3M Strategic Toxicology Laboratory

Study Title: Acute Inhalation Toxicokinetic Study of Perfluoroctanesulfonyl Fluoride (POSF) T-7098.4

Test Number (sample): T-7098.4 (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 acute inhalation toxicokinetic 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. All animals gained weight during the recovery period and showed no clinical pathology.

One animal (N=1) per time point from the POSF-treated animals was sacrificed on day 1, day 6 and day 14 post-exposure. All animals tolerated the 4-hour inhalation exposure and gained weight. Organ weights were normal and histological evaluation of the liver, lungs, bladder, kidney and brain revealed no significant findings.

Perfluorooctanesulfonate (PFOS) was found in the serum from each animal (N=1) at 8.6, 9.3 and 5.0 ppm of days 1, 6 and 14 post dose, respectively. The percentage of inhaled POSF represented by these serum PFOS concentrations were 0.18%, 0.23%, and 0.10%, respectively.

Conclusion: This study demonstrates that there was a small percentage (<0.5%) of total POSF inhaled found as PFOS in serum collected over 14 days. Results show that there was no significant change in PFOS in serum concentration over time. POSF can be absorbed from the lung, and metabolized to PFOS, which is present in serum for greater than 14 days.

I. Study Objective:

This study was done to establish toxicokinetic parameters data on an acute inhalation of perfluoroctanesulfonyl fluoride (POSF). The goals of this study were to test the compound via inhalation in rats at 1000 ppm for 4 hours for distribution and metabolism, and to use the results as a 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. This POSF sample was lab fractionated and acid/water washed. Chemical characterization of $C_8F_{17}SO_2F$ 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 a 13 liter glass exposure chamber (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 the Ideal Gas Law equation:
((target concentration)(MW)/(24.79L) x (1g/10^6 µg) x (airflow rate) x (1/density) x (1000 µl/mL))

Given:
Target concentration (ppm)
MW = g/mole
24.79L: The volume of one mole of an ideal gas at 25 °C and 1 atmosphere (760 mm Hg)
conversion factor: (1 gram)/(1 x 10^6µg)
airflow rate: (L/min)
density: (mL/g)
conversion factor: 1000 µl/mL

The target concentrations of each test compound, the syringe flow rate and the airflow rate were calculated using the Ideal Gas Law and are provided in the following table:

Dose Group	C (ppm)	Syringe Flow Rate (µl/min)	Airflow Rate (L/min)
Treated: POSF Toxicokinetic group	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). Airflow was increased when necessary to maintain a level of at least 1000 ppm. Control animals were exposed to room air under equivalent chamber conditions as the POSF dose group animals. The control rats used in this study were also used for the control group in the limit test of POSF (T-7098.3) conducted at the same time.

Animals were handled in accordance with an animal usage protocol approved by LARC protocol # 2000-0068. The day of dosing was designated day zero of the study. 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.

Blood serum was collected from one animal/time point of the toxicokinetic group sacrificed at day 1, day 6, and at day 14 post exposure 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).

Serum from the rat sacrificed day one post dose (Animal #RO1468) was initially extracted and quantified by LC/MS/MS based on a PFOS standard curve extracted from urine collected from the limit toxicity study in rats (T-7098.3). This was done to get an estimate of serum PFOS levels on day one post-dose assuming an equal extraction of urine PFOS std curve and serum PFOS. Serum from this animal was later extracted and quantified against a standard curve extracted from serum, and therefore this data was not used in any calculations, but it is included here for completeness (Table 4).

Lungs, liver, and bladder were collected at sacrifice. 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 of POSF metabolites levels.

IV. Results and Discussion:

Test Atmosphere

The POSF atmosphere generated in the exposure chamber, measured continuously for four hours by FTIR, averaged 932 ± 93 ppm for the toxicokinetic group (4). The average measured concentration in the chamber was 93% of the target concentration for the toxicokinetic group. The airflow was increased from 2 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 (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 is 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 Toxicokinetic Group:

The three POSF group male rats showed no adverse clinical observations during the in-life phase of the rats. All animals gained weight during the study. At necropsy, no abnormal gross observations were made. Liver weights were equivalent to the control liver weights. Histological analysis in one of the animals revealed that one macroscopic focus of atelectasis (collapsed alveoli) was present in the lung lobe. The change appeared chronic in duration and was not considered to be compound-related. There were no significant changes in the bladder.

