3M Specialty Materials

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VIA HAND DELIVERY

January 18, 2001

Document Processing Center (7407)
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
401 M Street SW
Washington, D.C. 20460

Attention: For Your Information Docket - Docket No. AR-226

Re: Information on Perfluorooctane Sulfonates and Related Compounds

Dear Sir or Madam:

This continues 3M's voluntary submissions of data on perfluorooctane sulfonates and related compounds, as part of our ongoing dialog with EPA regarding fluorochemistry.

Please find enclosed a supplemental submission containing a toxicokinetic study of perfluorooctane sulfonamide (FOSA). We just realized that this report had not yet been executed in final, and thus it was omitted from our recent December production.

We will provide an electronic copy of this material with a future submission. We will continue to provide information on a regular basis as it becomes available.

Very truly yours,

Michael A. Santoro
Director of Environmental, Health
Safety and Regulatory Affairs
Specialty Materials Markets

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Enclosures - index and study

cc: Dr. Charles Auer
Dr. Oscar Hernandez

ATTACHMENT TO LETTER OF JANUARY 18, 2001
SUPPLEMENTAL SUBMISSION

TOXICOLOGY AND MEDICAL SURVEILLANCE

FOSA	**Perfluorooctanesulfonamide**

Pharmacokinetic

1. Final Report, Toxicokinetic Study of Perfluorooctane Sulfonamide (PFOSA; T-7132.2) in Rats, 3M Strategic Toxicology Laboratory, 3M Reference Nos. T-7132.2; ST39, signed January 16, 2001 (cover date August 11, 2000).

FINAL REPORT

Study Title:

Toxicokinetic Study of Perfluorooctane Sulfonamide (PFOSA; T-7132.2) in Rats

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Date: August 11, 2000

Testing Facility

3M Strategic Toxicology Laboratory
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Summary:

Purpose: The purpose of this study was to assess the relative absorption, metabolism, and elimination kinetics of Perfluorooctane Sulfonamide (PFOSA) in the rat following a single oral dose.

Methods: Two groups of fifteen male rats each received a single dose of either 2% Tween 80, as the vehicle control, or 5 mg/kg PFOSA suspended in 2% Tween 80 by oral gavage. Perfluorooctanoate (POAA) was found at a level of 2.5 ng/ml in the dosing solution. The PFOSA was found to be 96 % pure Five rats from each dose group were sacrificed on days 1, 4, and 29 post dose. Liver and sera were analyzed for PFOSA and its predicted metabolites using high-pressure liquid chromatography/electrospray tandum mass spectrometry (HPLC/ESMSMS).

Results: The vehicle control group had no detectable levels of PFOSA or perfluorooctanesulfonate (PFOS) in liver or sera. The PFOSA dose group had decreasing levels of PFOSA, with evidence of conversion of PFOSA to PFOS in liver and sera throughout the study. Liver concentrations of PFOSA averaged 7.0 ppm, 3.7 ppm, and 0.1 ppm on days 1, 4, and 29 post-dose, respectively, with an apparent liver half-life of elimination of 5.2 days. Serum concentrations of PFOSA averaged 0.3 ppm and 0.1 ppm on days 1 and 4 post dose respectively, and were undetectable by day 29 post-dose, with an apparent serum half-life of elimination estimated to be less than 4 days. Liver PFOS concentrations were 28.2 ppm, 37.4 ppm and 23.8 ppm, representing 22.0%, 32.3%, and 25.2% of the PFOSA dosed, on days 1, 4 and 29 post-dose respectively. Sera PFOS averaged 8.2 ppm, 8.6 ppm, and 4.6 ppm on days 1, 4 and day 29 post dose respectively. The average total concentrations of PFOS equivalents (PFOS ≒ PFOSA) in liver were 35.2, 41.1, and 23.9 ppm on days 1, 4, and 29 post-dose, respectively. The average concentrations of PFOS equivalents in the sera increased from 8.5 ppm on day 1 post dose to 8.7 ppm on day 4 post dose and decreased to 4.6 ppm on day 29 post dose. The liver to sera PFOS concentration ratio increased throughout the study with values of 4.2. 4.8, and 5.4 on days 1, 4, and 29 post dose, respectively. Total PFOS equivalents in the liver and sera peaked on day 4 post dose at 35.4% and 6.0 % of the PFOS equivalents dosed, respectively. An average of 0.1 ppm POAA was found in the sera on day 1 and day 4 post dose, and equaled 31.2% and 24.1%of the POAA residual present in the dose, respectively.

