6/00
David S. Bell Date
Scientist, Centre Analytical Laboratories

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

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INTERIM CERTIFICATE OF ANALYSIS

Revision 1(9/7/00)

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

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

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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018A

Date of Last Analysis: 08/31/00

Expiration Date: 08/31/01

Storage Conditions: Frozen ≤-10°C

Re-assessment Date: 08/31/01

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

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

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

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

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

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

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

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

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INTERIM CERTIFICATE OF ANALYSIS

Centre Analytical Laboratories COA Reference #: 023-018A

LC/MS Purity Profile:

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

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

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

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

Appendix I: Report Signature Page

Marvin T Case — 4 May 2001

Marvin T. Case, D.V.M., Ph.D., *Study Director* — Date

John L. Butenhoff — 5/4/01

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

05/03/01

William Reagen, Ph.D., *Laboratory Manager* — Date

05/04/01

Kris J. Hansen, Ph.D., *Principle Analytical Investigator* — Date