Study No. T-6316.9; DT21
Fluorochemical (FC) Levels in Naïve Rats
Final Report, Revision A
January 09, 2002

In-Life Start Date: July 8, 1998
In-Life End Date: July 28, 1998

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Purpose of Revision:

The purpose of this revision is to incorporate all data available to date on the Naïve Rat Study (T-6316.9) and the rat chow/fishmeal analysis. This includes data reported in the initial final Naïve Rat Study report written by Corporate Toxicology in 1998 (1), a summary report on the liver PFOS levels written by 3M Environmental in 1998 (Appendix 1), and a summary report of the liver PFOS levels and rat chow/fishmeal analyses written by 3M Environmental in 2001 (Appendix 3).

Study Background:

In an attempt to determine the source of low-level PFOS body burden found in control rats involved in some 3M contract dietary studies, a comprehensive plan with the following objectives and responsibilities was designed:

> Objective 1 - To investigate potential sources of contamination within the study housing area of the current dietary studies on perfluorinated test compounds at Covance Madison. Andrew Seacat was appointed study director with Marv Case as alternate. Jim Wolters, 3M Environmental, is responsible for coordinating and conducting direct air monitoring and wipe samples.
> Objective 2 - To determine if contamination of feed is leading to the low levels of PFOS seen in control rats in the two-year dietary study of N-Ethyl FOSE conducted at Covance Madison. Andrew Seacat was appointed study director with Marv Case as alternate.
> **Objective 3** - To investigate the background serum and liver PFOS levels in naïve rats of different age groups from different sources. Information on the various diets supplied by the different vendors is to be obtained. Deanna (Nabbefeld) Luebker was appointed study director with Marv Case as alternate.
> Objective 4 - To investigate the possibility that PFOS exposure is stemming from tainted feed, exposure in rat rooms or a combination of both. Marv Case was appointed study director.

3M Environmental Analytical Laboratory was deemed responsible for chemical analysis of samples gathered in objectives 1-4.

The study to examine objective 3 is complete and is the focus of this report. Once data addressing each objective are available, reports of the various studies will be generated.

Report Summary:

The purpose of this study was to assess "endogenous" levels of fluorochemicals (FCs) in untreated Sprague Dawley rats of three different age groups acquired from three different breeders to determine what perfluorooctane sulfonate (PFOS) levels, if any, were present. Each vendor was contacted to provide information on the feed provided to their rats while at their facilities. Levels of PFOS in the rodent feed supplied by the different vendors and in fishmeal were determined.

The livers of male and female untreated Sprague Dawley rats 6-8 weeks old, 10-14 weeks old, and approximately 9-12 months old (old retired breeders, ORB) from Charles River, Harlan, and Taconic Farms Laboratories were examined for liver PFOS levels. Male rat livers from Charles River and Taconic Farms test animals showed significant endogenous levels of PFOS that roughly correlated with age. Livers collected from the oldest group of male rats from Taconic Farms contained significantly more PFOS than any other group in the study. The livers of the female rats from Charles River and Taconic Farms contained very consistent levels of PFOS, showing no correlation with age. The livers of test animals from Harlan did not contain PFOS above the limit of detection (15 ng/g). Based on these data, PFOS had bioaccumulated to a much great degree in male Charles River and Taconic Farms than in female rats from the same vendors. This difference may have been a result of PFOS being released in the milk and/or transferred *in utero* to the pups in females that had bore and nursed multiple litters. Female rats may also more readily excrete PFOS through urinary excretion than male rats. Results of the vendor feed/diet history survey revealed that fishmeal was the primary ingredient in feed supplied to Taconic Farms rats and the 5th listed ingredient in feed provided to Charles River rats. Fishmeal was not listed as an ingredient feed supplied to Harlan rats. Detectable levels of PFOS were found in samples of the chow fed to both Charles River and Taconic Farms rats. No PFOS could be detected in chow provided to Harlan rats (fishmeal free). Analyses of fishmeal (six types arising from a minimum of 3 kinds of fish) suggest that it may be the source of PFOS in the chow. To definitively make this conclusion, analysis of the actual fishmeal incorporated into each of the chow samples analyzed is required. Another possible source of PFOS is the paper coating used in feed bags. Further information on feed packaging may be gathered in a future study.

Methods:

This study was performed in the 3M Strategic Toxicology Laboratory under a defined protocol (2). All in-life procedures were approved by the 3M Laboratory Animal Review Committee (3M LARC).

Thirty Sprague Dawley rats, five male and five female from each of the following age groups: 6-8 weeks old, 10-14 weeks old, and ~ 9-12 weeks old (old retired breeder, ORBs) were ordered from Charles River, Harlan, and Taconic Farms. Each vendor was asked to provide the name of the feed provided to their rats, name and location of feed vendor, feed ingredient list and information on feed packaging. All animals remained in their shipping containers between arrival at 3M and euthanization. No food or water, other than that provided in the shipping containers, was furnished. Within one hour of arrival at 3M, rats were weighed, grossly examined, and euthanized by CO_2 asphyxiation. Sera and liver were harvested and sent to Kris Hansen, 3M Environmental Laboratory - Fluorine Analytical Chemistry Team (FACT), for PFOS analysis.

Liver specimens were analyzed as described in the Experimental Section of Appendix 1. Sera samples have not been analyzed but will be retained for possible future analysis. Statistical analysis to detect significant differences ($p \leq 0.05$) in liver PFOS concentration

between age groups, sexes, and vendors in the Charles River and Taconic Farms rats was performed using a one-tailed, homoscedastic T-test. The rate of change in PFOS levels (ppb/week) was estimated by dividing the change in PFOS concentration by the number of weeks evaluated (approximately 38.5 weeks).

Rat chow from each of the vendors and fish meal (six types arising from a minimum of 3 kinds of fish) were analyzed for PFOS concentrations as described in Appendix 2.

Results:

In Life Data & Liver PFOS Analysis

In life data including body weights, liver weights, and liver/body weight ratios as well as liver PFOS concentrations can be found in Tables 1-3. Detailed results of the FC analysis, including graphs and a full data table, can be found in Appendix 1.

Liver PFOS levels in Charles River male and female rats at 6-8 weeks of age averaged 43.0 ± 4.07 and 66.6 ± 19.27 ppb, respectively. At 10-14 weeks of age, liver PFOS levels in male and female Charles River rats had increased to 103.0 ± 21.33 and 79.9 ± 12.06 ppb, respectively. Liver PFOS levels in Charles River male and female ORBs were 145.0 ± 12.42 and 67.8 ± 29.63 ppb, respectively (See Table 1 and Appendix 1).

Liver PFOS levels in all Harlan rats were below the method detection limit of 15 ppb (See Table 2 and Appendix 1).

