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3M

February 5, 1999

Frank D. Kover, Chief
Chemical Information and Testing Branch
Chemical Control Division
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
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Washington, D.C. 20460

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Dear Mr. Kover:

Enclosed you will find two papers entitled, "The Science of Organic Fluorochemistry" and "Perfluorooctane Sulfonate: Current Summary of Human Sera, Health and Toxicology Data". An additional paper on a preliminary exposure assessment is being finalized and will be sent to you shortly.

I will talk with you in the near future to schedule a meeting between 3M and the EPA where we can discuss in more detail the information enclosed. I look forward to our continued dialog.

If you have any questions, please feel free to contact me.

Best regards,

William A. Weppner

EPA-OTS
000811762P

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Enclosures

THE SCIENCE OF ORGANIC FLUOROCHEMISTRY

3M

February 5, 1999

Introduction

The science of fluorochemistry begins with fluorine. Fluorine is the most abundant member of the halogen family and is one of the most reactive of all the elements. It is capable of combining with nearly every other element in the periodic table, which is why elemental fluorine is rarely if ever found in nature. The strength of the fluorine bond with other elements also made the discovery of elemental fluorine a difficult task. Elemental fluorine was not isolated until 1886, a relatively late date, as chemical discoveries go. Therefore, the science of fluorochemistry is relatively young. Because of its strong, electronegative properties, ionic fluorine will form weak bonds with other electronegative atoms and very strong bonds with electropositive atoms. Ionic metal fluorides are the most common chemical forms of fluorine found in nature, such as fluorspar (CaF_2). Naturally occurring fluorinated hydrocarbon molecules rarely occur in nature because of the energy required to make or break the carbon-fluorine bond in biological systems. However, partially and fully fluorinated organic molecules can be synthesized.

3M Experience in Fluorochemistry

3M Company began its history in fluorochemistry with the licensing of specific intellectual property from Dr. Simons of Penn State University in 1945. Dr. Simons had developed a process, now referred to as Simons Electro-Chemical Fluorination (ECF), to synthesize organofluorine molecules. In this process, organic feedstocks are dispersed in liquid, anhydrous hydrogen fluoride, and an electric current is passed through the solution, causing the hydrogen atoms on the molecule to be replaced with fluorine. The predominant component of the products produced by this process have the same carbon skeletal arrangement as the feedstock used with all of the hydrogen atoms replaced by fluorine. However, fragmentation and rearrangement of the carbon skeleton can also occur and significant amounts of cleaved, branched and cyclic structures may be formed. The degree of fluorination of the organic feedstock is also dependent upon the specific carbon chain length of the feedstock and parameters of the ECF process such as electrical current and the length of time the process is run. It is possible to synthesize fully fluorinated or perfluoroorganic molecules where all of the hydrogen atoms of the hydrocarbon feedstock have been replaced by fluorine atoms. 3M built the first manufacturing pilot scale ECF process in 1949 and has continued to develop and improve the Simons ECF process for the production of fluorochemical products. Currently, 3M has three manufacturing sites in the United States using the ECF process (Cottage Grove, MN, Cordova, IL, and Decatur, AL).

3M Production of Sulfonyl-based Fluorochemicals

3M has produced sulfonyl based fluorochemicals commercially for over 40 years using the ECF process. A basic building block of such products and the highest production volume fluorochemical 3M manufactures is perfluorooctanesulfonyl fluoride (POSF). The starting feedstock for this reaction is 1-octanesulfonyl fluoride. (Reaction 1)

Reaction 1

$$2\ C_8H_{17}SO_2F + 34\ HF \xrightarrow[ECF]{4.5\text{-}7.0\ V} 2\ C_8F_{17}SO_2F + 17\ H_2$$

1-Octanesulfonyl fluoride — Perfluorooctanesulfonyl fluoride (POSF)

It is important to understand that perfluorooctane sulfonic acid (PFOS) will result from the chemical or enzymatic hydrolysis of POSF. Under appropriate conditions, the perfluorooctane sulfonate anion can form salts with monovalent metallic cations. Current information strongly supports that PFOS or its salts cannot be broken down further chemically. Therefore PFOS is the ultimate degradation product from POSF derived fluorochemicals and will persist in that form.

The electrochemical fluorination process yields about 35%-40% straight chain (normal) POSF, as a mixture of biproducts and waste of unknown and variable composition comprised of the following:

1) higher and lower straight-chain homologs, i.e., $n\text{-}C_8F_{2n+1}SO_2F$, e.g., $C_4F_9SO_2F$, $C_7F_{15}SO_2F$, $C_9F_{19}SO_2F$ which comprise about 7% of the process output

2) branched-chain, perfluoroalkylsulfonyl fluorides with various chain lengths, about 18-20% of the output

3) straight-chain, branched, and cyclic (non-functional) perfluoroalkanes and ethers, which comprise about 20-25% of the output

4) "tars" (high molecular weight fluorochemical byproducts) and other byproducts, including molecular hydrogen, which comprise about 10-15% of the output.

Because of slight differences in process conditions, raw materials, and equipment, the mixture produced by the electrochemical fluorination process varies somewhat from lot-to-lot and from plant-to-plant. The product that results from electrochemical fluorination is thus not a pure chemical but rather a mix of isomers and homologues. The commercialized POSF derived products are a mixture of approximately 70% linear POSF derivatives and 30% branched POSF derived impurities.

During production, byproducts and waste products are formed. The volatile waste products, such as perfluoromethane, have been vented to the atmosphere in the past, but improvements are underway to capture and destroy these releases by thermal oxidation. The tars are incinerated at an in-house, hazardous waste incinerator. The byproducts, many of which are incompletely fluorinated with hydrogen atoms still present, can be recycled back into the ECF process or are partially degraded in stabilization processes, and discharged to controlled, in-house, wastewater treatment systems. The treatment sludge is either landfilled or land-incorporated. Some of the non-POSF byproducts are recovered and sold for secondary uses.

POSF is itself a commercially viable product, but is primarily an important intermediate in the synthesis of substances used in many other 3M fluorochemical products. The majority is used to produce functionally derivatized fluorochemicals and high molecular weight polymeric products. Table 1 identifies some fluorochemicals, their acronyms, chemical name, and formulas. To a lesser extent, some homologues of POSF, [$C_nF_{(2n+1)}SO_2F$ where n=other than 8], principally perfluorohexanesulfonyl fluoride, are also intermediates in the formation of other 3M products. PFOS is also a commercialized product for a variety of specific applications.

Using POSF as a basic building block, unique chemistries can be created by derivatizing POSF through the sulfonyl fluoride moiety of the molecule using conventional hydrocarbon reactions. Chart 1 outlines the general classes of fluorinated materials made by 3M. The major intermediates are represented by the trunk of the "tree". POSF is reacted with methyl or ethyl amine to produce either N-methyl or N-ethylperfluorooctanesulfonamide (FOSA). FOSA is subsequently reacted with ethylene carbonate to form either N-methyl or N-ethylperfluorooctanesulfonamidoethanol (FOSE).

Chart 1

POSF Fluorochemical Reaction Tree

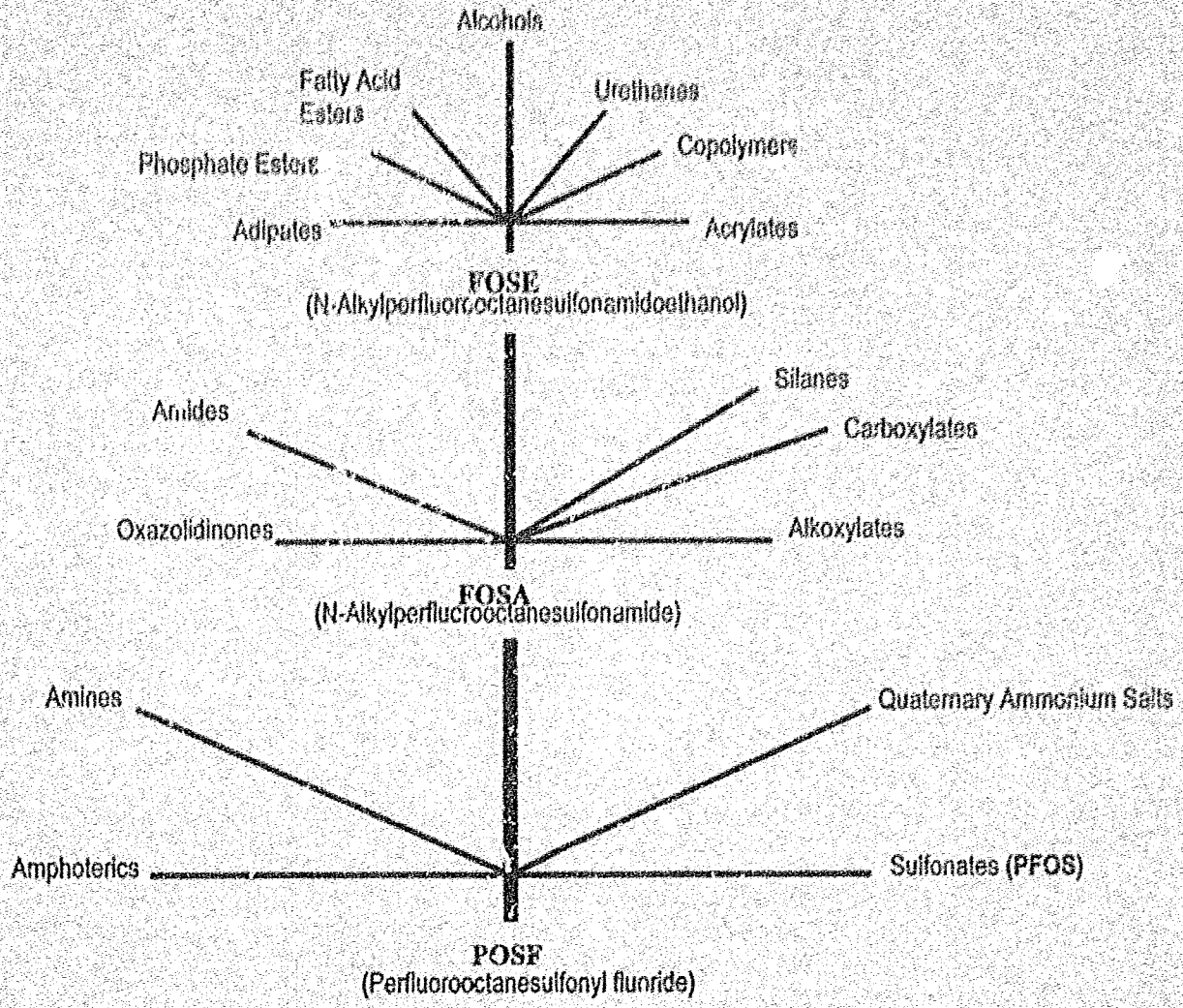


Table 1. Fluorochemical Glossary

Designation	Molecular Formula	Technical Name (CAS Name)
POSF	$C_8F_{17}SO_2F$	Perfluorooctanesulfonyl fluoride (1-Octanesulfonyl fluoride, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-)
PFOS	$C_8F_{17}SO_3^-$	Perfluorooctanesulfonate (1-Octanesulfonic acid anion, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-)
PFOSH	$C_8F_{17}SO_3H$	Perfluorooctanesulfonic acid (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-)
PFOS NH_4 salt	$C_8F_{17}SO_3NH_4$	Ammonium perfluorooctanesulfonate (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, ammonium salt)
PFOS DEA salt	$C_8F_{17}SO_3NH(CH_2CH_2OH)_2$	Perfluorooctanesulfonate, diethanolamine salt (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, compd. with 2,2'-iminobis[ethanol] (1:1))
PFOS K salt	$C_8F_{17}SO_3K$	Potassium perfluorooctanesulfonate (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, potassium salt)
PFOS Li salt	$C_8F_{17}SO_3Li$	Lithium perfluorooctanesulfonate (1-Octanesulfonic acid, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-, lithium salt)
FOSA	$C_8F_{17}SO_2NH_2$	Perfluorooctanesulfonamide (1-Octanesulfonamide, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-)
N-EtFOSGE	$C_8F_{17}SO_2N(CH_2CH_3)CH_2COO^-$	N-perfluorooctylsulfonyl-N-ethylglycinate (Glycine, N-ethyl-N-[(heptadecafluorooctyl)sulfonyl]-, anion)
N-EtFOSA	$C_8F_{17}SO_2NH(CH_2CH_3)$	N-Ethylperfluorooctanesulfonamide (1-Octanesulfonamide, N-ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-)
N-MeFOSA	$C_8F_{17}SO_2NHCH_3$	N-Methylperfluorooctanesulfonamide (1-Octanesulfonamide, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-N-methyl-)
N-EtFOSE	$C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OH$	N-Ethylperfluorooctanesulfonamidoethanol (1-Octanesulfonamide, N-ethyl-1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-N-(2-hydroxyethyl)-)
N-MeFOSE	$C_8F_{17}SO_2N(CH_3)CH_2CH_2OH$	N-Methylperfluorooctanesulfonamidoethanol (1-Octanesulfonamide, 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluoro-N-(2-hydroxyethyl)-N-methyl-)
N-EtFOSEA	$C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OCOCH=CH_2$	N-Ethylperfluorooctanesulfonamidoethyl acrylate (2-Propenoic acid, 2-[ethyl[(heptadecafluorooctyl)sulfonyl]amino]ethyl ester)
N-EtFOSEMA	$C_8F_{17}SO_2N(CH_2CH_3)CH_2CH_2OCOC(CH_3)=CH_2$	N-Ethylperfluorooctanesulfonamidoethyl methacrylate (2-Propenoic acid, 2-methyl-, 2-[ethyl[(heptadecafluorooctyl)sulfonyl]amino]ethyl ester)
N-MeFOSEA	$C_8F_{17}SO_2N(CH_3)CH_2CH_2OCOCH=CH_2$	N-Methylperfluorooctanesulfonamidoethyl acrylate (2-Propenoic acid, 2-[[(heptadecafluorooctyl)sulfonyl]methylamino]ethyl ester)

The secondary reactions producing all of these derivatives are single or sequential batch processes that do not necessarily produce pure products. There may be varying amounts of fluorochemical residuals (unreacted or partially reacted starting materials or intermediates, such as FOSA or FOSE) that are carried forward to the final product. Typically, these residuals are present at a concentration of 1% or less. Such processes are under a continuous improvement plan to reduce or eliminate the presence of unnecessary residuals in the production of commercializable product. It is important to understand that the non-fluorochemical moieties added to the sulfonlyl fluoride group of POSF can also be removed through a variety of degradation processes (chemical, environmental, and metabolic). Again, the terminal product of such degradation will be PFOS.

Physical-Chemical Properties

Fluorinated organics are less well described in the scientific literature than organic molecules bearing other halogens, i.e. bromine, and chlorine, which have been more thoroughly investigated by many researchers in published reports. To understand fluorinated organic properties, it is necessary to describe in more detail the properties of fluorine. Fluorine has several characteristics which differ from the other halogens and contribute to the unusual properties of fluorochemicals.

Fluorine has a van der Waals radius of 1.35 Å, more comparable to that of oxygen and smaller than other halogens, and is isosterically similar to a hydroxyl group. Fluorine has the highest electronegativity (4.0 – Pauling scale) of all the halogens, and the highest in the periodic table. This confers a strong polarity to the carbon-fluorine bond. The carbon-fluorine bond is one of the strongest in nature (~110 kcal/mol). See Table 2 and 3. This very strong, high energy bond contributes to the stability of fluorochemicals. Such stability may also lead to the persistence of certain fluorochemicals. That stability confers a variety of unique properties to fluorocarbons as described in Table 4.

TABLE 2
Fluorocarbon Structure Considerations

Structure	Bond Length A°	Atomic Van Der Waals' Radius A°	Bond Strength Kcal/Mole
H_3C-H	1.11	1.20	101
H_3C-F	1.385	1.35	107
H_3C-Cl	1.78	1.80	81
H_3C-Br	1.93	1.95	67
H_3C-I	2.13	2.15	55

TABLE 3
Effect of Increasing Fluorination

Structure	Bond Length A°	Bond Strength Kcal/Mole
H_3C-F	1.385	107.0
H_2FC-F	1.358	109.6
HF_2C-F	1.332	114.6
F_3C-F	1.317	116.0

TABLE 4
Stability of Fluorocarbons

Chemical
- Unaffected by any normal reagent
- React with alkali metals at high temperatures

Thermal and Oxidative
- Stable in air at high temperatures
- Non-flammable

Electrical
- High electric strength
- Low dielectric constant

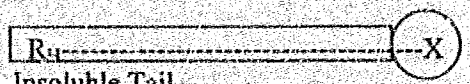
The high ionization potential of fluorine (401.8 kcal/mole) and its low polarizability leads to weak inter- and intramolecular interactions. This is demonstrated in the low boiling points of fluorochemicals relative to molecular weight, and their extremely low surface tension and low refractive index. Table 5 compares the physical properties of a perfluoroalkane with its hydrocarbon analog to demonstrate the effect of low polarizability.

TABLE 5
Physical Properties
(Effect of Low Polarizability)

	C_8F_{18}	C_8H_{18}
Low Boiling Point:		
MW	438	114
Bp (°C)	97	125
Low Heat of Vaporization		
G-Cal/Gram	20	86.8
Low Refractive Index		
N_D20	1.280	1.3975
Low Surface Tension		
Dynes/cm	15.0	21.8

The partitioning behavior of perfluoroalkanes is also unique. Some perfluoroalkanes, when mixed with hydrocarbons and water, form three immiscible phases, demonstrating that perfluorinated chains are both oleophobic and hyorophobic. A charged moiety, such as carboxylic acid, sulfonic acid, phosphate or a quaternary ammonium group, when attached to the perfluorinated chain, makes the molecule more water soluble because of the hydrophilic nature of these charged moieties. Therefore, such functionalized fluorochemicals can have surfactant properties.

A conventional hydrocarbon surfactant generally may be represented as:

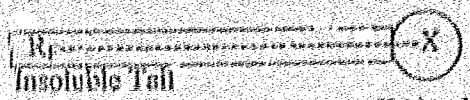


Insoluble Tail

Hydrophilic Group

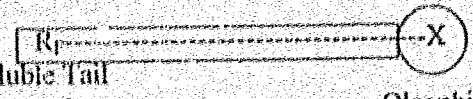
Where R.... represents the hydrocarbon "tail" and ---X represents a solubilizing group.

In a similar fashion, fluorochemical surfactants can typically be described by the following chemical structure:



Where the Rf.... portion is the stable fluorocarbon tail, -X represents a solubilizing group. It is this unique fluorochemical "tail", modified in length and structure to meet end use needs, which provides the exceptional resistance to thermal and chemical attack characteristic of the fluorochemical. This fluorochemical portion of each is basically responsible for its capability to dramatically reduce surface tension, as well as being the major difference between these materials and conventional surfactants.

The solubilizing group, -X is commonly water soluble, but can be designed to be oil soluble for use in nonaqueous systems.



The nature of the oleophilic group varies among the fluorochemical surfactants. By altering it, fluorochemical surfactants have been prepared which are extremely surface active in a number of environments, including many systems which would degrade hydrocarbon or silicone surfactants.

Physical properties available on 3M fluorochemical products are principally those parameters needed for quality control use and material handling. It is important to remember that these physiochemical properties have been obtained from products that are not highly refined, and that may have more than one fluorochemical component. Some products may have nonfluorochemical components which contribute to the determination of the values. One observes a wide range in values for physiochemical parameters among low molecular weight, POSF based, fluorochemicals. Typically these low molecular weight chemistries tend to have higher water solubility and lower vapor pressure than polymeric products containing them. In addition to being intermediates in the formation of products, some of these low molecular weight fluorochemicals are also likely intermediates in the degradation of polymeric compounds.

3M Sulfonyl-based Fluorochemical Products

The 3M product lines that use POSF-based fluorochemicals are summarized below. In some cases, 3M manufactures the final commercialized product. In other cases, 3M sells a fluorochemical which another company incorporates into their final product. (Product lines using fluorochemicals which contain no sulfonyl groups are not listed.)

Surface Treatments

Fabric/Upholstery Protector (High molecular weight [MW] polymers)
Carpet Protector (High MW polymers)
Leather Protector (High MW Polymers)
Paper and Packaging Protector (High MW phosphate esters or high MW polymers)

Surfactants (Low MW chemical substances)
- Specialty Surfactants
- Household Additives
- Electroplating and Etching Bath Surfactants
- Coatings and [illegible]ing Additives
- Chemical Intermediates
- Carpet Spot Cleaners
- Fire Extinguishing Foam Concentrates
- Mining and Oil Surfactants

Other Uses
- Insecticides (Low MW chemical substances)

Some of the POSF derived chemistries are relatively low molecular weight (< 500 daltons). These fluorochemicals can be intermediates that 3M or our customers use in making other finished products. Such fluorochemical intermediates can be covalently bound to a variety of polymeric hydrocarbon backbones to make products with unique performance characteristics. The majority of 3M fluorochemicals produced for commercialization are used in such polymeric form for treatment of surfaces and materials. For example, fluorochemical containing polymers (urethane and acrylate) plus fluorochemical adipates can provide soil, stain, and water resistance to personal apparel and home furnishings. Such protective products function through the fluorocarbon moiety on the polymer lowering the surface energy of the material to which they are applied.

The 3M paper protectors can be divided into two general classes of chemistries. One class is based on phosphate esters of N-EtFOSE. The other class is a N-MeFOSEA-acrylate copolymer. Applied to paper, the perfluorocarbon moiety in these products has the previously described effect of lowering the surface energy of the individual paper fibers. This lowered surface energy greatly contributes to the holdout of low surface energy liquids such as greases and oils.

As previously described, the POSF-derived fluorochemical products have surfactant properties. Such fluorochemical surfactants differ greatly from conventional hydrocarbon and silicone surfactants. In most systems they are far more efficient in reducing surface tension to levels that are unreachable with these other types. In some aqueous systems, surface tensions as low as 15 to 16 dynes/cm can be attained. The fluorochemical surfactants normally produce these extremely low values at concentrations as low as 100 parts per million, or less. Equally important is the fact that certain of these fluorochemical surface active agents are stable and effective in many extremely hostile environments, including strongly acidic, strongly alkaline and even strongly oxidizing systems. Table 6 summarizes the features of fluorochemical surfactants.

Table 6
Features of Fluorochemical Surfactants

SURFACE ACTIVITY

AQUEOUS SYSTEMS

Some of these surfactants can lower surface tension to less than 16 dynes/cm and function at low concentrations. They are effective in dramatically reducing surface tension in a wide variety of aqueous media, including acidic and basic systems.

NON AQUEOUS SYSTEMS

Fluorochemical Surfactants have been developed which uniquely reduce surface tensions of many organic media to about 20 dynes/cm, including solvents such as esters, alcohols and ethers and resin systems including epoxies, polyesters, urethanes and acrylics.

WETTING

Reduced surface tensions result in the ability to improve the wetting of a variety of materials, including such hard to wet surfaces as plastics and oily metals

BETTER SPREADING

Low surface tension in combination with low interfacial tension affects spontaneous spreading of a liquid over various surfaces. This is important in reducing pinholes, craters, and edge crawling of coatings applied to unclean surfaces.

REDUCED WATER SPOTTING

Because of reduced droplet formation, the need for distilled or deionized water in rinsing operations may be eliminated.

