3M MEDICAL DEPARTMENT, TOXICOLOGY SERVICES

Report for Study No. T-6316.9; DT21

Fluorochemical (FC) Levels in Naïve Rats

In-Life Start Date: July 8, 1998

In-Life End Date: July 28, 1998

Background:

In an attempt to determine the source of low-level perfluorooctanesulfonate (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. Dr. Andrew Seacat was appointed study director with Dr. 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 Perfluorooctanesulfonamido ethanol (N-Ethyl FOSE) conducted at Covance Madison. Dr. Andrew Seacat was appointed study director with Dr. 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 breeders is to be obtained. Deanna Nabbefeld was appointed study director with Dr. 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. Dr. Marv Case was appointed study director.

3M Environmental Analytical Laboratory is 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.

Study Objective:

This study was designed to determine what perfluorooctanesulfonate (PFOS) levels, if any, can be detected in untreated Sprague Dawley rats of three different age groups acquired from three different breeders. In addition, information on the rodent feed provided by the various animal suppliers was obtained.

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Summary:

The livers of male and female untreated Sprague Dawley rats of three different age groups (6-8 weeks old, 10-14 weeks old and old retired breeders (ORB) / ≈ 9-12 months old) from three different breeders (Breeder A, Breeder B and Breeder C) were examined for PFOS and other known fluorochemical (FC) metabolites. Each breeder was contacted to provide information on the feed provided to their rats while at their facilities.

Livers from Breeder A and Breeder C rats contained detectable levels of PFOS (≥15ppb). Male rat livers from both sources increased significantly ($p = 0.05$) in PFOS concentration with age, (slope = 51 Breeder A male; slope = 133 Breeder C male). The concentration of PFOS in livers from female Breeder A and Breeder C rats, however, did not increase with age (slope = 0.6 Breeder A female; slope = -3.7 Breeder C female). The livers of Breeder C male ORBs had the highest PFOS levels (average = 327 ± 115 ppb), significantly higher than the levels found in Breeder A male ORBs (average = 145 ± 12 ppb). No other FC metabolites could be detected in the Breeder A or Breeder C rat livers. No PFOS or other FC metabolites could be detected in the livers of Breeder B rats.

Breeder A rats are fed feed A supplied by Company A (City A, State A). Breeder B rats are fed feed B supplied by Company B (City B, State B). Breeder C rats are fed feed C supplied by Company C (City C, State C). Fish meal is the primary ingredient in feed C, the fifth listed ingredient in feed A and not present in feed B.

From these data, it appears as though PFOS greatly bioaccumulates in male rats, while in female rats it does so to a lesser degree. Possible explanations for this difference are that PFOS is released in the milk and/or transferred *in utero* to the pups in females that have bore and nursed multiple litters. Also, female rats may more readily excrete PFOS through urinary excretion than male rats. It is postulated that fish meal, an ingredient in some rodent chow, may contain FC and thus lead to the PFOS found in untreated rats. The possibility that 3M material is used to coat the feed bags and that this is a source of PFOS is also being considered.

Methods:

Thirty Sprague Dawley rats (five male, five female per age group; age groups = 6-8 weeks old, 10-14 weeks old and old retired breeder (ORB) / ≈ 9-12 months old) were ordered from each of the following breeders: Breeder A, Breeder B and Breeder C. Each breeder was asked to provide the name of the feed provided to their rats, name and location of feed breeder, 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. Sera and liver were harvested and sent to Kris Hansen, 3M Environmental Laboratory - Fluorine Analytical Chemistry Team (FACT), for FC analysis. (A description of the analysis method can be found in Appendix 1, Experimental section.) Statistical significance of results was determined using the students T-Test ($p = 0.05$). The rate of change of PFOS levels was analyzed by plotting age in months versus PFOS concentration (ppb) and calculating the slope of the line.

Results:

Laboratory Data

Raw data, including body weights, liver weights, liver/body weight ratios and PFOS concentrations can be found in Appendix 1. Results of the FC analysis, including graphs and a full data table, can be found in Appendix 2 - a Summary Report prepared by Kris Hansen.

Liver samples were deemed most meaningful and thus analyzed first. Sera samples have not yet been analyzed. Depending on the outcomes of objectives 1, 2 and 4 of the overall study plan (see background section, pg. 1), sera PFOS analysis may not performed.

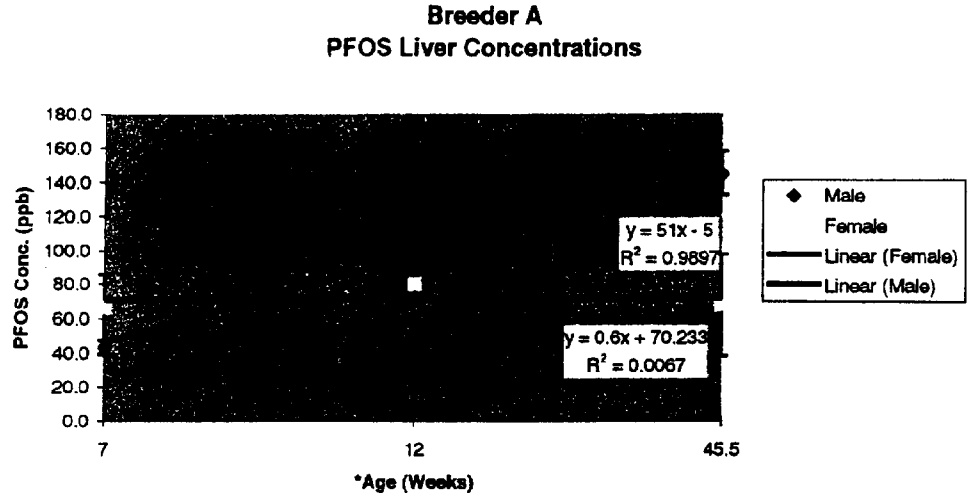
Average PFOS liver levels ± standard deviation for the various age / sex / breeder groups

included in this study are as follows:

Breeder A 6-8 week old Male	43.0 ± 4.07 ppb
Breeder A 6-8 week old Female	66.6 ± 19.27
Breeder A 10-14 week old Male	103.0 ± 21.33
Breeder A 10-14 week old Female	79.9 ± 12.06
Breeder A ORB Male	145.0 ± 12.42
Breeder A ORB Female	67.8 ± 29.63
Breeder B all groups	< method detection limit (15 ppb)
Breeder C 6-8 week old Male	60.2 ± 12.21 ppb
Breeder C 6-8 week old Female	73.0 ± 12.29
Breeder C 10-14 week old Male	92.2 ± 22.75
Breeder C 10-14 week old Female	75.7 ± 24.40
Breeder C ORB Male	327.0 ± 115.37
Breeder C ORB Female	65.6 ± 27.24

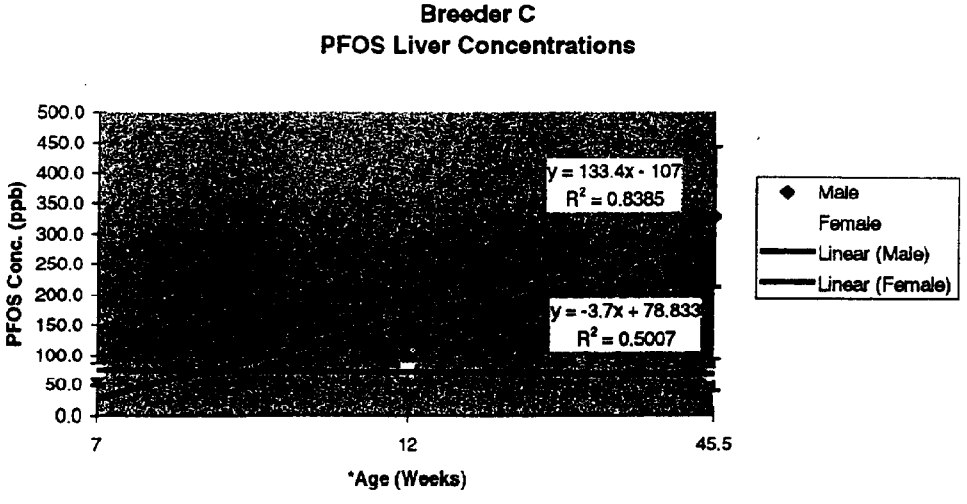
The rate of change of PFOS liver concentration with age is depict in Figures 1 and 2 for Breeder A and Breeder C rats respectively. The slope of the line for male Breeder A rats is 51 while that for female Breeder A rats is 0.6. The slope of the line for male and female Breeder C rats is 133 and -3.7 respectively.

Figure 1



* 7 weeks = 6-8 weeks old; 12 weeks = 10-14 weeks old, 45.5 weeks = ORB.

Figure 2



* 7 weeks = 6-8 weeks old; 12 weeks = 10-14 weeks old, 45.5 weeks = ORB.

The increase in PFOS concentration found in the livers of male Breeder A rats was significant between all three age groups ($p = 0.05$). The increase in PFOS levels in male Breeder C rats was significant between the 10-14 week old group and the ORB group, but not between the 6-8 and 10-14 week old groups. Comparing males to females at each age

group the Breeder A 10-14 week old groups differed significantly and the ORB groups from both Breeder A and Breeder C differed significantly. Comparing Breeder A rats to Breeder C rats at each age/sex group, male Breeder C ORB rats had significantly higher PFOS liver concentrations than did male Breeder A rats of the same age group. All other age/sex groups did not significantly differ in PFOS levels between the two breeders. Results of the T-Tests are shown in Table 1.

Table 1
STATISTICS
T-TEST - two-tailed distribution, paired, p = 0.05 ,
*** = statistically significant**

Breeder	Age/Sex Group Comparison								
	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*
A	*0.0123	*0.0388	*0.0008	0.1139	0.4702	0.9459	0.1043	*0.0478	*0.0125
C	0.1213	*0.0112	*0.0079	0.8620	0.6474	0.3421	0.2564	0.5374	*0.0045
	Breeder Comparison								
	Male			Female			xxxxxxxxxxxxxxxxxxxx		
	6-8 wk & 6-8 wk	*10-14wk & 10-14wk*	*ORB & ORB*	*6-8 wk & 6-8 wk*	*10-14wk & 10-14wk*	*ORB & ORB*			
A vs. C	0.0997	0.4305	*0.0232	0.5157	0.7749	0.9004			

Breeder Feed/Diet History

Breeder A rats are fed feed A supplied by company A (city A, state A). Feed A is packaged in paper bags coated with a paper coating material produced by 3M. The feed ingredients, as listed by company A, 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.

Breeder B rats are fed feed B supplied by company B (city B, state B). Feed B 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 feed B ingredients, as listed by company B, 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.

Breeder C rats are fed feed C supplied by company C (city C, state C). Feed C 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 feed C, as listed by company C., 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.

Conclusions/Future Directions:

The livers of Breeder B rats appear free of FC. Livers from male rats supplied by Breeder A and Breeder C increase in PFOS concentration with age. Livers from female rats supplied by Breeder A have detectable levels of PFOS but these levels plateau, slightly increasing from 6-8 weeks old to 10-14 weeks old and decreasing slightly in old retired breeders. Female rats supplied by Breeder C actually decrease slightly, although not significantly, in PFOS liver concentration with age. From these data, it appears as though PFOS greatly bioaccumulates in male rats, while in female rats it does so to a lesser degree. One hypothesis is that PFOS is released in the milk and/or transferred *in utero* to the pups in females that have bore and nursed multiple litters. Another possibility is that female rats are more readily able to clear PFOS through urinary excretion than are male rats.

Although the sources of PFOS leading to the liver concentrations identified in this study are unknown, one hypothesis is that PFOS is contained in the rat chow (Objective 2). It is thought that fish meal may be an ingredient containing PFOS. While not present in the

Breeder B rat chow, fish meal is the primary ingredient listed for Breeder C rat chow and the 5th ingredient listed for Breeder A chow. Analysis of the FC content of the rat chow used by each animal breeder is currently underway. In addition, samples of the fish meal may be obtained and analyzed for FC content.