Conclusions: Perfluorooctanesulfonamide was readily absorbed from the gut and found in the liver and sera following an oral exposure. PFOSA was progressively metabolized to PFOS, which accumulated in the liver as evidenced by the high concentration of PFOS detected in the liver, representing 22.0% 32.3% and 25.2% of the PFOSA dosed, on days 1,4 and 29 post dose respectively. The apparent half-live of elimination of PFOSA in the liver was approximately 5.2 days. The POAA present in the sera on days 1 and 4 post-dose could be accounted for by the residual POAA in the dosing solution, therefore, no direct evidence for metabolism of PFOSA to POAA was found in this study.

Introduction

The objective of this study was to assess the potential for oral absorption, urinary and fecal clearance, and biological persistence of Perfluorooctanesulfonamide (PFOSA) in male Sprague Dawley rats after a single oral dose. Analysis of the serum and liver for PFOSA and potential metabolites of PFOSA was performed by LCMS. Urine and feces were also collected and may be analyzed by LCMS and perhaps other methods as deemed necessary.
Previous studies on N-ethylperfluoroctanesulfonamide (1), the N-ethyl derivative of PFOSA, mistakenly concluded that PFOSA was the ultimate metabolite in rats (1,2); however, the gas chromatography analytical technique used in those studies was unable to detect PFOS. Dietary administration of N-ethylperfluoroctanesulfonamidoethanol (N-EtFOSE) in rats, and *in-vitro* metabolism by hepatocytes results primarily in the formation of PFOS with a minor amount of PFOSA (3, 4, and 5). Furthermore, PFOSA has been identified as a potent direct uncoupler of mitochondrial respiration *in-vitro* (6). Prior to the current study, direct metabolism of PFOSA to PFOS had not been demonstrated and the relative toxicokinetics of PFOSA were unknown. Methods were recently validated for the quantitation of PFOSA and its potential metabolite, PFOS, in serum and liver down to the low part per billion level (7). *In-vivo* studies were, thus, warranted to investigate the toxicokinetics of PFOSA.

Materials

The test material was identified as Perfluorooctanesulfonamide (PFOSA); Lot number L-10009. Initial analysis by GCMS determined that the staring material was over 99% pure (8). Qualitative and quantitative compositional results derived from ^{1}H and ^{19}F-NMR analysis revealed that the isomer distribution was approximately 65.8% CF3 (CF2) x-SO2-NH2 (Normal chain), 18.7% internal monomethyl branch and 11.2% Isopropyl branch (9). HPLC/MS characterization revealed low-level impurities of 9,600 ppm PFOS (0.96 %), and lower concentrations of several other amides (10). Perfluorooctanoate (POAA) was found in the dosing solution at a level of 2.5 µg/ml (0.25% of the nominal dose) (12). Based on the sum of the impurities, the purity of the PFOSA sample was determined to be approximately 96 % (8, 9 & 10).

Methods

The protocol (11) and analytical methods (12) used in this study are briefly described below.

Dose and Dosing Procedures:

A single 5mg/kg dose of PFOSA was administered via oral gavage to 15 male Sprague Dawley rats (obtained from Harlan Laboratories) on day zero of the study. The PFOSA was prepared as a 1% (1 mg/ml) uniform suspension in 2% Tween 80. A volume of 5 ml suspension / kg body weight was administered to each rat. The vehicle control group, consisting of 15 male Sprague Dawley rats (also obtained from Harlan Laboratories), received 2% Tween 80 in deionized water at a volume of 5 ml/kg on day zero of the study.

Specimen Collection:

Urine and feces collections were made on days 1 – 4 post dose from the animals designated for sacrifice on day 29 post dose group. Five animals from each dose group were euthanized by CO_2 on days 1, 4, and 29 post dose and gross necropsy was performed. Sera and liver were collected from each animal and flash-frozen in liquid nitrogen.

Specimen Handling:

Specimens (urine, feces, liver, and serum) were maintained at -70°C and sent to Kris Hansen, Ph.D., 3M Environmental Technology and Safety Services, for analysis. Liver and sera samples were analyzed by 3M Environmental for the parent compound and metabolites. The limits of detection (LODs) for PFOSA were 3.5 ng/ml in the liver and 1.51ng/ml in the sera. The LODs for PFOS were 8.45 ng/ml in liver and 1.74 ng/ml in the sera (12). Urine and feces were retained for possible future analysis as appropriate.

