AR226-1358

3M Strategic Toxicology Laboratory

Study Title: Acute Inhalation Limit Toxicity Study of Perfluoroctanesulfonyl Fluoride (POSF) T-7098.3

Test Number (sample): T-7098.3 (Perfluoroctanesulfonyl Fluoride (POSF)
Laboratory Project Identification: ST64
In-Life Initiation Date: 5/1/2001
In-Life Completion Date: 5/14/2001

Research Client: 3M Specialty Materials Division
3M Center Building 236
Saint Paul, MN 55133-3220

Testing Laboratory: 3M Strategic Toxicology Laboratory
3M Center Building 270-SB-181
Saint Paul, MN 55133-3220

Study Director: Andrew M. Seacat, Ph.D.
Toxicologist Specialist
3M Medical Department/Toxicology Services
3M Center Building 220-2E-02
Saint Paul, MN 55133-3220

Study Toxicologist: Tommie Yvette Turner, Ph.D.
Post Doctoral Research Fellow
3M Medical Department/Toxicology Services
3M Center Building 220-2E-02
Saint Paul, MN 55133-3220

Summary:

An inhalation toxicity screen was conducted using 3 male rats for control (air-only) and 3 male rats exposed to perfluoroctanesulfonyl fluoride (POSF) at a nominal concentration of 1000 ppm for 4 hours. Overnight urines were collected on day zero to analyze for perfluorooctane sulfonate (PFOS) in the urine. Animals were observed for any adverse effects for up to 14 days after cessation of exposure. At necropsy, serum was collected for PFOS determination.

The control animals significantly gained weight ($p<0.05$) during the recovery period and showed no clinical pathology.

The POSF treated animals tolerated the 4 hour inhalation exposure, had significant ($p<0.05$) body weight gain over the 14 day recovery period. Organ weights were normal and histological evaluation of the liver, lungs, bladder, kidney and brain revealed no significant findings. The amount of PFOS in urine collected overnight on the day of dosing, and the amount of PFOS in serum on day 14 post-dose were 3.08 +- 1.60 ppm (N=3) and 4.57 +- 0.70 ppm (N=3), respectively. These data indicate that POSF was absorbed from the lungs, metabolized to PFOS and found present in the serum then excreted in urine as PFOS. The average percentage of inhaled POSF in the serum or urine, as PFOS, was <1%

Conclusion: The 4-hour LC50 of POSF is greater than 1000 ppm..

I. Study Objective:

This study was done to establish data on acute inhalation toxicity and to approximate the 4-hour LC50 value of POSF. The results of this range finder study will be used as guidance for a 13-week inhalation study.

II. Test Articles:

A. Identification:

T#	Name	Structure	MW (g/mole)	Density (g/ml)
7098.3	POSF	$C_8F_{17}SO_2F$	502	1.69

B. Purity and Stability: Responsibility of research client. The POSF sample has been lab-fractionated and acid/water washed. Chemical characterization by ^{1}H-NMR and ^{19}F-NMR Spectroscopy was conducted by 3M Specialty Adhesives & Chemicals Analytical Laboratory/ SMD to determine the purity of the nominal product and to characterize as many impurity components as possible. The sample was found to be a high purity form of the nominal POSF product (1).

C. Handling and Storage: Upon receipt, the test article was stored tightly sealed at room temperature.

D. Disposition of Test Material: Any remaining unused portion of the test article was returned to the research client after completion of the study.

III. Test System:

Male rats were exposed to test compound in air for 4 hours for one day in 13-liter glass exposure chambers (3 rats/chamber/compound). The flow-through atmosphere was generated by mixing test compound from a syringe pump into a stream of fresh air flowing at 3.0 L/min into the chamber and exhausted through a vent. The syringe flow rate was calculated based on using the Ideal Gas Law equation:
((target concentration)(MW)/24.79) x (1g/10^6 µg) x (airflow rate) x (1/density) x (1000 µl/mL)

Given:
target concentration (ppm)
MW = g/mole)
conversion factor: (1 gram)/(1 x 10^6µg)
airflow rate: (L/min)
1/density: (mL/g)
conversion factor: 1000 µl/mL

The target concentrations of each test compound, the syringe flow rate and the airflow are provided in the following table:

Dose Group	C (ppm)	Syringe Flow Rate (µl/min)	Airflow Rate (L/min)
Treated: POSF	1000	30	2.0 - 3.0
Control: Air only			2.0 - 3.0

Standard target concentrations for each compound were prepared and analyzed by Fourier Transform Infrared Spectrometry (FTIR). Chamber air was monitored by FTIR for concentrations of test compound and oxygen (2). The day of dosing was designated day zero of the study. On day zero, an atmosphere of appropriate concentration was generated by adding a calculated amount of the test material to a test chamber of known volume containing the animals. Airflow was increased when necessary to maintain a level of 1000 ppm. Control animals were exposed to room air under equivalent chamber conditions as the POSF dose group animals.