SMALLER GAS BUBBLES

These smaller gas bubbles produced at the surface of metal during chemical etching will have less tendency to adhere, grow and cause surface imperfections.

SMALLER DROP FORMATION

Smaller drops are desired in fine aerosol mists.

BETTER LIQUID PENETRATION

The force required to cause liquids to move through small pore spaces can be greatly reduced.

IMPROVED FILM UNIFORMITY

Smoother, more even films are produced from polishes, finishes and coatings.

LEVELING

Emulsion coatings applied to difficult to wet surfaces can show greatly improved leveling with the addition of small quantities of these materials.

FOAMING

Stable foams can be produced in hostile media such as chromic acid or sodium hydroxide, where conventional surface active agents would be destroyed.

EMULSIFICATION

While generally not effective as emulsifiers in water-organic systems, these materials can be quite efficient emulsifiers in specialty applications, where fluorinated materials comprise either the continuous or the dispersed phase.

STABILITY

Chemical

Some of these surface active agents are stable in such rigorous environments as hot chromic acid, anhydrous hydrazine, hot concentrated sulfuric acid, hot concentrated hydrofluoric acid and hot concentrated sodium hydroxide solutions.

Thermal

While all of these materials have very good stability at moderate temperatures, a few can withstand temperatures in excess of 300°F in air.

LOW CONCENTRATION

These materials are normally effective at extremely low concentrations, and often are utilized at concentrations of 100 parts per million active solids or less.

Many applications involve more than just air-liquid interfaces where surface tension alone might be important. More often liquid-liquid or solid-liquid systems are encountered. In these cases, interfacial tension, as well as surface tension, plays a significant role in the wetting or leveling process. Quite often in these cases, a combination of a suitable hydrocarbon surfactant can produce a degree of wetting which cannot be accomplished by either type alone. Normally, in such a combination, it is the fluorochemical surfactant which reduces the surface tension, while the hydrocarbon material aids in the reduction of the interfacial tension. The net result can be a system that easily wets and spreads on otherwise hard to wet surfaces.

Another unique physical characteristic of fluorochemicals is their ability to form tough, yet resilient foams. Such foams have been formulated to resist the action of high temperature or aggressive chemicals and vapors. These formulations have found commercial application in suppressing flammable liquid, chemical and organic fires or toxic and obnoxious vapors and odors.

Perfluorooctane Sulfonate: Current Summary of Human Sera, Health and Toxicology Data

3M

January 21, 1999

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January 21, 1999

Executive Summary

3M has prepared this document to summarize the data related to the biological effects of perfluorooctane sulfonate (PFOS). It also presents current thinking on human health risk related to PFOS and includes information about future study plans. 3M Medical Department scientists and physicians, in consultation with outside experts, are the authors.

PFOS has been found at tens of parts per billion levels in serum samples of nonoccupationally exposed employees, in commercially available human serum and in pooled samples from multiple blood banks. PFOS is an eight-carbon molecule that is perfluorinated except for the sulfonate group on the terminal carbon. 3M has manufactured PFOS and molecules that may be metabolic precursors to it since 1948. Routes of exposure to PFOS or precursor molecules are not well understood at this time.

PFOS is an example of an "organic" fluorine molecule. Human serum has been known to contain organic fluorine molecules for over 30 years. The primary constituent of this organic fluorine fraction was tentatively identified as another molecule (perfluorooctanoate) in 1976. Current analysis of stored sera samples from a variety of sources are more consistent with PFOS being a major fraction of this organic fluorine. Improved analytic techniques allowing a relatively rapid analysis at low levels of detection make the current analyses possible. These analytic techniques were first available for use in medical surveillance of exposed workers in 1992. Detection limits have been lowered to allow the more recent analysis of serum from those without occupational exposure.

Medical surveillance has been done among 3M employees occupationally exposed to PFOS precursors for over 20 years. To date, no adverse health effect associated with PFOS exposure has been found in these employees. This conclusion applies at serum levels up to 6 parts per million, about 100 times higher than levels seen in the general

population. PFOS has a long residence time in the human body. In three retirees, the half-life in human sera ranges from 1100 to 1500 days. A mortality study at the U.S. plant primarily involved with production of PFOS related materials has found no significantly elevated standardized mortality ratios (SMR's).

Toxicology studies show that PFOS is well absorbed orally and distributes primarily in the serum and liver. It does not appear to be further metabolized. Some enterohepatic circulation of PFOS occurs, based on the observation of increased excretion in rats given cholestyramine. Elimination from the body is slow and occurs via both urine and feces.

Mutagenicity testing is negative in five salmonella species. It is not genotoxic in a mouse bone marrow micronucleus assay. The acute LD50 in rats is 250 mg/kg (moderately toxic). It does not produce dermal or ocular toxicity.

Subchronic studies have been done in rats and primates. PFOS causes liver enzyme elevations and hepatic vacuolization in rats, and hepatocellular hypertrophy at higher doses. Higher doses also cause other GI toxicity, hematological abnormalities, weight loss, convulsions, tremors and death. Monkeys show anorexia, emesis, diarrhea, hypoactivity and at higher doses prostration, convulsions and death. Atrophy of exocrine cells in salivary glands and the pancreas, and lipid depletion in the adrenals is found at high doses in the monkey.

The serum levels at which these compound related effects occurred in these early rhesus monkey studies are unknown. In a recently completed rangefinder study in cynomolgus monkeys the first observed biological effect was a decrease in serum cholesterol, first observed at a serum level of 72 ppm in one of the two monkeys in the high dose group. Using the relationship between cumulative dose and serum level found in this study, it can be estimate that significant toxicity occurred at 700 to 800 ppm in the early rhesus monkey studies, and death at 1100 ppm and above. More complete quantitative absorption, distribution and excretion data for PFOS is being obtained.

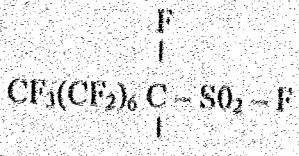
Available information therefore suggests that no identifiable health risk to humans would be expected to occur at the PFOS levels found in blood bank or commercial serum samples.

Extensive further research, which includes epidemiological and laboratory studies, is planned or underway. The purpose of this research is to explore the potential for chronic and reproductive effects, understand toxic mechanisms and obtain a better understanding of absorption, distribution, metabolism and excretion. The plan is to make as much use as possible of observational data in exposed workers and to establish no effect levels in both rats and primates for endpoints of importance.

I. INTRODUCTION

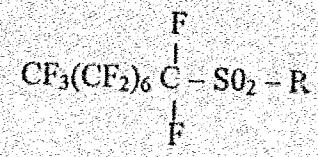
Evidence that organic compounds containing the element fluorine covalently bonded to carbon (organic fluorine compounds, OF) can be found in human sera has been available for 30 years. Although all of the specific compounds contributing to the total amount of OF present are not identified, it now appears that a compound called perfluorooctane sulfonate constitutes a significant fraction. Recent data provide evidence that PFOS is present at tens of parts-per-billion (ppb) levels in serum samples from the general population, averaging 30 ppb in blood bank samples from diverse locations in the U.S. Single digit parts per million (ppm) levels (approximately 100 times greater) are found in individuals occupationally exposed to PFOS and its precursors, averaging 2.0 ppm among participating employees at the primary U.S. manufacturing location for these compounds.

3M produces perfluorinated molecules by mixing anhydrous HF and hydrocarbon feed stock in an electrochemical cell (electrochemical fluorination). Perfluorooctane sulfonyl fluoride (POSF) is the cell product from which a group of products is developed:



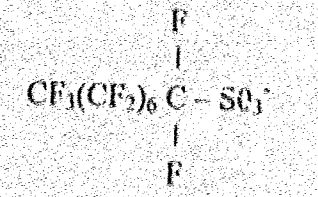
POSF

Other moieties ("R") are added to the sulfur, which leads to the creation of materials that may be polymerized or esterified. The vast majority of POSF produced is used in this way.



POSF derived molecule

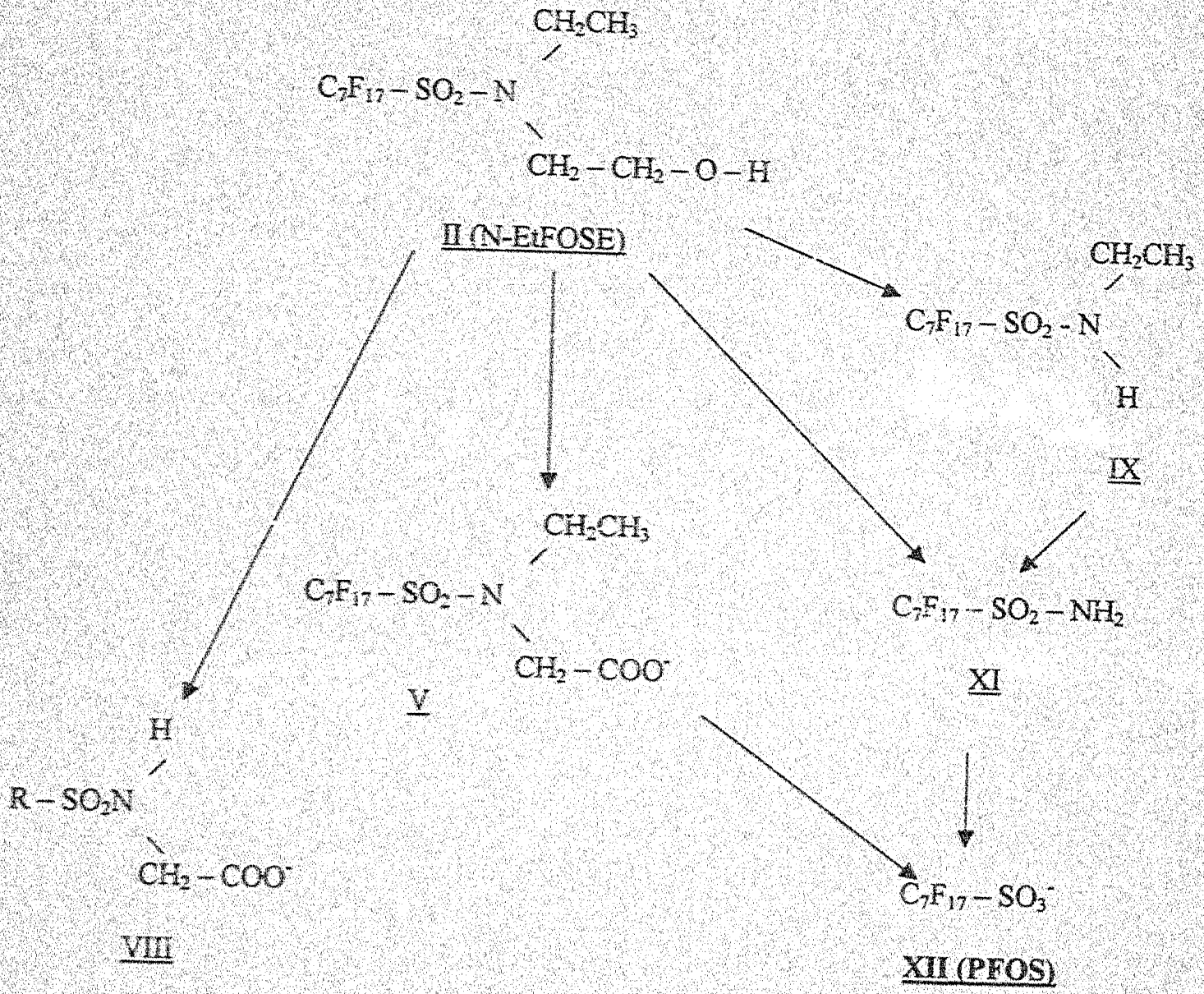
PFOS may result from the loss of the "R" moiety through metabolic processes. Current evidence would indicate that PFOS is not further metabolized. Some PFOS is produced and sold directly into industrial applications as a surfactant. This, however, amounts to only a small fraction of total POSF production.



PFOS

Most POSF that is produced is used in 2-(N-ethylperfluoroctanesulfonamido)-ethyl alcohol (N-Et-FOSE) and 2-(N-methylperfluoroctanesulfonamido)-ethyl alcohol (N-Me-FOSE) based products. Figure I.1 shows the chemical structure of N-Et-FOSE and metabolites that have been found in rat serum. All except compound VIII have been verified to metabolize further to PFOS. (Missing Roman numerals represent hypothesized intermediates not shown in this figure.)

Figure I.1 N-EtFOSE(*) Metabolites Identified in Rat Serum



In vitro studies in both rat and human hepatocytes lead to identification of the same compounds and hypothesized intermediates. Compound VIII has been detected in some samples of pooled human sera. A single dose absorption, distribution, metabolism and excretion study of N-Et-FOSE in cynomolgus monkeys is through the in life phase and tissue analysis is pending. N-Et-FOSE is esterified to produce larger molecules that are used on paper and packaging for oil and water repellency.

It is presumed that N-Me-FOSE, in which a methyl group replaces the ethyl group on the nitrogen, has a similar metabolism. N-Me-FOSE becomes part of very large molecules that act as protective chemicals on fabrics, leather and rugs.

Outside of the occupational setting, routes of human exposure to PFOS or its metabolic precursors are not understood, but are the subject of intense study. Exposure could occur from environmental releases of PFOS or its precursors at the Decatur, Alabama and Antwerp, Belgium manufacturing sites. It could occur from the environmental or biological degradation of products to PFOS or molecules metabolized to PFOS. Products also contain small amounts (generally less than a few percent) of residuals, such as N-Et-FOSE and other molecules found in Figure I.1, which are known or suspected metabolic precursors to PFOS. These residuals represent a source of PFOS that would not require environmental or biological degradation of large molecules. Downstream industrial users of POSF based products are also potential sources of environmental releases of PFOS or its precursors. The relative contribution of these various sources to population exposure is currently unknown.

Another surfactant is known to be found in the sera of employees and was reported in general population sera samples in 1976 (Taves). This is perfluorooctanoic acid, or PFOA:

$CF_3(CF_2)_6COOH$

PFOA is also made by electrochemical fluorination. It is mentioned here because following Taves' report in 1976 it was presumed to be a significant fraction of the total organic fluorine found in the sera samples analyzed by Taves and others. This report was a stimulus for investigation and subsequent medical surveillance of employees in fluorochemical production, including those producing POSF based materials. It should be recognized, however, that PFOA is a different and unrelated compound. It does not metabolize to or become PFOS. It is likely that PFOA was misidentified as a major fraction of organic fluorine in the 1976 Taves paper. The evidence for this is discussed in Section II.

The purpose of this report is to describe the data on PFOS levels in human sera, and to discuss the potential for those levels to affect health based on current scientific knowledge. A review of current findings and historical information on PFOS levels in sera is presented in Section II. This is followed in Section III with a description of 3M's epidemiology and medical monitoring database obtained from studies of its workers in plants in the United States and Belgium. The animal and other laboratory toxicology data available on PFOS are presented in Section IV. Section V offers a preliminary evaluation of the serum findings in light of the available health effects data.

3M is actively developing further human health and toxicological information. Section VI outlines the current research agenda.

II. HISTORICAL REVIEW AND CURRENT FINDINGS OF FLUOROCHEMICALS IN HUMAN SERA

The data on fluorochemicals in human sera is presented in this section. The presence of organic forms of fluorine in human serum was observed 30 years ago, and PFOA was thought to account for most of this fraction. 3M finds little current evidence to support this view. Evidence is presented below that PFOS is more likely to be consistently found in sera. Based on the limited data provided by historical samples, there is no evidence of significant change in PFOS concentration in serum samples taken over the last two to three decades.

The advancement of analytical chemistry technology has had a significant influence on our knowledge of fluorocarbons in human sera. The techniques developed and used by researchers in the 1960's and 1970's were time intensive, requiring hours for a single analysis. The methods were also nonspecific, measuring organic fluorine (fluorine covalently bonded to carbon) rather than specific molecules. The development of a rapid analytic technique in the late 1970's decreased analytic time to under an hour, allowing large scale medical surveillance of production employees at higher detection limits (about 0.5 parts per million organic fluorine) that were adequate for the levels found in occupationally exposed individuals. The advancement of chromatographic/mass spectroscopy technology enabled rapid analysis of specific fluorochemicals from small volumes of sera in the early 1990's. This technology was first used in medical surveillance in 1992. Detection limits for PFOS were lowered to 50 parts per billion by 1997. The first report to 3M of PFOS in commercially available pooled sera occurred in late summer of 1997, prompting more research into the technique, and confirming its validity over a period of several months.

Since older published data described organic fluorine content rather than a specific molecule, it is useful to understand the relationship between PFOS levels currently observed and organic fluorine content. Fluorine is 65% of the molecular weight of PFOS. The contribution of a PFOS value to organic fluorine, in ppb, will therefore be [0.65 x (PFOS value in ppb)]. Conversely, if the measured organic fluorine is entirely PFOS, the value of PFOS in ppb will be [1.54 x (OF level in ppb)].

This section presents 1) a brief summary of the historical information regarding organic fluorine in human sera, 2) data from 3M employees involved in fluorochemical production, 3) data from a small group of non-occupationally exposed 3M employees, 4) data from commercially available human pooled serum samples, 5) data from pooled sera from 18 regional blood banks and 6) data from current analysis of stored serum samples.

Historical Finding of the Organic Form of Fluorine in Blood

Taves (1968a) described two forms of fluorine in serum, one that was exchangeable with radioactive fluorine-18 and one that was not. Pothapragada et. al. (1971) also described two forms, ionic and nonionic. Taves (1968b) showed that the non-exchangeable fluorine was bound to albumin. This finding, along with results of extraction and precipitation and the need for ashing to release this form of fluorine, led to the conclusion that the non-exchangeable or nonionic fluorine was "organic", i.e. covalently bound to carbon (Taves et. al., 1976). Using NMR spectroscopy, these authors tentatively identified a component of the organic fluorine as perfluorooctanoic acid (PFOA). There was some variation in the observed spectra from an authentic sample of PFOA, however, leading the arthors to suggest that branching, or the presence of a sulfonate, was possible.

A number of studies over the past 25 years reported levels of organic fluorine in human blood serum. Table II.1 presents the study author, level measured, population studied and methods of analysis. The variety of methods used for determination of

fluorine suggests that some caution be used in interpreting results. All reported means were in the tens of part per billion levels. The average of reported values from United States sources is 37.6 ppb.

Table II.1.
Historical Findings of Serum Organic Fluorine Levels

Year	Author	OF* (ppb)	N	Method**	Source
1972	Guy	30	65	ash	US
1975	Venkateswarlu	36	2	O bomb	US
1976	Guy, Taves	25	106	ash	US
1978	Belisle	20	9	O bomb	US
1979	Singer	45	264	ash	US
1980	Paez	85	pooled	ash	Argentina
1980	Ubel	45	4	mod O bomb	US
1981	Belisle	11	8	O bomb	China
1989	Yamamota	32	11	LOPA	Japan

* Organic fluorine, specific identities not provided.
** Varied methods were used to measure organic fluorine. See papers for details.

Occupationally Exposed Employees

3M has produced PFOA (the ammonium salt) by electrochemical fluorination since the early 1950's. It is a surfactant used in fluoropolymer production. The company began medical monitoring of employees involved in FOA production in 1976, by measuring serum levels of organic fluorine (OF) and performing medical assessments. Employee monitoring was expanded significantly in 1980 following the development of a more rapid test for organic fluorine. Measured serum levels of OF in these employees averaged less than 10 parts per million (ppm).

As noted earlier, PFOS is a surfactant used as a wetting and foaming agent in industrial and commercial processes. Certain fluorochemicals that may transform metabolically to PFOS have been produced since the early 1950's by electrochemical fluorination. Since 1980 this has occurred at primarily one location in the United States (Decatur, Alabama). This 3M site consists of a fluorochemical plant and a film plant that are physically separate entities. Chemical plant employees have been offered a medical monitoring program that includes standard medical testing as well

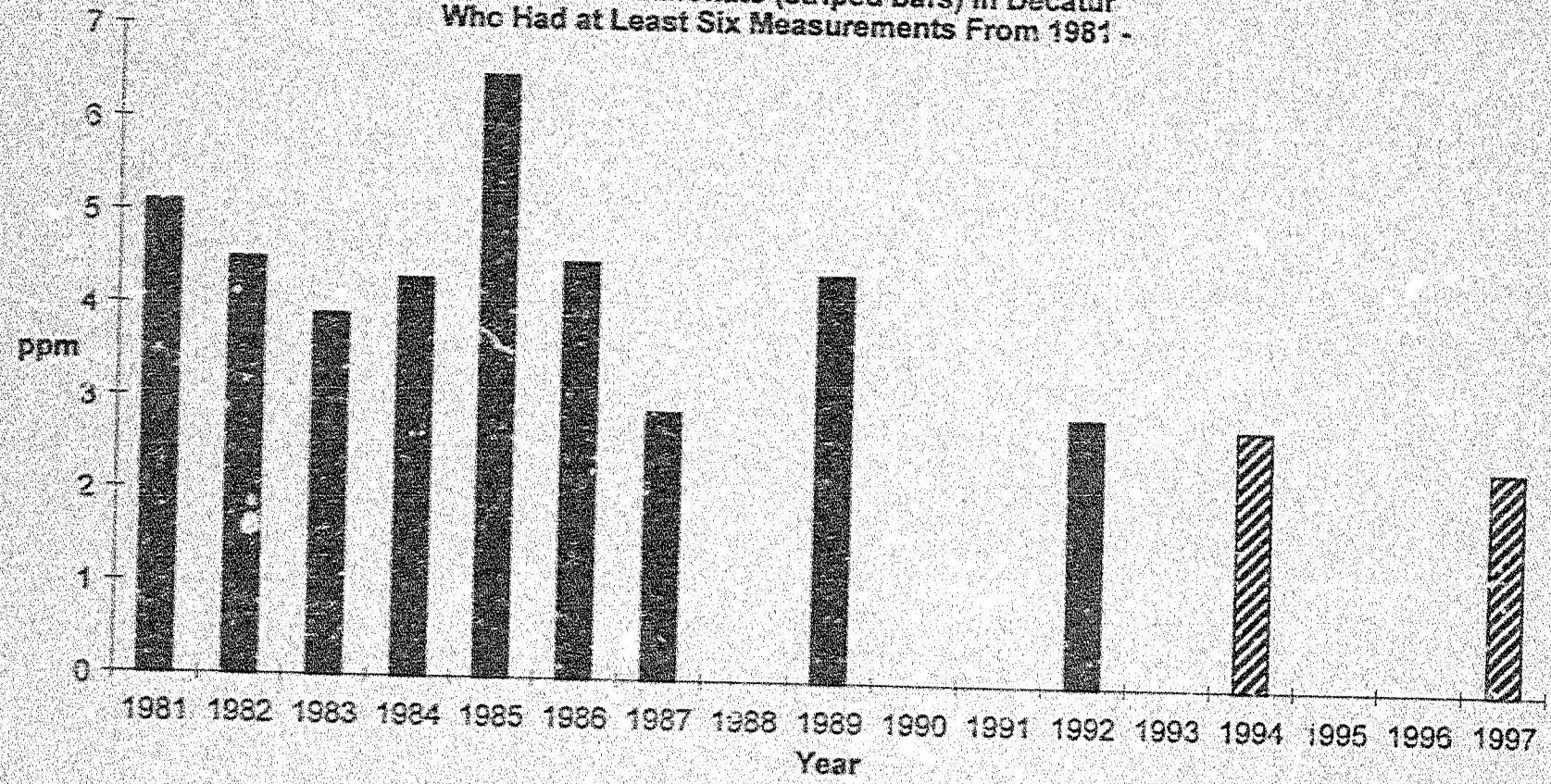
as measurement of serum levels of OF. Among employees with six or more measurements, OF levels averaged 2.9 to 6.5 ppm from 1981 to 1992[1] (Figure II.1). With the introduction of high performance liquid chromatography-mass spectrometry, serum PFOS was measured in 1994 and 1997 (see also Figure II.1). In these two years, mean PFOS levels were 2.44 ppm (range 0.25 – 12.83 ppm) and 1.96 ppm (range 0.10 – 9.93 ppm), respectively.