Liver PFOS levels in Taconic Farms male and female rats at 6-8 weeks of age averaged 60.2 ± 12.21 and 73.0 ± 12.29 ppb, respectively. At 10-14 weeks of age, liver PFOS levels in male and female Taconic Farms rats had increased to 92.2 ± 22.75 and 75.7 ± 24.40 ppb, respectively. Liver PFOS levels in Charles River male and female ORBs were 327.0 ± 115.37 and 65.6 ± 27.24 ppb, respectively (See Table 3 and Appendix 1).

The average rate of change in PFOS liver concentration for male and female Charles River rats from week 7 to week 45.5 was estimated to be approximately 2.6 and 0.031 ppb/week, respectively. The average rate of change in PFOS liver concentration for male and female Taconic Farms rats over the same time-period was estimated to be approximately 6.9 and –0.19 ppb/week, respectively.

Results of the T-test are included in Table 4. The change in liver PFOS concentration between age groups in male Charles River and Taconic Farms rats was significant at all timepoints ($p \leq 0.05$). Livers from Charles River male rats contained significantly more PFOS at all age groups than did livers of Charles River female rats of the same age group. Livers of male Taconic Farms ORBs contained significantly more PFOS than livers of female Taconic Farms ORBs. Livers of male Taconic Farms 6-8 week and ORB rats contained significantly higher PFOS concentrations than did male Charles River rats of the same age groups.

Vendor Feed/Diet Survey

Charles River rats are fed PMI 5L79 Rat and Mouse Diet supplied by PMI Nutrition International Inc., Brentwood, MO. PMI feed is packaged in paper bags coated with a paper coating material produced by 3M. The feed ingredients, as listed by PMI Nutrition International Inc., are as follows:

> ground yellow corn, wheat middlings, soybean meal, animal fat preserved with BHA, **fish meal,** alfalfa meal, cane molasses, calcium carbonate, salt, cyanocobalamin (source of vitamin B-12), biotin, DL methionine, calcium
>
> pantothenate, folic acid, riboflavin, cholecalciferol (source of vitamin D-3), vitamin A acetate, di-alpha tocopheryl acetate (source of vitamin E), thiamin, magnesium oxide, sodium selenite, nicotinic acid, pyridoxine hydrochloride, menadione dimethylprimidinol bisulfite (source of vitamin K activity), silicon dioxide, calcium iodate, manganous oxide, copper sulfate, cobalt carbonate, ferrous carbonate, zinc sulfate, zinc oxide.

Harlan rats are fed Harlan Teklad LM-485 Mouse/Rat Sterilizable Diet supplied by Harlan Teklad, Madison, WI. Teklad feed is also packaged in paper bags. It is unknown at this time, however, whether or not these bags are coated with a 3M material. The Teklad feed ingredients, as listed by Teklad, are as follows:

> ground corn, soybean meal, ground oats, wheat middlings, alfalfa meal, soybean oil, corn gluten meal, calcium carbonate, dicalcium phosphate, brewers dried yeast, iodized salt, L-lysine, DL-methionine, vitamin A-acetate, D-activated animal sterol (source of vitamin D_3), vitamin E supplement, niacin, calcium pantothenate, riboflavin, thiamin mononitrate, pyridoxine hydrochloride, menadione sodium bisulfite complex (source of vitamin K), folic acid, biotin, vitamin B_{12} supplement, calcium carbonate, manganous oxide, ferrous sulfate, copper sulfate, zinc oxide, calcium iodate, cobalt carbonate.

Taconic Farms rats are fed TAC #31 Rodent Diet supplied by Zigler Co., Gardner, PA. TAC #31 is packaged in a "commercially acceptable 3 ply laminated paper bag". It is unknown at this time whether or not a 3M material is used in these bags. The feed ingredients in TAC #31, as listed by Zigler Co., are as follows:

> **fish meal,** soybean meal, alfalfa meal, corn gluten meal, ground whole wheat, ground #2 yellow corn, ground whole oats, wheat middlings, Brewer's dried yeast, soybean oil, salt, dicalcium phosphate, ground limestone, vitamin and mineral premixes.

Rodent Feed PFOS Analyses

PMI Lab diet, fed to rats obtained from Charles River contained approximately 18 ng/g PFOS. Rodent diet from Zigler, fed to rats purchased from Taconic Farms contained approximately 12 ng/g of PFOS. Teklad rat/rodent diet, fed to rats obtained from Harlan (fishmeal free), did not contain PFOS above the limit of quantitation (LOQ) (see Appendix 2).

Fish Meal PFOS Analyses

Six types of fishmeal arising from at least three types of fish were analyzed for PFOS (see Appendix 2). PFOS was detected in 50% of the fishmeal samples analyzed. It is not known, however, what type of fishmeal was incorporated into the various rat chows. Therefore, it cannot be definitively concluded that fishmeal is the source of PFOS in the rat chow. Analysis of the actual fishmeal incorporated into the chow is required to provide a direct link.

Conclusions:

Based on these data, PFOS had bioaccumulated to a much great degree in male rats from Charles River and Taconic Farms than in female rats from these same vendors. This difference may have been a result of PFOS being released in the milk and/or transferred *in utero* to the pups in females that had bore and nursed multiple litters. Female rats may also more readily excrete PFOS through urinary excretion than male rats. The livers of test animals from Harlan did not contain PFOS above the limit of detection (15 ng/g). Results of the feed analyses revealed detectable levels of PFOS in samples of the chow fed to both Charles River and Taconic Farms rats. No PFOS could be detected in chow provided to Harlan rats (fishmeal free). Analyses of fishmeal (six types arising from a minimum of 3 kinds of fish) suggest that it may be the source of PFOS in the chow. To definitively make this conclusion, analysis of the actual fishmeal incorporated into each of the chow samples analyzed is required. Another possible source of PFOS is the paper coating used in feed bags. As stated in the results section, 3M supplies paper coating to PMI, the manufacturer of Charles River PMI chow. It is not known, however, if Harlan and Taconic Farms feed suppliers also use 3M material in their bags. Further information on feed packaging may be gathered in a future study.

List of Tables:

1) Charles River In-Life and Liver PFOS Data

2) Harlan In-Life and Liver PFOS Data

3) Taconic Farms In-Life and Liver PFOS Data

4) T-Test Results (p values) – Charles River and Taconic Farms Age, Sex, and Vendor Liver PFOS Comparision.