At necropsy for each animal, there was a small percentage of POSF measured in the serum for each individual animal (Table 3). Perfluorooctanesulfonate (PFOS) was found in the serum from each animal (N=1) at 8.6, 9.3 and 5.0 ppm of days 1, 6 and 14 post dose, respectively. The percentage of inhaled POSF represented by these serum PFOS concentrations were 0.18%, 0.23%, and 0.10%, respectively. These data suggest that the serum PFOS concentrations were at or near their apparent peak concentrations for the first week following exposure and then PFOS began to clear from the serum over the following week. Although there is no statistical power in the present study, the data are consistent with a plasma elimination half-life of ^{14}C following single oral administration of [^{14}C]FC-95 (mean dose 4.2 mg/kg) to male rats of 7.5 days (5).

Rat # RO1468 was sacrificed on day 1 post dose and sera was collected and analyzed for PFOS. The calculation of serum PFOS was based on urine PFOS standard curve, assuming equal extraction (Figure 1). The amount of PFOS in serum averaged over time, collected on days 1, 6, and 14 post-exposure was 25.90 $\pm$ 1.10 ppm (Table 4). Serum from this animal was later extracted and quantified against a standard curve extracted from serum. The data dervived from the PFOS standard curve (shown in table 4) extracted from urine was not used in any calculations, but it is included here for completeness.

This toxicokinetic study confirmed early findings of a POSF 4-hour rat LC_{50} greater than 1000 ppm in an inhalation limit toxicity study of POSF (6). In the limit study, rats were exposed to 1000 ppm POSF for four hours. All animals survived the duration of the study. The concentration of PFOS that was found in the serum on day 13 post –dose was 4.57 $\pm$ 0.70 ppm (N=3) which represented approximately 0.10 $\pm$ 0.01% of the inhaled dose of POSF. The average amount of PFOS in the day-one overnight urine was 3.08 $\pm$ 1.60 ppm (N=3) which represented approximately 0.05 $\pm$ 0.03% on the inhaled dose of POSF (6). Thus, the data from the POSF limit test and the data from the toxicokinetic test presented herein are in agreement with each other and support the same conclusions.

V. Conclusion:

This study demonstrates that there was a small percentage (<0.5%) of total POSF inhaled found as PFOS in serum collected over 14 days. Results show that there was no significant change in POSF in serum concentration over time.

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.

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

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

5. Johnson, J. D. and Ober, R.F . 1979. Absorption of FC-95-14C in rats after a single oral dose. Project No. 8900310200, Riker Laboratories, Inc., St. Paul, MN.

6. Seacat, A. and Turner, T. (2001) Acute Inhalation Limit Toxicity Study of Perfluooctanesulfonyl Fluoride (POSF) T-7098.3.

VI. Signature Page

Authors:

Tommie Yvette Turner 11/1/01

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

Andrew M Seacat 11/1/01

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

Reviewed by:

John L. Butenhoff 11/9/01

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 Students' T-test, assuming equal variance. Predose vs. day 6 body weight. Statistically significant if $P<0.05$

Table 2: Toxicokinetic Group Biological Data

Sacrifice date			Day 0 (5-1-01)	Inhaled Dose POSF(1)	Body Weight day 1 post dose	Body Weight day 6 post dose	Body Weight day 14 post dose	Organ Weight
	5/2/01 (POSF ppm)	Animal #	Predose Body weight	(mg/kg)	(g)	(g)	(g)	Liver (g)
5/2/2001 (day 1 post dose)	1000	R01468	382	150.79	378.2			14.6
5/7/2001 (day 6 post dose)	1000	R01471	426.4	135.08	425	431		15.6
5/14/2001 (day 13 post dose)	1000	R01472	351	164.10	350	359	376	13.6
	Avg		**386**	**149.99**	**384**	**395**		**14.6**
	STD		**38**	**14.53**	**38**	**50.91**		**1**

(1) Available dose (Amount inhaled over time) = alpha x VT x f x C x t. For this calculation assume that alpha is 100%, (I.e. = 1) so that all of the inhaled volume of the test atmosphere (1000 ppm POSF) is available for absorption (I.e. the available dose n the exposure to the "lungs").