Another possible source of PFOS is the paper coating used in feed bags. As stated in the results section, 3M supplies paper coating to Company A, the manufacturer of Feed A. It is not known at this time if 3M material is also used in bags for Feeds B and C. Further information on feed packaging will be gathered in a separate study.

Signatures:

Prepared By:

Deanna Nabbefeld, MS
Advanced Toxicologist

Date

Reviewed By:

Dr. Marv Case
Corporate Scientist

Date

Dr. Andrew Seacat
Senior Research Toxicologist

Date

Appendix 1

Breeder	ID	Sex	Age Grp.	Body wt (g)	Liver wt (g)	liver wt/ body wt	PFOS Liver Conc. (ng/g or ppb) Individual	Ave/Grp	Std/Grp
A									
	A1	M	6-8 wks	171.7	8.30	0.048	**305	43.0	4.07
	A2	M	6-8 wks	179.6	8.30	0.046	48.8		
	A3	M	6-8 wks	161.5	7.05	0.044	42.6		
	A4	M	6-8 wks	151.6	6.60	0.044	39.5		
	A5	M	6-8 wks	166.8	8.06	0.048	41.1		
	A6	F	6-8 wks	173.7	8.69	0.050	54.9	66.6	19.27
	A7	F	6-8 wks	168.3	7.13	0.042	62.0		
	A8	F	6-8 wks	165.1	7.46	0.045	65.4		
	A9	F	6-8 wks	141.7	4.91	0.035	99.6		
	A10	F	6-8 wks	159.7	6.47	0.041	51.2		
	A11	M	10-14 wks	324.3	10.55	0.033	100.0	103.0	21.33
	A12	M	10-14 wks	367.9	12.66	0.034	140.0		
	A13	M	10-14 wks	356.7	13.10	0.037	96.8		
	A14	M	10-14 wks	358.5	13.23	0.037	87.6		
	A15	M	10-14 wks	365.6	13.65	0.037	90.1		
	A16	F	10-14 wks	226.1	10.49	0.046	72.2	79.9	12.06
	A17	F	10-14 wks	199.5	6.32	0.032	93.9		
	A18	F	10-14 wks	203.4	6.13	0.030	71.6		
	A19	F	10-14 wks	196.4	7.14	0.036	92.2		
	A20	F	10-14 wks	209.8	7.08	0.034	69.6		
	A21	M	*ORB	406.2	9.75	0.024	131.0	145.0	12.42
	A22	M	ORB	342.3	10.09	0.029	133.0		
	A23	M	ORB	395.1	10.83	0.027	151.0		
	A24	M	ORB	363.7	9.24	0.025	149.0		
	A25	M	ORB	460.2	13.93	0.030	160.0		
	A26	F	ORB	394.2	12.70	0.032	119.0	67.8	29.63
	A27	F	ORB	412.5	16.09	0.039	67.1		
	A28	F	ORB	482.9	14.80	0.031	45.0		
	A29	F	ORB	485.3	14.96	0.031	55.5		
	A30	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

Appendix 1

Breeder	ID	Sex	Age Grp.	Body wt (g)	Liver wt (g)	liver wt/ body wt	PFOS Liver Conc. (ng/g or ppb) Individual	Ave/Grp	Std/Grp
B									
	B1	M	6-8 wks	198.4	9.45	0.048	<MDL	<MDL	
	B2	M	6-8 wks	201.7	9.34	0.046	<MDL		
	B3	M	6-8 wks	203.4	8.50	0.042	<MDL		
	B4	M	6-8 wks	203.9	8.38	0.041	<MDL		
	B5	M	6-8 wks	204.2	9.17	0.045	<MDL		
	B6	F	6-8 wks	214.3	7.62	0.036	<MDL	<MDL	
	B7	F	6-8 wks	208.0	7.19	0.035	<MDL		
	B8	F	6-8 wks	212.8	7.14	0.034	<MDL		
	B9	F	6-8 wks	209.3	7.30	0.035	<MDL		
	B10	F	6-8 wks	209.0	8.08	0.039	<MDL		
	B11	M	10-14 wks	300.6	10.69	0.036	<MDL	<MDL	
	B12	M	10-14 wks	307.9	12.33	0.040	<MDL		
	B13	M	10-14 wks	304.0	11.26	0.037	<MDL		
	B14	M	10-14 wks	299.8	10.09	0.034	<MDL		
	B15	M	10-14 wks	306.0	11.38	0.037	<MDL		
	B16	F	10-14 wks	237.7	6.65	0.028	<MDL	<MDL	
	B17	F	10-14 wks	241.1	7.20	0.030	<MDL		
	B18	F	10-14 wks	239.7	7.14	0.030	<MDL		
	B19	F	10-14 wks	236.3	8.54	0.036	<MDL		
	B20	F	10-14 wks	233.4	7.33	0.031	<MDL		
	B21	M	*ORB	470.7	17.00	0.036	<MDL	<MDL	
	B22	M	ORB	462.8	14.90	0.032	<MDL		
	B23	M	ORB	446.6	15.55	0.035	<MDL		
	B24	M	ORB	458.2	14.52	0.032	<MDL		
	B25	M	ORB	478.9	11.19	0.023	<MDL		
	B26	F	ORB	337.8	10.45	0.031	<MDL	<MDL	
	B27	F	ORB	326.2	10.27	0.031	<MDL		
	B28	F	ORB	285.7	8.54	0.030	<MDL		
	B29	F	ORB	286.2	9.01	0.031	<MDL		
	B30	F	ORB	306.9	11.00	0.036	<MDL		