Table 1: Charles River In-Life and Liver PFOS Data

Vendor	ID	Sex	Age Grp.	Body wt (g)	Liver wt (g)	liver wt/ body wt	PFOS Liver Conc. (ng/g or ppb) Individual	Ave	Std Dev
CHARLES									
RIVER	CR1	M	6-8 wks	171.7	8.30	0.048	**305	43.0	4.07
	CR2	M	6-8 wks	179.6	8.30	0.046	48.8		
	CR3	M	6-8 wks	161.5	7.05	0.044	42.6		
	CR4	M	6-8 wks	151.6	6.60	0.044	39.5		
	CR5	M	6-8 wks	166.8	8.06	0.048	41.1		
	CR6	F	6-8 wks	173.7	8.69	0.050	54.9	66.6	19.27
	CR7	F	6-8 wks	168.3	7.13	0.042	62.0		
	CR8	F	6-8 wks	165.1	7.46	0.045	65.4		
	CR9	F	6-8 wks	141.7	4.91	0.035	99.6		
	CR10	F	6-8 wks	159.7	6.47	0.041	51.2		
	CR11	M	10-14 wks	324.3	10.55	0.033	100.0	103.0	21.33
	CR12	M	10-14 wks	367.9	12.66	0.034	140.0		
	CR13	M	10-14 wks	356.7	13.10	0.037	96.8		
	CR14	M	10-14 wks	358.5	13.23	0.037	87.6		
	CR15	M	10-14 wks	365.6	13.65	0.037	90.1		
	CR16	F	10-14 wks	226.1	10.49	0.046	72.2	79.9	12.06
	CR17	F	10-14 wks	199.5	6.32	0.032	93.9		
	CR18	F	10-14 wks	203.4	6.13	0.030	71.6		
	CR19	F	10-14 wks	196.4	7.14	0.036	92.2		
	CR20	F	10-14 wks	209.8	7.08	0.034	69.6		
	CR21	M	*ORB	406.2	9.75	0.024	131.0	145.0	12.42
	CR22	M	ORB	342.3	10.09	0.029	133.0		
	CR23	M	ORB	395.1	10.83	0.027	151.0		
	CR24	M	ORB	363.7	9.24	0.025	149.0		
	CR25	M	ORB	460.2	13.93	0.030	160.0		
	CR26	F	ORB	394.2	12.70	0.032	119.0	67.8	29.63
	CR27	F	ORB	412.5	16.09	0.039	67.1		
	CR28	F	ORB	482.9	14.80	0.031	45.0		
	CR29	F	ORB	485.3	14.96	0.031	55.5		
	CR30	F	ORB	424.2	11.34	0.027	53.0		

* ORB = old retired breeders
** Outlier - not used in calculations.
Method detection limit (MDL): PFOS = 15ng/g or ppb

Table 2: Harlan In-Life and Liver PFOS Data

Vendor	ID	Sex	Age Grp.	Body wt (g)	Liver wt (g)	liver wt/ body wt	PFOS Liver Conc. (ng/g or ppb) Individual	Ave	Std Dev
HARLAN									
	H1	M	6-8 wks	198.4	9.45	0.048	<MDL	<MDL	
	H2	M	6-8 wks	201.7	9.34	0.046	<MDL		
	H3	M	6-8 wks	203.4	8.50	0.042	<MDL		
	H4	M	6-8 wks	203.9	8.38	0.041	<MDL		
	H5	M	6-8 wks	204.2	9.17	0.045	<MDL		
	H6	F	6-8 wks	214.3	7.62	0.036	<MDL	<MDL	
	H7	F	6-8 wks	208.0	7.19	0.035	<MDL		
	H8	F	6-8 wks	212.8	7.14	0.034	<MDL		
	H9	F	6-8 wks	209.3	7.30	0.035	<MDL		
	H10	F	6-8 wks	209.0	8.08	0.039	<MDL		
	H11	M	10-14 wks	300.6	10.69	0.036	<MDL	<MDL	
	H12	M	10-14 wks	307.9	12.33	0.040	<MDL		
	H13	M	10-14 wks	304.0	11.26	0.037	<MDL		
	H14	M	10-14 wks	299.8	10.09	0.034	<MDL		
	H15	M	10-14 wks	306.0	11.38	0.037	<MDL		
	H16	F	10-14 wks	237.7	6.65	0.028	<MDL	<MDL	
	H17	F	10-14 wks	241.1	7.20	0.030	<MDL		
	H18	F	10-14 wks	239.7	7.14	0.030	<MDL		
	H19	F	10-14 wks	236.3	8.54	0.036	<MDL		
	H20	F	10-14 wks	233.4	7.33	0.031	<MDL		
	H21	M	*ORB	470.7	17.00	0.036	<MDL	<MDL	
	H22	M	ORB	462.8	14.90	0.032	<MDL		
	H23	M	ORB	446.6	15.55	0.035	<MDL		
	H24	M	ORB	458.2	14.52	0.032	<MDL		
	H25	M	ORB	478.9	11.19	0.023	<MDL		
	H26	F	ORB	337.8	10.45	0.031	<MDL	<MDL	
	H27	F	ORB	326.2	10.27	0.031	<MDL		
	H28	F	ORB	285.7	8.54	0.030	<MDL		
	H29	F	ORB	286.2	9.01	0.031	<MDL		
	H30	F	ORB	306.9	11.00	0.036	<MDL		

* ORB = old retired breeders
Method detection limit (MDL): PFOS = 15ng/g or ppb

Table 3: Taconic Farms In-Life and Liver PFOS Data

Vendor	ID	Sex	Age Grp.	Body wt (g)	Liver wt (g)	liver wt/ body wt	PFOS Liver Conc. (ng/g or ppb) Individual	Ave	Std Dev
TACONIC									
FARMS	TF1	M	6-8 wks	200.2	9.64	0.048	63.6	60.2	12.21
	TF2	M	6-8 wks	202.4	10.18	0.050	63.0		
	TF3	M	6-8 wks	184.1	9.28	0.050	47.7		
	TF4	M	6-8 wks	187.5	10.26	0.055	49.2		
	TF5	M	6-8 wks	183.6	9.34	0.051	77.5		
	TF6	F	6-8 wks	201.2	9.73	0.048	67.0	73.0	12.29
	TF7	F	6-8 wks	189.9	8.40	0.044	89.3		
	TF8	F	6-8 wks	172.5	9.40	0.055	78.8		
	TF9	F	6-8 wks	207.9	10.28	0.049	73.2		
	TF10	F	6-8 wks	199.5	10.54	0.053	56.6		
	TF11	M	10-14 wks	454.3	17.97	0.040	86.5	92.2	22.75
	TF12	M	10-14 wks	334.6	13.07	0.039	54.7		
	TF13	M	10-14 wks	459.9	17.65	0.038	84.9		
	TF14	M	10-14 wks	407.5	14.17	0.035	119.0		
	TF15	M	10-14 wks	317.8	12.75	0.040	86.2		
	TF16	F	10-14 wks	214.4	9.33	0.044	52.9	75.7	24.40
	TF17	F	10-14 wks	226.1	8.94	0.040	71.5		
	TF18	F	10-14 wks	216.1	8.47	0.039	72.6		
	TF19	F	10-14 wks	241.0	11.21	0.047	64.4		
	TF20	F	10-14 wks	246.5	8.53	0.035	117.0		
	TF21	M	*ORB	687.8	22.22	0.032	151.0	327.0	115.37
	TF22	M	ORB	561.2	18.24	0.033	441.0		
	TF23	M	ORB	456.0	14.96	0.033	379.0		
	TF24	M	ORB	525.7	17.91	0.034	390.0		
	TF25	M	ORB	586.6	18.24	0.031	276.0		
	TF26	F	ORB	281.5	19.56	0.069	59.5	65.6	27.24
	TF27	F	ORB	281.4	18.81	0.067	105.0		
	TF28	F	ORB	247.5	15.65	0.063	71.3		
	TF29	F	ORB	289.5	14.95	0.052	63.0		
	TF30	F	ORB	338.7	17.58	0.052	29.0		