Results

Control Groups:

Body and Liver Weight
The average initial body weight ± SD of the fifteen vehicle control male rats was 273.7 ± 7.3 g. These rats lost an average of 2.2 g body weight from day zero to day 1 post dose . From day zero to day 4 post dose, the average body weight gain was 24.2 g and from day zero to day 29 post dose. body weight increased an average of 114.1 g . Liver weights and relative liver to body weight percentages are shown in Table 1A.

Liver and Sera Concentrations
Control animals had no detectable levels of PFOSA or PFOS in liver or sera at any time during this study (data not shown).

PFOSA Dose Groups:

Body and Liver Weight
The average initial body weight ± SD of the fifteen PFOSA dosed male rats was 270.0 ± 3.6 g. These rats lost an average of 4.5 g body weight from day zero to day 1 post dose . From day zero to day 4 post dose, the average body weight gain was 20.0 g and from day zero to day 29 post dose, body weight increased an average of 100.9 g . Liver weights and relative liver to body weight percentages were equivalent to control values (Table 1B).

Liver and Sera Concentrations

PFOSA and PFOS were detected in the liver and sera of all animals dosed with PFOSA. Liver and sera levels of PFOSA were highest on day 1 post dose and dropped significantly between

days 1 and 4 post dose and again between days 4 and 29 post dose (Tables 2 and 3). The average liver concentrations of PFOSA decreased from approximately 7.0 ppm to 3.7 ppm to 0.1 ppm from days 1 to 4, to 29 post dose, respectively (Table 2). The half-life of elimination of PFOSA in the liver was calculated from a log linear regression of the liver PFOSA concentrations to be 5.2 days. The sera concentrations of PFOSA were 0.3 ± 0.1 ppm, 0.1 ± 0.0 and <LOD (1.74 ng/ml) on days 1, 4, and 29 post dose, respectively (Table 3). The half-life of elimination of PFOSA in the serum cannot be calculated from this data, but is estimated to be less than 4 days. Average PFOS liver and sera levels peaked on day 4 post dose and declined by day 29 post dose to lower levels than found on day one. Average ± SD liver PFOS levels increased significantly from 28.2 ± 6.1 ppm on day 1 post dose to 37.4 ± 3.6 ppm on day 4 post dose. Levels then decreased significantly to 23.8 ± 4.1 ppm by day 29 post dose (Table 2). Average sera PFOS values were approximately 8 ppm on day 1 and day 4 post dose, then decreased significantly to approximately 4.6 ppm on day 29 post dose (Table 3).

Liver and Sera Concentrations as a Percentage of Dose

The percentage of PFOSA dosed detected as PFOSA in the liver and sera peaked on day one post dose at 5.4% and 0.2 % respectively. By day 4 post dose, these levels had dropped to 3.2% and 0.0 % and by day 29 post dose the liver levels dropped to 0.1 % and the sera levels remained at 0.0% (Table 6).

The percent PFOS equivalents dosed detected as PFOS in the liver and sera was 22.0% and 5.2 % on day 1 post dose and increased to 32.3% and 6.0% by day 4 post dose. On day 29 post dose, these levels had dropped to 25.2% and 4.1%, respectively (Table 6). Residual PFOS in the dose could account for 3.5%, 2.5%. and 5.6% of the PFOS found in sera, and 3.6%, 2.7%, and 3.9 % of the PFOS found in liver on days 1, 4, and 29 post dose, respectively (Tables 4 and 5).

The percent PFOS equivalents dosed detected as PFOS equivalents in the liver and sera was 27.2 % and 5.3 % n day 1 post dose and increased to 35.4 % and 6.0 % by day 4 post dose. On day 29 post dose these levels decreased to 25.4 % and 4.1 % in the liver and sera respectively (Table 6).

Sera POAA Concentrations

Perfluorooctanoate (POAA) was found at a level of 2.5 ng/ml in the dosing solution (12). Sera samples in the PFOSA dose group contained POAA at average values of 0.1 and 0.8 ppm POAA on days 1 and 4 post-dose respectively. These values equal 30.8% and 23.5%, respectively, of the POAA dosed (Table 7).

Conclusions:

No compound related effects were observed on body weight, liver weight, or liver/body weight ratios under treatment conditions.