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

Appendix 1

Breeder	ID	Sex	Age Grp.	Body wt (g)	Liver wt (g)	liver wt/ body wt	PFOS Liver Conc. (ng/g or ppb) Individual	Ave/Grp	Std/Grp
C									
	C1	M	6-8 wks	200.2	9.64	0.048	63.6	60.2	12.21
	C2	M	6-8 wks	202.4	10.18	0.050	63.0		
	C3	M	6-8 wks	184.1	9.28	0.050	47.7		
	C4	M	6-8 wks	187.5	10.26	0.055	49.2		
	C5	M	6-8 wks	183.6	9.34	0.051	77.5		
	C6	F	6-8 wks	201.2	9.73	0.048	67.0	73.0	12.29
	C7	F	6-8 wks	189.9	8.40	0.044	89.3		
	C8	F	6-8 wks	172.5	9.40	0.055	78.8		
	C9	F	6-8 wks	207.9	10.28	0.049	73.2		
	C10	F	6-8 wks	199.5	10.54	0.053	56.6		
	C11	M	10-14 wks	454.3	17.97	0.040	86.5	92.2	22.75
	C12	M	10-14 wks	334.6	13.07	0.039	54.7		
	C13	M	10-14 wks	459.9	17.65	0.038	84.9		
	C14	M	10-14 wks	407.5	14.17	0.035	119.0		
	C15	M	10-14 wks	317.8	12.75	0.040	86.2		
	C16	F	10-14 wks	214.4	9.33	0.044	52.9	75.7	24.40
	C17	F	10-14 wks	226.1	8.94	0.040	71.5		
	C18	F	10-14 wks	216.1	8.47	0.039	72.6		
	C19	F	10-14 wks	241.0	11.21	0.047	64.4		
	C20	F	10-14 wks	246.5	8.53	0.035	117.0		
	C21	M	*ORB	687.8	22.22	0.032	151.0	327.0	115.37
	C22	M	ORB	561.2	18.24	0.033	441.0		
	C23	M	ORB	456.0	14.96	0.033	379.0		
	C24	M	ORB	525.7	17.91	0.034	390.0		
	C25	M	ORB	586.6	18.24	0.031	276.0		
	C26	F	ORB	281.5	19.56	0.069	59.5	65.6	27.24
	C27	F	ORB	281.4	18.81	0.067	105.0		
	C28	F	ORB	247.5	15.65	0.063	71.3		
	C29	F	ORB	289.5	14.95	0.052	63.0		
	C30	F	ORB	338.7	17.58	0.052	29.0		

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

Appendix 2

3M Environmental Laboratory- Fluorine Analytical Chemistry Team

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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
Breeder A	City A, State A	* Feed A	10	10	10	1:1
Breeder B	City B, State B	Feed B	10	10	10	1:1
Breeder C	City C, State C	Feed C	10	10	10	1:1

*3M is/was a supplier of paper coating material to company A, supplier of feed A. Currently, it is not known if 3M supplies material to company B or C, suppliers of feeds B and C respectively.

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.

Appendix 2

Results Summary

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

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

PFOS levels in the livers of male rats from Breeder A and Breeder C 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 Breeder A and Breeder C 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.

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.

Appendix 2

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

Cone voltage:	30-60V
Collision gas energy:	40 eV
Mode:	electrospray negative
Source block temperature:	115°C
Desolvation temperature:	250°C
Primary Ion:	499

Appendix 2

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 2

Study:	FC Levels using Native Rats T-6316.9			
Product Number/Test Substance:	T-6316 (EtFOSE-OH)			
Matrix:	Rat Liver			
Method/Revision:	FACT-M-1.0 & FACT-M-2.0			
Analytical Equipment System Number:	Amelia 062498	Madeline 041098	Madeline 041098	
Instrument Software/Version:	MassLynx 3.0	MassLynx 3.0	MassLynx 3.0	
Date of Extraction/Analyst:	7/14/98 IAS/SAJ	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	

Filename: See Attachments, full report only
R-Squared Value: See Attachments, full report only
Slope: See Attachments, full report only
Y-Intercept: See Attachments, full report only

Sample Data

RAT LIVER

Group Dose	Sample #	PFOS Calc. Conc. ng/g	Amount of PFOS ug/g	Average PFOS ug/g	RSD Std. Dev.	Sample #	PFOS Calc. Conc. ng/g	Amount of PFOS ug/g	Average PFOS ug/g	RSD Std. Dev.	Sample #	PFOS Calc. Conc. ng/g	Amount of PFOS ug/g
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
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
QC - 100 ppb	A-1M-MS	107	90%			S398-178-MS	86.0	72%			C-1M-MS	87.7	73%
	A-1M-MSD	98.0	82%	86%	0.0652	S398-178-MSD	105	88%	80%	0.114	C-1M-MSD	80.3	67%
Group 1	A-1M	305	0.305			B-1M	<MDL	<MDL			C-1M	63.6	0.0636
Week 6-8	A-2M	48.8	0.0488		9.50**	B-2M	<MDL	<MDL			C-2M	63.0	0.0630
	A-3M	42.6	0.0426	0.0430**	0.00408**	B-3M	<MDL	<MDL			C-3M	47.7	0.0477
	A-4M	39.5	0.0395		123	B-4M	<MDL	<MDL	<MDL		C-4M	49.2	0.0492
	A-5M	41.1	0.0411	0.0954	0.117	B-5M	<MDL	<MDL	<MDL	<MDL	C-5M	77.5	0.0775
	A-6F	54.9	0.0549			B-6F	<MDL	<MDL			C-6F	67.0	0.0670
	A-7F	62.0	0.0620			B-7F	<MDL	<MDL			C-7F	89.3	0.0893
	A-8F	65.4	0.0654			B-8F	<MDL	<MDL			C-8F	78.8	0.0788
	A-9F	99.6	0.0996		28.9	B-9F	<MDL	<MDL		<MDL	C-9F	73.2	0.0732
	A-10F	51.2	0.0512	0.0666	0.0193	B-10F	<MDL	<MDL	<MDL	<MDL	C-10F	56.6	0.0566
Group 2	A-11M	100	0.0999			B-11M	<MDL	<MDL			C-11M	86.5	0.0865
Week 10-14	A-12M	140	0.140			B-12M	<MDL	<MDL			C-12M	84.7	0.0847
	A-13M	96.8	0.0968			B-13M	<MDL	<MDL			C-13M	84.9	0.0849
	A-14M	87.6	0.0876		20.6	B-14M	<MDL	<MDL		<MDL	C-14M	119	0.119
	A-15M	90.1	0.0901	0.103	0.0212	B-15M	<MDL	<MDL	<MDL	<MDL	C-15M	86.2	0.0862
	A-16F	72.2	0.0722			B-16F	<MDL	<MDL			C-16F	52.9	0.0529
	A-17F	93.9	0.0939			B-17F	<MDL	<MDL			C-17F	71.5	0.0715
	A-18F	71.6	0.0716			B-18F	<MDL	<MDL			C-18F	72.6	0.0726
	A-19F	92.2	0.0922		15.1	B-19F	<MDL	<MDL		<MDL	C-19F	64.4	0.0644
	A-20F	69.6	0.0696	0.0799	0.0121	B-20F	<MDL	<MDL	<MDL	<MDL	C-20F	117	0.117
Group 3	A-21M	131	0.131			B-21M	<MDL	<MDL			C-21M	151	0.151
Old Retired Breeds	A-22M	133	0.133			B-22M	<MDL	<MDL			C-22M	441	0.441
	A-23M	151	0.151			B-23M	<MDL	<MDL			C-23M	379	0.379
	A-24M	149	0.149		8.69	B-24M	<MDL	<MDL		<MDL	C-24M	390	0.390
	A-25M	160	0.160	0.145	0.0126	B-25M	<MDL	<MDL	<MDL	<MDL	C-25M	276	0.276
	A-26F	119	0.119			B-26F	<MDL	<MDL			C-26F	59.5	0.0595
	A-27F	67.1	0.0671			B-27F	<MDL	<MDL			C-27F	105	0.105
	A-28F	45.0	0.0450			B-28F	<MDL	<MDL			C-28F	71.3	0.0713
	A-29F	55.5	0.0555		43.4	B-29F	<MDL	<MDL		<MDL	C-29F	63.0	0.0630
	A-30F	53.0	0.0530	0.0678	0.0295	B-30F	<MDL	<MDL	<MDL	<MDL	C-30F	29.0	0.0290