* ORB = old retired breeders
Method detection limit (MDL): PFOS = 15ng/g or ppb

Table 4: T-Test Results (p values) – Charles River and Taconic Farms Age, Sex, and Vendor Liver PFOS Comparision

Vendor	Age/Sex Group Comparision								
	Male			Female			Male/Female		
	6-8 & 10-14 wk	*10-14wk & ORB*	*6-8wk & ORB*	*6-8 & 10-14 wk*	*10-14wk & ORB*	*6-8wk & ORB*	*6-8 wk & 6-8 wk*	*10-14wk & 10-14wk*	*ORB & ORB*
Charles River	**0.00**	**0.00**	**0.00**	0.11	0.21	0.47	**0.02**	**0.03**	**0.00**
Taconic Farms	**0.03**	**0.00**	**0.00**	0.42	0.28	0.30	0.07	0.25	**0.00**
	Vendor Comparision								
	Male			**Female**			**xxxxxxxxxxxxxxxxxxxxx**		
Charles River	*6-8 wk & 6-8 wk*	*10-14wk & 10-14wk*	*ORB & ORB*	*6-8 wk & 6-8 wk*	*10-14wk & 10-14wk*	*ORB & ORB*			
vs Taconic Farms	**0.03**	0.13	**0.00**	0.28	0.37	0.45			
T-TEST - one-tailed distribution, homoscedastic									

References:

1) 3M Medical Department, Toxicology Services Report for Study No. T-6316.9; DT21. Fluorochemical (FC) Levels in Naïve rats. Sept., 1998.

2) 3M Medical Department, Toxicology Services Protocol for Study No. T-6316.9; DT21. Fluorochemical (FC) Levels in Naïve rats. July, 1998.

Signatures:

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Appendix 1

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Study of PFOS levels in Naïve Rats
Summary report

Experimental Summary

In order to assess "endogenous" levels of PFOS in test animals, the livers of ninety rats from three different suppliers were quantitatively analyzed for PFOS. Three distinct ages of rats were represented in the group of animals received from each supplier: 6-8 weeks old, 10-14 weeks old, and retired breeders (> 14 weeks). The test animals, received by the Toxicology Department at 3M, were sacrificed upon receipt; tissue samples were delivered to the 3M Environmental Lab for extraction and analysis by FACT.

Supplier	Location	Chow	# at 6-8 wks	# at 10-14 wks	#>14 wks	Ratio male:female
Charles River	Raleigh, NC	Purina*	10	10	10	1:1
Harlan	Indianapolis, IN	TEKlad	10	10	10	1:1
Taconic Farms	Germantown, NY	TAC #31	10	10	10	1:1

*3M is/was a supplier of paper coating material to Purina Mills, Inc. Currently, it is not known if 3M supplies material to TEKlad or Taconic Farms.

Analytical Summary

Liver samples were homogenized and extracted using an ion-paring reagent. The extracts were analyzed quantitatively using high-pressure liquid chromatography-electrospray tandem mass spectrometry (HPLC-ESMSMS) and evaluated versus an extracted curve. Analytical details are available in the full report. The presence or absence of other known fluorochemical contaminants and metabolites was ascertained by inspection.

Results Summary

Rat livers from Charles River and Taconic Farms test animals showed significant endogenous levels of PFOS. Livers collected from oldest group of male rats from Taconic Farms contained significantly more PFOS than any other group in the study.

The livers of test animals from Harlan did not contain PFOS above the limit of detection (15 ppb).

PFOS levels in the livers of male rats from Charles River and Taconic Farms roughly correlated with the age of the animals. That is, livers collected from the youngest male rats, 6-8 weeks old, contained the least PFOS, while the old, retired breeder male rats contained the highest concentration.

The livers of the female rats from Charles River and Taconic Farms were determined to contain very consistent levels of PFOS, showing no correlation with age.

No other known fluorochemical contaminants or metabolites were identified in the liver samples analyzed in this study.

Graphical results and a full table of results are attached.

Appendix 1

Currently, methods are being developed for the analysis of low levels of PFOS and ethyl-FOSE alcohol in samples of chow from each supplier.

Experimental

Sample preparation-aqueous samples, HPLC-ESMS: Ion-pairing extraction

Analyte is extracted from a sample matrix with an ion-pairing reagent (tetrabutyl ammonium hydrogen sulfate (TBA)) in a pH-controlled environment. The cationic reagent selectively targets anionic fluorochemicals. Once the anion-TBA pair is formed, the analyte is transferred into a non-polar organic solvent (ethyl acetate), dried, and reconstituted in methanol for MS analysis.

HPLC-ESMS and HPLC-ESMSMS: For detailed qualitative work

In HPLC, an aliquot of extract is injected and passed through a reverse-phase liquid chromatographic column. Based on the affinity of the analyte for the stationary phase in the column relative to the liquid mobile phase, the analyte is retained for a characteristic amount of time. For example, in a standard solution PFOS may elute at 10.5 minutes. Retention times between a standard PFOS solution and the analyte extracted from groundwater in this analysis were matched to within 1% on the HPLC system.

Following HPLC separation, ESMS provides a rapid and accurate means for analyzing a wide range of organic compounds, including fluorochemicals. Electrospray, an ionization technique used primarily for the detection of molecular ions, is generally operated at relatively mild temperatures. Molecules are ionized, possibly fragmented, and detected.

ESMSMS adds an additional dimension of certainty to compound identification. As in ESMS, a characteristic primary ion is selected. However, instead of simply monitoring the primary ion, in ESMSMS the ion is bombarded with high-energy gas. As a result of high- energy collisions, smaller secondary ionic fragments unique to the primary ion are created and detected.

For example, for PFOS ($C_7F_{15}SO_3^-$) analysis, ion 499 is selected as the characteristic primary ion. This ion is fragmented into other ions such as 80 amu (corresponding to SO_3^-), 99 amu (corresponding to FSO_3^-), 130 amu (corresponding to $CF_2SO_3^-$), 180 amu ($C_2F_4SO_3^-$), and 230 amu ($C_3F_6SO_3^-$). Each of these secondary fragments is detected and can be used to differentiate PFOS from other compounds that might have the same characteristic 499 amu primary ion but different chemical compositions and secondary ion fragmentation patterns.