Both PFOSA and PFOS were found at high concentrations in the liver and sera of rats one day following an oral dose. Approximately 38% of the PFOSA dosed was converted to PFOS four

days post-dose. PFOSA levels diminished in both sera and liver over time, with a liver half-life of approximately 5.2 days, and a much shorter residence period in the sera. Significantly more PFOS was found in the liver and sera at all time points than were present in the dose, therefore metabolic conversion of PFOSA to PFOS occurred. No direct evidence for metabolism of PFOSA to POAA was found in this study. The route of elimination of PFOSA in this study was not determined. No glucuronide or other conjugates were analyzed for, and further investigation of possible conjugates in the urine and feces is warranted. It is possible that some of the PFOSA was eliminated through the lung via expiration, but this has not been determined. The present results suggest that the PFOSA from the dose is progressively converted to PFOS and that the resulting PFOS is accumulated in the liver.

Signatures

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List of Tables:

Table 1A: Biological Parameters. Control Group							
Time	Dose	animal # 9R03-xxx	Initial BW (g)	Terminal BW (g)	BW Gain (g)	Liver wt (g)	Liver wt as % of BW
Day 1	Control	230	282.5	282.0	-0.5	12.0	4.3%
Day 1	Control	231	272.1	268.4	-3.7	11.2	4.2%
Day 1	Control	232	277.4	277.1	-0.3	12.4	4.5%
Day 1	Control	233	274.1	274.8	0.7	11.5	4.2%
Day 1	Control	234	267.6	260.5	-7.1	11.2	4.3%
	Avg		**274.7**	**272.6**	**-2.2**	**11.7**	**4.3%**
	SD		**5.6**	**8.3**	**3.2**	**0.5**	**0.1%**
Day 4	Control	235	266.9	294.5	27.6	11.6	3.9%
Day 4	Control	236	275.8	301.8	26.0	11.4	3.8%
Day 4	Control	237	261.9	283.4	21.5	11.9	4.2%
Day 4	Control	238	279.0	302.7	23.7	11.3	3.7%
Day 4	Control	239	268.3	291.2	22.9	10.7	3.7%
	Avg		**270.4**	**294.7**	**24.3**	**11.4**	**3.9%**
	SD		**6.9**	**8.0**	**2.4**	**0.4**	**0.2%**
Day 29	Control	240	273.5	371.8	98.3	13.9	3.7%
Day 29	Control	241	270.5	380.5	110.0	14.4	3.8%
Day 29	Control	242	280.2	389.4	109.2	14.2	3.6%
Day 29	Control	243	289.9	444.0	154.1	17.5	3.9%
Day 29	Control	244	266.3	365.2	98.9	14.3	3.9%
	Avg		**276.1**	**390.2**	**114.1**	**14.9**	**3.8%**
	SD		**9.2**	**31.4**	**23.0**	**1.5**	**0.1%**

Table 1B: Biological Parameters. PFOSA Dose Group							
Time	Dose	animal # 9R03-xxx	Initial BW (g)	Terminal BW (g)	BW Gain (g)	Liver wt (g)	Liver wt as % of BW
Day 1	5 mg/kg	245	263.2	257.8	-5.4	10.4	4.0%
Day 1	5 mg/kg	246	266.1	259.2	-6.9	10.2	3.9%
Day 1	5 mg/kg	247	266.5	264.1	-2.4	10.9	4.1%
Day 1	5 mg/kg	248	267.2	264.0	-3.2	10.1	3.8%
Day 1	5 mg/kg	249	268.7	264.3	-4.4	10.7	4.0%
	Avg		**266.3**	**261.9**	**-4.5**	**10.5**	**4.0%**
	SD		**2.0**	**3.1**	**1.8**	**0.3**	**0.1%**
Day 4	5 mg/kg	250	266.6	286.3	19.7	11.3	3.9%
Day 4	5 mg/kg	251	272.1	294.6	22.5	11.7	4.0%
Day 4	5 mg/kg	252	271.7	294.5	22.8	12.1	4.1%
Day 4	5 mg/kg	253	271.8	291.4	19.6	11.3	3.9%
Day 4	5 mg/kg	254	272.9	288.4	15.5	11.7	4.1%
	Avg		**271.0**	**291.0**	**20.0**	**11.6**	**4.0%**
	SD		**2.5**	**3.7**	**2.9**	**0.3**	**0.1%**
Day 29	5 mg/kg	255	275.2	385.4	110.2	16.7	4.3%
Day 29	5 mg/kg	256	273.6	380.3	106.7	14.4	3.8%
Day 29	5 mg/kg	257	271.4	373.9	102.5	13.9	3.7%
Day 29	5 mg/kg	258	274.5	371.4	96.9	14.6	3.9%
Day 29	5 mg/kg	259	268.7	356.7	88.0	12.7	3.6%
	Avg		**272.7**	**373.5**	**100.9**	**14.5**	**3.9%**
	SD		**2.6**	**10.9**	**8.7**	**1.5**	**0.3%**