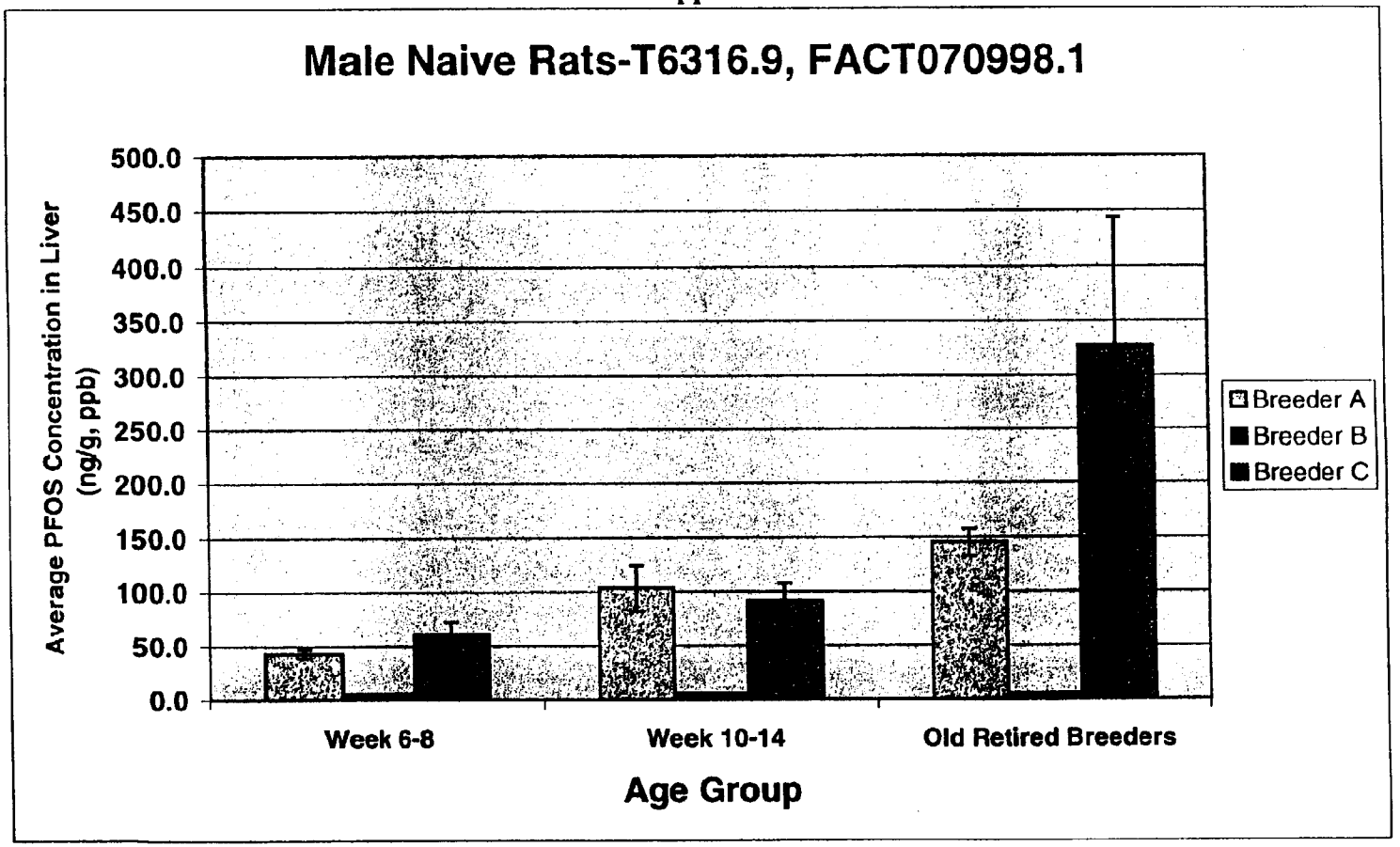
Practical Quantitation Limit (PQL) = PFOS = 30 ng/g, PFOSA = 10 ng/g, PFOSAA = 60 ng/g, EtFOSE = 60 ng/g
Method Detection Limit (MDL): PFOS = 15 ng/g, PFOSA = 5 ng/g, PFOSAA = 30 ng/g, EtFOSE = 60 ng/g
PFOS = Perfluorooctanesulfonate
PFOSA = Perfluorooctanesulfonamide
PFOSAA = Perfluorooctanesulfonamidoacetate
EtFOSE = Narrow Range N-Ethyl Perfluorooctanesulfonamido ethyl alcohol