HPLC system: Hewlett-Packard Series 1100 Liquid Chromatograph

Column:	Keystone Betasil C18 column
	2 X 100 mm, 5 µm particle size
Flow rate:	300 µl/min
Solvent A:	2.0 mM ammonium acetate
Solvent B:	Methanol
Solvent Gradient:	40% to 90% B in 8.5 minutes
	Hold at 90% B for 3 minutes
	Return to 40% B in 1 minute
	Hold at 40% B for 1 minute
Injection volume: 10 µL	
Run time:	13.5 minutes

Electrospray Tandem Mass Spectrometer
Micromass Quattro II API mass spectrometer mass spectrometer
Mass Lynx 3.1 software

Appendix 1

Cone voltage:	30-60V
Collision gas energy:	40 eV
Mode:	electrospray negative
Source block temperature:	115°C
Desolvation temperature:	250°C
Primary Ion:	499
Daughter Ions:	80, 99, 130, 180
Electrode:	Z-spray

Quality control summary

All analyses were conducted with a moderate to high level of quality control. Duplicate matrix spike analyses were conducted for one animal from each group of animals. Except as noted in the results table, recoveries were within the acceptable range of 80-120%.

A calibration check standard was analyzed every 5-10 samples to monitor instrumental drift. Quantiation was based on linear regression analysis of two curves bracketing each group of samples. Quantitation of PFOS was based on the response of 3-4 daughter ions of the primary ion.

Results

See spreadsheet attached to this report.

FACT members participating:

K. Hansen
L. Clemen
H. Johnson
M. Ellefson
G. Langenburg
R. Wynne
I.A. Smith
S. Heimdahl

Appendix 1

Study:	FC Levels using Naïve Rats T-6316.9					
Product Number(Test Substance):	T-6316 (EtFOSE-OH)					
Matrix:	Rat Liver			Filename:	See Attachments, full report only	
Method/Revision:	FACT-M-1.0 & FACT-M-2.0			R-Squared Value:	See Attachments, full report only	
Analytical Equipment System Number:	Amelia 062498	Madeline 041098	Madeline 041098	Slope:	See Attachments, full report only	
Instrument Software/Version:	MassLynx 3.0	MassLynx 3.0	MassLynx 3.0	Y-Intercept:	See Attachments, full report only	
Date of Extraction/Analyst:	7/14/98 IAS/SAH	7/30/98 SAH	8/03/98 IAS			
Date of Analysis/Analyst:	7/27/98 KJH	7/31/98 MEE	8/04/98 KJH			
Date of Data Reduction/Analyst:	7/28/98 KJH	8/03/98 KJH	8/05/98 KJH			

Sample Data

RAT LIVER

Group Dose	Sample #	PFOS Calc. Conc. ng/g	Amount of PFOS ng/g	Average PFOS ng/g	RSD Std. Dev.	Sample #	PFOS Calc. Conc. ng/g	Amount of PFOS ng/g	Average PFOS ng/g	RSD Std. Dev.	Sample #	PFOS Calc. Conc. ng/g	Amount of PFOS ng/g	Average PFOS ng/g	RSD Std. Dev.
Method Blk	H2O Blk-1	<MDL	<MDL	0	0	H2O Blk-1	<MDL	<MDL			H2O Blk-1	<MDL	<MDL		
Method Blk	H2O Blk-2	<MDL	<MDL	<MDL	<MDL	H2O Blk-2	<MDL	<MDL	<MDL	<MDL	H2O Blk-2	<MDL	<MDL	<MDL	<MDL
Matrix Blk	Rat Liver Blk-1	<MDL	<MDL	0	0	Rat Liver Blk-1	<MDL	<MDL			Rat Liver Blk-1	<MDL	<MDL		
Matrix Blk	Rat Liver Blk-2	5.82	0.00582	0.00582	NA	Rat Liver Blk-2	<MDL	<MDL	<MDL	<MDL	Rat Liver Blk-2	<MDL	<MDL	<MDL	<MDL
QC - 100 ppb	CR-1M-MS	107	90%			S398-178-MS	86.0	72%			TF-1M-MS	87.7	73%		
	CR-1M-MSD	98.0	82%	86%	0.0552	S398-178-MSD	105	88%	80%	0.114	TF-1M-MSD	80.3	67%	70%	0.0439
Group 1	CR-1M	305	0.305			H-1M	<MDL	<MDL			TF-1M	63.6	0.0636		
Week 6-8	CR-2M	48.8	0.0488		9.50**	H-2M	<MDL	<MDL			TF-2M	63.0	0.0630		
	CR-3M	42.6	0.0426	0.0430**	0.00408**	H-3M	<MDL	<MDL			TF-3M	47.7	0.0477		
	CR-4M	39.5	0.0395		123	H-4M	<MDL	<MDL		<MDL	TF-4M	49.2	0.0492		20.3
	CR-5M	41.1	0.0411	0.0954	0.117	H-5M	<MDL	<MDL	<MDL	<MDL	TF-5M	77.5	0.0775	0.0602	0.0122
	CR-6F	54.9	0.0549			H-6F	<MDL	<MDL			TF-6F	67.0	0.0670		
	CR-7F	62.0	0.0620			H-7F	<MDL	<MDL			TF-7F	89.3	0.0893		
	CR-8F	65.4	0.0654			H-8F	<MDL	<MDL			TF-8F	78.8	0.0788		
	CR-9F	99.6	0.0996		28.9	H-9F	<MDL	<MDL		<MDL	TF-9F	73.2	0.0732		16.8
	CR-10F	51.2	0.0512	0.0666	0.0193	H-10F	<MDL	<MDL	<MDL	<MDL	TF-10F	56.6	0.0566	0.0730	0.0123
Group 2	CR-11 M	100	0.0999			H-11 M	<MDL	<MDL			TF-11 M	86.5	0.0865		
Week 10-14	CR-12M	140	0.140			H-12M	<MDL	<MDL			TF-12M	84.7	0.0847		
	CR-13M	96.8	0.0968			H-13M	<MDL	<MDL			TF-13M	84.9	0.0849		
	CR-14M	87.6	0.0876		20.6	H-14M	<MDL	<MDL		<MDL	TF-14M	119	0.119		16.2
	CR-15M	90.1	0.0901	0.103	0.0212	H-15M	<MDL	<MDL	<MDL	<MDL	TF-15M	86.2	0.0862	0.0922	0.0149
	CR-16F	72.2	0.0722			H-16F	<MDL	<MDL			TF-16F	52.9	0.0529		
	CR-17F	93.9	0.0939			H-17F	<MDL	<MDL			TF-17F	71.5	0.0715		
	CR-18F	71.6	0.0716			H-18F	<MDL	<MDL			TF-18F	72.6	0.0726		
	CR-19F	92.2	0.0922		15.1	H-19F	<MDL	<MDL		<MDL	TF-19F	64.4	0.0644		32.3
	CR-20F	69.6	0.0696	0.0799	0.0121	H-20F	<MDL	<MDL	<MDL	<MDL	TF-20F	117	0.117	0.0757	0.0244
Group 3	CR-21M	131	0.131			H-21M	<MDL	<MDL			TF-21M	151	0.151		
Old Retired Breeder	CR-22M	133	0.133			H-22M	<MDL	<MDL			TF-22M	441	0.441		
	CR-23M	151	0.151			H-23M	<MDL	<MDL			TF-23M	379	0.379		
	CR-24M	140	0.140		8.69	H-24M	<MDL	<MDL		<MDL	TF-24M	390	0.390		35.3
	CR-25M	160	0.160	0.145	0.0126	H-25M	<MDL	<MDL	<MDL	<MDL	TF-25M	276	0.276	0.327	0.116
	CR-26F	119	0.119			H-26F	<MDL	<MDL			TF-26F	59.5	0.0595		
	CR-27F	67.1	0.0671			H-27F	<MDL	<MDL			TF-27F	105	0.105		
	CR-28F	45.0	0.0450			H-28F	<MDL	<MDL			TF-28F	71.3	0.0713		
	CR-29F	55.5	0.0555		43.4	H-29F	<MDL	<MDL		<MDL	TF-29F	63.0	0.0630		41.6
	CR-30F	53.0	0.0530	0.0678	0.0295	H-30F	<MDL	<MDL	<MDL	<MDL	TF-30F	29.0	0.0290	0.0656	0.0273