Table 3: POSF Toxicokinetic Serum PFOS Data

T-7098.1 POSF Inhalation toxicokinetic Study
Date of Exposure : 5/2/01
Exposure: 1000 ppm POSF/4hrs, 3 male rats

Post Dose	Dose (POSF ppm	Animal #	Day 0 (5-1-01) Predose Body weight	Inhaled Dose POSF(2) (mg/kg)	Serum volume (1) (ml)	serum ppm (PFOS) (ug/ml)	PFOS in serum (mg)	Available POSF in inhaled air (mg)	% of inhaled POSF in serum at necropsy (% of dose in serum)
Day 1	1000	R01468	382	150.79	12.22	8.6	0.105	57.600	0.182%
Day 6	1000	R01471	426.4	135.08	13.92	9.3	0.129	57.600	0.225%
Day13	1000	R01472	351	164.10	11.60	5	0.058	57.600	0.101%
Avg			386	149.99	12.58	7.63	0.097	57.600	0.169%
STD			38	14.53	1.20		0.036	0.000	0.063%

(1) Given: (32.3 mls serum)/(kg of body weight). Serum volume =(bw in grams/1000) x 32.3
(2) Serum ppm: Analytical work done by Dave Ehresman. 1:100 dilution of PFOS in serum measured by HPLC/MS.
(3) PFOS in serum (mg): [(Serum volume)(Serum PFOS)] /1000
(4) 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
(5) % inhaled POSF in serum: [(PFOS in serum)/(POSF in inhaled air)]*100

Table 4: POSF Toxicokinetic Serum Data, day one post-dose, based on urine std curve.

Serum from the rat sacrificed day one post dose (Animal #RO1468) was initially extracted and quantified by LC/MS/MS based on a PFOS standard curve extracted from urine collected from the limit toxicity study in rats (T-7098.3). This was done to get an estimate of serum PFOS levels on day one post-dose assuming an equal extraction of urine PFOS std curve and serum PFOS. Serum from this animal was later extracted and quantified against a standard curve extracted for serum, and therefore this data was not used in any calculations, but it is included here for completeness.

Serum PFOS from day 1 post dose toxicokinetic group animal based on urinary PFOS standard curve.

	Y (AUC)	PFOS in serum (ppb) using Standard curve equation form combination of two urine extracted PFOS standard curves. X = ppb of PFOS in serum = (Y-61687)/564.53	Diluted serum PFOS (ppm)	Dilution factor	PFOS in undiluted serum (ppm)
RO1468 d1 PD	3827145	6670	6.67	4	26.68
RO1468 d1 PD	3608110	6282	6.28	4	25.13
Average	**3717628**	**6476**	**6.48**		**25.90**
SD	**154881**	**274**	**0.27**		**1.10**

(1) Assuming a 1:1 relationship between POSF and PFOS. 100% inhaled dose of POSF could be PFOS in urine and serum.