A = breeder A
B = breeder B
C = breeder C

** A1 was confirmed an outlier and not included in these calculations; data is OK.

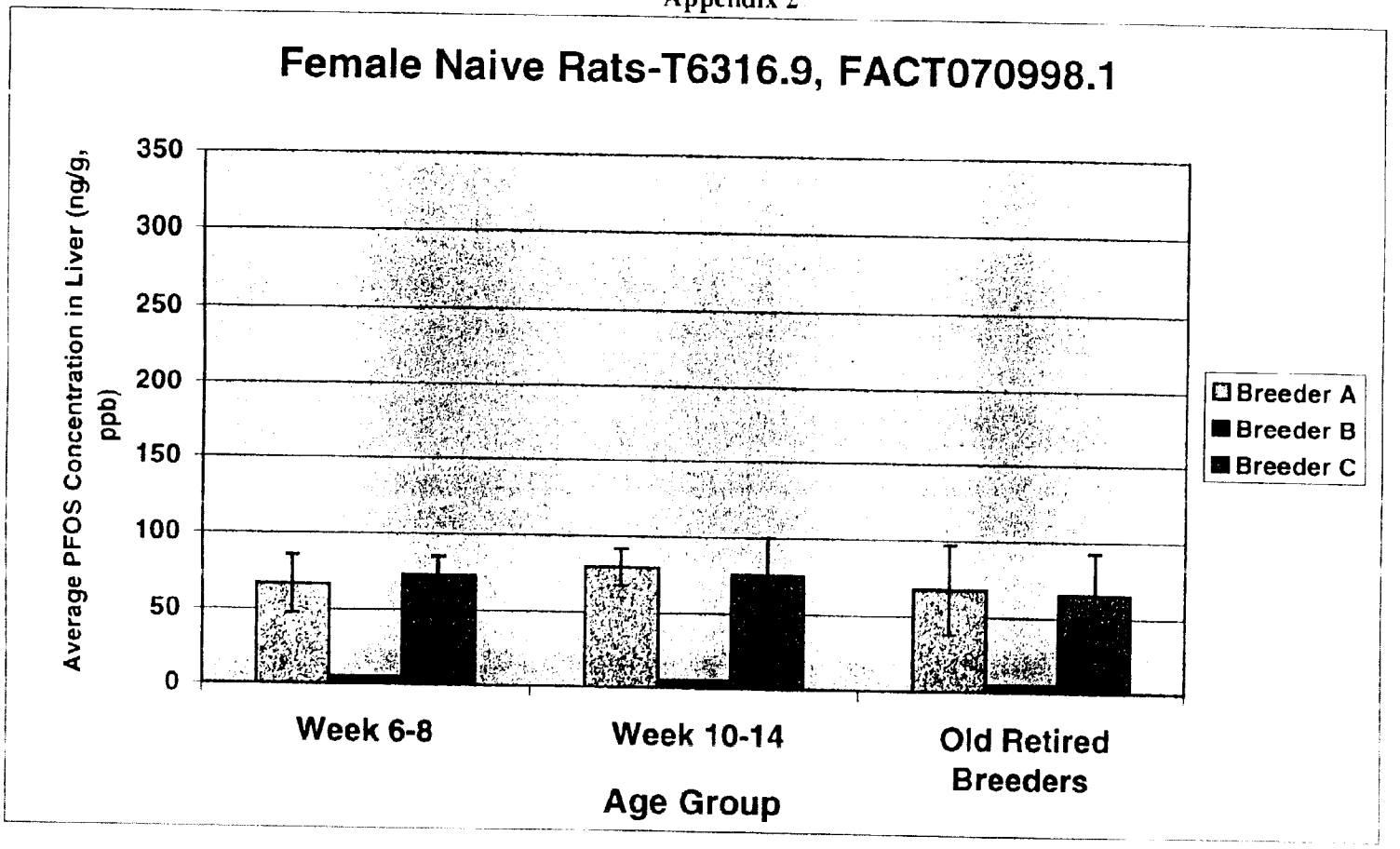
Date Entered/By: 7/28/98 LAC 8/03/98 LAC 8/06/98 LAC
Date Verified/ By: 8/07/98 KJH 8/07/98 KJH 8/07/98 KJH