Animals were handled in accordance with an animal usage protocol approved by the 3M LARC. Body weights were determined immediately prior to dosing and immediately prior to euthanasia. At the termination of the study, all animals were humanely euthanized by CO_2 asphyxiation and necropsies were performed.

Serum was collected at necropsy for PFOS analysis. Analysis of PFOS levels by high-pressure liquid chromatography/tandem electrospray mass spectroscopy (HPLC-MS/MS) was performed according to published methods (3). Urine was collected overnight on day zero from rats of the limit test group. Urine PFOS levels were determined following a validated extraction procedure (4), with a slight modification in that no surrogate standard (THPFOS) was added.

Lungs, liver, kidney, bladder and brain were collected at necropsy on day 14 from the limit test group. A portion of each tissue was immediately fixed in a 10% formalin solution for subsequent histological evaluation. The remaining sections were placed directly into propylene tubes filled with liquid nitrogen for possible future evaluation.

IV. Results and Discussion:

Test Atmosphere

The POSF atmosphere generated in the exposure chamber averaged 912 ± 91 ppm for the limit test group (5). The measured concentration in the chamber was 91% of the target concentration for the limit test group. Factors which may account for this include the fact that the calculated concentration based on the Ideal Gas Law and the actual concentration in the flow-through chambers when the system reaches equilibrium may not be the same. The airflow was increased to 3 liters/ minutes to adjust chamber concentration.

Control Group:

The three control group male rats had no adverse clinical observations noted during the 4 hr air-only exposure. The average body weight was 378 ± 12 g at the initiation of the study. The control group gained an average of 22 g (5.5%) of body weight during the 14 day study period. Liver weights averaged 14.1 ± 1.0 g and the average liver to body weight ratio was 0.035 ± 0.001 (Table1).

Necropsy findings and the histopathology report by Dr. Elden Lamprecht, 3M Surgical and Histopathology Services, is found in Appendix 1. All rat lungs (both control and treated groups) had mild to moderate congestion of alveoli with occasional extravasation of blood around arterioles and bronchioles. This was possibly an agonal change associated with CO_2 euthanasia. It was noted that some of the lungs were not well insufflated. Other than these artifacts, histological analysis revealed no significant changes in lungs and urinary bladder in all three of the controls.

POSF Limit Group:

The three POSF group male rats were exposed to 912 ± 91 ppm POSF measured continuously for four hours. There were no biologically significant effects over a 14-day recovery period after exposure for the POSF treated group. As a gross observation, the POSF treated animals had an increase of urine and feces output on day one post-exposure as compared to control. The average body weight was 404 ± 31 g (N =3) fourteen days post exposure. The limit group gained an average of 19g (4.9%) of body weight during the 14-day study period. Liver weights averaged 14.2 ± 2.0g and the average liver to body weight ratio was 0.035 ± 0.002 (Table 2), similar to controls.

Day zero, overnight urine samples were collected and extracted, to measure the levels of PFOS. Each urine sample was prepared in duplicate. Independent dilutions of the urine were done with each value calculated from the average of the duplicate determination. The average amount of PFOS in urine was 3.08 ± 1.60 ppm and the average percentage of PFOS in urine was 0.05 ± 0.03% (Table 3). The standard curve

used to calculate the levels of PFOS in extracted urine samples is shown in Figure 1, and the area under the curve (AUC) for these extracted urine standards are in Table 4.

Serum samples were collected at necropsy on day 13 post-dose to measure the concentration of PFOS (Table 5). The average amount of PFOS in serum was 0.058 ± 0.004 mg. The average percentage of inhaled POSF in serum at necropsy was 0.101 ± 0.007% (Table 5).

These findings are similar to the PFOS levels observed in an accompanying toxicokinetic study of POSF (T-7098.4) in which a small percentage of PFOS was also found in the serum and urine (4).