Table 2: Liver PFOSA and metabolites in the PFOSA dose Group

Time	Dose	animal # 9R03-xxx	Liver [PFOS] ppm		Liver [PFOSA] ppm		Liver PFOSA PFOS eq ppm[1]
Day 1	5 mg/kg	245	23.7		6.02		6.02
Day 1	5 mg/kg	246	32.3		7.02		7.02
Day 1	5 mg/kg	247	36.2		6.24		6.24
Day 1	5 mg/kg	248	21.2		6.66		6.66
Day 1	5 mg/kg	249	27.8		8.81		8.81
	Avg		28.2		6.95		6.95
	SD		6.1		1.11		1.11
	Range	Min	21.2		6.02		6.02
		Max	36.2		8.81		8.81
	n	5	5		5		5
Day 4	5 mg/kg	250	36.8		3.52		3.52
Day 4	5 mg/kg	251	32.6		3.47		3.47
Day 4	5 mg/kg	252	42.6		3.96		3.96
Day 4	5 mg/kg	253	36.9		3.45		3.45
Day 4	5 mg/kg	254	38.3		4.11		4.11
	Avg		37.4	*	3.70	*	3.70
	SD		3.6		0.31		0.31
	Range	Min	32.6		3.45		3.45
		Max	42.6		4.11		4.11
	n	5	5		5		5
Day 29	5 mg/kg	255	20.3		0.09		0.09
Day 29	5 mg/kg	256	20.6		0.02		0.02
Day 29	5 mg/kg	257	22.0		0.10		0.10
Day 29	5 mg/kg	258	26.2		0.14		0.14
Day 29	5 mg/kg	259	29.9		0.30		0.30
	Avg		23.8	**	0.13	* / **	0.13
	SD		4.1		0.11		0.11
	Range	Min	20.3		0.02		0.02
		Max	29.9		0.30		0.30
	n	5	5		5		5

1 Assuming a 1:1 ratio of PFOSA:PFOS equivalents; Liver PFOSA PFOS equivalents =Liver PFOSA
* = significantly different from day 1 values by a two tailed T-test ($p<0.05$)
** = significantly different from day 4 values by a two tailed T-test ($p<0.05$)
PQL = practical quantitation limit in liver (PFOS = 30 ng/g; PFOSA 10ng)
MDL = method detection limit in liver: (PFOS = 15 ng/g, PFOSA = 5 ng/g)

Table 3: Serum PFOSA and metabolites in the PFOSA Group .

Time	Dose	animal # 9R03-xxx	Sera [PFOS] ppm		Sera [PFOSA] ppm		Sera PFOSA PFOS eq ppm[1]
Day 1	5 mg/kg	245	6.9		0.26		0.26
Day 1	5 mg/kg	246	10.8		0.44		0.44
Day 1	5 mg/kg	247	9.4		0.26		0.26
Day 1	5 mg/kg	248	6.7		0.33		0.33
Day 1	5 mg/kg	249	7.3		0.28		0.28
	Avg		**8.2**		**0.31**		**0.31**
	SD		**1.8**		**0.08**		**0.08**
	Range	Min	6.7		0.26		0.26
		Max	10.8		0.44		0.44
	n .	5	5		5		5
Day 4	5 mg/kg	250	7.8		0.06		0.06
Day 4	5 mg/kg	251	7.5		0.06		0.06
Day 4	5 mg/kg	252	11.3		0.08		0.08
Day 4	5 mg/kg	253	8.6		0.06		0.06
Day 4	5 mg/kg	254	7.9		0.06		0.06
	Avg		**8.6**		**0.06**	*	**0.06**
	SD		**1.6**		**0.01**		**0.01**
	Range	Min	7.5		0.06		0.06
		Max	11.3		0.08		0.08
	n	5	5		5		5
Day 29	5 mg/kg	255	3.6		<LOD		
Day 29	5 mg/kg	256	4.0		<LOD		
Day 29	5 mg/kg	257	4.0		<LOD		
Day 29	5 mg/kg	258	4.1		<LOD		
Day 29	5 mg/kg	259	7.4		0.0		
	Avg		**4.6**	* / **	**0.0**		
	SD		**1.5**				
	Range	Min	3.59				
		Max	7.36				
	n		5		5		5