Practical Quantitation Limit (PQL) = PFOS = 30 ng/g, PFOSA = 10 ng/g, PFOSAA = 60 ng/g, EtFOSE = 60 ng/g

Method Detection Limit (MDL): PFOS = 15 ng/g, PFOSA = 5 ng/g, PFOSAA = 30 ng/g, EtFOSE = 60 ng/g

PFOS = Perfluorooctanesulfonate

PFOSA = Perfluorooctanesulfonamide

PFOSAA = Perfluorooctanesulfonamidoacetate

EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol

CR = Charles River

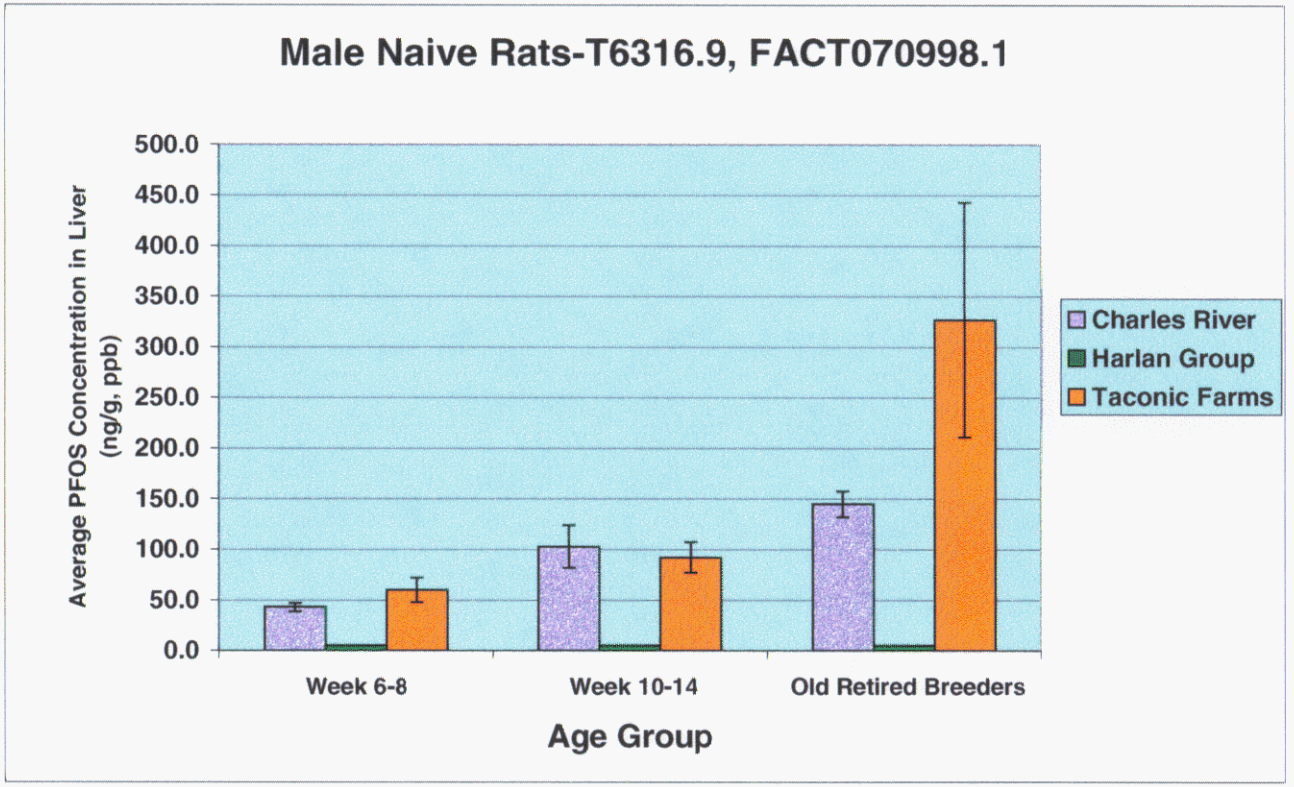
H = Harlan Group

TF = Taconic Farms

** CR1 was a confirmed outlier and not included in these calculations; data is OK.

Date Entered/By:	7/29/98 LAC	8/03/98 LAC	8/05/98 LAC
Date Verified/ By:	8/07/98 KJH	8/07/98 KJH	8/07/98 KJH