V. Conclusion:

The apparent inhalation 4-hour LC50 in rats for POSF is greater than 1000 ppm.

VI. References:

1. Kestner, T. Chemical Characterization of POSF (FM-3270) by ^{1}H-NMR & ^{19}F-NMR Spectroscopy. 3M Specialty Materials & Manufacturing Division Analytical Laboratory. Request No. 6370. May 15, 2001.

2. Modified EPA Method 320. Pace Analytical. (2001).

3. Hansen, K.J., Clemen, L. A., Ellefson, M. E., Johnson, H.O. (2001). Compound-Specific, Quantitative Characterization of Organic Fluorochemicals in Biological Matrices. *Environ. Sci. and Tech.*.**35**, 766-770.

4. Method Number: ETS-8-96.0. Extraction of potassium perfluorooctanesulfonate or other fluorochemical compounds from urine for analysis using HPLC-electrospray/mass spectrometry/mass spectrometry. 3M Environmental Laboratory.

5. Gutzkow, T. (2001) POSF Animal Exposure Test Report. Pace Analytical Services, Inc. 3M Laboratory Request No. E01-0648. 3M ET&SS Field Analytical Services and Technologies.

6. Seacat, A. and Turner, T. (2001) Acute Inhalation Toxicokinetic Study of Pefluooctanesulfonyl Fluoride (POSF) T-7098.4.

VI. Signature Page

Authors:

Tommie Yvette Turner 9/24/01

Tommie Yvette Turner, Ph.D. Date
Post Doctoral Research Fellow
Study Toxicologist

Andrew M. Seacat 9/25/01

Andrew Seacat, Ph.D. Date
Toxicologist Specialist
Study Director

Reviewed by:

John L. Butenhoff 10/19/01

John L. Butenhoff, Ph.D, CIH, DABT Date
Toxicologist Specialist
Study Director

Table 1: Control Group Biological Data

Exposure	animal number	5/1/01 Animal weight Pre exposure Day 0 (g)	5/2/01 Animal weight Post exposure Day 1 (g)	5/3/01 Animal weight Post exposure Day 2 (g)	5/7/01 Animal weight Post exposure Day 6 (g)	5/14/01 Animal weight (g)	T-Test p-value Predose vs day 6 BW (1)	5/14/01 Necropsy Liver weights (g)	Liver/BW ratio	notes
Control 1	R01462	374	372	369.1	376	391	0.04	14.1	0.036	
Control 2	R01463	368	364.5	360.1	373	386		13.1	0.034	
Control 3	R01464	392	376	386	401	422		15	0.036	(overnight fast)
avg		378	371	372	383	400		14.1	0.035	
SD		12	6	13	15	20		1	0.001	

(1) p-value for a 2-tailed Student's T-test, assuming equal variance. Predose vs. day 6 body weight. Statistically significant if $P<0.05$.

Table 2: POSF Limit Dose Group Liver Weights

Exposure POSF (ppm)	animal number	5/1/01 Animal weight Pre exposure Day 0 (g)	5/2/01 Animal weight Post exposure Day 1 (g)	5/3/01 Animal weight Post exposure Day 2 (g)	5/7/01 Animal weight Post exposure Day 6 (g)	5/14/01 Animal weight (g)	T-Test p-value Prexpo vs day 6 BW (1)	Liver weights (g) 5/14/01 Necropsy	Liver/BW ratio
1000	R01465	374	372	370.7	379	390	0.01	12.8	0.033
1000	R01466	364	355	360.7	368	383		13.6	0.036
1000	R01467	416	392.6	409.8	429	440		16.1	0.037
avg		**385**	**373**	**380**	**392**	**404**		**14.2**	**0.035**
SD		**28**	**19**	**26**	**33**	**31**		**2**	**0.002**
P-value (2)		0.72	0.85	0.63	0.70	0.84		0.93	0.880

(1) p-value for a 2-tailed Students' T-test, assuming equal variance. Predose vs. body weight on day 6. Statistically significant if $p<0.05$
(2) p-value comparing control vs. treated group body weights. Statistically significant if $p<0.05$