Another 3M plant in the United States where PFOS has been measured in employees' serum is Cottage Grove, Minnesota. Some PFOS is manufactured at this plant. In 1997, the mean serum level of PFOS among 74 Cottage Grove fluorochemical production employees was 0.82 ppm (range 0.05 - 6.25 ppm). Outside the United States, 3M manufactures PFOS related materials at its Antwerp, Belgium plant. PFOS levels were measured in Antwerp employees in 1995 (mean = 1.9 ppm, range 0.0 - 9.9 ppm) and in 1997 (mean 1.5 ppm, range 0.1 - 4.8) ppm.

The cross-sectional stratified analysis presented in section III, examining the relationship between PFOS sera level and various clinical chemistry and hormone parameters, was conducted at the Decatur, Alabama and Antwerp, Belgium plants. The Cottage Grove facility was not included because little PFOS related product is manufactured there. It is also the primary site for PFOA production.

[1] We are aware of one occasion in 1979 where the serum of 5 Decatur employees was measured for PFOS by electron capture gas chromatograph and microwave plasma detection methods [Central Analytical Laboratory, 1979]. Total serum organic fluorine levels for these five employees were 10.1, 5.7, 9.4, 11.8 and 4.1 ppm. The percent of PFOS found was 60%, 70%, 80%, 55% and 65% of the total serum organic fluorine levels, respectively.

Figure II.1.
Mean Levels (ppm) of Total Serum Organic Fluorine (solid black or Perfluorooctane Sulfonate (striped bars) in Decatur Who Had at Least Six Measurements From 1981 -



Non-Occupationally Exposed 3M Employees

A total of 31 3M employees were tested for PFOS in their serum in 1998 (Table II.2). All were corporate staff or division managers. None had worked in fluorochemical production or in fluorochemical research and development. Samples were from five females and 26 males. Employees ranged in age from 37 to 62 years. All employees had measurable PFOS in their blood serum (mean = 47 ppb; range = 28 to 96 ppb). Age was significantly associated with increased serum PFOS and accounted for 24% of the variance in PFOS levels. There was no gender-related difference if age was considered. Only four employees had PFOA measured above the detection limit of 10 ppb. The average of these four PFOA measurements was 12.5 ppb. Twelve employees were re-tested eight weeks later to check for reliability of the analytical method. The findings suggested reliability ($R^2 = .94$) in the range of quantification (Figure A1 in the Appendix).

Table II.2.
Summary of Mean and Range of PFOS (ppb) Levels in Current and Historical Human Populations,
All Data Analyzed in February - April, 1998

Populations	Description of Sample	Mean* (ppb)	Range* (ppb)
A. Current Populations (blood collected in 1998)			
1. Non-occupationally exposed 3M employees	31 individuals	47	28-96
2. Commercial pooled serum samples			
Intergen Laboratory	3 samples each with ≥ 100 donors	44	43-44
Sigma Laboratory	3 samples each with ≥ 100 donors	33	26-45
3. Pooled serum from 21 separate U.S. blood banks (see Figure 1 for more detail)	3 to 6 pooled samples per location with 5 to 10 donors per sample	30	9 - 56
B. Historical Samples (chronological order)			
1. Korean War era U.S. military recruits, 1948 to 1951	10 pooled samples with 10 donors per sample	N.D.**	N.D.
2. Swedish samples, 1957	10 individual samples	2	N.D. - 2
3. Michigan Breast Cancer Study, 1969-1971	5 individual samples	33	N.D. - 59
4. Swedish samples, 1971	10 individual samples	1	N.D. - 1
5. MRFIT pooled calibration samples, 1976 (Multiple Risk Factor Intervention Trial)	6 pooled samples with unknown number of donors per sample	31	14 - 56
6. MRFIT pooled calibration samples, 1980	3 pooled samples with unknown number of donors per sample	23	14 - 41
7. China samples (Linxian, rural province), 1984	6 individual samples	N.D.	N.D.
8. MRFIT individual samples, 1985	3 individual samples	31	N.D. - 44
9. China samples (Shandong, rural province), 1994	6 individual samples	N.D.	N.D.

* Rounded to nearest ppb
** Not detected

Commercial Pooled Serum Samples

Six pooled sera samples, obtained from two commercial sources (Intergen and Sigma), were tested in 1998. No information was available about the donor pool, such as age, sex or geographical location. Samples from Intergen came from donor pools consisting of approximately 500 individuals. Three Intergen pools showed PFOS levels of 43, 44 and 44 ppb (Table 2A.2). Pools from another source, Sigma, were from an unknown number of donors. The pools from which the samples were drawn were 50 liters, suggesting a minimum of 200 donors. The three Sigma pools contained PFOS at levels of 26, 28 and 45 ppb.

Blood Bank Pooled Sera

Eighteen blood banks from various geographic areas across the continental United States and Alaska each donated three to six pooled samples that had from 5 to 10 donors per pool (Table II.3). Altogether there were 68 total pools, representing 340 to 680 individual donors. All pools contained detectable levels of PFOS in tests performed in 1998. The range found in the pooled serum was 9 to 56 ppb of PFOS (see table on next page). The location means ranged from 14 ppb in Santa Barbara, California to 52 ppb in Greenville, South Carolina. PFOA levels were detected in 20 (about one-third) of the pooled samples but quantifiable in only two samples (12 and 22 ppb).

The blood bank samples from the regional blood banks are not a statistically valid sample of the U.S. population, but do provide, to date, the best estimate of mean PFOS sera levels. The overall mean was 29.7 ppb of PFOS. If one wishes to make a comparison with the organic fluorine levels in the published literature, this level of PFOS would contribute 19.3 ppb to the total serum organic fluorine level (0.65 x 29.7). An approximate mean of historic organic fluorine measurements from published literature is 37.6 ppb. If this were all PFOS, a PFOS sera level would have been 57.9 ppb (1.54 x 37.6).

Table II.3 **PFOS (ppb) Levels from 18 Blood Banks**

City	# of Pooled Samples	Range	Average
Anchorage, AK	4	19-31	24
Billings, MT	3	18-26	23
Cheyenne, WY	3	13-35	23
Corpus Christi, TX	3	26-32	29
Davenport, IA	3	28-40	32
East Orange, CA	3	20-27	24
Grand Rapids, MI	4	18-31	26
Greenville, SC	3	47-56	52
Kansas City, MO	3	24-35	30
Lafayette, LA	3	39-50	46
Las Vegas, NV	3	25-28	23
Meridian, MS	3	27-56	39
Minneapolis, MN	6	41-54	46
Newark, DE	5	21-32	24
Omaha, NE	5	9-27	17
Santa Barbara, CA	3	13-16	14
Santa Rosa, CA	3	23-26	24
Scottsdale, AZ	3	28-47	37

Overall mean = 29.7ppb

Historical Samples

Nine sets of historical samples have been analyzed for PFOS in 1998. These are summarized in chronological order below.

Korean War era U.S. military recruits, 1948 to 1951. Ten pooled samples consisting of ten individual samples each were measured. All were below 1 ppb, the limits of detection.

Swedish samples, 1957. Ten individual samples ranged from below limit of detection to 4.1 ppb. Two were below detection limit (1 ppb). The mean was 1.98 ppb.

Michigan Breast Cancer Study, 1969 - 1971. Five individual samples ranged from 11.8 to 59.4 ppb. The mean was 33.4 ppb.

Swedish samples, 1971. Ten individual samples ranged from below detection limit (1 ppb) to 2.8 ppb. Three were below detection limit. The mean was 1.13 ppb.

MRFIT pooled calibration samples, 1976. MRFIT (Multiple Risk Factor Intervention Trial) was a cardiovascular risk reduction program conducted in the 1970's and early 1980's. The number of donors per pool is unknown. Six pooled calibration samples ranged from 13.7 to 55.5 ppb. The mean was 30.9 ppb.

MRFIT pooled calibration samples, 1980. Three pooled calibration samples ranged from 13.8 to 40.5 ppb. The number of donors per pool is unknown. The mean was 25.5 ppb.

China samples, 1984. Six individual samples from Linxian province in rural China were all below limits of detection or quantitation. The samples were from an NCI study on nutrition and cancer prevention.

MRFIT individual samples, 1985. Three individual samples from participants in the MRFIT study were obtained. One was below limits of quantitation (5 ppb), the other two were 43.3 and 43.9. Assuming the LOQ for the low sample, the mean was 30.7.

China samples, 1994. Six individual samples from Shandong province in rural China were all below limits of detection or quantitation. The samples were from an NCI study on nutrition and cancer prevention.

In addition to the PFOS measurements described above, PFOA was also analyzed in these historical samples. PFOA was not found in any historical sample at a detection limit of 10 ppb.

Comment

Organic fluorine has been noted in human serum since the late 1960's. We have now identified PFOS as a part of this organic fluorine fraction. PFOS-related materials were not produced commercially prior to 1948, and only in small quantities for several years thereafter. Therefore, it is not surprising that samples from 1948 to 1951 show undetectable levels. There was clearly an increase 20 years later; however the very limited data shows no further upward trend despite steadily increasing production volumes since this time. Due to limited knowledge on distribution of this chemical in the body it is uncertain that body burden is adequately reflected by serum levels. Information on distribution and kinetics is needed to shed further light. The available data on this topic will be presented in the toxicology section. It would also be of interest to measure total organic fluorine and PFOS in the same sample to determine how much PFOS currently contributes to the total organic fluorine content.

PFOA may have been misidentified as a major component of organic fluorine in 1976 (Taves, 1976). Although detectable in some samples, neither historic nor current samples confirm this as a major fraction, except in occupationally exposed employees.

III. SUMMARY OF MEDICAL SURVEILLANCE AND EPIDEMIOLOGY STUDIES

Epidemiologic Investigation of Clinical Chemistries, Hematology and Hormones in Relation to Serum PFOS Levels in Male Fluorochemical Production Employees

Medical surveillance has been routinely performed on 3M fluorochemical production workers (in Decatur, Alabama and Antwerp, Belgium) with potential exposure to PFOS and/or to perfluorinated precursors that may metabolically degrade to PFOS. A recent study (Olsen et al., 1998) provided an analysis of hematology, clinical chemistries and hormonal parameters in relation to serum PFOS as determined by high performance liquid chromatography mass spectrometry methods. These relationships were assessed in fluorochemical production employees from two time periods, 1995 (N = 178) and 1997 (N = 149). [2] In 1995, for Antwerp and Decatur, the mean serum PFOS levels were 1.93 and 2.44 ppm, respectively. In 1997, the mean serum PFOS levels were 1.48 and 1.96 ppm, respectively.

Descriptive simple and stratified analyses, Pearson correlation coefficients, analysis of variance and multivariable regression were used to evaluate for possible associations between PFOS and each hematological and clinical chemistry test and hormonal assay. Age, body mass index, current alcohol consumption (drinks per day) and cigarette use (cigarettes smoked per day) were potential confounding factors that were considered in the analyses.

Four categorizations of serum PFOS levels were assessed in relation to the response variables: 0 - < 1 ppm; 1 - < 3 ppm; 3 - < 6 ppm; and ≥ 6 ppm (Table III.1). (Note: other PFOS categorizations were used with comparable findings). In 1995, mean serum PFOS levels by category were 0.49 ppm, 1.82 ppm, 4.12 ppm and 8.17 ppm,

[2] Hematocrit, hemoglobin, red blood cells, white blood cells and platelet count, alkaline phosphatase, gamma glutamyl transferase, aspartate aminotransferase, alanine aminotransferase, total and direct bilirubin, blood urea nitrogen, creatinine, glucose, cholesterol, low density lipoproteins, high density lipoproteins and triglycerides, cortisol, dehydroepiandrosterone sulfate, estradiol, follicle stimulating hormone, 17-alpha

respectively. In 1997, mean serum PFOS levels by category were 0.52 ppm, 1.78 ppm, 3.87 ppm and 7.20 ppm, respectively. For both years, 95 percent of the employees' serum PFOS levels were below 6 ppm. The two plant populations differed by age, body mass index and alcohol consumption which resulted in differences, as expected, in several clinical chemistry parameters (Table III.2). When analyzed in aggregate, the two plant populations showed no consistent significant associations for both years between the clinical chemistries and hematology parameters and the employees' serum PFOS levels (Figure A1, Appendix). Total bilirubin levels appeared to trend downwards but upon further analysis this was restricted to Decatur employees and the values were all within the reference range.

Multivariable regression models were fitted with PFOS analyzed as a continuous variable using linear as well as non-linear transformations in order to maximize the possibility of finding associations between PFOS and the parameters of interest adjusting for potential confounders. No consistent associations were observed by plant and/or by year. In 1995, hormone values were also obtained from a sub-sample of employees with the higher PFOS measurements. After adjusting for age and body mass index, no significant associations were observed between hormones and serum PFOS levels.

The findings from this study suggest that, among these Antwerp and Decatur male fluorochemical production employees, significant hematological, clinical chemistry and hormonal abnormalities were not associated with serum PFOS levels up to 6 ppm. It was not possible to derive inferences from the few employees with serum PFOS levels $\geq$ 6 ppm. Limitations of this study included its cross-sectional design, the voluntary participation rates and the few subjects with levels $\geq$ 6 ppm.

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hydroxyprogesterone, luteinizing hormone, prolactin, sex hormone binding globulin, free testosterone, bound testosterone, and thyroid stimulating hormone.

Table III.1

Distribution of Employees by Year, Location and PFOS Exposure Level (ppm)

	1995 Data						1997 Data					
	All Employees		Antwerp		Decatur		All Employees		Antwerp		Decatur	
PFOS	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
0 - < 1 ppm	45	25	34	39	11	12	60	40	31	48	29	35
1 - < 3 ppm	91	51	32	36	59	66	63	43	25	38	38	45
3 - < 6 ppm	35	20	19	22	16	18	21	14	9	14	12	14
>= 6 ppm	7	4	3	3	4	4	5	3	0	0	5	6
	178	(100)	88	(100)	90	(100)	149	(100)	65	(100)	84	(100)

Table III.2

Mean Values of PFOS, Demographic, Serum Chemistry and Hematologic Parameters for Antwerp and Decatur, 1995 and 1997 Examinations

Variable	1995 Data		1997 Data	
	Antwerp	Decatur	Antwerp	Decatur
PFOS (ppm)	1.93	2.44	1.48	1.96
Age	37***	45	33***	44
BMI	23.9***	29.2	23.5***	30.0
Cigarettes	4.7*	7.9	5.5	6.6
Alcohol	1.3***	0.2	1.1***	0.1
Alk Phosphatase	75***	97	70***	87
GGT	41	48	26*	36
AST	26*	29	27	26
ALT	44	47	31	34
Total bilirubin	0.86***	0.58	0.80***	0.58
Direct bilirubin	0.22	0.21	0.15***	0.12
BUN	17.0***	14.8	14.9	14.1
Creatinine	0.9***	1.1	0.9**	1.0
Glucose	81***	92	81***	98
Cholesterol	214	218	206	215
LDL	138	136	134	137
HDL	54***	43	50***	42
Triglycerides	115***	187	111***	192

Table III.2 (Continued)

Variable	1995 Data		1997 Data	
	Antwerp	Decatur	Antwerp	Decatur
Hematocrit	47***	46	46*	45
Hemoglobin	15.4	15.2	15.4	15.3
RBC	4.9	5.0	5.1	5.1
MCH	31.4***	30.7	30.5	30.4
MCHC	32.9***	33.4	33.3***	33.7
MVC	95.7***	91.8	91.6*	90.0
WBC	6.4***	7.5	6.5	6.4
Platelets	224	229	237*	217

* $p < .05$; ** $p < .01$; *** $p < .001$ (Antwerp compared to Decatur)

Mortality Study of Employees at the 3M Plant in Decatur, Alabama

A retrospective cohort mortality study of 1,957 employees who worked at least one year at the 3M plant in Decatur, Alabama was conducted by epidemiologists at the University of Minnesota School of Public Health (Mandel and Johnson, 1995). The purpose of this study was to determine whether the mortality experience of these employees was significantly different from that which would be expected in a comparable population. The cohort was followed from March ., 1961 through December 31, 1991. Table A2 (Appendix) provides a description of the final employment and vital status for the male (n = 1,639) and female (n = 318) employees. These totals include both the Decatur chemical and film plant employees. The two plants are physically distinct entities (approximately 300 yards apart) located at the Decatur site. The vital status of 99.7% of the cohort was identified. The male and female employees contributed 33,108 and 4,807 person-years experience, respectively (Table A3, Appendix). The majority of the male employees were hired between 1965 and 1974 (Table A4, Appendix) whereas the majority of the female employees were hired between 1975 and 1979 (Table A5, Appendix). Altogether there were 74 deaths (70 males and 4 females). Among males, SMRs were below the null value for all major causes of death regardless of the comparison population used to calculate the expected values (Table III.3). Data analyses were also restricted to examining the mortality experience of employees who ever (n = 1,050) and only (n = 485) worked in the Decatur chemical plant (Tables A6 and A7, Appendix). Neither analysis resulted in significantly ($p < .05$) elevated SMRs. There were no analyses conducted by duration of employment within the chemical plant. The data were also restricted to employees who were ever (n = 1,116) and only (n = 547) employed at the Decatur film plant (data not shown). None of these analyses resulted in significantly elevated SMRs. There were four deaths among female employees (SMR = 62.6; 95% CI 17.1 – 160.4). Of these four deaths, three were from external causes (SMR = 213.4; 05% CI 44.0 - 623.6). By examining the death certificates for the specific causes of death and

circumstances surrounding the deaths, it was determined that the excess mortality among females due to external causes was not work-related. The only recommendation by the University of Minnesota investigators was that the mortality experience of Decatur plant employees be updated in 1998 which would extend the vital status search, via National Death Index records, through 1996.

In addition to the Decatur mortality study, the University of Minnesota investigators have also followed the mortality experience of a cohort of employees associated with fluorochemical production at the 3M Cottage Grove (Gilliland and Mandel, 1993). Unlike the Decatur plant, the greatest potential for exposure at Cottage Grove is to PFOA (perfluorooctanoic acid) although the plant does produce PFOS. The total cohort consisted of 2,788 male and 749 female workers employed between 1947 and 1983. There was no significantly increased cause-specific SMR for either men or women. In a proportional hazard regression model, ten years of employment in jobs associated with the chemical division was associated with a 3.3-fold increase (95% CI 1.02 – 10.60) in prostate cancer mortality compared to no employment in the chemical division. The authors suggested that this finding may be biologically plausible with exposure to PFOA as animal toxicology and human data had associated PFOA with reproductive hormone changes (Gilliland, 1992). However, the authors urged caution in the interpretation of this result due to the nonspecificity of the exposure index as well as the few subjects (n = 4) considered 'exposed.' Subsequent research on the Cottage Grove male workforce involved with PFOA production has provided reasonable assurance that there are no significant hormonal changes associated with PFOA at the serum levels measured (Olsen et al., 1998).

Current and proposed human health and exposure research initiatives are found in Section VI.

Table III.3. Selected Cause - Specific SMRs for Men
by Comparison Population (n = 1,639)

Cause of Death	Observed Deaths	SMR (95% Confidence Interval)		
		U.S.	Alabama	AL Regional Counties
All Causes of Death	70	62.9 (49.0, 79.5)	54.8 (42.7, 69.3)	52.0 (40.5, 65.7)
All Malignant Neoplasms	15	68.4 (38.3, 112.8)	60.9 (34.1, 100.4)	59.9 (33.5, 98.8)
Cerebrovascular Disease	1	33.5 (0.8, 186.8)	27.8 (0.7, 155.2)	26.5 (0.7, 147.5)
All Heart Disease	17	59.1 (34.4, 94.7)	53.2 (31.0, 85.1)	49.1 (28.6, 78.6)
Respiratory Disease	0	--- (0.0, 91.2)	--- (0.0, 80.2)	--- (0.0, 73.9)
External Causes	25	74.5 (48.2, 110.0)	59.0 (38.2, 87.1)	55.0 (35.6, 81.3)

IV. SUMMARY OF TOXICOLOGY DATA ON PFOS

Introduction

This section summarizes the existing database with respect to the potential mammalian toxicity of PFOS. Toxicology data are valuable in identifying potential health effects and provide a basis for risk characterization. The use of this information in risk characterization will be described in Section V.

With few exceptions, past toxicology studies either did not characterize blood and tissue concentrations associated with exposure or were not completely specific in characterizing blood and tissue concentrations. For these past studies, blood and tissue concentrations can only be estimated based on limited pharmacokinetic data and results from more recent studies, most of which have been designed to include a pharmacokinetic component to obtain highly sensitive and specific identification of PFOS in blood and tissues. Also, the extent of potential interspecies and intraspecies variability in pharmacokinetic handling of PFOS is not presently well defined. Comparison of serum concentrations of PFOS from toxicity studies to measured human serum concentrations may be an interesting and convenient comparison; however, body burden and/or target organ tissue concentration may be more meaningful in characterizing risk. The available body of animal toxicity data cannot be related quantitatively and without uncertainty and speculation to the concentrations of PFOS in human blood reported in Section II and the results of epidemiologic investigation as reported in Section III.

Despite the current limitations in the ability to characterize risk based on the relationship of blood and tissue PFOS concentrations from toxicity studies to human blood concentrations of PFOS, the existing toxicology database on PFOS does provide valuable insight into potential health hazards and forms a foundation for risk characterization.

It should be mentioned that the specific sources of PFOS identified in the serum of non-occupationally exposed humans are currently unknown. The PFOS molecule itself may not be the primary source. PFOS in the serum of non-occupationally exposed humans may derive from metabolism of higher molecular weight molecules present in industrial, commercial and consumer products or manufacturing by-products.

The studies described in the following discussion reflect the toxic responses resulting from direct dosing with PFOS. The studies that are summarized in this section were performed with the potassium salt of PFOS unless otherwise annotated. Studies on related compounds are considered outside the scope of this discussion and will only be mentioned when they provide valuable insight into the toxicity of PFOS. Some data will be discussed from studies which are currently in progress or incomplete. Tabular summaries of rodent repeated-dose toxicity studies, primate repeated-dose toxicity summaries and developmental and reproduction studies will be found at the end of this section (Table IV.9, IV.10, and IV.11, respectively). In addition to these summary tables, Tables IV.12 presents the results of rodent and primate 90-day sub-chronic studies organized by increasing nominal mg/kg dose level. A list of studies initiated in 1998 can be found at the end of this section in Table IV.8 and summary descriptions of these studies can be found in the Appendix.