1. Assuming a 1:1 ratio of PFOSA :PFOS equivalents, Sera PFOSA PFOS equivalents =Sera PFOSA concentration

* = significantly different from day 1 values by a two tailed T-test ($p<0.05$)

** = significantly different from day 4 values by a two tailed T-test ($p<0.05$)

MDL = method detection limit (PFOS = 4.75 ng/ml; PFOSA = 2.89 ng/ml)

LOD = limit of detection (PFOS = 1.74 ng/ml; PFOSA = 1.51 ng/ml)

Table 4: Percent Dose Liver PFOS equivalents in PFOSA Group

Time	Dose	animal # 9R03-xxx	PFOS eq concentration in liver (ppm) [1]		Total PFOS eq in whole liver (ug) [2]		PFOS eq in dose (ug) [3]	% PFOS eq dosed found in liver [4]		Residual PFOS in dose (ug) [5]	% of PFOS in liver from residual PFOS in dose (%) [6]
Day 1	5 mg/kg	245	29.7		309.1		1316.0	23.5		12.6	4.09%
Day 1	5 mg/kg	246	39.3		401.1		1330.5	30.1		12.8	3.18%
Day 1	5 mg/kg	247	42.4		462.6		1332.5	34.7		12.8	2.77%
Day 1	5 mg/kg	248	27.8		281.4		1336.0	21 1		12.8	4.56%
Day 1	5 mg/kg	249	36.6		391.7		1343.5	29.2		12.9	3.29%
	Avg		35.2		369.2		1331.7	27.7		12.8	3.58%
	SD		6.2		73.4		10.1	5.5		0.1	0.73%
	Range	Min	27.8		281.4		1316.0	21.1		12.6	2.77%
		Max	42.4		462.6		1343.5	34.7		12.9	4.56%
	n	5	5		5		5	5		5	5
Day 4	5 mg/kg	250	40.3		455.6		1333.0	34.2		12.8	2.81%
Day 4	5 mg/kg	251	36.1		422.0		1360.5	31.0		13.1	3.09%
Day 4	5 mg/kg	252	46.6		563.4		1358.5	41.5		13.0	2.31%
Day 4	5 mg/kg	253	40.4		456 0		1359.0	33.6		13.0	2.86%
Day 4	5 mg/kg	254	42.4		496.2		1364.5	36.4		13.1	2.64%
	Avg		41.1		478.6	*	1355.1	35.3	*	13.0	2.74%
	SD		3.8		54.2		12.6	3.9		0.1	0.29%
	Range	Min	36.1		422.0		1333.0	31.0		12.8	2.31%
		Max	46.6		563.4		1364.5	41.5		13.1	3.09%
	n	5	5.0		5.0		5.0	5.0		5	5
Day 29	5 mg/kg	255	20.4		340.4		1376.0	24.7		13.2	3.88%
Day 29	5 mg/kg	256	20.6		296.9		1368.0	21.7		13.1	4.42%
Day 29	5 mg/kg	257	22.1		307.2		1357.0	22.6		13.0	4.24%
Day 29	5 mg/kg	258	26.3		384.6		1372.5	28.0		13.2	3.43%
Day 29	5 mg/kg	259	30.2		383.6		1343.5	28.6		12.9	3.36%
	Avg		23.9	**	342.5	**	1363.4	25.1	**	13.1	3.87%
	SD		4.2		41.2		13.2	3.1		0.1	0.47%
	Range	Min	20 4		296.9		1343.5	21.7		12.9	3.36%
		Max	30.2		384.6		1376.0	28.6		13.2	4.42%
	n		5		5		5	5		5	5

1. PFOS equivalents in liver (ppm) = Liver PFOS (ppm) + Liver PFOSA PFOS equivalents (ppm); 2. Total PFOS equivalents in whole liver (ug) = PFOS equivalent concentration in liver (ppm) * liver weight (g); 3. Assuming a 1:1 ratio of PFOSA:PFOS equivalents, PFOS equivalents in dose (ug) = Initial BW (g) * dose (mg/kg) = BW*5; 4. % PFOS eq dosed as PFOSA in liver =(PFOS equivalents in whole liver (ug)/ PFOS equivalents in dose)*100; 5. Residual PFOS in dose (ug) = dose*0.96% = (5mg/kg*BW)0.0096; 6. % of PFOS in liver from residual PFOS in dose = (residual PFOS in dose / total PFOS eq in liver+V14)*100; * Significantly different from day 1 values by a two tailed T-test ($p<0.05$) ; ** Significantly different from day 4 values by a two tailed T-test ($p<0.05$); PQL = practical quantitation limit (PFOS = 30 ng/g; PFOSA 10ng)