Table 3: Limit Group PFOS in Urine

Samples	Area under the curve (AUC) Abundance x min	Concentration of PFOS in urine (ppb) (ppb)	ppm PFOS	Dilution factor	Undiluted urine PFOS (ppm)	Collected Volume of Urine (ml)	Amount Average Total PFOS (ug)	Amount of PFOS in Overnight Urine Average Total PFOS (mg)	% PFOS in urine (3)
MeOH Solvent Blank	8888	-94							
RO1645 d1PD	1154557	1936	1.94	2	3.88				
RO1645 d1PD	949883	1573	1.57	2	3.14				
avg	**1052220**	**1755**	**2**		**3.51**	10	35.1	0.04	0.06
SD	**144726**	**256**	**0.26**		**0.52**				
RO1646 d1PD	394606	590	0.59	2	1.18				
RO1646 d1PD	460644	707	0.71	2	1.42				
avg	**427625**	**648**	**1**		**1.3**	17	22.1	0.02	0.04
SD	**46696**	**83**	**0.08**		**0.17**				
RO1647 d1PD	1384246	2343	2.34	2	4.68				
RO1647 d1PD	1238595	2085	2.08	2	4.16				
avg	**1311420.5**	**2214**	**2**		**4.42**	18	79.56	0.08	0.14
SD	**102991**	**182**	**0.18**		**0.37**				
Overall AVG					**3.08**			**0.05**	**0.08**
Overall SD					**1.60**			**0.03**	**0.05**

(1) Standard curve equation where Y= area under the curve (AUC) in units of relative abundance multiplied by time (minutes) , and X = ppb of PFOS in urine. X = (Y-61687)/564.53

(2) Mean + SD of PFOS in urine are shown for N=3, where each of the 3 values are the mean of duplicate determinations.

(3) Inhaled Dose of POSF: (2.0 ml/breath) x (120 breaths/min) x (1 mg POSF/ L of air) x (240 min)/[(Body weight day 0 (g)/(1000g/Kg)

Table 4: Extracted Urine Combined Standard Curve for PFOS

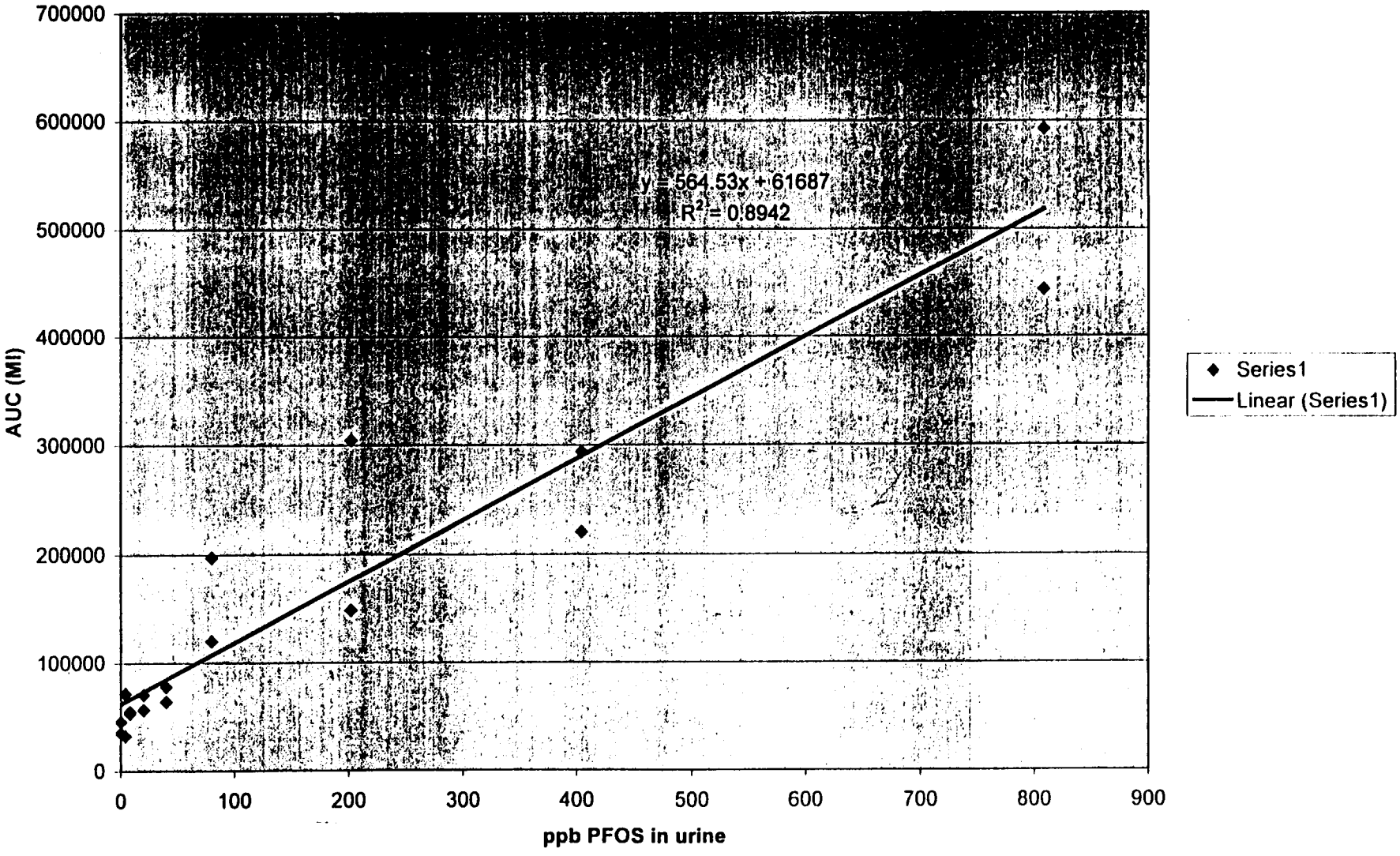
Sample	POSF AUC (MI)
MeOH Solvent Blank	11952
Milli Q water	36312
Control urine Matrix blank	35330
MS 4 ppb	32394
MS 8 ppb	53129
MS 20 ppb	56366
MS 40 ppb	77325
MS 80 ppb	119932
MS 202 ppb	147773
MS 404 ppb	220143
MS 808 ppb	442924
Control urine Matrix blank	45441
MS 4 ppb	71331
MS 8 ppb	54846
MS 20 ppb	70255
MS 40 ppb	63918
MS 80 ppb	197266
MS 202 ppb	304118
MS 404 ppb	293452
MS 808 ppb	592511