Pharmacokinetics of PFOS

The absorption, tissue distribution, potential metabolism and excretion of PFOS has been studied most extensively in rats by both radiolabel and direct quantitation. Limited data relating oral dose to serum and liver concentrations of PFOS in the cynomolgus monkey is available from a recent 28-day rangefinder capsule dosing study. In addition, serum PFOS concentrations in three retired male 3M chemical workers have been followed in an attempt to estimate an elimination rate constant for the human.

In general, PFOS:

- is well absorbed from the digestive system;
- has some limited potential for dermal absorption;
- distributes predominantly to the blood and liver, with liver concentrations being several times higher than serum concentrations;
- appears to have a serum half-life shorter than the elimination half-life after a single dose;
- appears to have extended elimination half lives in rats, monkeys and humans;
- shows slight but consistent sex differences in serum and liver concentrations on repeated dosing in the rat but not the cynomolgus monkey;
- appears to undergo enterohepatic recirculation;
- does not appear to be metabolized, including conjugation reactions.

Absorption: At least 95% of a single oral dose averaging 4.2 mg/kg [^{14}C]PFOS administered to two groups (24 hour and 48 hour sacrifice) of 3 male Charles River CD rats (248-315 g, mean = 285 g) was absorbed within 24 hours (Johnson and Ober, 1979). The radiochemical purity of the [^{14}C]PFOS used in this and the other radiolabel studies listed below was >99% (Johnson and Behr, 1979).

PFOS was applied to skin that had been clipped free of hair as a suspension in water at 5,000 mg/kg covering 40% of the total body surface area of ten male and ten female rabbits. An impervious plastic sheet occluded the skin for 24 hours and was then removed. Animals were maintained and observed for a 28-day period. Blood samples were obtained on days 1, 7, 14, and 28. Analysis for total blood fluoride was performed on the day one and day 28 samples from a single male and single female. Total serum fluoride values for the male were 10.3 ppm for day one and 130 ppm for day 28. The respective values for the female were 0.9 ppm and 128 ppm. Although this study indicated some dermal absorption, it is limited in that the values from only

two animals were measured, and only from the day one and day 28 samples (O'Malley and Ebbens, 1980).

Three male and three female albino rabbits per dose group were exposed dermally under occlusion for 24-hours to an aqueous suspension of PFOS (0.06 %) at doses equal to 0, 0.003, 0.06, and 0.3 mg PFOS / kg and held for 28 days (Glaza, 1995). Liver samples taken at term were analyzed for total organic fluorine. No quantifiable organic fluorine could be detected in the 28-day livers by combustion or by electrospray mass spectroscopy (Johnson, 1995).

Distribution: By 89 days after a single iv dose of PFOS-^{14}C (mean dose, 4.2 mg/kg) six Charles River CD male rats (initial body weights 262-303 g, mean = 288 g) excreted a mean of 30.2% of the total carbon-14 via urine. Mean cumulative fecal excretion was 12.6%. At 89 days, mean tissue concentration of total carbon-14 expressed as µg PFOS-^{14}C equivalents/g were: liver, 20.6; plasma, 2.2; kidney, 1.1; lung, 1.1; spleen, 0.5; and bone marrow, 0.5. Lower concentrations (≤0.5) were measured in adrenals, skin, testes, muscle, fat and eye. No radioactivity (<0.05) was detected in brain. The carbon-14 in liver and plasma represents 25 and 3 percent of the dose, respectively (Johnson etal, 1979).

Serum PFOS concentrations were measured throughout a 28-day oral rangefinder study in which male and female cynomolgus monkeys weighing 2.1 to 2.4 kg were given capsules containing PFOS at either 0.0 mg/kg/day (two males and two females), 0.02 mg PFOS/kg/day (three males and three females), or 2.0 mg/kg/day (one male and one female) (Thomford, PJ, 1998). The monkeys dosed with PFOS (0.02 and 2.0 mg/kg/day) demonstrated an extremely linear (r-squared > 0.99) increase in serum concentration throughout the exposure period (28 days). There was no apparent sex difference and the individual slopes of the cumulative PFOS dose versus serum PFOS concentration curve appeared to be virtually identical between the monkeys in the two dose groups. The average slope of the curve in the 0.02 mg/kg/day group (n = 6) was

5.22 ± 0.74 ppm PFOS in serum per mg/kg cumulative dose, and for the two monkeys in the 2.0 mg/kg/day dose group, the average was 5.40 ± 0.61 ppm PFOS in serum per mg/kg PFOS cumulative dose. At the end of the 28-day dosing period, serum concentration in the 0.02 mg/kg/day dose group reached approximately 3 ppm and in the 2.0 mg/kg/day dose group, serum concentrations reached approximately 300 ppm.

This data suggests a volume of distribution of 0.19 L/kg for continuous dosing over a dose range of two orders of magnitude for the male and female cynomolgus monkey. This is calculated as follows:

One (1) mg/kg results in 5.3 ppm serum PFOS;
This is equivalent to 5.3 mg/L of serum (mg/L = ppm weight/volume);
One (1) mg/kg given to 2.2 kg monkey results in a total dose of 2.2 mg;
V_d (L) is calculated as 2.2 mg / 5.3 mg/L = 0.41 L;
Normalizing this for body weight gives 0.41 L / 2.2 kg = 0.19 L / kg = V_d (L / kg);
V_d for a 60 kg woman is estimated to be 0.2 L / kg x 60 kg = 12 L = V_d (L).

Metabolism: Preliminary data from analysis of urine, feces and tissues of rats as well as the inherent stability of perfluorinated anions suggest that PFOS is not metabolized (Johnson et al., 1984). Exposure of primary human and rat hepatocytes to PFOS did not result in further metabolism (Gordon, 1998). Analysis by LC/MS of serum and liver samples from studies currently in progress have not revealed any evidence of metabolism.

Excretion: In the previously mentioned study (Johnson etal, 1979) single intravenous doses (mean 4.2 mg/kg) of [^{14}C]PFOS in 0.9% NaCl were administered to male rats. By 89 days after dosing, 30.2% of the administered ^{14}C had been excreted in the urine and 12.6% had been excreted in the feces.

Whole body elimination in the male rat appeared to be biphasic. Initial redistribution from the plasma yielded a plasma elimination half-life of ^{14}C of 7.5 days following single oral administration of [^{14}C]PFOS (mean dose 4.2 mg/kg) to male rats (Johnson and Ober, 1979). In the aforementioned intravenous study, elimination of only 42.8 % of the dose through urine and feces after 89 days indicates that the half-life of elimination from the body is > 89 days in the male rat.

Serum PFOS levels in three retired male 3M chemical workers have been followed for five and one-half years and suggest a mean serum elimination half-life of 1,428 days. Over that time period that these serum samples were taken and analyzed, the analytical method changed from thermo-spray mass spectrometry to electro-spray mass spectrometry, and the analytical laboratories changed. These changes should not have affected the values reported to any appreciable extent. Since urine and feces have not been followed, it is difficult to ascertain if this represents a true elimination half-life from the body; however, this is the closest value for elimination half-life applicable to humans in existence. The actual data from these three retired chemical workers is presented in Table IV.1.

Table IV.1 Serum Elimination of PFOS in Retired 3M Chemical Workers.

	Serum [PFOS] in mg/L (ppm)					
	Retiree 1		Retiree 2		Retiree 3	
	3/4/92	5.8	3/18/92	1.6	3/18/92	4.2
	9/20/93	6.0	11/15/93	1.4	11/29/93	3.3
	3/1/94	4.0	5/5/95	0.7	5/4/95	1.7
	6/19/95	3.0	11/18/97	0.7	10/23/97	1.4
	12/5/97	2.6				
Serum Elim. Constant (λ)	- 0.0132/month		- 0.0136/month		- 0.0176/month	
Serum Elim. $T_{1/2}$	52.5 mo/1575 d		50.9 mo/1527 d		39.4 mo/1182 d	

Fecal and total excretion of ^{14}C were markedly increased in male rats administered cholestyramine (~2.7 g/kg/d) in their diet following single intravenous doses of [^{14}C]PFOS. The results suggest that there was significant enterohepatic circulation of PFOS (Johnson and Gibson, 1980, 1984). Cholestyramine administered at 4% by weight in feed to male rats decreased the retention of carbon-14 in liver, plasma, and red blood cells and increased the elimination of carbon-14 via feces after iv dosing with PFOS-^{14}C. Groups of five rats (twelve-week old Charles River CD averaging 320 g) were dosed intravenously with PFOS-^{14}C (mean dose, 3.4 mg/kg). Groups of five control rats were dosed similarly but were not treated with cholestyramine. Rats were sacrificed at 21 days post dose. The mean liver, plasma, and red blood cell concentration as well as fecal and urinary excretion of ^{14}C for cholestyramine-treated

rats were compared to mean control rat values. Mean cholestyramine-treated rat ^{14}C concentrations in liver (9.4µg/g), plasma (0.9µg/ml), and red blood cells (0.3 µg/g) represent a decrease from mean control rat concentrations of 3.8, 7.7, and 6.0 fold, respectively. Fecal elimination (75.9% with cholestyramine treatment) was increased 9.5 fold. The extent of urinary ^{14}C elimination, as a result of the relatively high rate of fecal elimination of ^{14}C was lower in cholestyramine-treated rats. The extent of total elimination of ^{14}C (urine plus feces) was higher in the cholestyramine-treated rats. Since cholestyramine is approved for use in humans as a cholesterol lowering agent, these results in rats support the concept of testing cholestyramine in humans to promote excretion of PFOS (Johnson et al, 1980).

Acute Toxicity

Acute Oral Toxicity: PFOS was administered by gavage in a 20 : 80, acetone: corn oil suspension to five male and five female rats a dose levels of 100, 215, 464, and 1000 mg/kg. Observations of toxicity were made over a 14-day period. All rats in the 1000 and 464 mg/kg dose groups died. The signs before death included hyperactivity, decreased limb tone and ataxia. At 215 mg/kg, three of ten animals died. At 100 mg/kg, no deaths were observed. The combined acute oral LD50 in male and female rats is 251 mg/kg (95% C.I.: 199-318 mg/kg). PFOS is considered moderately toxic on acute oral administration under the conditions of this study (Dean et al., 1978).

Acute Dermal Toxicity: Ten male and ten female albino rabbits were clipped free of hair and an aqueous suspension equivalent to 5 g/kg PFOS was placed over 40% of the total body surface area, occluded with an impervious plastic sheet and left in contact with the skin for 24 hours, then removed. Animals were maintained and observed for a 28-day period after which they were necropsied. Blood samples were obtained on days 1, 7, 14 and 28. Day 0, 7, 14 and 28 body weights were recorded. Hyperactivity was noted in 5 of 10 males on day 6. All animals recovered by day 7 and remained asymptomatic throughout the study period. Weight gains were observed for all rabbits. No visible lesions were noted at necropsy. Analysis of total blood fluoride from day 1 and day 28 blood samples of one male and one female indicated that PFOS was absorbed through the skin, reaching blood concentrations of approximately 130 ppm after 28 days (see section above on absorption). PFOS can be considered practically non-toxic on single dermal contact (O'Malley and Ebbens, 1980).

Acute Inhalation Toxicity: Groups of five male and five female Sprague-Dawley rats were exposed by inhalation for one-hour to nominal exposure concentrations of 24, 7.1, 6.5, 4.9, 2.9, 1.9 and 0.0 milligrams PFOS per liter of air. The rats were observed hourly for the first four hours and daily thereafter for 14 days. All rats

exposed to 24 milligrams per liter died. Partial mortality was produced at nominal exposure concentrations of 7.1 (80%), 6.5 (80%), 4.9 (20%) and 2.9 (10%) milligrams per liter. Symptoms observed during the exposure and post-exposure periods were labored breathing, reduced activity, excessive salivation and lacrimation, mucoid and red nasal discharge, yellow staining of the ano-genital fur, and dried red material on the facial area. The most frequent abnormal necropsy observations were of lung and liver discoloration. Lung discoloration was also observed in a high number of control rats and thus may not be treatment-related. The nominally determined median lethal concentration (LC50) for a one-hour exposure to PFOS was determined to be 5.2 milligrams per liter with 95% confidence limits of 4.4 milligrams per liter and 6.4 milligrams per liter (Rusch and Rinehart, 1979).

Primary Irritation

Ocular Irritation: PFOS was found to be mildly irritating to the eyes of albino rabbits when tested according to standard Federal Hazardous Substances Act guidelines. The ocular irritation was limited to the conjunctivae in the six test rabbits. Irritation was noted at the 1, 24 and 48 hour post-instillation reading times. The maximum irritation score was 9.3 out of a highest possible score of 110 at the 24 hour reading. By 72 hours post-instillation all readings were zero (Biesemeier and Harris, 1974).

Dermal Irritation: PFOS was found to be non-irritating to the skin of albino rabbits when tested under conventional Draize procedures. No signs of dermal irritation were observed in any of the test animals at any time during the study period. The primary skin irritation score was 0.0 out of a highest possible score of 8.0 (Biesemeier and Harris, 1974).

Genotoxicity

Gene Mutation: PFOS was not mutagenic in *Salmonella typhimurium* strains TA-1535, TA-1537, TA-1538, TA-98, TA-100 or in *Saccharomyces cerevisiae* strain D4

in a standard plate incorporation assay with or without metabolic activation. (Jagannath and Brusic, 1978).

Chromosomal Effects: PFOS did not cause chromosomal aberrations in an *in vivo* mouse bone marrow micronucleus assay. PFOS in water was administered by oral gavage at 0, 237.5, 475, and 950 mg/kg to five male and five female mice for each dose/ harvest time group. The mice were euthanized 24, 48 and 72 hours after dosing for extraction of bone marrow. No increase in bone marrow polychromatic erythrocytes was observed (Murli, 1996).

Repeated-Dose Toxicity

A number of repeated-dose toxicity studies have been conducted with PFOS and will be discussed in this sub-section. A tabular summary of these studies will be found at the end of this section in Table IV.9 for rodent studies and Table IV.10 for primate studies. In addition to these summary tables, Table IV.12 presents the results of rodent and primate 90-day sub-chronic studies organized by increasing nominal mg/kg dose level.

90-Day Dietary Study in Rats: PFOS was fed in the diet at levels of 0, 30, 100, 300, 1,000 and 3,000 ppm to groups of five male and five female Charles River CD rats for 90 days. These doses represent approximately 0, 2.6, 18, 60 and 180 mg/kg/day. The rats were observed twice daily for overt signs of toxicity and mortality. Individual body weight and sex group food consumption were recorded weekly. Hematological, biochemical and urinalysis studies were conducted prior to commencement of dosing, at one month and at study termination.

At the 30 ppm (approximately 2 mg/kg/day) dosage level, no rats showed any compound related changes in appearance or behavior. Mean body weights were slightly lower when compared to the controls. At one month, one female rat showed a

slightly elevated blood glucose, and one male rat showed a slightly elevated serum alkaline phosphatase. At three months, one male rat had slight to moderate elevations in blood glucose, blood urea nitrogen and gamma-glutamyl transpeptidase activity.

At the 100 ppm (approximately 6 mg/kg/day) dosage level, mean body weight and food consumption was significantly lower than the control group. One male and two females died. Slight increases in creatinine phosphokinase (CPK) and serum alkaline phosphatase activity, slight to moderate increases in blood glucose and blood urea nitrogen, and slight to marked increases in plasma transaminase activities (AST and ALT) were seen at one month. At three months, all rats in the 100 ppm dose group had slight to moderate decreases in hemoglobin, hematocrit and erythrocyte counts, and slight to moderate increases in transaminase activities were seen for two of the three surviving female rats.

At dosage levels of 300, 1,000 and 3,000 ppm (approximately 18, 60 and 180 mg/kg/day) all rats died prior to scheduled termination of the study. Time of death was dose-related. Overt clinical observations of toxicity included emaciation, convulsions, altered posture, red material (right eye and/or mouth), yellow material in the ano-genital region, increased sensitivity to external stimuli and reduced motor activity. Compound-related gross changes such as emaciation and areas of discoloration involving the stomach and liver were observed among treated rats that died prior to sacrifice. Similar changes were also observed in the liver of a few rats sacrificed at termination of study from the 30 and 100 ppm groups.

Morphological changes consisting of centrilobular to midzonal cytoplasmic enlargement (hypertrophy) of hepatocytes and necrosis of liver cells was present in all PFOS dose groups. The incidence and relative severity of the above lesions were more evident among male rats. In addition, rats from the 300, 1,000 and 3,000 ppm dosage levels displayed compound-related changes involving the primary (thymus,

bone marrow) and secondary (spleen, mesenteric lymph nodes) lymphoid organs, stomach, intestines, muscle and skin (Goldenthal et al, 1978a).

Two-Year Dietary Study in Rats, Four and Fourteen Week Results: Results are available from four-week and fourteen-week interim sacrifices in Sprague-Dawley rats which are part of an on-going 104-week dietary study (Covance, 1998). In these sub-studies, groups of five male and five female rats (four and 14-week sacrifice groups) and ten male and ten female rats (14-week sacrifice groups) were exposed to either 0, 0.5, 2, 5, or 20 ppm PFOS in the diet for four or 14 weeks. Clinical observations were made twice daily. Body weights and food consumption were measured once a week. Organ weights were measured at term. Samples were taken at both sacrifices for PCNA, liver palmitoyl CoA oxidase activity, serum and liver PFOS concentrations and at the 14-week sacrifice for hematology, clinical chemistry (including urinalysis), and histopatholgy. During weeks 4 and 14, blood and urine were collected for hematology, clinical chemistry, urinalysis, and urine chemistry tests from ten animals/sex in Groups 1 through 5. Five animals/sex in Groups 1 through 5 were sacrificed during Week 4; livers were collected and weighed. A portion of the liver was prepared for PFOS analysis, a portion was shipped to Pathology Associates International for hepatocellular proliferation rate (PCNA) measurements, and a portion was used for determination of palmitoyl-CoA oxidase activity. At week 14, necropsies were performed on five animals/sex in Groups 1 through 5. At necropsy, macroscopic observations were recorded, organ weights were obtained, and tissues were placed in fixative as specified by the protocol. In addition, liver samples were collected for PFOS analysis, hepatocellular proliferation rate measurement, and palmitoyl-CoA oxidase determination. Microscopic examinations were done on selected tissues from animals necropsied during week 14. The tissues were adrenals, brain, eyes, kidney, liver, mesenteric lymph node, pancreas, spleen, testes, and ovaries. In addition, microscopic examinations were done on tissues from animals that died or were sacrificed due to poor health.

There were relatively few statistically significant or otherwise notable differences for clinical pathology results between the control and treated groups. Although none of the statistically significant differences for high dose animals were consistent at bo testing intervals, it was considered likely that administration of the test material was associated with mildly higher urea nitrogen at Week 14 for males and females fed 20.0 ppm and moderately lower cholesterol and mildly higher alanine aminotransferase at Week 14 for males fed 20.0 ppm. There were no correlative microscopic renal findings for the minor change in urea nitrogen. The findings for alanine aminotransferase and cholesterol were likely associated with the histopathological findings of hepatocellular hypertrophy and vacuolation.

Of uncertain relationship to the test material was mildly, but statistically, higher absolute neutrophil count for males fed 20.0 ppm. Females were unaffected, and there were no correlative microscopic findings for this small difference.

All other statistically significant differences for clinical pathology results between the control and treated groups were considered incidental. Lower glucose at Week 4 for high-dose males and lower aspartate aminotransferase at Week 4 for high dose females were considered incidental because they were not present at Week 14. Higher albumin at Week 14 for high dose females was considered incidental because the low dose females had a similar, but higher, statistically significant difference for albumin.

Table IV.2 and IV.3 summarize key clinical pathology results at 14 weeks for males and females, respectively.

Table IV.2 Clinical chemistry and hematology in Male rats at week 14 (n=10)

	Average Value and Std Dev. by Dose Group (ppm PFOS in Diet)					ANOVA
Parameter	0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm	Pr > F
ALB	5 ± 0.17	5 ± 0.2	4.8 ± 0.35	5 ± 0.3	5 ± 0.16	0.32
ALT (SGPT)	38 ± 31	42 ± 7	40 ± 6	51 ± 15	81 ± 74	0.28
AST (SGOT)	109 ± 31	121 ± 14	139 ± 14	137 ± 10	159 ± 77	0.34
CHOL	64 ± 13	53 ± 22	50 ± 16	57 ± 8	36 ± 18*	0.13
GLU	103 ± 8	104 ± 13	83 ± 15	93 ± 8	92 ± 14	0.062
N_SEG	1 ± 0.4	1.2 ± 0.3	0.94 ± 0.2	1.3 ± 0.46	1.7 ± 0.3	NA
UN	13 ± 2	14 ± 2	14 ± 2	14 ± 0	17 ± 1*	0.0007
PCoAO	4.6 ± 1.34	4.8 ± 3.3	5.4 ± 3	1.8 ± 1.8	5.4 ± 1.9	0.14

* Mean significantly different (Dunnetts' T-test, $p < 0.05$) than the control group value

Table IV.3 Clinical chemistry and hematology in female rats at week 14 (n=10)

	Average Value and Std Dev. by Dose Group (ppm PFOS in Diet)					ANOVA
Parameter	0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm	Pr > F
ALB	5.5 ± 0.25	6.1 ± 0.5*	5.7 ± 0.17	6.2 ± 0.2*	6.1 ± 0.24*	0.0057
ALT (SGPT)	34 ± 2	39 ± 11	30 ± 5	36 ± 5	33 ± 5	0.24
AST (SGOT)	103 ± 21	108 ± 23	98 ± 8	109 ± 22	79 ± 9	0.092
CHOL	78 ± 19	79 ± 14	70 ± 12	68 ± 19	65 ± 16	0.64
GLU	102 ± 16	100 ± 12	105 ± 3	90 ± 8	100 ± 7	0.31
N_SEG	0.8 ± 0.36	0.9 ± 0.4	0.53 ± 0.13	0.56 ± 0.1	0.64 ± 0.1	NA
UN	14 ± 1	16 ± 4	14 ± 2	16 ± 1.7	16 ± 1.8	0.26
PCOAO	1.8 ± 1.6	3.0 ± 2.6	1 ± 0.8	1.6 ± 2.6	5 ± 2.9	0.10

* Mean significantly different (Dunnetts' T-test, $p < 0.05$) than the control group value

Body and Organ Weights: Terminal body weights at 14 weeks for the 2 ppm dose group were reduced in comparison to controls by 4.5% in the males and 11.7% in the females. Absolute and relative liver weights were significantly increased by approximately 35% in the males given 20.0 ppm PFOS in the diet. In females given

20.0 ppm, the absolute liver weights were increased by approximately 30% over control values, but only the liver-to-body weight percentage was significantly increased. The absolute spleen weight was significantly decreased in the females given 20.0 ppm, as was the absolute lung weight in females given 2.0, 5.0, or 20.0 ppm. Spurious significant increases in left thyroid/parathyroid-to-body weight ratios were seen in females given 5.0 or 20.0 ppm.

Macroscopic Observations: There were no macroscopic observations that could be attributed to the administration of the test material.

Microscopic Observations: Test material related histomorphologic changes were limited to the liver in the males given 5.0 or 20.0 ppm and in the females given 20.0 ppm. The changes consisted of hypertrophy of hepatocytes in centrilobular areas, and midzonal to centrilobular hepatocytic vacuolation. The incidence and severity of the changes tended to be greater in the males.

