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

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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

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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.