Table 5: POSF Limit Dose Group Serum PFOS Data

Dose (ppm)	Animal #	Predose Body weight Day 0 (g)	Inhaled Dose POSF(1) (mg/kg)	Body weight day 1 post dose (g)	Body weight day 2 post dose (g)	B.W. day 5 post dose (g)	Body weight day 13 post dose (at necropsy) (g)	Serum volume (2) (ml)	serum ppm (PFOS) (3) (ug/ml)	Amount of PFOS in serum (4) (mg)	Available POSF in inhaled air (5) (mg)	% of inhaled POSF in serum as PFOS at necropsy serum)
1000 ppm POSF	R01645	374	154.01	372	370.7	379	390	12.02	5.1	0.061	57.60	0.11
1000 ppm POSF	R01646	384	158.24	355	360.7	368	383	12.37	4.8	0.059	57.60	0.10
1000 ppm POSF	R01647	416	138.46	392.6	409.8	429	440	14.21	3.8	0.054	57.60	0.09
Avg		385	150.24	373	380	392	411.6	12.87	4.57	0.058	57.60	0.10
STD		28	10.42	19	26	32.5	40	1.18	0.7	0.004	0.00	0.01

(1) Inhaled Dose of POSF: (2.0 ml/breath) x (120 breaths/min) x (1 mg POSF/ 1000ml air) x (240 min)/[(Body weight day 0 (g)/(1000g/Kg)]
(2) Given: (32.3 mls serum)/(kg of body weight). Serum volume =(BW day 13 (g)/1000g/Kg) x 32.3 ml/Kg
(3) Serum ppm: Analytical work done by Dave Ehresman PFOS in serum measured by HPLC/MS (see appendix 2).
(4) PFOS in serum (mg): [(Serum volume ml)(Serum PFOS ug/ml)] /1000 ug/mg
(5) POSF in inhaled air (mg): (2.0 ml/breath) x (120 breaths/min) x (1 mg/ 1000ml) x (240 min) = 57.6 mg POSF in inhaled air
(6) % inhaled POSF in serum: [PFOS in serum (mg)/POSF in inhaled air (mg)]*100

Figure 1: extracted urine combined standard curve for PFOS



Appendix 1

Histopathology Results

Necropsy 5/3/01

All rats lungs have generalized mild to moderate congestion of alveoli with occasional extravasation of blood around arterioles and bronchioles. This is possibly an agonal change associated with CO2 euthanasia. Some lungs were not well insufflated.