There were no apparent test material-related lesions in the remaining tissues examined.

Serum and liver concentrations of PFOS increased with dose and with length of exposure, and liver values were significantly higher than corresponding serum values. These analytical results are presented in Tables IV.4 and IV.5 for serum and liver, respectively.

Table IV.4 Mean Serum PFOS Concentrations (ppm) After 4 and 14 Weeks Dietary Exposure

Serum [PFOS] in ppm by Dietary Dose Group (ppm PFOS in Diet) $n = 5$ per sex per dose group						
Time Interval	Dose Group	0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm
4 Weeks	Male	< MDL**	1.14 ± 0.07	5.05 ± 1.2	8.95 ± 2.31	46.4 ± 8.46
	Female	0.04 ± 0.003	1.90 ± 0.22	7.50 ± 0.053	14.3 ± 1.85	59.4 ± 8.46
14 Weeks	Male	< MDL**	4.22 ± 0.83	17.9 ± 1.23	45.6 ± 5.73	152 ± 13.3
	Female	2.45 ± 4.19*	6.65 ± 1.08	26.9 ± 2.26	62.9 ± 6.19	216 ± 21.9

*0 ppm female [PFOS] in serum at 14 weeks without one outlying value (n=4) is 0.59 ± 0.59 ppm.

**MDL = Method Detection Limit, which was 15 ppb.

Table IV.5 Mean Liver PFOS Concentrations (ppm) After 4 and 14 Weeks Dietary Exposure

Liver [PFOS] in ppm by Dietary Dose Group (ppm PFOS in Diet) $n = 5$ per sex per dose group						
Time Interval	Dose Group	0 ppm	0.5 ppm	2 ppm	5 ppm	20 ppm
4 Weeks	Male	0.11 ± 0.74	11 ± 1.70	33 ± 8.0	49 ± 12	295 ± 45
	Female	0.11 ± 0.05	8.6 ± 0.57	25 ± 6.2	80 ± 17	357 ± 43
14 Weeks	Male	0.44 ± 0.05	26 ± 4.4	77 ± 7.5	387 ± 33	600 ± 116
	Female	1.58 ± 2.1	19 ± 3.4	68 ± 3.0	362 ± 26	618 ± 50

90-Day Drinking Water Study with Lithium Perfluorooctane Sulfonate (LPOS) in Rats: The lithium salt of perfluorooctane sulfonate was administered to male and female Sprague-Dawley rats in drinking water for 90 consecutive days. With the exception of controls, male and female doses did not directly correspond and each dose group was either ten males or ten females. Female LPOS doses, expressed as mg/kg body weight, were: 0 (two separate controls, one added later as a concurrent control for the 0.6 mg/kg dose group); 0.02; 0.06; 0.2; and 0.6 mg/kg (added later). Male LPOS doses, also expressed as mg/kg body weight, were: 0; 0.3; 1.0; and 3.0.

weight, kidney weight and testes weight relative to body weight (3.0 mg/kg dose group). Gross pathology revealed the following organ-weight effects in female rats: 1) increases in absolute liver weights and liver weight relative to body weight (0.06, 0.2 and 0.6 mg/kg dose groups); 2) increased liver weight relative to brain weight (0.2 and 0.6 mg/kg dose groups); 3) decreases in absolute heart weight, heart weight relative to brain weight and heart weight relative to body weight (0.02, 0.06, 0.2 and 0.6 mg/kg dose groups); 4) increased brain weight relative to body weight (0.02 and 0.06 mg/kg dose groups) and 5) increased kidney weight relative to body weight (0.2 mg/kg dose group). With the exception of liver, these organ weight changes were related to reduced body weight gain and were not associated with histopathologic alterations. The dose-related increases in liver weights and reductions in serum cholesterol and triglycerides are supported by the histopathologic finding of hepatic vacuolization in the 1.0 and 3.0 mg/kg male rats.

90-Day Oral Gavage Study in Rhesus Monkeys: In an initial study, five groups consisting of two male and two female rhesus monkeys initially weighing between 2.75 and 4.10 kg were given daily doses of 0, 10, 30, 100 and 300 mg PFOS / kg by gastric intubation (gavage) as distilled water suspension. This study was terminated after 20 days due to the death of all PFOS-dosed monkeys (Goldenthal, 1978). Time of death was dose-related. Monkeys in the 300 mg/kg/day dose group died between the second and fourth day. The 100 mg/kg dose group died between the third and fifth day. At 30 mg/kg, deaths occurred between the seventh and tenth day. The 10 mg/kg dose group died between days 11 and 20. All PFOS-treated monkeys lost weight. Clinical observations of toxicity were similar for all PFOS dose groups, and time of onset of toxic effects was dose-related. These toxic symptoms included anorexia, slight to severe decreased activity, frothy or food-like emesis, and occasional diarrhea. Prior to death, body and limb stiffening, general body tremors, convulsions and prostration were observed. The 10 mg/kg dose group included one monkey who had black stools and one who developed facial erythema. Gross

pathology revealed a yellowish-brown discoloration of the livers of several monkeys in the 100 mg/kg and 300 mg/kg dose groups. Organ weights appeared within normal limits; however, due to early deaths of PFOS-treated monkeys and the reuse of control monkeys on a follow-up study at lower dose levels, concurrent control data was not available. Histopathologic examination of tissues from treated monkeys did not reveal any consistent observations which could be directly related to an effect of PFOS. Congestion and lipid depletion of the adrenal cortex was seen in all dose groups and was considered agonal. The study was terminated after the death of the last 10 mg/kg dose-group monkey.

After termination of the initial study after early deaths of all PFOS-treated monkeys, the control group monkeys were used in a follow-up study at lower doses (Goldenthal etal, 1978b). In this study, five groups consisting of two male and two female rhesus monkeys initially weighing between 2.55 and 3.75 kg were given daily doses of 0, 0.5, 1.5, and 4.5 mg PFOS / kg by gastric intubation (gavage) as distilled water suspension for 90 days. The monkeys were observed twice daily for general physical appearance, behavior and other clinical signs of toxicity. Body weights were recorded weekly. Hematological and biochemical studies and urinalysis were conducted once in the control period and at the end of the first and third month of the study.

The monkeys treated at the 4.5 mg/kg/day dosage level died or were sacrificed in extremis between weeks 5 and 7 of the study. These monkeys exhibited gastrointestinal symptoms including anorexia, emesis, black stool and dehydration from the first or second day of study. These monkeys also exhibited decreased activity and showed marked to severe rigidity, convulsions, generalized body trembling, prostration and loss of body weight prior to death. The mean body weight decreased from 3.44 kg at the beginning of the study to 2.70 kg at week 5 of the study. All monkeys at the 4.5 mg/kg/day dosage level had decreased serum cholesterol values and serum alkaline phosphatase activity at one month.

All monkeys at the 1.5 mg/kg/day dosage level survived to the end of the study. These monkeys exhibited slightly decreased activity from the first week of the study which occasionally became moderate to marked. In addition, they occasionally had black stools, diarrhea, mucous in the stool and bloody stool and exhibited dehydration or general body trembling at the end of study. The monkeys from this group had a slight decrease in mean body weight. Slight decreases in serum alkaline phosphatase activity and serum inorganic phosphate concentrations were evident at the end of the study in addition to a marked decrease in serum cholesterol.

All monkeys at the 0.5 mg/kg/day dosage level survived to the end of the study. Monkeys at this dosage level exhibited an occasional soft stool, diarrhea, anorexia and emesis, all of which also occurred occasionally in the control group. Slighily decreased activity was noted intermittently in three monkeys at this dosage level. At three months of study a statistically significant decrease in the serum alkaline phosphatase activity was noted in the males as compared to control values; however, the toxicologic significance of this finding is questionable when compared to individual pre-dose values. The 0.5 mg/kg dose has been considered to present a LOEL by past reviewers. Recent evaluation of the study by 3M toxicologists lend to a conclusion that this dose more likely represents a NOAEL, if not a NOEL.

No treatment-induced gross or microscopic pathological lesions were seen in tissues other than the adrenals, pancreas, and submandibular salivary glands of male and female rhesus monkeys at the 4.5 mg/kg/day dosage level. Microscopically, the adrenals from male and female monkeys at the 4.5 mg/kg/day dosage level had compound-related marked diffuse lipid depletion; the pancreas from male and female monkeys at the 4.5 mg/kg/day dosage level had compound-related moderate diffuse atrophy of exocrine cells; the submandibular salivary glands from male and female monkeys had compound-related moderate diffuse atrophy of the serous alveolar cells. No statistically significant variations in sex group mean weights of organs occurred between the control and experimental groups.

Table IV.6 summarizes the individual cholesterol values for all dose groups.

Table IV.6: Longitudinal analysis of serum cholesterol and PFOS concentrations of individual male and female rhesus monkeys during 90 days dosing with either 1.5 or 4.5 mg/kg PFOS (n = 2 per sex per dose group) (Goldenthal etal, 1978b).

Individual Serum Cholesterol (mg /100 ml) by Study Period and Cumulative Dose (CD in mg/kg)

Dose Group	Individual	Pre-Dosing	1 Month	3 Months	% of Pre-Dosing Value at Term
		CD = 0	*CD = 0*	*CD = 0*	
0	♂ (ID # 7355)	192	212	179	93.2
mg/kg/day	♂ (ID # 7358)	174	184	144	82.8
Dose Group	♀ (ID # 7368)	155	188	144	92.9
Monkeys	♀ (ID # 7372)	204	208	185	90.7
		CD = 0	*CD = 15*	*CD = 45*	
0.5	♂ (ID # 7463)	182	208	160	87.9
mg/kg/day	♂ (ID # 7483)	161	192	143	88.8
Dose Group	♀ (ID # 7466)	208	230	178	85.6
Monkeys	♀ (ID # 7504)	208	211	160	76.9
		CD = 0	*CD = 45*	*CD = 135*	
1.5	♂ (ID # 7462)	196	222	132	67.3
mg/kg/day	♂ (ID # 7486)	174	204	112	64.4
Dose Group	♀ (ID # 7500)	172	154	128	74.4
Monkeys	♀ (ID # 7501)	236	184	96	40.7
		CD = 0	*CD = 135*	No Survivors	
4.5	♂ (ID # 7484)	186	76	-	40.9
mg/kg/day	♂ (ID # 7485)	194	97	-	50.0
Dose Group	♀ (ID # 7502)	182	128	-	70.3
Monkeys	♀ (ID # 7503)	170	96	-	56.4

28-Day Range-Finding, Oral Capsule-Dosing Study in Cynomolgus Monkeys: Male and female cynomolgus monkeys weighing 2.1 to 2.4 kg were given capsules placed directly in the stomach that contained PFOS at either 0.0 mg/kg/day (two males and two females), 0.02 mg PFOS/kg/day (three males and three females), or 2.0 mg/kg/day (one male and one female) for 28 days in a range-finding study to determine doses for a six-month chronic oral capsule-dosing study (Thomford, PJ, 1998). Blood was collected for clinical chemistry on study days -7 (baseline values), 2, 7, 14 and 29. In addition to standard hematologic parmeters and serum chemistry determinations, sex and thyroid hormones, cholecystokinin (CCK) and pancreatic amylase were measured. At the same time points and on day 3, blood was also obtained for determination of serum PFOS concentration. Tissues were obtained at necropsy, weighed, fixed and prepared for histopathologic analysis. In addition to histopathologic samples, liver specimens were obtained for analysis for proliferating cell nuclear antigen (PCNA), determination of PFOS concentration, and determination of palmitoyl CoA oxidase activity. Serum PFOS concentrations increased with a high degree of linearity at both dose levels, with no difference between males and females and at a linear rate of 5.3 ppm serum PFOS per mg/kg (for details please see subsection on toxicokinetics). At the end of the 28-day dosing period, serum PFOS concentration in the 0.02 mg/kg/day dose group reached approximately 3 ppm and in the 2.0 mg/kg/day dose group, serum concentrations reached approximately 300 ppm. The only treatment-related effect observed in the study was a dramatic reduction in serum cholesterol in the male and female that received a dose of 2.0 mg/kg/day. Serum cholesterol dropped from baseline values of 150 and 141 mg/dl for the male and female, respectively, to 91 and 62 mg/dl at termination on day 29. The first evidence of a significant decrease occurred between day 2 and day 7 for the female, with a day 2 value of 136 mg/dl and a day 7 value of 117 mg/dl. The male cholesterol value fell from to 151mg/dl on day 2 to 137 on day 7. The day 7 values for the male and female corresponded to a serum PFOS concentration of 72 ppm. This cholesterol data and corresponding cumulative dose and serum PFOS concentrations are summarized in Table IV.7. There were no other significant findings.

Table IV.7: Longitudinal analysis of serum cholesterol and PFOS concentrations in male and female cynomolgus monkeys (n = 6 per sex) during 28 days dosing with 2.0 mg/kg PFOS.

Study Day	-7	2	7	14	29
Cumulative Dose (mg/kg)	0	2	12	26	56
♂ Serum Cholesterol (mg/dl)	150	131	137	132	91
♂ [PFOS] in serum (ppm)	0.013	12.6	71.8	129	313
♀ Serum Cholesterol (mg/dl)	141	136	117	107	62
♀ [PFOS] in serum (ppm)	0.014	14.4	72.7	143	299

Six-Month Oral (Capsule) Study in Cynomolgus Monkeys

Unaudited clinical pathology and clinical observations through 90 days of dosing are available from an ongoing six-month oral (capsule) dosing study in cynomolgus monkeys (Covance Study Number 6329-223, in progress). In this study cynomolgus monkeys are being dosed by capsule with either 0, 0.03, 0.15, or 0.75 mg/kg/day PFOS for a period of six months. Dose groups include six monkeys per sex per group with the exception of the 0.03 mg/kg/day dose group which includes four monkeys per sex. The only significant finding through 90 days is a reduction in serum total cholesterol in the males and females of the high dose group. While serum PFOS concentrations have not yet been analyzed and reported, previous experience in the range-finder for this study suggests that the serum concentrations are expected to center on approximately 350 ppm PFOS as 90 days for the high-dose monkeys.

Reproductive and Developmental Toxicity

This sub-section discusses the available information on developmental and reproductive toxicity. A summary of this data in tabular form can be found at the end of this section in Table IV.11.

Oral Developmental Toxicity (Teratology) in Rats: A rat PFOS oral teratology study was conducted at Riker Laboratories (Gortner etal, 1980). Dose levels (oral)

given to the pregnant rat dams were 0, 1, 5, and 10 mg/kg. Maternal toxicity (reduced weight gain) occurred at the high dose of 10 mg/kg on days 6 through 15 of gestation. Evidence of fetal toxicity was not found at any dose level. No skeletal and soft tissue teratogenic changes were found at any dose level with one exception. A change in the lens of the eye was found in all dose groups including the control but the incidence in high dose group was significantly higher. This change was reported out as a developmental eye abnormality and the summary of the report states the compound was teratogenic. An outside consultant and teratology expert, Dr. E. Marshall Johnson from Jefferson Medical College, visited 3M and reviewed the rat pup eye specimens in question. He concluded that the eye/lens changes were, in fact, sectioning artifacts and not compound related teratology abnormalities. Thus, the weight of the evidence indicates that PFOS does not cause teratogenic effects in rats when dosed at levels which are not maternally toxic. The lens change observed in rat pups in Riker Laboratories studies was a sectioning artifact and was not found upon repeat studies at independent laboratories.

Oral Developmental Toxicity (Teratology) in Rats: In a subsequent study, PFOS (suspended in corn oil) was administered on gestational days 6-15 by oral gavage to groups of 25 pregnant Sprague-Dawley CD rats at doses of 0 (control), 1, 5, and 10 mg/kg/day (Wetzel, etal, undated). Severe maternal toxicity occurred in the 5 mg/kg and 10 mg/kg dose groups, as evidenced by significant reductions in mean body weight gain, terminal body weight minus gravid uterine weight and food consumption compared to control dams, actual losses in body weight on commencement of treatment among numerous dams and death in two dams in the 10 mg/kg dose group prior to gestational day 20. Mean body weight gains (days 0-20) at 5 and 10 mg/kg were 104 ± 35 (S.D.) and 34 ± 73 (S.D.), respectively, as compared to 125 ± 24 (S.D.) in the control group. Mean food consumption values (days 0-20) at 5 and 10 mg/kg were 363 ± 60 (S.D.) and 264 ± 90 (S.D.), respectively, as compared to 421 ± 28 for the control group. Mean terminal body weight minus gravid uterine weight at 5 and 10 mg/kg was 293 ± 28 (S.D.) and 241 ± 60 (S.D.), respectively, as compared to 321

± 23 (S.D.) in the control group. Clinical signs in surviving dams included hunching, lower body weight, alopecia, rough haircoat, anorexia. Gastrointestinal and kidney lesions were noted in the high-dose dams.

Treatment-related fetal effects that were attributed to maternal toxicity included: increased resorptions and fetal death, decreased fetal body weight, delayed skeletal ossification, cleft palate, subcutaneous edema and cryptorchism (undescended testicles). These effects occurred primarily in the high-dose group. The maternal and fetal NOAELs for this study were both 1 mg/kg day.

Oral Developmental Toxicity (Teratology) in Rabbits: A final draft report of an oral developmental toxicity study in rabbits was recently received (York, 1998). In this study, dose groups of 22 pregnant new zealand white rabbits were dosed on days 7 through 20 of gestation with either 0, 0.1, 1.0, 2.5, or 3.75 mg/kg/day PFOS.

Maternal toxic effects included. 1) decreased body weight at the highest three dose levels with a minimal effect at the 1.0 mg/kg dose; 2) decreased food consumption at the highest two doses; 3) frequent scant feces at the highest dose, and 4) increased abortions at the highest two doses.

Fetal toxic effects included reduced fetal weight and an increase in delayed ossification at the highest two doses. No teratogenic events were observed in the study.

Based on this draft final report, PFOS was not teratogenic under conditions of the study and the maternal and fetal NOELs are 0.1 mg/kg/day and 1.0 mg/kg/day, respectively.

Two-Generation Reproductive Toxicity in Rats by Oral Gavage: Interim results are available from an on-going two-generation reproduction study in rats by oral

gavage (Argus, 1998). In this study, groups of 35 male and 35 female rats were exposed to 0, 0.1, 0.4, 1.6 and 3.2 mg/kg by daily oral intubation six weeks prior to and during mating. For the females, treatment continued during gestation, parturition and lactation.

A pre-mating reduction in mean body-weight gain as compared to controls occurred in females and possibly in the males at the high dose level. At the 3.2 mg/kg dose level, male body-weight gain was 97.2 % of control and female body-weight gain was 91.5 % of control. There was no effect on the number of pregnancies.

During gestation, females showed a reduction in mean body-weight gain at the three highest dose levels, reaching 86.9% of the control at 3.2 mg/kg. A group of ten dams per dose group was sacrificed on day 10 of gestation. No increases in resorptions occured, and there were no decreases in the number of implantations or number of live fetuses.

At parturition in the 3.2 mg/kg high-dose group, the mean number of pups delivered was decreased compared to the control (10 versus 14) and the percent of pups delivered stillborn was increased significantly (24.9 % as compared to 2.2 %.) Survival of pups during days one through four of lactation was severely affected at 1.6 and 3.2 mg/kg (66 % and 0% survival, respectively.) Most deaths at the high dose occured within the first 24 hours after birth. Surviving pups in the 1.6 mg/kg dose group showed severely depressed mean body-weight gains through day 21 of lactation (72.1 % of controls.)

A series of follow-up studies are to be initiated in November, 1998 to gain a better understanding of the reduced perinatal survival at the high dose levels. These will include a complete cross-fostering study, and two pharmacokinetic studies with pregnant dams.

The post-weaning F_1 generation currently exhibits no signs of toxicity at 0.1 and 0.4 mg/kg/day.

Mechanistic Studies

The specific mechanisms relating to PFOS toxicity are not completely understood. Several studies provide clues to the potential operative mechanism(s) of toxicity. Effects on lipid and lipoprotein processing, cholesterol synthesis and bioenergetics have been studied.

Mitochondrial Bioenergetics: Studies in isolated rat liver mitochondria at PFOS concentration in the range of 10 μM demonstrate: 1) detergent type effects on mitochondrial membranes; 2) stimulation of mitochondrial respiration, and 3) fluidization of the inner mitochondrial membrane (Wallace and Starkov, 1998).

Interference with Fatty Acid Binding and Transport: Interference of PFOS (1-10 μM) with endogenous fatty acid binding to carrier protein substrates, liver fatty acid binding protein (L-FABP) and albumin (BSA) has been investigated and show a 66% reduction of initial fluorescence when added to solutions containing 1mM L-FABP and 1mM DAUDA and an IC_{50} of 4.9 μM (Nabbefeld et al., 1998; Nabbefeld, 1998).

Peroxisome Proliferation: Liver biochemical effects associated with peroxisome proliferation have been investigated in two published studies.

Sohlenius et al. (1993) exposed mice to 30 mg/kg/day PFOS for five days (0.05% in diet). In addition to weight loss, increases in each of the following hepatic parameters were observed: 1) relative liver weight (slight elevation); 2) mitochondrial and microsomal protein; 3) palmitoyl-CoA oxidation; 3) catalase in mitochondrial and cytosolic fractions; 4) glutathione transferase; 5) epoxide hydrolase and 6) DT-diaphorase, Ω- and Ω-1-hydroxylation (Sohlenius et al., 1993).

Ikeda et al. (1987) exposed male rats were to 0.02% PFOS in the diet for 2 weeks. The prominent induction of peroxisome proliferation was demonstrated by electron microscopy. Activities of catalase, fatty acyl-CoA oxidizing system, carnitine acetyl transferase and cytochrome P450s responsible for the Ω-hydroxylation of lauric acid were increased (Ikeda et al., 1987).

Hypolipidemia: The mechanism of the hypolipidemic effect of PFOS has been studied. Rats were fed 12 mg/kg/day for 7 – 14 days (0.02% in diet). Decreased body weight, increased liver weight, increased liver triacylglycerol, increased liver free cholesterol, decreased liver cholesterol ester, decreased serum cholesterol and triacylglycerols were observed. Hepatocytes isolated from treated rats showed reduced synthesis of cholesterol from acetate, pyruvate and hydroxymethylglutarate but not from mevalonate, increased oxidation of palmitate and reduced fatty acid synthesis. Activities of liver hydroxymethyl glutaric acid-CoA reductase and acyl-CoA:cholesterol acyltransferase were reduced. These results suggest that the hypolipemic effect of PFOS may be due to impaired production of lipoprotein particles due to reduced synthesis and esterification of cholesterol together with enhanced oxidation of fatty acids in the liver (Haughom and Øystein, 1992).

On-Going Research Program

A number of studies were initiated in 1998 to gain better insight into the potential health hazards of PFOS and to provide a strong foundation for risk characterization. These studies are discussed in Section VI, and outlines of them can be found in the Appendix.