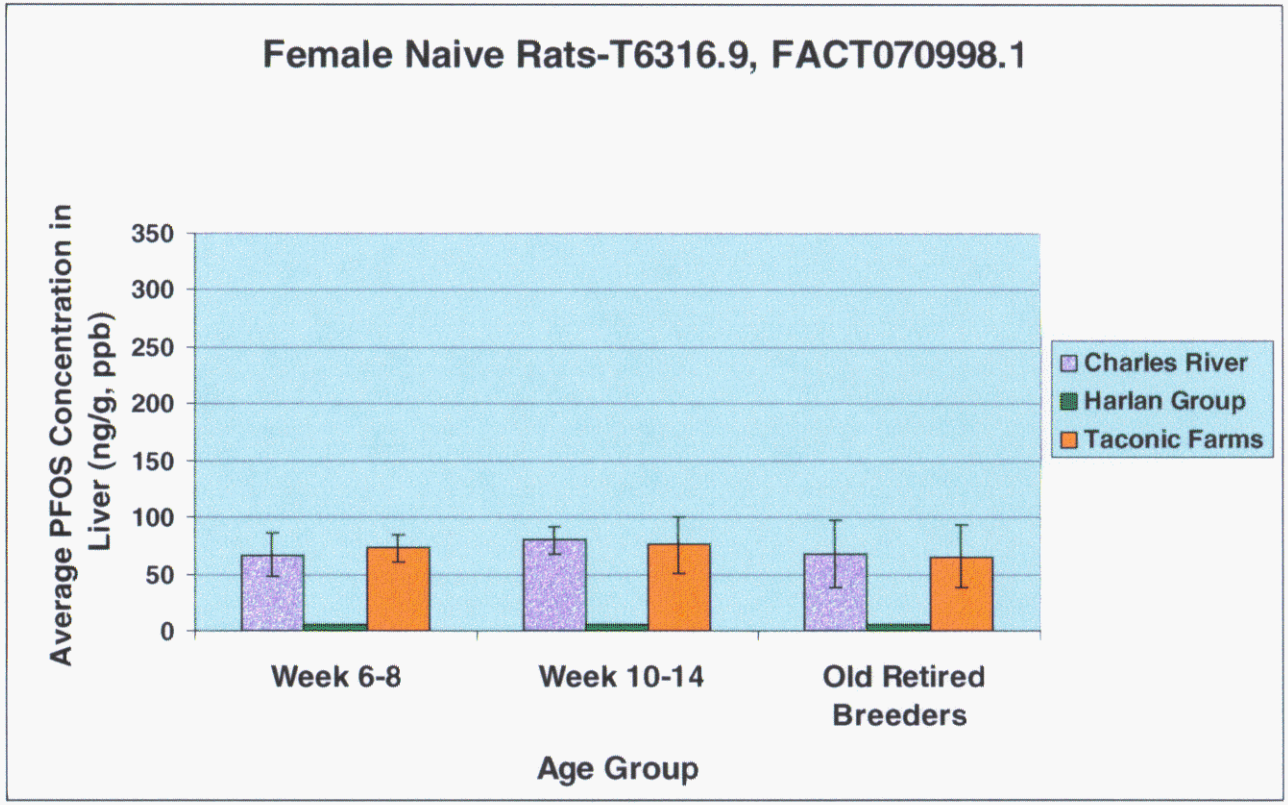
Appendix 1



Charles River Rats (Raleigh, North Caroline) were fed PMI (Purina Mills) Chow
Harlan Group Rats (Indianapolis, Indiana) were fed TEKlad Chow
Taconic Farms Rats (Germantown, New York) were fed TAC#31 Chow

Average PFOS levels determined from population n = 5 (per Age Group, per Source), except Charles River 6-8 Week Male Rats
This data set used only 4 samples in calculation of average PFOS levels due to an outlier.
Harlan Group PFOS results were all < PFOS MDL (15 ppb)

Appendix 1



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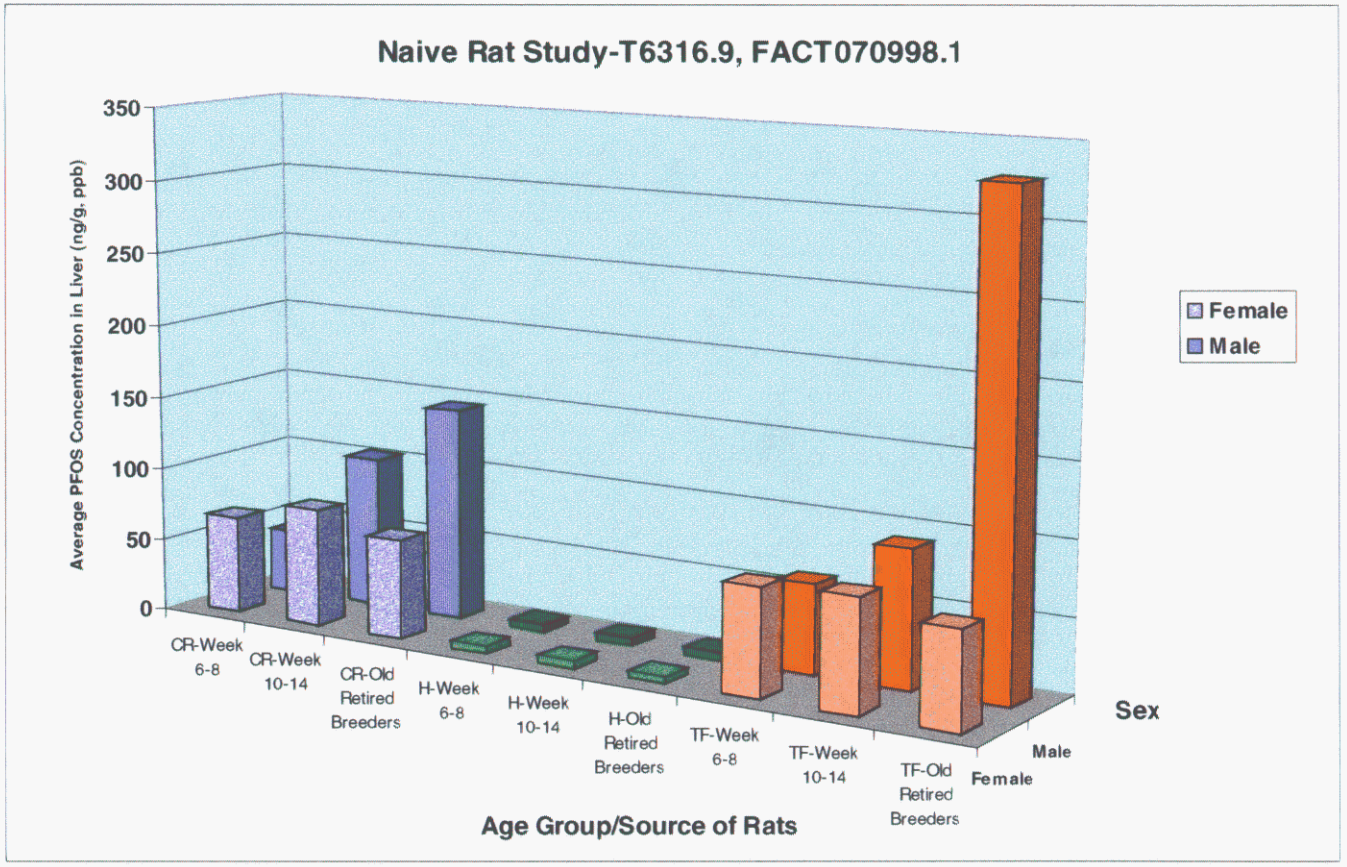

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This data set used only 4 samples in calculation of average PFOS levels due to an outlier.
Harlan Group PFOS results were all < PFOS MDL (15 ppb)

Appendix 2

SUMMARY OF NAÏVE RAT/RAT CHOW/FISH MEAL STUDY ANALYSES
Conducted by the 3M Environmental Lab (prepared 03/01/01)

Naïve Rats

(Taken from summary report issued in 1998.) In order to assess "endogenous" levels of PFOS in test animals, the livers of ninety rats from three different suppliers were quantitatively analyzed for PFOS. Three distinct ages of rats were represented in the group of animals received from each supplier: 6-8 weeks old, 10-14 weeks old, and retired breeders (> 14 weeks).