Animal #
Control RO1462:
No significant changes in lung and bladder.

Toxicokinetic RO1468:
No significant changes in lung and bladder.

Toxicokinetic RO1471:
One macroscopic focus of atelectasis (collapsed alveoli) is present in one lung lobe. The change was chronic in duration. No significant change is present is bladder.

Toxicokinetic RO1472:
No significant changes in lung and bladder

Appendix 2
Serum PFOS

To: Andrew Seacat/US-Corporate/3M/US@3M-Corporate

Tommie Turner/US-Corporate/3M/US
cc: John L. Butenhoff/US-Corporate/3M/US@3M-Corporate
Paul Lieder/US-Corporate/3M/US@3M-Corporate
Sue Tanaka/US-Corporate/3M/US@3M-Corporate
Kathy Thompson/US-Corporate/3M/US
Deanna J. Luebker/US-Corporate/3M/US@3M-Corporate
Subject: PFOS Levels from rats exposed to POSF via inhalation T-7098.3

Andrew and Tommie:

The 1 to 100 estimate for the serum levels of PFOS was right on target. The following results are based on the rat serum being diluted 1:100 prior to extraction. The extraction was carried out at pH 3.0 using a single shake out in Ethyl Acetate. The EA was transferred to a clean polypropylene tube and blown to dryness using nitrogen gas. The resultant residue was re-dissolved in 100 uL of 25% acetonitrile and 75% 10 mm ammonium acetate. Of this solution 20 uL were injected onto a Betasil C18 reversed phase HPLC column (50 x 2 mm, 5 micron particle size). The flow rate was maintained at 0.5 ml/min. A gradient flow of acetonitrile was used to elute the compounds of interest starting with 15% acetonitrile and ending with 65% acetonitrile over 5.5 minutes. Return to initial operational parameters and column equilibration requires and additional 2.5 minutes for a run time cycle of 8.0 minutes per run.

The MS was operated in the Electro Spray negative ionization mode with a constant 3.0 kV potential applied to the source. The method used was an MS/MS method involving the transition of the following ions:

Compound	Base Peak	MS/MS Ion	Q-2 Offset Voltage
Internal Standard (PFHS)	299 amu	80 amu	60 volts
PFOS	499 amu	80 amu	60 volts

The capillary heater was held at a constant 300 degrees C. All quantitative calculations were based on the MS/MS ion rations between the compound of interest (PFOS) and the internal standard (PFHS). The standard curve ranged from 5 ng/mL (ppb) to 250 ng/mL (ppb). A quadratic curve fit was used with the standard value weighted 1/X . This curve fit produced an R squared value of 0.9935.

The first tube of two available for control animal number 1R01464 was diluted and run with the initial extractions. This tube (1:100 dilution) had a PFOS level of 2.3 ppm. Repeat of this tube dilution produced comparable results (2.1 ppm). The second tube labeled 1R01464 was diluted 1:100 and extracted. The second tube on this control animal had a result of less than the lowest standard (5.0 ppb on the diluted serum).

Only one serum tube was available for animal 1R01465, unfortunately this tube was labeled 1R0465 with the correct date and T # recorded. I diluted and extracted this tube and recorded results under the animal number 1R01465.

The sample for 1R01468 was noted as hemolyzed (2+ hemolysis). The initial result of 1R01472 appeared to be significantly lower than 1R01468 and 1R01471. This sample was also re-extracted and the analysis repeated. No significant difference was noted on re-extraction.

Results:

Rat #	Control	PFOS Level		
1R01462	yes	< 5.0	ppb (on diluted serum)	
1R01463	yes	<5.0	"	
1R01464	yes	<5.0	"	
1R01465	no	5.1	ppm	
1R01466	no	4.8	ppm	
1R01467	no	3.8	ppm	
1R01468	no	8.6	ppm	
1R01471	no	9.3	ppm	
1R01472	no	5.0	ppm	(5.1 ppm on duplicate)