Table IV.8: Summary of Rodent Repeated-Dose Studies

Study	Species (strain)	Dose (units)	*n* (Male = M Female = F)	Clinical Observations; Clinical Pathology; Gross Pathology; Histopathology
90-Day Dietary (Goldenthal et al., 1978a)	Rats/CD	0 (ppm)	5 M / 5 F	No effect
		30	5 M / 5 F	↓ body wgt, ↑ glutamate-pyruvate transaminase & glutamate-oxalacetate transaminase, liver discoloration
		100	5 M / 5 F	3 deaths; ↑sensitivity to stimuli, red material around the eyes or mouth, ↓ food consump., ↑ CPK, alk phos, glucose & BUN, ↓ hemoglobin, hematocrit, erythrocyte count, reticulocyte count (in females) and leucocyte count, liver enlargement, necrosis and hepatocellular hypertrophy and stomach discoloration and hemorrhage
		300	5 M / 5 F	Death; emaciation, convulsions, stomach mucosal hyperkeratosis, bone marrow hypocellularity, thymic follicular atrophy, splenic lymphoid follicular atrophy, atrophy of mesenteric lymph nodes, atrophy of villi in small intestines, skeletal muscle atrophy and dermal acanthosis and hyperkeratosis.
		1000	5 M / 5 F	Death; hunched posture
		3000	5 M / 5 F	Death; hypoactivity
14-Week Dietary (Part of on-going 2-yr study)	Rats (Sprague Dawley	0 (ppm)	10 M / 10 F	No effect
		0.5	10 M / 10 F	
		2	10 M / 10 F	
		5	10 M / 10 F	
		20	10 M / 10 F	↓ body wgt (♂ & ♀), ↓ cholesterol (♂),↑ liver wgt (♂ & ♀), enlarged & vacuolated liver cells, ↑ Palmitoyl CoA oxidase activity
90-Day Drinking Water (Limoges, 1995)	Rats (Sprague Dawley)	0	10 M / 10 F	No effect
		(mg/kg/d)	10 F	No effect
		0	10 F	No effect
		0.02	10 F	↑ liver weight
		0.06	10 F	↑ liver weight
		0.2	10 F	↑ liver weight, hematology and serum chemistry effects
		0.6	10 M	↓ serum triglycerides
		0.3	10 M	↑ liver weight, ↓ serum cholesterol and triglycerides, hepatic vacuolization
		1.0	10 M	↑ liver weight, ↓ serum cholesterol and triglycerides, hepatic vacuolization
		3.0		

Table IV.9: Summary of Primate Repeated-Dose Studies

Study	Species (strain)	Dose (units)	*n* (Male = M Female = F)	Clinical Observations; Clinical Pathology; Gross Pathology; Histopathology
90-Day Gavage (Goldenthal et al., 1978)	Rhesus Monkey	0 (mg/kg/d)	2 M / 2 F	No effect
		10	2 M / 2 F	Death within 11 - 20 days, weight loss, weakness anorexia, ↓ activity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage and lipid depletion of adrenal cortex
		30	2 M / 2 F	Death within 7 - 10 days; weight loss, marked weakness anorexia, hypoactivity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage and lipid depletion of adrenal cortex
		100	2 M / 2 F	All dead in 3 – 5 days, weight loss, marked weakness anorexia, hypoactivity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage and lipid depletion of adrenal cortex
		300	2 M / 2 F	All dead in 2 – 4 days, weight loss, marked weakness, anorexia, hypoactivity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage and lipid depletion of adrenal cortex
90-Day Gavage (Goldenthal et al., 1978b)	Rhesus Monkey	0 (mg/kg/d)	2 M / 2 F	No effect
		0.5	2 M / 2 F	Slight & intermittent ↓ activity in 3 of 4 monkeys
		1.5	2 M / 2 F	Blood & mucous in stools, diarrhea, dehydr., tremors, ↓ body wgt, ↓ cholesterol, marked ↓ activity
		4.5	2 M / 2 F	marked ↓ cholesterol Death within 7 weeks; black or bloody stool, dehydration, rigidity, convulsions, prostration, decreased serum cholesterol, diffuse lipid depletion of adrenals, atrophy of pancreatic exocrine cells and atrophy of submandibular salivary gland serous alveolar cells, decreased serum alkaline phosphatase, increased SGOT.
28-Day Capsule Range-finding	Cyno. Monkey	0 (mg/kg/d)	2 M / 2 F	No effect
		0.02	3 M / 3 F	No Effect
		2.0	1 M / 1 F	↓ cholesterol (♂ & ♀)

Table IV.10: Summary of Developmental and Reproductive Studies

Study	Species (strain)	Dose (units)	*n* (Male = M Female = F)	Clinical Observations; Clinical Pathology; Gross Pathology; Histopathology
Teratology (Riker, 1986)	Rat (CD)	0 (mg/kg/d)	20 F (preg.)	No effect
		1	20 F (preg.)	No effect
		5	20 F (preg.)	No effect
		10	20 F (preg.)	↓ Body wgt gain
Teratology (Wetzel, 1980 & Wetzel, et al., 1983)	Rat (CD)	0 (mg/kg/d)	25 F (preg.)	No effect
		1	25 F (preg.)	No effect
		5	25 F (preg.)	↓ maternal (♀), body wgt gain, ↓ food consumption, thinness, hunching, rough hair coat, anorexia, ↓ pup weight
		10	25 F (preg.)	↑ subcutaneous edema, cleft palate & cryptorchism, ↓ maternal (♀), body wgt gain, ↓ food consumption, slight ↓ pups per litter, ↓ pup weight
Two-Gen Reproduct. (in progress, 10/98)	Rat (CD)	0 (mg/kg/d)	35 M / 35 F	No effect
		0.1	35 M / 35 F	No effect
		0.4	35 M / 35 F	No effect
		1.6	35 M / 35 F	↑ stillborn pups & ↓ pup survival, (♀), body weight gain
		3.2	35 M / 35 F	↓ body wgt gain (♀), slight ↓ body wgt gain (♂), ↑ stillborn pups & ↓ pup survival

Table IV.11: 90-Day Sub-Chronic Study Results Organized by Increasing Nominal mg/kg Dose Level

Dose as mg/kg/day	Dosage form	Species	Outcome
0.02	Drink. Water	F rat	No effect (Limoges, 1995)
0.06	Drink. Water	F rat	No adverse effect; increased liver weight (Limoges, 1995)
0.20	Drink. Water	F rat	No effect; increased liver weight (Limoges, 1995)
0.30	Drink. Water	M rat	Reduced serum triglycerides (Limoges, 1995)
0.50	Gavage	Monkey	anorexia, emesis, diarrhea and slightly decreased serum alkaline phosphatase. (Goldenthal et al., 1978b)
0.60	Drink. Water	F rat	Hematology and serum chemistry effects; increased liver weights (Limoges, 1995)
1.0	Drink. Water	M rat	Reduced serum cholesterol and triglycerides; hepatic vacuolization; increased liver weights (Limoges, 1995)
1.5	Gavage	Monkey	hypoactivity, tremors, weight loss, decreased serum alkaline phosphatase and inorganic phosphate (Goldenthal et al., 1978b)
2.0	Dietary	Rat	Weight loss, elevated plasma glutamate-pyruvate transaminase, elevated plasma glutamate-oxalacetate transaminase and liver discoloration. (Goldenthal et al., 1978a)
3.0	Drink. Water	M rat	Reduced serum cholesterol and triglycerides; hepatic vacuolization; increased liver weights (Limoges, 1995)
4.5	Gavage	Monkey	Death within 7 weeks; black or bloody stool, dehydration, rigidity, convulsions, prostration, decreased serum cholesterol, diffuse lipid depletion of adrenals, atrophy of pancreatic exocrine cells and atrophy of submandibular salivary gland serous alveolar cells, decreased serum alkaline phosphatase, increased SGOT. (Goldenthal et al., 1978b)
6.0	Dietary	Rat	3 deaths; increased sensitivity to external stimuli, red material around the eyes or mouth, decreased food consumption, elevated plasma creatinine phosphokinase, alkaline phosphatase, blood glucose and blood urea nitrogen, decreased hemoglobin, hematocrit, erythrocyte count, reticulocyte count (in females) and leucocyte count, liver enlargement, necrosis and hepatocellular hypertrophy and stomach discoloration and hemorrhage (Goldenthal et al., 1978a).
10	Gavage	Monkey	Death within 11 - 20 days; clinical signs included weight loss, marked weakness anorexia, hypoactivity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage and lipid depletion of adrenal cortex. (Goldenthal, 1978)
18	Dietary	Rat	Death; emaciation, convulsions, stomach mucosal hyperkeratosis, bone marrow hypocellularity, thymic follicular atrophy, splenic lymphoid follicular atrophy, atrophy of mesenteric lymph nodes, atrophy of villi in small intestines, skeletal muscle atrophy and dermal acanthosis and hyperkeratosis. (Goldenthal et al., 1978a)
30	Gavage	Monkey	Death within 7 - 10 days; clinical signs included weight loss, marked weakness anorexia, hypoactivity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage and lipid depletion of adrenal cortex (Goldenthal, 1978)
60	Dietary	Rat	Death; hunched posture. (Goldenthal et al., 1978a)
100	Gavage	Monkey	Death within 3 – 5 days; clinical signs included weight loss, marked weakness anorexia, hypoactivity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage and lipid depletion of adrenal cortex (Goldenthal, 1978)
180	Dietary	Rat	Death; hypoactivity. (Goldenthal et al., 1978a)
300	Gavage	Monkey	Death within 2 – 4 days; clinical signs included weight loss, marked weakness anorexia, hypoactivity, emesis, diarrhea, tremors, prostration, congestion, hemorrhage and lipid depletion of adrenal cortex. (Goldenthal, 1978)

V. RISK CHARACTERIZATION

The principal issue of concern is the potential health risk associated with the levels of PFOS identified in serum samples from non-occupationally exposed employees, commercially available serum and blood bank samples. The purpose of this section is to evaluate this risk using available human and animal data. Sources of exposure and their control are discussed in a companion document.

As noted previously (Section III), 3M has conducted medical surveillance programs at manufacturing locations where employees are exposed to PFOS or precursor molecules. These employees have PFOS serum levels that range from less than one ppm up to 12 ppm in the most recent testing. The 1997 mean among participating employees at the Decatur, Alabama plant, the U. S. location where PFOS related materials are principally produced, was 2.0 ppm. The best current estimate of serum levels in non-occupationally exposed individuals comes from the pooled samples collected from regionally diverse blood banks. These values ranged from 14 to 56 ppb, and averaged 30 ppb. It should be noted that while this is our best estimate, it cannot be considered representative of the U.S. population since blood donors are not necessarily representative of the U.S. population. In addition this data, since it is from pooled samples, does not reflect the variability and range one would expect to find among individuals.

Analysis of data collected in 1995 and 1997 reveals that the serum concentrations of PFOS identified in production workers are not associated with alterations in the hematological or clinical chemistry parameters that were evaluated. This includes measurement of the parameters that would reflect the pathology found in high dose animal studies (see below). In addition, no differences from normal were detected in ten different hormone levels. These analyses included employees of the 3M Belgium manufacturing facility, where serum levels are slightly lower than Decatur. The findings apply for PFOS serum levels up to 6 ppm, approximately two orders of

magnitude above the upper end of the range seen in the U.S. regional blood bank samples. The mean serum concentration in participating Decatur plant employees in 1997 was 68 times the mean of these blood bank samples.

In 1995, epidemiologists from the University of Minnesota completed a mortality study of the Decatur employee cohort. No higher than expected mortality rates were found for any cancer or for any other cause of mortality. This study involved 1,957 employees who had worked at least one year at 3M's Decatur, Alabama plant, and their mortality experience was traced from 1961 through the end of 1991. Details of this study can be found in Section III.

Animal toxicology data has been reviewed in Section IV. An older data set from rhesus monkeys exists and new data from a rangefinder study in cynomolgus monkeys is available. Serum PFOS concentrations were not measured in the older rhesus monkey studies, but can be estimated based on data from the cynomolgus monkey studies. It is recognized that uncertainties are introduced by such estimates, and that derived numbers need to be confirmed in ongoing or planned studies.

Data from the cynomolgus monkey rangefinder study itself documents that a decrease in cholesterol, an early biological effect in both rodents and primates, was observed at a PFOS serum level of 72 ppm in one of the two high dose animals. In the rhesus monkey studies, lowered cholesterol was observed after 90 days of oral dosing at 1.5 mg/kg/day. Severe toxicity, resulting in death, occurred at doses equal to and greater than 4.5 mg/kg/day, in seven weeks or less. Serum concentrations of PFOS that might have been achieved in the older rhesus monkey studies have been estimated from the cumulative dose - serum concentration of PFOS relationship seen in the recent cynomolgus study. These estimates suggest that severe toxicity occurred at serum levels of 700 to 800 ppm and death at 1000 to 1200 ppm.

Other available toxicity data also provide valuable information. PFOS is not mutagenic in five different bacterial strains. It fails to cause chromosomal aberrations in the mouse bone marrow micronucleus assay. Developmental studies in rats show that the compound causes adverse effects on fetal structural development only at doses that are clearly toxic to the mothers.

Preliminary results from an ongoing two generation reproductive study in rats, through F1 weaning, show no interference with sexual maturation, mating behavior, fertilization, number of resorptions, or litter size. At the highest two dose groups (1.6 and 3.2 mg/kg/day) there was significant perinatal mortality in the F1 generation. This effect was not seen at 0.4 mg/kg/day. The study cannot be used in risk assessment until it is completed and fully evaluated, but is discussed here because we are aware of these interim results. The doses observed to cause effect are in the range where toxicity is observed in 90 day rat studies.

Several 90-day studies in rats, involving PFOS administration over a wide range of doses in the diet, in drinking water, and by stomach tube, reveal that no significant toxic effects are produced at or below a dose of 0.2 mg/kg/day. Above this dose adverse effects on the liver, body weight, and changes in several indicators of fat metabolism are demonstrable.

Possible explanations for the absence of detectable toxicity in production workers are that the workers' serum and tissue levels of PFOS are significantly below those achieved in the animal studies, as estimates would indicate, or human beings are less sensitive to the effects of PFOS than are laboratory animals. Of these two explanations, the former seems more likely, because both primates and non-primates were shown to be susceptible to PFOS exposure in the animal studies, so there is no obvious reason to expect large interspecies differences in sensitivity. The second possible explanation – that humans are less sensitive – cannot be ruled out based on available data. It is also possible that the human studies were insufficiently powerful,

because of sample size limitations, to detect the toxicity of PFOS. This seems unlikely because the animal studies showing toxicity involved even fewer subjects, and, at a given dose, all or most animals responded in similar ways. Sample size would not seem to be a significant factor for evaluating clinical, hematological, or hormonal effects.

Excess mortality, including that associated with cancer, has not been observed in production workers. The experimental data showing that PFOS is not mutagenic (Ames tests) and does not cause chromosomal aberrations (mouse bone marrow micronucleus assay) is consistent with these findings.

Non-occupationally exposed populations appear to exhibit serum levels that are approximately two orders of magnitude lower than those exposed occupationally, more than three orders of magnitude below levels estimated to show minimum biological effects (cholesterol reduction) in primate studies and three to four orders of magnitude below levels associated with significant toxic effects. None of the health effects evaluated and found to be absent in the worker studies would be expected to occur in the non-occupationally exposed populations. The additional information provided by the two generation reproductive study in rats is not interpretable at this time, but the dose at what currently appears to be a no effect level is comparable to doses that produce no effect for other endpoints. The animal data revealing adverse effects are not yet fully useful for quantifying human risks, but estimates from current and ongoing studies suggest that PFOS serum levels achieved in those earlier animal studies with compound related effects must have been greater than those experienced by workers and therefore much greater than those experienced by non-occupationally exposed populations.

The currently available evidence does not suggest there is health risk associated with the levels of PFOS found in the serum of occupationally or non-occupationally exposed employees, blood bank samples or commercially available serum.

Several avenues of further investigation are needed. The long-term consequences of PFOS exposure require additional study. Although the available worker studies do not suggest a health problem or evidence of increased mortality rates, animal data reflecting chronic exposures are not yet available. Such data are recommended because worker studies, involving relatively small populations, are generally not sufficiently powerful to detect small excess risks. A two year PFOS feeding study in rats was started in April of 1998. The utility of the available animal data is limited because serum levels associated with adverse events can only be estimated at this time. This limitation is being remedied by collecting additional animal data, including sufficient kinetic information to assess the relationship between administered doses and serum and tissue levels, as cited above for cynomolgus monkeys, as well as more definitive data on compound distribution and elimination.

There is data suggesting that PFOS can, at sufficiently high doses, induce peroxisome proliferation in rodents and alter metabolic processes in laboratory models. Further study of these effects is desirable to determine whether they can occur at serum and tissue levels relevant to those observed or estimated in humans. Section VI of this report describes additional 3M studies that will address these issues. Further follow-up on worker mortality experience is also underway, including an attempt to better classify workers with regard to PFOS exposure.

Finally, studies are underway to improve knowledge about possible sources of PFOS, and the pathways by which non-occupationally exposed individuals are exposed. Although there is no current evidence that the reported serum levels, and corresponding body burdens, represent a health risk, it is appropriate to identify and act on possible ways to reduce exposure. To date this has been done through reduction of residual monomer levels in products and reduced manufacturing emissions.

VI. CURRENT AND PLANNED RESEARCH

Toxicology Studies

Fluorochemical Study Purposes and Outlines

A number of studies were initiated in 1998 to gain better insight into the potential health hazards of PFOS and to provide a strong foundation for risk characterization. These studies include those conducted with PFOS as well as studies which are conducted with N-Ethyl Perfluorooctane Sulfonamido Ethanol (N-EtFOSE), N-Methyl Perfluorooctane Sulfonamido Ethanol (N-MeFOSE) and other related compounds which are presumed to degrade metabolically to PFOS. N-EtFOSE and N-MeFOSE represent major starting points for additional synthesis and are regulated by FDA as contaminants of indirect food additives. These compounds are also known to be absorbed well from the gastrointestinal tract and will produce PFOS as a major metabolite. Therefore, it is appropriate to use these two compounds as models in further investigating the toxicity of this class of chemicals which have the perfluorooctane sulfonyl moiety as a base. The goals of this research program are as follows:

- To understand the relationship between measured concentrations of PFOS in serum and potential adverse health effects;
- To understand the kinetics of increases in body burden of PFOS as reflected in serum PFOS measurements;
- To determine if a critical cumulative body burden exists and how this is related to toxicokinetics;
- To define the metabolic relationship of PFOS to other Perfluorooctanesulfonyl-based chemicals;
- To understand the primary mechanism of toxicity responsible for early toxic effects;

- To establish reasonable and substantiated no significant risk levels for risk assessment.

Studies initiated in 1998 with PFOS and N-EtFOSE are identified in Tables VI.1 and VI.2, respectively. In addition to these, a 90-day dietary study with N-MeFOSE has been completed and studies have been undertaken to better understand the potential for metabolic degradation of perfluorooctanesulfonamide-based chemicals to PFOS. Descriptive summaries of many of these studies are included in the Appendix.

Table VI.1: PFOS Toxicity Studies Initiated in 1998

Study Title	Start Date	Completion Date (In-Life)
Four-Week Capsule Range-Finder Study in Cynomolgus Monkeys	4/23/98	5/22/98
Six-Month Capsule Feeding Study in Cynomolgus Monkeys	8/5/98	2/8/99 (histo.) 5/7/99 (recov.)
Two-Year Dietary Study in Rats	5/20/98	4/24/2000
Hepatic Peroxisome Induction and Cell Proliferation Study	12/1/98	1/7/98
Biochemical and Molecular Biology Mechanistic Studies	7/1/97	On-going
Segment II Teratology in Rabbits	8/28/98	9/29/98
Two-Generation Reproduction Study in Rats	5/26/98	12/31/98
One-Generation Cross-Fostering Reproduction Study in Rats	11/1/98	2/15/99
One-Generation Reproduction PK Study in Rats (through Gestation)	11/7/98	1/30/99
One-Generation Reproduction PK Study in Rats (through Lactation)	11/23/98	3/1/99

Table VI.2: N-EtFOSE Toxicity Studies Initiated in 1998

Study Title	Start Date	Completion Date (In-Life)
Two-Year Dietary Study in Rats	1/26/98	1/25/2000
Single Dose ADME in Cynomolgus Monkeys	5/14/98	10/1/98
Biochemical and Molecular Biology Mechanistic Studies	7/1/97	On-going
Segment II Teratology in Rats	8/11/98	9/4/98
Segment II Teratology in Rabbits	8/28/98	9/29/98
Two-Generation Reproduction Study in Rats	6/8/98	12/31/98

104-week dietary carcinogenicity study with perfluorooctane sulfonic acid, potassium salt in rats. In-life Start date: 4/20/98; In-life completion date: 4/24/2000

The purpose of this two year assay is to determine: carcinogenic potential, bioaccumulation with repeated doses, threshold effect levels, a no observable adverse effect level (NOAEL), and mechanisms of toxicity. Proliferating cell nuclear antigen (PCNA) will be measured at early time points in both liver and pancreas as an indicator of preneoplastic lesions. There are six dose groups: 0, 0.5, 2.0, 5.0, 20 and 20 (recovery) ppm PFOS in the feed. Interim sacrifices at weeks 4, 14 and 52 will be performed to determine PCNA, palmitoyl Co-A oxidation, clinical chemistry, and histopathology. Animals in group 6 will be treated for 52 weeks, then treatment will be discontinued and the animals will be observed for reversibility, persistence, or delayed occurrence of toxic effects for at least 26 weeks post-treatment.

Serum samples will be collected for PFOS detection from the scheduled sacrifices and in-life blood draws. Hematology, serum chemistry, urinalysis, urine chemistry, and serum sampling (In-life blood draws) will be done at weeks 14, 27 and 53.

104 -Week Dietary Carcinogenicity Study with Narrow Range (98.1%) N-Ethyl Perfluorooctanesulfonamido Ethanol in Rats. In-life Start Date: 1/ 26/1998.

The purpose of this study is to assess the carcinogenicity of the test material, N-ethyl perfluorooctanesulfonamido ethanol (N-EtFOSE) when administered in the diet to rats for at least 104 weeks. The test material was administered at dose levels of 0, 1, 3, 30, 100, and 300 ppm. Necropsies were performed during Week 4 and during Week 14 for hepatocellular proliferation rate measurements, hepatic palmitoyl-CoA oxidase activity, and serum and liver PFOS levels. Due to the morbidity and morbundity, the 300 ppm group was terminated at 8 weeks. During Week 8 and 14, blood and urine were collected for hematology, clinical chemistry, urinalysis, and urine chemistry. At necropsy, macroscopic observations were recorded, organ weights were obtained, and tissues were placed in fixative as specified by the

protocol. Microscopic examinations were done on selected tissues from animals necropsied during Weeks 8 and 14. A recovery group of animals in the 100 ppm dose group will be treated for at least 52 weeks then treatment will be discontinued, and the animals will be observed for reversibility, persistence, or delayed occurrence of toxic effects for at least 26 week post-treatment.

Six-Month Capsule Feeding Study in Cynomolgus Monkeys. In-life Start Date: 8/5/1998. In-life completion date: 5/7/1999.