Table 5: Percent Dose: Serum PFOS equivalents in PFOSA Group

Time	Dose	animal # 9R03-xxx	PFOS eq conc in Serum (ppm) [1]	PFOS eq conc in liver (ppm)	Liver/serum PFOS ratio (ppm/ppm)	Total PFOS Eq in whole liver (ug)	Serum volume (ml) [2]	Total PFOS Eq in Serum (ug) [3]	Liver/ Serum PFOS Eq ratio [4]	PFOS eq in dose (ug) [5]	% PFOS eq dosed found in serum [6]	Residual PFOS in dose (ug) [7]	% of PFOS in serum from residual PFOS in dose (%) [8]
Day 1	5 mg/kg	245	7.2	29.7	4.1	309.3	8.3	60.0	5.2	1316.0	4.6	12.6	21.1%
Day 1	5 mg/kg	246	11.2	39.3	3.5	401.4	8.4	94.1	4.3	1330.5	7.1	12.8	13.6%
Day 1	5 mg/kg	247	9.6	42.5	4.4	462.9	8.5	81.9	5.6	1332.5	6.1	12.8	15.6%
Day 1	5 mg/kg	248	7.0	27.9	4.0	281.7	8.5	59.6	4.7	1336.0	4.5	12.8	21.5%
Day 1	5 mg/kg	249	7.6	36.6	4.8	392.1	8.5	65.0	6.0	1343.5	4.8	12.9	19.8%
	Avg		8.5	35.2	4.2	369.5	8.5	72.1	5.2	1331.7	5.4	12.8	17.7%
	SD		1.8	6.2	0.5	73.4	0.1	15.3	0.7	10.1	1.1	0.1	3.5%
	Range	Min	7.0	27.9	3.5	281.7	8.3	59.6	4.3	1316.0	4.5	12.6	13.6%
		Max	11.2	42.5	4.8	462.9	8.5	94.1	6.0	1343.5	7.1	12.9	21.5%
	N	5	5	5.00	5	5	5	5	5	5	5	5	5
Day 4	5 mg/kg	250	7.8	40.3	5.1	455.8	9.2	72.5	6.3	1333.0	5.4	12.8	17.6%
Day 4	5 mg/kg	251	7.5	36.1	4.8	422.2	9.5	71.7	5.9	1360.5	5.3	13.1	18.2%
Day 4	5 mg/kg	252	11.4	46.6	4.1	563.6	9.5	108.2	5.2	1358.5	8.0	13.0	12.1%
Day 4	5 mg/kg	253	8.7	40.4	4.7	456.1	9.4	81.6	5.6	1359.0	6.0	13.0	16.0%
Day 4	5 mg/kg	254	8.0	42.4	5.3	496.4	9.3	74.2	6.7	1364.5	5.4	13.1	17.6%
	Avg		8.7	41.2	4.8	478.8	9.4	81.7	5.9	1355.1	6.0	13.0	15.9%
	SD		1.6	3.8	0.5	54.2	0.1	15.3	0.6	12.6	1.1	0.1	2.5%
	Range	Min	7.5	36.1	4.1	422.2	9.2	71.7	5.2	1333.0	5.3	12.8	12.1%
		Max	11.4	46.6	5.3	563.6	9.5	108.2	6.7	1364.5	8.0	13.1	18.2%
	N	5	5	5	5	5	5	5	5	5	5	5	5
Day 29	5 mg/kg	255	3.6	20.4	5.7	340.4	12.4	44.7	7.6	1376.0	3.2	13.2	29.6%
Day 29	5 mg/kg	256	4.0	20.6	5.2	296.9	12.3	48.9	6.1	1368.0	3.6	13.1	26.9%
Day 29	5 mg/kg	257	4.0	22.1	5.5	307.3	12.1	48.8	6.3	1357.0	3.6	13.0	26.7%
Day 29	5 mg/kg	258	4.1	26.3	6.4	384.6	12.0	49.5	7.8	1372.5	3.6	13.2	26.6%
Day 29	5 mg/kg	259	7.4	30.2	4.1	383.6	11.5	84.8	4.5	1343.5	6.3	12.9	15.2%
	Avg		4.6 **	23.9 **	5.4	342.6	12.1	55.3	6.5	1363.4	4.1	13.1	23.7%
	SD		1.5	4.2	0.8	41.2	0.4	16.6	1.3	13.2	1.3	0.1	5.6%
	Range	Min	3.6	20.4	4.1	296.9	11.5	44.7	4.5	1343.5	3.2	12.9	15.2%
		Max	7.4	30.2	6.4	384.6	12.4	84.8	7.8	1376.0	6.3	13.2	29.6%
	N		5	5	5	5	5	5	5	5	5	5	5