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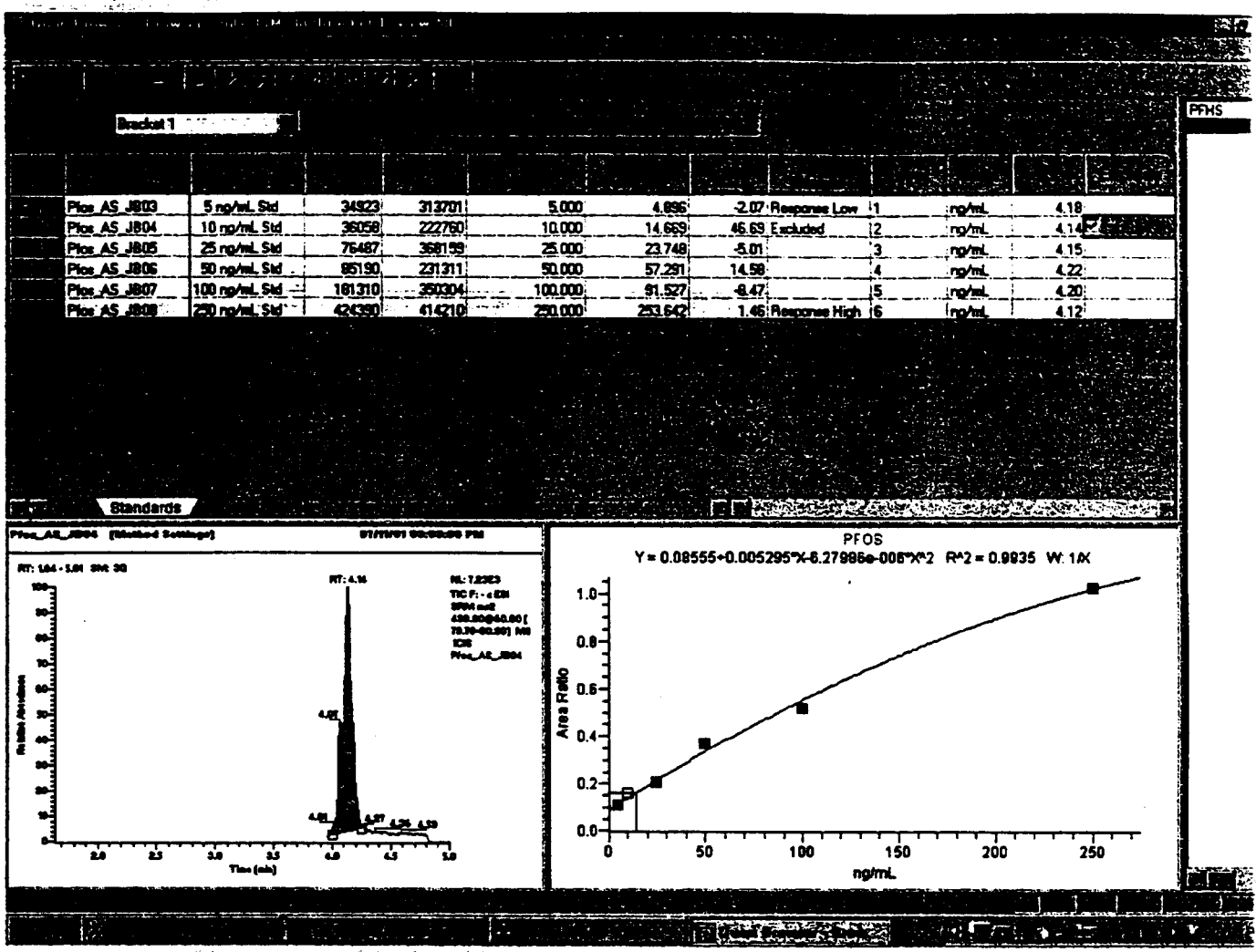
David J. Ehresman
3M Corporate Toxicology
Bldg. 236-1B-22
Phone 651-733-5070
FAX 651-737-4754
djehresman@mmm.com



Plos_AS_J803	5 ng/mL Std	34923	313701	5.000	4.896	-2.07	Response Low	1	ng/mL	4.18
Plos_AS_J804	10 ng/mL Std	36058	222760	10.000	14.669	46.69	Excluded	2	ng/mL	4.14
Plos_AS_J805	25 ng/mL Std	76487	368159	25.000	23.748	-5.01		3	ng/mL	4.15
Plos_AS_J806	50 ng/mL Std	85190	231311	50.000	57.291	14.58		4	ng/mL	4.22
Plos_AS_J807	100 ng/mL Std	161310	350304	100.000	91.527	-8.47		5	ng/mL	4.20
Plos_AS_J808	250 ng/mL Std	424350	414210	250.000	253.642	1.46	Response High	6	ng/mL	4.12