Figure 1: extracted urine combined standard curve for PFOS

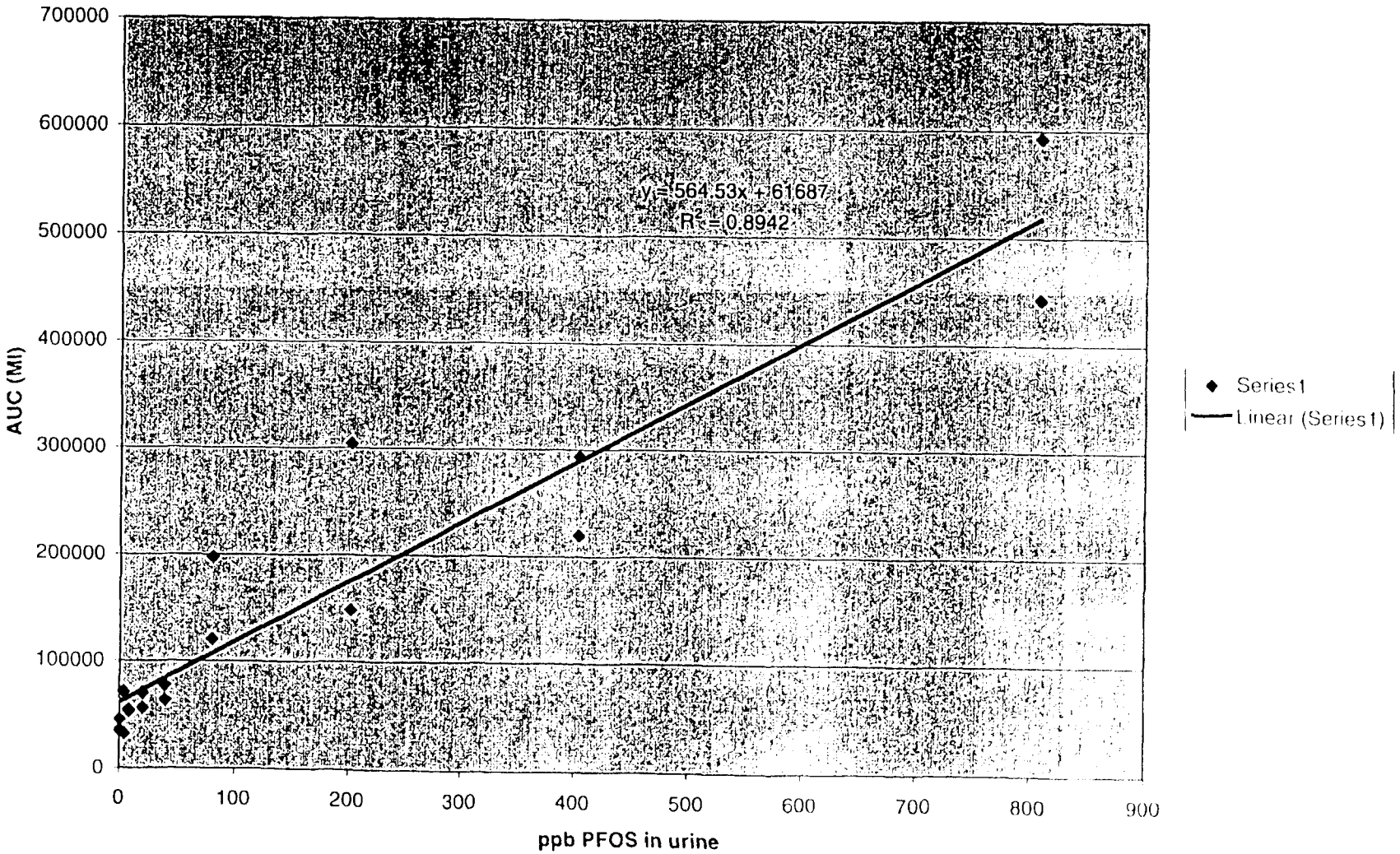
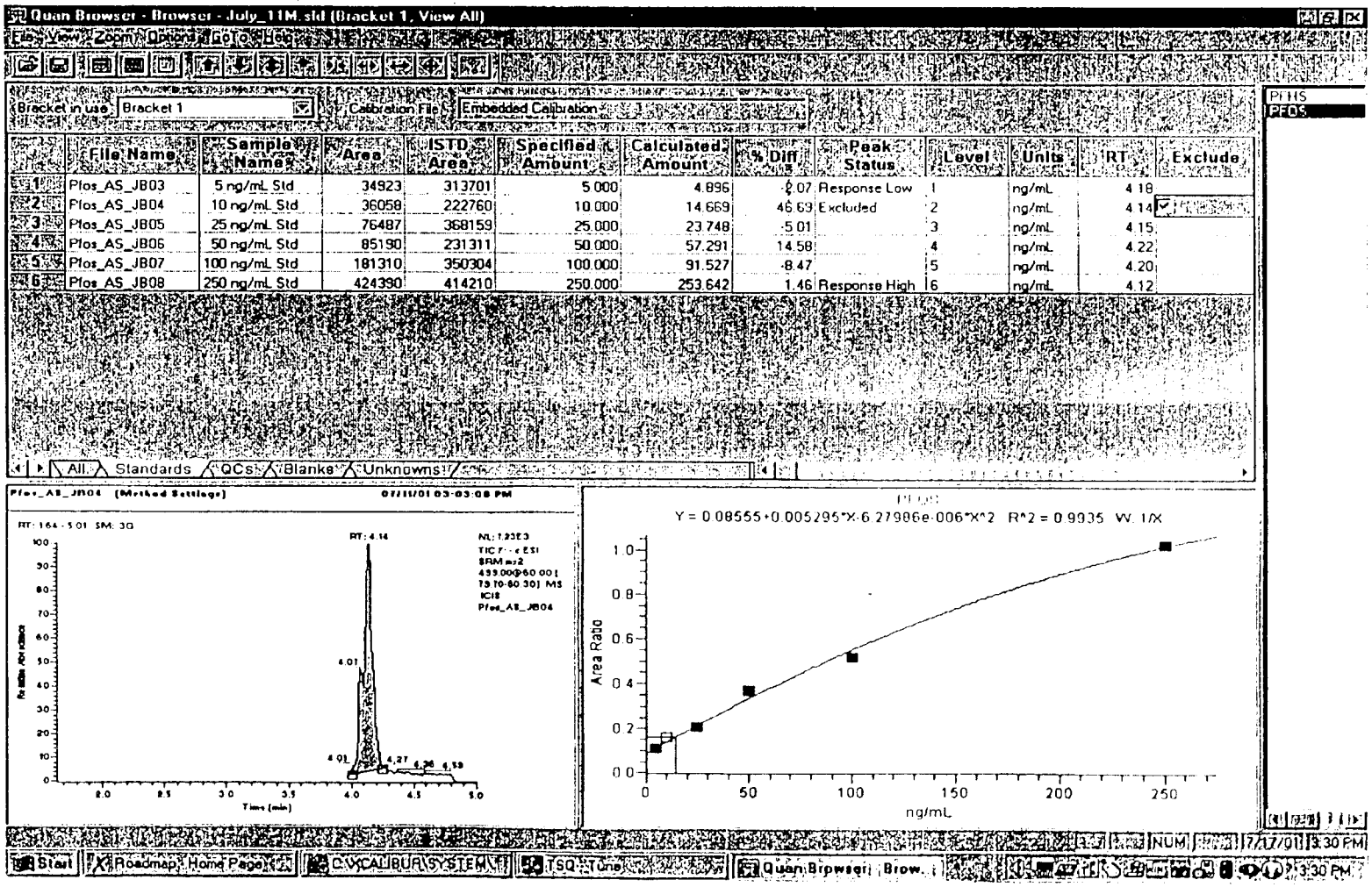


Figure 2: Extracted PFOS standard curve in serum



	File Name	Sample Name	Area	ISTD Area	Specified Amount	Calculated Amount	% Diff	Peak Status	Level	Units	RT	Exclude
1	Pfos_AS_JB03	5 ng/mL Std	34923	313701	5.000	4.896	-2.07	Response Low	1	ng/mL	4.18	
2	Pfos_AS_JB04	10 ng/mL Std	36058	222760	10.000	14.669	46.69	Excluded	2	ng/mL	4.14	
3	Pfos_AS_JB05	25 ng/mL Std	76487	368159	25.000	23.748	-5.01		3	ng/mL	4.15	
4	Pfos_AS_JB06	50 ng/mL Std	85190	231311	50.000	57.291	14.58		4	ng/mL	4.22	
5	Pfos_AS_JB07	100 ng/mL Std	181310	350304	100.000	91.527	-8.47		5	ng/mL	4.20	
6	Pfos_AS_JB08	250 ng/mL Std	424390	414210	250.000	253.642	1.46	Response High	6	ng/mL	4.12	

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