Rat livers from Charles River and Taconic Farms test animals showed significant endogenous levels of PFOS. Livers collected from oldest group of male rats from Taconic Farms contained significantly more PFOS than any other group in the study.

The livers of test animals from Harlan did not contain PFOS above the limit of detection (15 ng/g).

PFOS levels in the livers of male rats from Charles River and Taconic Farms roughly correlated with the age of the animals. That is, livers collected from the youngest male rats, 6-8 weeks old, contained the least PFOS, while the old, retired breeder male rats contained the highest concentration.

The livers of the female rats from Charles River and Taconic Farms were determined to contain very consistent levels of PFOS, showing no correlation with age.

Limits of Data:

Screening for other known metabolites of Et-FOSE-OH (e.g. PFOSA, PFOSAA) was performed. The original summary report document stated that "No other known fluorochemical contaminants or metabolites were identified in the liver samples analyzed in this study." However, based on the levels of PFOS observed in the liver samples, the analytical LOQ may not have been sufficient to see expected metabolite levels. (Based on the 2 years Et-FOSE-OH feeding study, PFOSAA levels would be expected to be approximately 9% of PFOS levels; PFOSA concentrations would be expected to be approximately 5% of PFOS levels. Back calculated, the expected concentrations of the metabolites are near the 1998 LOQ. Data from the relatively high-dose 2 year feeding study may or may not provide an acceptable model of this low-level exposure.)

Spike recovery studies were conducted for PFOS in rat livers and were acceptable. Matrix spike studies conducted for other analytes were, for the most part, acceptable.

Rat Chow

Rat chow from 4 separate vendors was investigated. Two of the sources of rat chow were determined to contain PFOS above the limit of quantitation.

NIH/PMI Lab diet, fed to rats obtained from Charles River was determined to contain approx 18 ng/g PFOS. Small animal diet from Zeigler, fed to rats purchased from Taconic Farms was determined to contain approximately 12 ng/g of PFOS. Teklad rat/rodent diet, fed to rats obtained from Harlan, did not contain PFOS above the LOQ.

Limits of the Data:

Appendix 2

Spike recoveries studies were conducted on each type of chow tested and all results were acceptable in all cases (77-98%). The LOQ for PFOS in rat chow is approximately 2-10 ng/g.

Fish Meal

There is great variability in both the source and types of fishmeal available commercially. This variability complicates the analysis of fishmeal and the interpretation of data concerning fishmeal.

Fishmeals used for animal and fish diet are typically generated from what are referred to as "industrial fish". Industrial fish, including menhaden and herring, are usually quite high in oil content and thus not fit for human consumption. Approximately 90% of the world's fishmeal is produced from industrial fish. Fishmeal is processed in factories or directly on the fishing vessel and results in the production of several separate fishmeal products. After the fish are cooked, a liquor (oil, water, and protein) is separated from the solids by "pressing". The solid that remains after pressing is called the "presscake", the liquid component, oil and soluble protein, is "stickwater". Subsequent to pressing, the oil is separated from the remaining components of the stickwater by centrifugation. After further distillation, this fish oil may be directly incorporated into animal diet. The remaining stickwater is evaporated down to a thick syrup of 30-50% solids and may be sold as "condensed fish solubles". The syrup may also be added back to the presscake and dried and sold as "whole meal". Presscake without the syrup is sold as "presscake meal." All of these components may be incorporated into animal feed.

According to the University of Florida Corporate Extension Service, good quality fishmeal averages 60-70% protein; the oil content ranges from 2-15%.

Fishmeal is incorporated into food for a variety of animals and fish including farm-raised salmon, poultry, and cows (particularly dairy cows).

In this study, six types of fishmeal, arising from at least 3 types of fish, were analyzed.

- Menhaden travel in large schools and migrate up the coast each year. They are found in warmer, near shore waters and in the US, are fished primarily off Atlantic Coast. Menhaden constitute 98% of the fishmeal produced in the US.

- Capelin, also a schooling fish, spend much of their lives off shore in the deep waters of the Northern Pacific and the Arctic and move in-shore only to spawn.

- The habitat for different classes of herring is much more far ranging than that of either menhaden or capelin. Different types of herring can be found from the Northern Pacific waters off the coast of Canada and down to the southeastern Atlantic coast. Like menhaden and capelin, herring are small, schooling fish and thus are targeted for large-scale netting.

Type of Fishmeal Analyzed	**PFOS (ng/g)**
Herring	< LOQ (approx 3 ng/g)
Capelin	< LOQ (approx 3 ng/g)
Protein Concentrate (type of fishmeal not specified)	< LOQ (approx 3 ng/g)
Omega Protein FAQ (type of fishmeal not specified)	15.7
Omega Protein Sealac (presumed to be menhaden)	9.87
Menhaden Fishmeal	6.10

Appendix 2

Limits of the data:

Matrix spike studies were conducted on a single type of fishmeal (menhaden). PFOS spiked into the menhaden fishmeal was recovered at approximately 66%. Matrix spike studies were not conducted for the remaining types of fishmeal tested.

The LOQ for PFOS determination in these fishmeal samples was approximately 3.5 ng/g.

Summary of the 3 components of this study:

Vendor of Rats	Rats with endogenous PFOS?	Rat Chow used by Vendor Contains PFOS?	Rat Chow used by Vendor Contains fishmeal?
Charles River	Yes	Yes	Yes
Taconic Farms	Yes	Yes	Yes
Harlan	No (LOQ=30 ng/g)	No (LOQ=2 ng/g)	No

Limits of the study:

Because it is not known what type of fishmeal was incorporated into the various rat chows, it cannot be definitively concluded that fishmeal is the source of PFOS in the rat chow. Although indirect evidence indicates this probability, because PFOS was only detected in 50% of the fishmeal samples analyzed, analysis of the actual fishmeal incorporated into the chow is required to provide a direct link.

Other variables affecting the rats prior to shipment to 3M (such as PFOS levels in water and in other components of the feed) have not been tested. To the best of my memory, this chow has not been tested for the potential of EtFOSE-OH transfer from packaging.

Should further work be conducted on the tissue samples, a lower level screen for metabolites and other known fluorochemicals is recommended. This information would provide additional evidence concerning the origin of PFOS identified in rat livers.

The motivation to pursue any of these additional investigations depends on the corporation's end use of this data. The original study objective: "... to determine what PFOS levels, if any, are present in Sprague Dawley rats of three different ages from three different suppliers" (Medical Dept. protocol for study # T-6319.9) was completed upon issuance of the Environmental Lab summary report in 1998.