1. PFOS equivalents in sera (ppm) = sera PFOS (ppm) + sera PFOSA PFOS equivalents (ppm); 2. There are 32.3 mL Plasma per Kg of rat. (9); 3. Total PFOS equivalents in sera (ug) = PFOS equivalent concentration in sera (ppm) * serum volume (ml); 4. Liver/ Serum total PFOS Eq ratio = total PFOS equivalents in whole liver (ug)/ total PFOS equivalents in serum (ug); 5. Assuming a 1:1 ratio of PFOSA:PFOSA PFOS equivalents, PFOS equivalents in dose (ug) = Initial BW (g) * dose (mg/kg) = BW*5; 6. % PFOS equivalents dosed in serum = (PFOS equivalents in sera (ug)/ PFOS equivalents in dose (ug)) *100; 5. Residual PFOS in dose (ug) = dose*0.96% = (5mg/kg*BW)0.0096; 6. % of PFOS in sera from residual PFOS in dose = (residual PFOS in dose / total PFOS eq in sera)*100; ** Sign. different from dy 4 values by a two tailed T-test ($p<0.05$); MDL = method detection limit in sera: (PFOS = 4.75 ng/ml; PFOSA = 2.89 ng/ml)

Table 6 - Summary: % + SD of Dose or of PFOS Equivalents Dosed Detected in Liver & Sera			
	Day 1 post dose	day 4 post dose	day 29 post dose
% PFOSA dosed detected as PFOSA in:			
liver	5.4 ± 0.9	3.2 ± 0.3	0.1 ± 0.1
sera	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
liver & sera	5.6 ± 0.9	3.2 ± 0.3	0.1 ± 0.1
% PFOS Equivalents dosed detected as PFOS in:			
liver	22.0 ± 5.2	32.3 ± 3.7	25.2 ± 3.0
sera	5.2 ± 1.1	6.0 ± 1.1	4.1 ± 1.2
liver & sera	27.1 ± 6.2	38.3 ± 4.8	29.3 ± 3.8
NA = not analyzed			
PFOS equivalents (ppm) = PFOS (ppm) + PFOSA PFOS equivalents (ppm)			

Table 7: Serum POAA in the PFOSA Group.

Time	Dose	animal # 9R03-xxx	Sera POAA (ppm)	Total POAA in total Serum (ug)	*POAA in dose (ug)	% POAA dosed found as POAA in sera
Day 1	5 mg/kg	245	0.11	0.90	3.29	27.3%
Day 1	5 mg/kg	246	0.15	1.23	3.33	37.0%
Day 1	5 mg/kg	247	0.13	1.09	3.33	32.8%
Day 1	5 mg/kg	248	0.10	0.88	3.34	26.3%
Day 1	5 mg/kg	249	0.12	1.02	3.36	30.5%
	Avg		0.12	1.02	3.33	30.8%
	SD		0.02	0.15	0.03	4.3%
	Range	Min	0.10	0.88	3.29	26.3%
		Max	0.15	1.23	3.36	37.0%
	n	5	5	5	5	5
Day 4	5 mg/kg	250	0.07	0.63	3.33	18.9%
Day 4	5 mg/kg	251	0.07	0.64	3.40	18.9%
Day 4	5 mg/kg	252	0.11	1.00	3.40	29.4%
Day 4	5 mg/kg	253	0.10	0.95	3.40	28.0%
Day 4	5 mg/kg	254	0.08	0.76	3.41	22.2%
	Avg		0.08	0.80	3.39	23.5%
	SD		0.02	0.17	0.03	5.0%
	Range	Min	0.07	0.63	3.33	18.9%
		Max	0.11	1.00	3.41	29.4%
	n	5	5	5	5	5

Limit of detection (LOD): POAA 24.7 ng/ml
NA. Not analyzed
* based on work by Kris Hansen finding 2.5 ug/ml POAA in dosing solution
† There are 32.3 mL Plasma per Kg of rat.(13)