Appendix 2



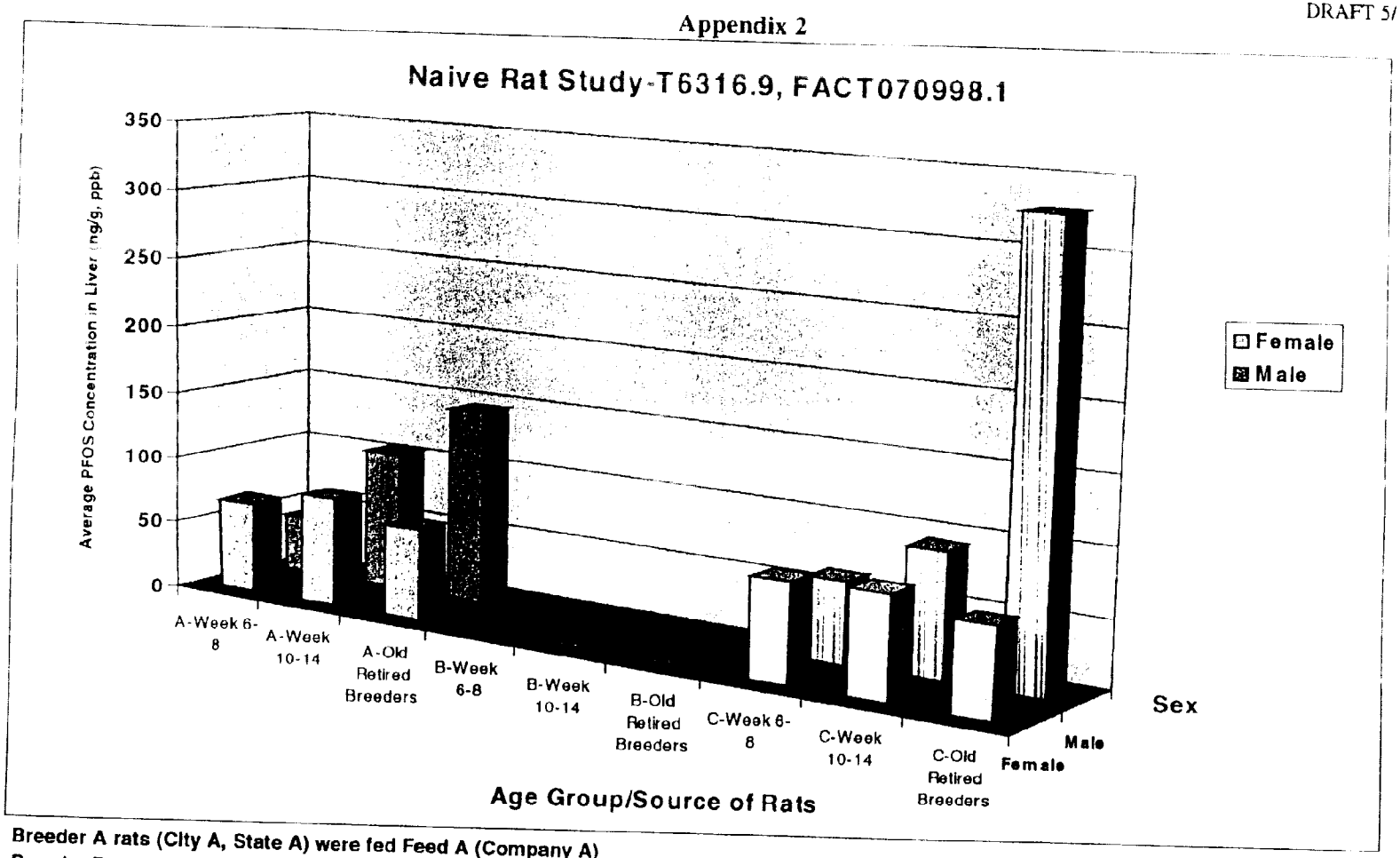
Breeder A rats (City A, State A) were fed Feed A (Company A)
Breeder B rats (City B, State B) were fed Feed B
Breeder C rats (City C, State C) were fed Feed C
Average PFOS levels determined from population n = 5 (per Age Group, per Source), except Breeder A 6-8 Week Male Rats
Breeder B Group PFOS results were all < PFOS MDL (15 ppb)

Appendix 2



Breeder A rats (City A, State A) were fed Feed A (Company A)
Breeder B rats (City B, State B) were fed Feed B
Breeder C rats (City C, State C) were fed Feed C
Average PFOS levels determined from population n = 5 (per Age Group, per Source), except Breeder A 6-8 Week Male Rats
Breeder B Group PFOS results were all < PFOS MDL (15 ppb)

Appendix 2



Breeder A rats (City A, State A) were fed Feed A (Company A)
Breeder B rats (City B, State B) were fed Feed B
Breeder C rats (City C, State C) were fed Feed C
Average PFOS levels determined from population n = 5 (per Age Group, per Source), except Breeder A 6-8 Week Male Rats
Breeder B Group PFOS results were all < PFOS MDL (15 ppb)

3M MEDICAL DEPARTMENT, TOXICOLOGY SERVICES
Report for Study No. T-6316.9; DT21
Fluorochemical (FC) Levels in Naïve Rats – Further Investigation of Rat Chow

Background:

In an attempt to determine the source of low-level perfluorooctanesulfonate (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. Dr. Andrew Seacat was appointed study director with Dr. 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 Perfluorooctanesulfonamido ethanol (N-Ethyl FOSE) conducted at Covance Madison. Dr. Andrew Seacat was appointed study director with Dr. 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 was appointed study director with Dr. 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. Dr. Marv Case was appointed study director.

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

The focus of this report is to cover the data to date examining the possibility of rat chow contamination. The study to examine objective 3 is complete and is covered in a separate report. Once further data addressing each objective are available, reports of the various studies will be generated.

The 3M Environmental Laboratory – Fluorine Analytical Chemistry Team (FACT) is currently in the process of analyzing rat chow samples for PFOS and its metabolites. Attached is a preliminary summary report generated by 3M Environmental Laboratory in August of 1998 (Appendix 1). Samples of rat chow from Covance Laboratories, Harlan Laboratories and NIH were analyzed for 2-(N-ethylperfluorooctanesulfonamido)-ethyl alcohol (EtFOSE-OH) by GCMS. Traces of EtFOSE-OH were found in a sample of Covance chow. This prompted the 3M Environmental Lab Field Group to collected air, airborne particle and wipe samples on site. Preliminary conclusions drawn by the Environmental Lab based on the data to date are that "Contamination in control animals may be due to respiration of airborne EtFOSE-OH, from transfer of test material from one animal to another during sample handling and possibly from sporadic contamination of the food source".

Appendix 1

3M Environmental Laboratory - Fluorine Analytical Chemistry Team
Lisa Dick / Kris Hansen
Fluorine Analytical Chemistry Team
Building 2-3E-09
612-778-7540 / 612-778-6018
ladick@mmm.com / kjhansen@mmm.com

Preliminary summary report: Further Investigation of Rat Chow

Summary:

In June 1998, several rat chow samples from Covance Laboratories and 3 from NIH were supplied to the Environmental Lab for characterization of fluorochemicals that are being monitored in animal studies conducted by 3M Toxicology. A single side of the bag chow sample from Covance was determined to be of significantly higher contamination than the samples from NIH. Because contaminants were found, air and airborne particle and wipe samples were collected on site by the 3M Environmental Lab Field Group. So that results could be based on more than a single sample, additional bags of chow from Covance and Vendor B were also analyzed and are reported in this summary.

In the chow samples, 2-(N-ethylperfluorooctanesulfonamido)-ethyl alcohol (EtFOSE-OH) was quantitated by GCMS. EtFOSE-OH was not present above the detection limit of 1 ng/g chow in the triplicate analyzed samples from three Covance bags (labeled meals for June 9, June 15, and June 21, respectively) or from the single Vendor B bag. Triplicate chow samples have not been analyzed for PFOS.