The purpose of this study is to establish a no observable effect level for PFOS in the Cynomolgus monkey. PFOS is a known hepatic peroxisome proliferator (PP) in the rat. Non-human primates such as the Cynomolgus monkey respond similarly to humans with no to low hepatic response to peroxisome proliferators, and therefore are the appropriate human surrogate species. Blood hormone levels will be determined pretreatment and after 30, 60, 90, and 180 days of treatment, and after 30, 60, and 90 days of recovery. Samples will be analyzed for estradiol, estrone, estriol, thyroid stimulating hormone, and triiodothryonine (T3), thyroxin (T4), cortisol and testosterone. Serum and liver PFOS levels will be determined and correlated to blood chemistries. Urine and feces PFOS determinations will be made day 0 of recovery and after 6, 30, and 90 days of recovery to track elimination kinetics. Four groups of four male and four female Cynomolgus monkeys will be orally dosed with PFOS triturated in lactose at 0, 0.03, 0.15 and 0.75 mg/kg/day daily by gelatin capsule. Dose levels were determined in a previously conducted rangefinder study. Two additional animals in the control, mid and high dose groups will be designated as recovery animals for which treatment will be discontinued at 26 weeks, and the animals will be observed for reversibility, persistence, or delayed occurrence of toxic effects for at least 13 weeks post-treatment. Hematology and clinical chemistry will be determined once before initiation, after 30, 60, 90, and 180 days of treatment, and after 30, 60, and 90 days of recovery. Clinical chemistry will include urea nitrogen, cholesterol, triglicerides, alkaline phosphatase, alanine aminotransferase, bile acids, total and direct bilirubin, lipase, and pancreatic-specific amylase. Palmitoyl CoA

Mono N-EtFOSE Phosphate – Absorption, Disposition, Metabolism, Excretion in Rats. In-life Start Date: 5/15/1998. In-life completion date: 6/15/1998.

The purpose of this study was to assess the potential for absorption and metabolism of the monophosphate ester of FC-807 in rats. FC-807 is the brand name of a fluorochemical based product sold by 3M and approved by the FDA for use as an oil and water repellant in paper and paperboard food packaging. The primary concern of this study was to determine if and to what extent the monoester is absorbed from the intestinal tract, and to what extent it can be metabolized ton N- ethyl (perfluorooctane)sulfonamido ethanol (N-Ethyl FOSE) and other metabolites, including PFOS, once in the blood stream. Both a time-course study following a single dose of compound, and a dose response study were conducted. In the time course study, rats were dosed orally, via gavage, or by intravenous (i.v.) injection, via the tail vein, with FC-807 monoester. One animal from each dosage route group was sacrificed two hours after dosing. Urine and feces were collected daily for four days. On day 4, one animal from each group was sacrificed, and the remainder of the animals were sacrificed 28 days post dose. The preliminary results indicated absorption of the oral 50 mg/kg dose and metabolism at day 4, and day 28. The i.v doses of 0.5 mg/kg were also extensively metabolized and were deemed to be too high. The dose-response study consisted of six dose groups. There was one control group, three groups dosed orally at 0.01, 0.1, and 5 mg/kg, via gavage, and two groups that received tail vein i.v. injections at 0.01, and 0.1 mg/kg at a volume of 5 ml/kg on day one of the study to each group. All groups were sacrificed on day 4 of the study.

13-week Dietary Toxicity Study with N-Methyl FOSE in Rats. In-life Start Date: 9/1/1998. In life completion date: 12//0/1998.

The purpose of this study was to develop sub-chronic toxicity data and the toxicokinetics for N-Methyl FOSE when administered in the diet to rats for at least 13 weeks. The study design included 20 males and 20 female animals/group fed 0, 3,

30 and 100 ppm N-MeFOSE in the diet. After 4 weeks of treatment, 5 rats/sex/group were sacrificed for Palmitoyl CoA oxidase, PCNA, liver and sera PFOS analysis.

Two-Generation Rat Reproduction Study with PFOS. In-life Start Date: 5/26/1998. In-life completion date: 12/31/1998.

The purpose of this study is to test for toxic effects of PFOS on reproductive function of dosed male and female rats and to assess whether rat pups exposed to the compound in utero & via milk have any developmental, learning and reproductive effects. A secondary objective will be to assess compound and/or metabolite levels in serum and liver from parent animals. Groups of 35 rats will be dosed daily via gastric gavage at dose levels of 0, 0.1, 0.4, 1.6 and 3.2 mg/kg/d. Dosing will start 4 weeks prior to mating, will continue during mating, and for females will continue during gestation and lactation. Reproductive performance will be assessed by fertility index, gestation index, number of pups/ litter, pup viability index and lactation index. The F1 generation pups at each dose level will be assessed for developmental objectives, undergo learning testing, and reproductive performance. Serum and liver samples will be collected at necropsy from five male and five female rats/dose group from F_0 (Parent) animals and analyzed for compound by 3M.

Two-Generation Rat Reproduction Study with N-EtFOSE. Start Date: 6/8/1998. In life completion date: 12/31/1998.

The purpose of this study is to test for toxic effects of N-EtFOSE on reproductive function of dosed male and female rats and to assess whether rat pups exposed to the compound in utero & via milk have any developmental, learning and reproductive effects. A secondary objective will be to assess compound and/or metabolite levels in serum and liver from parent animals. Groups of 35 rats will be dosed daily via gastric gavage at dose levels of 0, 1, 5, 10 and 15 mg/kg/d. Dosing will start six weeks prior to mating, will continue during mating, and for females will continue during gestation and lactation. Reproductive performance will be assessed by fertility index, gestation index, number of pups/ litter, pup viability index and lactation

index. The F1 generation pups at each dose level will be assessed for developmental objectives, undergo learning testing and reproductive performance. Serum and liver samples will be collected at necropsy from 5 male and 5 female rats/dose group from F_0 (Parent) animals and analyzed for compound by 3M.

Segment II Teratology in Rabbits with PFOS. In-life Start date: 8/28/1998. In-life end date 9/29/1998.

The purpose of this study is to detect adverse effects of PFOS on New Zealand White pregnant female rabbits and development of the embryo on fetus consequent to exposure of the doe from implantation to closure of the hard palate. Dose groups of 22 presumed pregnant female rabbits were dosed with 0, 0.1, 2.0, 2.5 and 3.75 mg/kg/day via a stomach tube. A toxicokinetic satellite group of female rabbits (5 at the control and high dose levels plus three at the other dose levels) were sacrificed at day 21 of gestation (the day following the last dosage) serum, liver, fetal and placental samples were analyzed for PFOS and possible metabolites. Rabbits were Caesarian sectioned on day 29 of presumed gestation. The fetuses were examined for body weight, gross alterations and skeletal alterations, number and distribution of corpora lutea, implantation sites, live and dead fetuses and early and late resorptions.

Segment II Teratology with N-EtFOSE in rats. In-life Start date: 9/4/1998. In life end date 9/11/1998.

The purpose of this study was to detect adverse effects of N-EtFOSE on presumed pregnant female rats and development of the embryo and fetus consequent to exposure of the dam from implantation to closure of the hard palate. Dose groups of 25 presumed pregnant female rats were dosed with 0, 1, 5, 10 and 20 mg/kg/day by oral gavage. A toxicokinetic satellite group of female rats (five at the control and high dose levels plus three at the other dose levels) were sacrificed at day 18 of gestation (the day following the last dosage) serum, liver, fetal and placental samples were analyzed for N-EtFOSE and possible metabolites. Rats were Caesarian

sectioned on day 20 of presumed gestation. The fetuses were examined for body weight, gross alterations and skeletal alterations, number and distribution of corpora lutea, implantation sites, live and dead fetuses and early and late resorptions.

Segment II Teratology in Rabbits with N-EtFOSE. In-life Start date: 8/28/1998. In life end date 9/29/1998.

The purpose of this study was to detect adverse effects of N-EtFOSE on New Zealand White pregnant female rabbits and development of the embryo and fetus consequent to exposure of the doe from implantation to closure of the hard palate. Dose groups of 22 presumed pregnant female rabbits were dosed with 0, 0.1, 2.0, 2.5 and 3.75 mg/kg/day via a stomach tube. A toxicokinetic satellite group of female rabbits (five at the control and high dose levels plus three at the other dose levels) were sacrificed at day 21 of gestation (the day following the last dosage) serum, liver, fetal and placental samples were analyzed for N-EtFOSE and possible metabolites. Rabbits were Caesarian section on day 29 of presumed gestation. The fetuses will be examined for body weight, gross alterations and skeletal alterations, number and distribution of corpora lutea, implantation sites, live and dead fetuses and early and late resorptions.

January 21, 1999

Current and Proposed Human Health and Exposure Research Initiatives

At present, there are several ongoing research studies and proposals. These are briefly outlined below.

1. Half-life Fluorochemical Determination Study. 3M retirees from the Decatur and Cottage Grove plants will be asked to participate in a study to determine the half-life of PFOA and PFOS. Retirees' serum will be analyzed semi-annually for the next five years for PFOS, PFOA and perfluorohexane sulfonate.

2. Decatur Serum Exposure Assessment Study. Employees at the 3M Decatur chemical plant have in the past voluntarily participated in a fluorochemical medical surveillance program. Analysis of the surveillance data has not shown significant associations between the employees' clinical chemistry and hematology tests and either total serum organic fluorine or serum PFOS levels. However, the voluntary nature of the medical surveillance program does not allow for a complete understanding of the distribution of employee fluorochemical serum levels. In order to address this issue, a random sample of approximately 80 Decatur film plant and 125 Decatur chemical plant employees will be asked to participate in a serum determination study for the following fluorochemicals: perfluorooctane sulfonate, perfluorooctane sulfonate amide, perfluorohexane sulfonate, perfluorooctanoic acid, N-ethyle perfluorooctnae sulfonamido ethanol and its acetate derivitive, and N-methyl perfluoroctanesulfonamido ethanol. A sub-sample of employees will also be tested for total serum organic fluorine. A brief questionnaire will also be administered to each employee inquiring about current and past work history as well as possible routes of oral ingestion.

3. An Epidemiologic Analysis of the Inpatient Claims Experience of 3M Decatur Employees, 1993-1997. The purpose of this study is to examine the inpatient claims database, as maintained by Corporate Health Strategies, from January 1, 1993 – December 31, 1997 of Decatur plant employees. The data will be stratified by whether the employees are in the chemical or film plants. The study population will include full-

time active employees who worked throughout the five year interval, all full-time active employees who quit, died or went on long-term disability within the five year interval, and all employees who have retired. Observed inpatient claims for each plant population will be compared to an expected experience based on the 3M normative database. Inpatient claims analysis will proceed in a sequential, hierarchical manner. First we will examine Medical Diagnosis Codes; next Diagnosis Related Groups, and finally selected ICD-9 codes within each DRG.

4. PFOS and PFOA Retrospective Cohort Mortality Studies of Employees at the 3M Decatur and Cottage Grove Plants. Previous retrospective cohort mortality studies have been conducted at the Decatur and Cottage Grove plants. However, neither of these studies utilized the employees' fluorochemical serum measurements to design job-, department- and calendar-year exposure matrices. The purpose of these two studies is to construct PFOS and PFOA exposure matrices based on previously collected employee serum PFOS and PFOA measurements in conjunction with their plant work history experiences. Person-years will be allocated based on the exposure matrices to calculate the traditional measures of risk, i.e., Standardized Mortality Ratios, for more than 50 causes of death. Vital status and cause of death will be ascertained through December 31, 1997.

5. Geographical Reliability Study. The purpose of this study is to determine whether there are geographical differences in human serum measurements in the United States. We will request serum samples from the 18 blood banks that originally participated in the fluorochemical determination study (see Table II.3) to determine whether comparable serum levels are measured.

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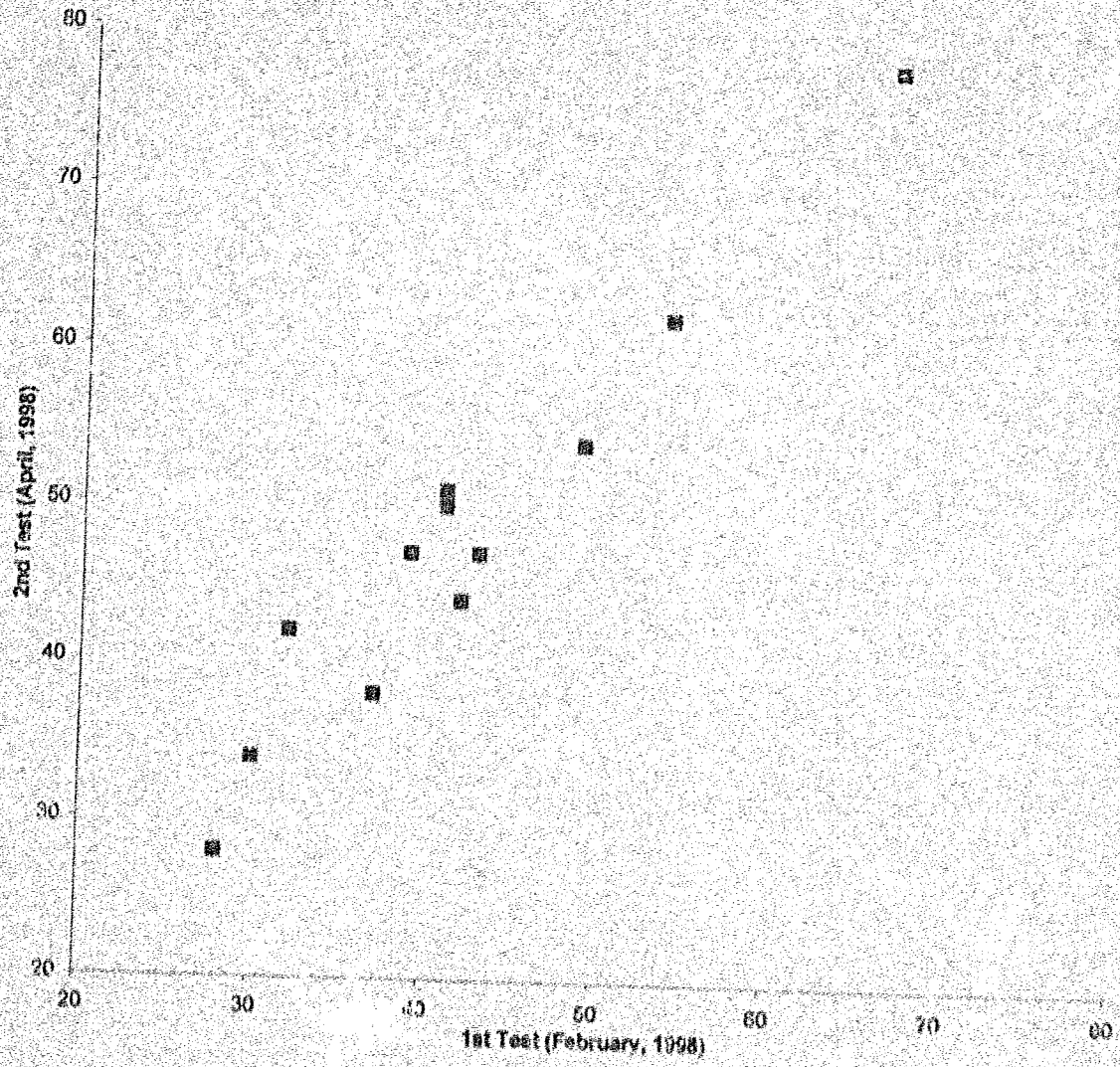
APPENDIX

FIGURE A1. Reliability of PFOS Serum Measurements in 12 3M Employees



Appendix – Table A1

Mean, Median, Standard Deviation (SD) of Mean and Range of PFOS, Demographic, Serum Chemistries and Hematological for Antwerp and Decatur Employees Combined, 1995 (N=178) and 1997 (N=147)

PFOS* (ppm)	1995 Data				1997 Data			
	Mean	Median	SD	Range	Mean	Median	SD	Range
PFOS (ppm)								
0 - < 1ppm	0.49[1]	0.5	0.27	0.00 - 0.90	0.52[1]	0.52	0.27	0.10 - 0.97
1 - < 3ppm	1.82[1]	1.77	0.58	1.00 - 2.91	1.78[1]	1.64	0.56	1.02 - 2.99
3 - < 6ppm	4.12[1]	3.97	0.81	3.00 - 5.80	3.87[1]	3.59	0.70	3.09 - 5.30
≥ 6ppm	8.17[1]	7.73	2.52	6.06 - 12.83	7.20[1]	6.68	1.59	6.05 - 9.3
	F value = 321.9, p < .0001				F value = 367.6, p < .0001			
Age (yrs)								
0 - < 1 ppm	37	36	8	21 - 58	36	34	11	21 - 62
1 - < 3 ppm	42[2]	41	9	25 - 60	42[2]	41	9	24 - 63
3 - < 6 ppm	40	40	7	26 - 55	41	42	5	32 - 54
≥ 6 ppm	45	43	7	37 - 56	42	45	9	29 - 52
	F value = 3.7, p = .02				F value = 5.1, p = .002			

Table A1 (Continued)

PFOS (ppm)	1995 Data Mean	Median	SD	Range	1997 Data Mean	Median	SD	Range
Alcohol (drinks/day)								
0 - < 1 ppm	0.8	0.6	0.9	0.0 - 3.6	0.5	0.1	0.8	0 - 4.3
1 - < 3 ppm	0.5	0.1	0.7	0.0 - 3.6	0.5	0.1	0.8	0 - 5.0
3 - < 6 ppm	1.2	0.3	1.9	0.0 - 6.0	1.0	0.1	1.8	0 - 7.1
≥ 6 ppm	0.7	0.0	1.1	0.0 - 2.9	0.2	0.1	0.2	0.1 - 0.8
	F value = 4.0, p = .009				F value = 1.8, p = .15			
BMI (kg/m^2)								
0 - < 1 ppm	25.5	24.8	4.2	17.9 - 38.7	26.0	24.9	4.9	20.1 - 41.7
1 - < 3 ppm	27.7	26.3	5.8	19.6 - 60.7	27.7	26.4	5.7	18.1 - 48.5
3 - < 6 ppm	24.9[3]	25.0	3.8	17.9 - 32.5	27.3	27.9	4.4	19.1 - 36.0
≥ 6 ppm	27.7	29.4	4.2	20.6 - 33.0	30.8	29.7	4.0	26.1 - 36.2
	F value = 3.7, p = .02				F value = 2.1, p = .10			
Cigarettes (per day)								
0 - < 1 ppm	2.6	0.0	6.4	0.0 - 25.0	4.7	0.0	9.4	0 - 40
1 - < 3 ppm	6.8	0.0	11.3	0.0 - 40.0	8.2	0.0	11.5	0 - 40
3 - < 6 ppm	10.6	8.0	12.4	0.0 - 40.0	4.1	0.0	8.3	0 - 30
≥ 6 ppm	0.4	0.0	1.1	0.0 - 3.0	6.0	0.0	13.4	0 - 30
	F value = 4.8, p = .003				F value = 1.5, p = .23			

Table A1 (Continued)

PFOS (ppm)	1995 Data Mean	Median	SD	Range	1997 Data Mean	Median	SD	Range
				BUN				
0 - < 1ppm	16.5	15	3.5	11 - 26	14.5	14.0	2.8	9.0 - 21.0
1 - <3 ppm	15.4	15.0	4.0	8.0 - 26.0	14.2	14.0	3.2	6.0 - 26.0
3 - < 6 ppm	16.4	16.0	3.7	10.0 - 23.0	15.0	15.0	2.9	9.0 - 20.0
≥ 6 ppm	15.1	14.0	4.2	10.0 - 21.0	13.8	12.0	4.1	9.0 - 19.0
	F value = 1.1, p = .36				F value = 0.5, p = 0.67			
				Creatinine				
0 - < 1ppm	1.0	1.0	0.2	0.7 - 1.6	0.9	0.9	0.1	0.6 - 1.2
1 - <3 ppm	1.0	1.0	0.2	0.7 - 1.6	0.9	0.9	0.1	0.7 - 1.3
3 - < 6 ppm	0.9	0.9	0.2	0.7 - 1.2	0.9	0.9	0.1	0.7 - 1.1
≥ 6 ppm	1.1	1.2	0.3	0.6 - 1.6	1.0	0.9	0.2	0.8 - 1.4
	F value = 2.3, p = .08				F value = 0.4, p = 0.78			
				Glucose				
0 - < 1 ppm	85	85	15	66 - 170	87	85	17	58 - 174
1 - <3 ppm	86	86	22	60 - 260	93	84	39	65 - 303
3 - < 6 ppm	84	83	12	66 - 114	95	89	25	74 - 192
≥ 6 ppm	84	83	14	71 - 105	89	88	7	80 - 97
	F value = 0.9, p = .44				F value = 0.6, p = .59			

Table A1 (Continued)

PFOS (ppm)	1995 Data				1997 Data			
	Mean	Median	SD	Range	Mean	Median	SD	Range
Alkaline Phosphatase								
0 - < 1 ppm	80	78	22	31 - 158	77	73	17	49 - 132
1 - <3 ppm	89	89	27	49 - 191	83	79	23	41 - 163
3 - < 6 ppm	86	85	21	32 - 124	76	74	22	29 - 120
≥ 6 ppm	88	85	24	63 - 136	88	84	18	65 - 114
	F value = 1.3, p = .28				F value = 1.17, p = .32			
GGT								
0 - < 1 ppm	43	31	28	16 - 155	28	22	20	10 - 142
1 - <3 ppm	47	36	39	2 - 293	36	25	33	10 - 179
3 - < 6 ppm	40	39	15	21 - 80	28	27	14	13 - 71
≥ 6 ppm	43	33	18	28 - 79	33	37	12	17 - 48
	F value = 0.5, p = .71				F value = 1.1, p = .34			
AST								
0 - < 1 ppm	27	25	13	15 - 96	27	25	7	13 - 53
1 - <3 ppm	29	26	12	14 - 90	26	25	7	15 - 56
3 - < 6 ppm	25	24	5	13 - 37	25	23	7	14 - 43
≥ 6 ppm	33	33	6	26 - 43	29	28	3	26 - 34
	F value - 1.8, p = .14				F value = 0.5, p = .67			

Table A1 (Continued)

PFOS (ppm)	1995 Data				1997 Data			
	Mean	Median	SD	Range	Mean	Median	SD	Range
ALT								
0 - < 1 ppm	48	43	20	27 - 118	31	30	11	13 - 60
1 - <3 ppm	46	42	21	18 - 183	33	29	16	10 - 89
3 - < 6 ppm	42	41	7	30 - 59	34	31	18	14 - 82
≥ 6 ppm	51	49	17	29 - 82	41	45	10	25 - 49
	F value = 1.0, p = .38				F value = 0.9, p = .46			
Total Bilirubin								
0 - < 1 ppm	0.88	0.70	0.50	0.40 - 2.90	0.77	0.60	0.40	0.30 - 2.30
1 - <3 ppm	0.66[2]	0.60	0.30	0.20 - 1.50	0.61[2]	0.60	0.21	0.30 - 1.30
3 - < 6 ppm	0.64[2]	0.60	0.28	0.20 - 1.40	0.63	0.50	0.31	0.40 - 1.30
≥ 6 ppm	0.76	0.70	0.23	0.50 - 1.20	0.58	0.50	0.24	0.40 - 1.00
	F value = 4.4, p = .005				F value = 2.9, p = .04			
Direct Bilirubin								
0 - < 1 ppm	0.22	0.20	0.05	0.02 - 0.40	0.15	0.10	0.07	0.10 - 0.40
1 - <3 ppm	0.21	0.20	0.06	0.10 - 0.40	0.12[2]	0.10	0.04	0.10 - 0.20
3 - < 6 ppm	0.21	0.20	0.04	0.20 - 0.30	0.12	0.10	0.04	0.10 - 0.20
≥ 6 ppm	0.20	0.20	0.02	0.10 0.30	0.10	0.10	0.00	0.10 - 0.10
	F value = 0.6, p = .58				F value = 3.5, p = .02			