Air and airborne particle samples were collected at Covance by Jim Wolter and Kurt Oldenburg of the 3M Environmental Lab Field Group. Air was passed through charcoal and glass fiber filters. Details of the sampling procedure can be found in a report by Jim Wolter and Kurt Oldenburg. The largest volume air samples from the backs of rooms were collected on glass fiber filters and analyzed while samples from the front of each room were collected on charcoal and analyzed. Wipe samples from all rat backs were analyzed but wipes from cages, doorhandles, and floors were not.

In air samples taken from Room 3045, EtFOSE-OH was detected by HPLC/ESMS at levels below the practical quantitation limit (approximately 0.17 ppb/L) but above the method detection limit (approximately 0.02 ppb/L) in some samples. Perfluorooctanesulfonate (PFOS) was not detected at levels above the blanks in any room. Analysis of wipes from the backs of rats in Room 3045 contained a measurable amount of EtFOSE-OH, whereas wipes from rats in other rooms did not. Due to variations in sampling technique, wipe samples were not quantifiable. Wipes from animal backs were not analyzed for PFOS.

Appendix 1

EtFOSE-OH Levels in Rat Chow

Chow source	Number of samples	Matrix Spike Pass	EtFOSE-OH
Vendor B	3	2/sample	Non-detect
Covance – June 9	3	2/sample	Non-detect
Covance – June 15	3	2/sample	Non-detect
Covance – June 21	3	2/sample	Non-detect

EtFOSE-OH Levels in Covance Lab Environment

Covance Sample Location	Number of samples	EtFOSE-OH detection
Room 3045 Air Samples (GF and charcoal)	4 out of 8	0.02 ppb/L < detect < 0.17 ppb/L
Room 3004, 349 Air Samples (GF and charcoal)	16	Non-detect
Room 3045 Rat Backs	6	Detect (8 ppb/wipe average)
Room 3004, 349 Rat Backs	12	Non-detect

Experimental summary:

Sample preparation: Methylene chloride extraction

Analytes were extracted from chow by addition of ether. Samples were weighed and then covered with 20 mL ether. Non-polar organic analytes transfer from the chow to the organic layer. Samples were shaken for 1 hour and then centrifuged for 30 minutes. Fifteen mL of ether was removed and blown down to 1 to 2 mL.

Glass filter fiber and charcoal adsorbates were split and then prepared by extraction with methanol or ether. All of the charcoal inside the sterile tubes was extracted. Final sample volumes were 1 mL.

GC: Characteristic retention times

In gas chromatography, an aliquot of sample is injected and vaporized onto a chromatographic column. Individual components of the sample adsorb to the stationary phase of the column. As the temperature is raised, components are eluted from the column based on physical and chemical characteristics. An inert gaseous mobile phase carries the components through the column. Carrier gas flow rate, column temperature and gas pressure are adjusted to optimize chromatographic separation.

MS: Electron Impact

One method for producing ions for mass spectra is by bombardment with energetic electrons. In addition to the formation of a molecular ion, a series of reactions leads to the formation of other fragment ions that may be larger or smaller than the molecular ion and are useful for compound identification and quantitation.

Appendix 1

HPLC: Characteristic retention times

In HPLC, an aliquot of extract is injected and passed through a liquid phase 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 filter fibers in this analysis were matched to within 1% on the HPLC system.

ES/MS: Detecting and monitoring molecular ions

Following HPLC separation, ES/MS provides a rapid and accurate means for analyzing a wide range of organic compounds, including fluorochemicals. Electrospray, one of the softest ionization techniques available, is generally operated at relatively mild temperatures. Molecules are ionized, fragmented, and detected. Initially, the mass to charge range m/z = 100 to 1210 is monitored following direct flow injection of the samples. Ions characteristic of known fluorochemicals were observed. These results are used to select ions that can be monitored selectively for quantitative results.

Analysis of organic fluorine standard compound indicates that the primary ion characteristic of EtFOSE-OH is m/z = 630 amu, corresponding to the mass of the compound complexed to acetate anion from the running buffer: $C_8F_{17}SO_2N(C_2H_5)(CH_2CH_2OH)/CH_3COO^-$. Single ion monitoring was used to determine the concentration of this ion in the samples.

Quality control summary:

Methanol blanks were analyzed periodically to ensure complete isolation of the sample. Charcoal and glass fiber filter blanks collected on site were also analyzed and found to be blank. Quantitation of HPLC-ESMS data for fluorochemicals is based on the linear regression of 5 point standard curves from 10 ppb to 1000 ppb or matrix spike recovery comparisons.

Quantitation of GC-MS peaks is based on the recovery of known spike amounts in the same sample matrix and on the linear regression of 5 point standard curves.

Instrumental specifics:

GC/Mass Spectrometers

Hewlett-Packard ATD 400 Gas Chromatograph and Mass Spectrometer

Column:	J& W DB-624 30m
Temperature ramp:	50°C to 250°C @ 20°C/min
	250°C hold 5 min
Carrier gas:	Helium
Spike volume:	25 µL
Oven temperature:	180°C
Trap low temp.:	-30°C
Desorb time:	10 min.
Trap fast:	Yes
Trap high temp.:	250°C

Appendix 1

Line temperature:	225°C
Pressure:	18.4 psi
Valve temperature:	225°C
Trap hold:	5 min.
Desorb flow:	25 mL/min
Ions monitored:	540, 448 amu
Source temperature:	250°C
Quad temperature:	125°C
EM volts:	2598 V
Interface temperature:	250°C

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 Mass Spectrometer

Micromass Platform II atmospheric pressure ionization (API) mass spectrometer

Mass Lynx 2.1 software

Cone voltage:	- 60V
Mode:	electrospray negative
Source temperature:	90°C
Analyzer pressure:	9.2×10^{-5} mBar
Ions:	630, 526, 499
Electrode:	cross-flow

Conclusions:

Low levels of PFOS found in control animals may be due to respiration of airborne EtFOSE-OH, from transfer of test material from one animal to another during sample handling, and possibly from sporadic contamination of the food source.