Table A1 (Continued)

PFOS (ppm)	1995 Data				1997 Data			
	Mean	Median	SD	Range	Mean	Median	SD	Range
Cholesterol								
0 - <1 ppm	219	215	47	100 - 340	198	197	40	110 - 280
1 - <3 ppm	216	213	45	118 - 315	216	219	42	116 - 365
3 - <6 ppm	214	214	35	128 - 278	229[2]	224	29	192 - 321
≥ 6 ppm	213	221	36	160 - 251	229	238	26	186 - 250
	F value = 0.1, p = .96				F value = 4.3, p = .006			
LDL								
0 - <1 ppm	140	137	43	29 - 261	124	128	34	50 - 205
1 - <3 ppm	134	134	40	44 - 234	141	134	38	61 - 290
3 - <6 ppm	137	135	34	65 - 190	148[2]	142	24	111 - 196
≥ 6 ppm	142	136	32	95 - 178	145	156	26	103 - 164
	F value = 0.2 , p = .87				F value = 3.7, p = .01			
HDL								
0 - <1 ppm	53	53	13	31 - 94	46	48	11	19 - 74
1 - <3 ppm	48	47	13	26 -94	44	45	10	28 - 69
3 - <6 ppm	45	46	11	23 - 74	48	47	10	32 - 69
≥ 6 ppm	45	46	9	34 - 61	40	38	4	37 - 45
	F value = 2.9, p = .04				F value = 1.1, p = .34			

Table A1 (Continued)

PFOS (ppm)	1995 Data				1997 Data			
	Mean	Median	SD	Range	Mean	Median	SD	Range
Triglycerides								
0 - <1 ppm	129	96	98	41 - 622	148	107	162	38 - 1209
1 - <3 ppm	161	133	107	41 - 651	156	122	108	44 - 534
3 - <6 ppm	158	142	88	34 - 413	166	158	92	45 - 394
≥ 6 ppm	132	151	45	64 - 187	220	191	83	149 - 352
	F value = 1.1, p = .35				F value = 0.5, p = .67			
Hematocrit								
0 - <1 ppm	47	47	2	43 - 51	46	46	2	40 - 52
1 - <3 ppm	46	46	3	38 - 52	45	46	3	39 - 53
3 - <6 ppm	47	47	2	41 - 52	46	44	3	39 - 50
≥ 6 ppm	47	48	2	44 - 49	45	44	2	42 - 47
	F value = 2.4, p = .07				F value = 2.1, p = .11			
Hemoglobin								
0 - <1 ppm	15.5	15.5	0.7	14.0 - 16.7	[illegible]	15.5	0.8	13.5 - 17.0
1 - <3 ppm	15.2	15.2	1.0	13.0 - 17.4	[illegible]	15.5	0.9	13.3 - 17.3
3 - <6 ppm	15.5	15.5	0.8	13.6 - 17.4	13.8	14.7	1.0	12.5 - 16.7
≥ 6 ppm	15.5	15.4	0.7	14.7 - 16.2	15.1	15.0	0.7	14.1 - 16.2
	F value = 2.2, p = .10				F value = 1.8, p = .15			

Table A1 (Continued)

PEOS (ppm)	1995 Data Mean	Median	SD	Range	1997 Data Mean	Median	SD	Range
RBC								
0 - <1 ppm	5.0	5.0	0.3	4.3 - 5.7	5.1	5.2	0.3	4.3 - 5.9
1 - <3 ppm	4.9	4.9	0.3	4.3 - 5.7	5.0	5.1	0.3	4.1 - 5.5
3 - <6 ppm	5.0	5.0	0.2	4.6 - 5.5	5.0	5.0	0.3	4.4 - 5.7
≥ 6 ppm	5.0	4.9	0.5	4.0 - 5.7	5.0	4.9	0.3	4.7 - 5.5
	F value = 0.4, p = .75				F value = 1.4, p = .25			
MCH								
0 - <1 ppm	31.3	31.2	1.6	27.7 - 34.3	30.4	30.3	1.5	27.6 - 34.1
1 - <3 ppm	30.9	30.9	1.4	26.4 - 34.3	30.6	30.5	1.2	26.7 - 33.8
3 - <6 ppm	31.2	31.3	1.5	26.0 - 33.6	30.2	31.2	1.9	26.2 - 32.9
≥ 6 ppm	31.2	30.5	2.8	28.2 - 36.9	30.5	30.5	2.1	27.5 - 33.4
	F value = 0.9, p = .45				F value = 0.6, p = .65			
MCHC								
0 - <1 ppm	33.1	33.2	0.7	31.9 - 34.7	33.6	33.6	0.5	32.2 - 34.9
1 - <3 ppm	32.2	33.1	0.6	31.7 - 34.5	33.6	33.6	0.5	31.8 - 34.6
3 - <6 ppm	33.1	32.6	0.7	31.3 - 34.3	33.5	33.5	0.7	32.0 - 34.4
≥ 6 ppm	33.1	33.3	0.6	32.2 - 34.0	33.9	33.9	0.5	33.4 - 34.6
	F value = 0.2, p = .90				F value = 0.7, p = .56			

Table A1 (Continued)

PFOS (ppm)	1995 Data Mean	Median	SD	Range	1997 Data Mean	Median	SD	Range
MCV								
0 - <1 ppm	94	95	5.2	85-106	90	90	4.4	83 – 101
1 - <3 ppm	93	94	4.3	80-104	91	91	3.6	81 – 99
3 - <6 ppm	94	94	4.8	81-104	90	91	5.2	80 – 97
≥ 6 ppm	94	92	9.7	85-115	90	91	5.7	81 – 96
	F value = 1.1, p = .35				F value = 0.6, p = .59			
WBC								
0 - <1 ppm	6.1	6.0	1.3	4.1 – 9.4	6.1	5.8	1.4	3.8 – 10.3
1 - <3 ppm	7.0[2]	6.8	2.0	3.6 – 15.5	6.9	6.6	1.9	3.8 – 13.2
3 - <6 ppm	7.6[2]	6.9	2.2	4.1 – 13.3	6.2	6.1	1.5	4.0 – 10.0
≥ 6 ppm	7.0	6.9	0.6	6.4 – 7.8	6.2	7.1	1.5	4.2 – 7.4
	F value = 4.3, p = .006				F value = 2.2, p = .09			
Platelets								
0 - <1 ppm	226	224	40	159 – 309	227	220	53	106 – 406
1 - <3 ppm	229	224	47	122 – 367	229	223	49	124 – 359
3 - <6 ppm	228	226	45	132 – 344	220	221	45	147 – 316
≥ 6 ppm	185	182	50	132 – 277	199	191	58	146 – 295
	F value = 2.1, p = .10				F value = 0.6, p = 0.60			

Table A1 (continued)

1. Mean is significantly different ($p < .05$, Bonferroni (Dunn) test) than the mean of the other PFOS categories.
2. Mean is significantly different ($p < .05$, Bonferroni (Dunn) test) than the mean of 0 - < 1 ppm PFOS category.
3. Mean is significantly different ($p < .05$, Bonferroni (Dunn) test) than the mean of 1 - < 3 ppm category.

* Sample Size	1995 Data	1997 Data
0 - <1 ppm	45	60
1 - <3 ppm	91	63
3 - <6 ppm	35	21
≥ 6 ppm	7	5

Table A2. Description of Final Employment and Vital Status for Male (n = 1,639) and Female (n = 318) Employees

Final Employment Status and Vital Status	Male Employees N	Male Employees %	Female Employees N	Female Employees %
Currently Employed	810	49.4	141	44.3
Retired				
Alive	59	3.6	9	2.8
Deceased	10	0.6	0	0.0
Unknown	0	0.0	0	0.0
Terminated				
Alive	694	42.3	164	51.6
Deceased	29	1.8	1	0.3
Unknown	6	0.4	0	0.0
Died While Employed	31	1.9	3	0.9
TOTAL	1,639	100.0*	318	100.0*

*percentages may not add to 100 due to rounding

Table A3. Characteristics of Male (n = 1,639) and Female (n = 318) Employees

Variable	Males	Females
Number of Employees	1,639	318
Number of Person-years	33,108	4,807
Number of Deaths	70	4
Average Age Started Work	25	26
Average Year of Entry	1971	1977
Average Age at Death	47	28
Average Year of Death	1984	1980

Table A4. Distribution of Men
by Age and Year of Entry into Follow-up (n = 1,639)

	Year of Entry into Follow-up							
Age	1960 to 1964	1965 to 1969	1970 to 1974	1975 to 1979	1980 to 1984	1985 to 1989	1990 to 1991	TOTAL
<20	18	34	86	31	4	2	0	175
20-24	114	238	301	96	28	18	3	798
25-29	64	139	65	37	21	14	5	345
30-34	21	58	33	19	8	10	4	153
35-39	22	25	9	15	6	5	1	83
40-44	19	18	4	10	8	2	0	61
45-49	3	3	4	5	0	2	0	17
50-54	0	1	0	0	2	3	0	6
55-59	0	1	0	0	0	0	0	1
60+	0	0	0	0	0	0	0	0
TOTAL	261	517	502	213	77	56	13	1,639

Table A5. Distribution of Women
by Age and Year of Entry into Follow-up (n = 318)

	Year of Entry into Follow-up							
Age	1960 to 1964	1965 to 1969	1970 to 1974	1975 to 1979	1980 to 1984	1985 to 1989	1990 to 1991	TOTAL
<20	2	12	18	16	4	0	0	52
20-24	4	22	23	50	4	9	1	113
25-29	3	4	4	47	4	5	3	70
30-34	2	3	2	15	1	4	2	29
35-39	1	2	3	16	3	3	2	30
40-44	0	0	0	8	3	2	0	13
45-49	0	0	0	5	1	1	0	7
50-54	0	0	0	3	0	0	0	3
55-59	0	0	0	1	0	0	0	1
60+	0	0	0	0	0	0	0	0
TOTAL	12	43	50	161	20	24	8	318

Table A6. Cause-Specific SMRs for Men
Ever Employed in Chemical Department (s)
Using the U.S. as Comparison Population (n = 1,050)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% CI
All Causes of Death	57	81.5	70.0	(53.0, 90.6)
All Malignant Neoplasms	13	16.9	76.9	(40.9, 131.5)
Cancer of Buccal Cavity and Pharynx	0	0.5	---	(0.0, 800.6)
Cancer of Digestive Organs and Peritoneum	1	3.6	27.5	(0.7, 153.2)
Cancer of Esophagus	0	0.4	---	(0.0, 913.3)
Cancer of Stomach	0	0.5	---	(0.0, 686.1)
Cancer of Large Intestine	1	1.3	76.9	(1.9, 428.5)
Cancer of Rectum	0	0.3	---	(0.0, 1,271.9)
Cancer of Liver and Biliary Passages	0	0.3	---	(0.0, 1,188.2)
Cancer of Pancreas	0	0.8	---	(0.0, 480.6)
Cancer of All Other Digestive Organs	0	0.1	---	(0.0, 3,203.2)
Cancer of Respiratory System	7	6.1	115.1	(46.3, 237.1)
Cancer of Larynx	0	0.2	---	(0.0, 1,891.9)
Cancer of Bronchus Trachea and Lung	7	5.8	120.7	(48.5, 248.7)
Cancer of Other Respiratory Organs	0	0.1	---	(0.0, 4,214.1)
Cancer of Prostate	0	0.5	---	(0.0, 805.9)
Cancer of Testes and Other Male Genital Organs	0	0.2	---	(0.0, 1,678.5)
Cancer of Kidney	0	0.5	---	(0.0, 768.2)
Cancer of Bladder and Other Urinary Organs	1	0.2	415.5	(10.4, 2,315.3)

Table A6. (Continued)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% CI
Cancer of Brain and Other Central Nervous System	1	0.9	117.2	(2.9, 653.0)
Cancer of Thyroid and Other Endocrine Glands	0	0.1	---	(0.0, 5,113.2)
Cancer of Bone	0	0.1	---	(0.0, 4,368.3)
Cancer of All Lymphatic and Hematopoietic Tissue	2	2.2	92.9	(11.2, 335.5)
Lymphoma	0	0.3	---	(0.0, 1,341.4)
Hodgkin's Disease	0	0.3	---	(0.0, 1,163.3)
Leukemia and Aleukemia	1	0.8	120.0	(3.0, 668.8)
Cancer of Other Lymphatic and Hematopoietic Tissue	1	0.7	137.2	(3.4, 764.5)
Malignant Melanoma of Skin	0	0.6	---	(0.0, 614.7)
All Other Malignant Neoplasms	1	1.5	68.9	(1.7, 383.7)
Diabetes Mellitus	0	1.1	---	(0.0, 323.9)
Cerebrovascular Disease	1	2.3	43.4	(1.1, 241.7)
All Heart Disease	11	22.5	48.8	(24.4, 87.4)
Hypertension	0	0.1	---	(0.0, 3,289.9)
Respiratory Disease	0	3.2	---	(0.0, 116.3)
Ulcer of Stomach and Duodenum	0	0.2	---	(0.0, 1,796.3)
Cirrhosis of Liver	3	3.0	100.2	(20.7, 292.7)
Nephritis and Nephrosis	0	0.3	---	0.0, 1,308.2)
External Causes	20	22.2	90.2	(55.1, 139.3)
Accidents	16	13.6	117.3	(67.0, 190.5)
Motor Vehicle Accidents	8	7.8	102.8	(44.4, 202.5)
All Other Accidents	8	5.9	135.7	(58.6, 267.4)
Suicides	3	5.2	57.7	(11.9, 168.6)
Homicides and Other External Causes	1	3.2	31.4	(0.8, 175.1)
Residual Causes*	7	9.0	77.6	(31.2, 159.9)
Unknown Causes &	2			

*all other causes of death combined

& no death certificate obtained; included only in all causes of death category

Table A7. Cause-Specific SMRs for Men
Only Employed in Chemical Department (s)
Using the U.S. as Comparison Population (n = 485)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% CI
All Causes of Death	32	44.1	72.5	(49.6, 102.4)
All Malignant Neoplasms	9	9.6	93.6	(42.8, 177.8)
Cancer of Buccal Cavity and Pharynx	0	0.3	---	(0.0, 1,396.4)
Cancer of Digestive Organs and Peritoneum	0	2.1	---	(0.0, 175.2)
Cancer of Esophagus	0	0.2	---	(0.0, 1,577.7)
Cancer of Stomach	0	0.3	---	(0.0, 1,195.2)
Cancer of Large Intestine	0	0.8	---	(0.0, 488.5)
Cancer of Rectum	0	0.2	---	(0.0, 2,175.6)
Cancer of Liver and Biliary Passages	0	0.2	---	(0.0, 2,072.6)
Cancer of Pancreas	0	0.5	---	(0.0, 827.2)
Cancer of All Other Digestive Organs	0	0.1	---	(0.0, 5,692.4)
Cancer of Respiratory System	5	3.6	141.0	(45.8, 329.0)
Cancer of Larynx	0	0.1	---	(0.0, 3,198.4)
Cancer of Bronchus, Trachea, and Lung	5	3.4	147.8	(48.0, 344.9)
Cancer of Other Respiratory Organs	0	0.1	---	(0.0, 7,763.4)
Cancer of Prostate	0	0.3	---	(0.0, 1,202.0)
Cancer of Testes and Other Male Genital Organs	0	0.1	---	(0.0, 3,501.4)
Cancer of Kidney	0	0.3	---	(0.0, 1,365.0)
Cancer of Bladder and Other Urinary Organs	1	0.2	669.9	(16.7, 3,732.7)

Table A7. (Continued)

Cause of Death	Observed Deaths	Expected Deaths	SMR	95% CI
Cancer of Brain and Other Central Nervous System	1	0.5	222.6	(5.6, 1,240.3)
Cancer of Thyroid and Other Endocrine Glands	0	0.0	---	(0.0, 9,544.3)
Cancer of Bone	0	0.0	---	(0.0, 8,697.5)
Cancer of All Lymphatic and Hematopoietic Tissue	2	1.2	174.0	(21.1, 628.7)
Lymphoma	0	0.2	---	(0.0, 2,466.1)
Hodgkin's Disease	0	0.2	---	(0.0, 2,346.1)
Leukemia and Aleukemia	1	0.4	226.3	(5.7, 1,260.7)
Cancer of Other Lymphatic and Hematopoietic Tissue	1	0.4	249.5	(6.2, 1,390.1)
Malignant Melanoma of Skin	0	0.3	---	(0.0, 1,200.4)
All Other Malignant Neoplasms	0	0.8	---	(0.0, 455.3)
Diabetes Mellitus	0	0.6	---	(0.0, 589.0)
Cerebrovascular Disease	0	1.3	---	(0.0, 274.4)
All Heart Disease	7	13.1	53.4	(21.5, 110.1)
Hypertension	0	0.1	---	(0.0, 5,834.4)
Respiratory	0	1.9	---	(0.0, 195.3)
Ulcer of Stomach and Duodenum	0	0.1	---	(0.0, 3,083.3)
Cirrhosis of Liver	1	1.6	62.3	(1.6, 347.3)
Nephritis and Nephrosis	0	0.2	---	(0.0, 2,346.9)
External Causes	10	10.5	95.0	(45.6, 174.7)
Accidents	9	6.5	139.4	(63.7, 264.5)
Motor Vehicle Accidents	5	3.6	138.0	(44.8, 322.0)
All Other Accidents	4	2.9	140.2	(38.2, 359.0)
Suicides	1	2.5	40.0	(1.0, 223.1)
Homicides and Other External Causes	0	1.5	---	(0.0, 247.4)
Residual Causes*	3	4.7	63.4	(13.1, 185.3)
Unknown Causes &	2			

*all other causes of death combined
& no death certificate obtained; included only in all causes of death category

Rat Oral (diet) Carcinogenicity on N-EtFOSE

Study Outline

Study Objectives: Main Objective – To determine the carcinogenicity of N-EtFOSE upon chronic oral administration.

Secondary Objectives – To determine chronic and subchronic toxicity of N-EtFOSE after 52 and 13 weeks, respectively, of compound administration. To determine compound's potential for perixosme proliferation (palmitoyl-CoA oxidase activity) and hepatocyte proliferation (PCNA, proliferative cell nuclear antigen). To measure compound and/or metabolite levels in the liver and serum after various time-points of exposure.

GLP Status: A GLP study with appropriate QA audits; a signed QUA statement to be included in the final report.

Animals: Male and female Charles River CD rats

Dose Groups: Eight – controls (two groups), 1, 3, 30, 100 & 300 ppm in diet plus a 100 ppm recovery group (receive compound for 52 weeks followed by 52 weeks without compound).

Number per Group: 50 males and 50 females per group plus additional animals for interim sacrifices at 4, 13 & 52 weeks. (Note the 100 ppm recovery group has 40 males and 40 females).

Test Article: N-EtPFOS furnished by Sponsor who is responsible for compound identity and purity.

Compound Administration: Daily administration for up to two-years (104 weeks) admixed in the diet.

Clinical Observations: Twice daily.

Body Weights: Once a week for the first 16 weeks; once every four weeks thereafter.

Food Consumption: Once a week for the first 16 weeks; once every four weeks thereafter.

Hematology and Clinical Chemistry: Ten rats/sex from 0, 1, 3, 30 & 100 ppm groups at weeks 14, 52 and 104.

Urinalysis: Ten rats/sex from 0, 1, 3, 30 & 100 ppm groups at weeks 14, 52 and 104.

Interim Necropsies: Five rats/sex from 0, 1, 3, 30, 100 & 300 ppm groups at 4 and 14 weeks for PCNA, palmitoyl CoA oxidase, and compound level samples. (Note samples for organ weights and histopathology taken at 14 weeks.) Ten rats/sex from 0, 1, 3, 30 &100 ppm groups at 52 weeks for compound levels, organ weight, histopathology samples.

Organ Weights: Measured at 14 and 52 week interim necropsies.

Histopathology: Microscopic examination of selected tissues at 14 and 52 week animals. Complete tissue examination of 0 (one control group), 3, 30, and 100 ppm 104 weeks animals. Selected tissue examination of 100 ppm recovery animals. Histopathologic results will indicate extent of tissue examination of 1 ppm and second control group.

Rat Oral (diet) Carcinogenicity on PFOS

Study Outline

Study Objectives: Main Objective – To determine the carcinogenicity of PFOS upon chronic oral administration.

Secondary Objectives – To determine chronic and subchronic toxicity of after 52 and 13 weeks, respectively, of compound administration. To determine compound's potential for perixosme proliferation (palmitoyl-CoA oxidase activity) and hepatocyte proliferation (PCNA, proliferative cell nuclear antigen). To measure compound and/or metabolite levels in the liver and serum after various time-points of exposure.

GLP Status: A GLP study with appropriate QA audits; a signed QUA statement to be included in the final report.

Animals: Male and female Charles River CD rats

Dose Groups: Six – 0, 0.5, 2, 5, & 20 ppm in diet plus a 20 ppm r overy group (receive compound for 52 weeks followed by 52 weeks without compound).

Number per Group: 50 males and 50 females per group plus additional animals for interim sacrifices at 4, 13 & 52 weeks. (Note the 20 ppm recovery group has 40 males and 40 females).

Test Article: PFOS furnished by Sponsor who is responsible for compound identity and purity.

Compound Administration: Daily administration for up to two-years (104 weeks) admixed in the diet.

Clinical Observations: Twice daily.

Body Weights: Once a week for the first 16 weeks; once every four weeks thereafter.

Food Consumption: Once a week for the first 16 weeks; once every four weeks thereafter.

Hematology and Clinical Chemistry: Ten rats/sex from 0, 0.5, 2, 5, & 20 ppm groups at weeks 14, 52 and 104.

Urinalysis: Ten rats/sex from 0, 0.5, 2, 5, & 20 ppm groups at weeks 14, 52 and 104.

Interim Necropsies: Five rats/sex from 0, 0.5, 2, 5, & 20 ppm groups at 4 and 14 weeks for PCNA, palmitoyl CoA oxidase, and compound level samples. (Note samples for

organs weights and histopathology taken at 14 weeks.) Ten rats/sex from 0, 0.5, 2, 5, & 20 ppm groups at 52 weeks for compound levels, organ weight, histopathology samples.

Organ Weights: Measured at 14 and 52 week interim necropsies.

Histopathology: Microscopic examination of selected tissues at 14 and 52 week animals. Complete tissue examination of 0, 2, 5, and 20 ppm 104 weeks animals. Selected tissue examination of 20 ppm recovery animals. Histopathologic results will indicate extend of tissue examination of 0.5 ppm group.

January 21, 1999

Rat Teratology Rangefinder on N-EtFOSE

Study Outline

Study Objective: Explore possible dose levels for an oral rat teratology study

GLP Status: Study should be conducted under GLP principles but since it is a rangefinder study QA audits will not be done

Animals: Mated female Charles River CD rats

Dose Groups: Seven - control, 0 mg/kg; low-1, 1 mg/kg; low-2, 5 mg/kg; mid-1, 10 mg/kg; mid-2, 20 mg/kg; high-1, 25 mg/kg; high-2, 35 mg/kg

Number per Group: 8 mated females per group

Test Article: N-EtPFOS will be furnished by the Sponsor who is responsible for compound identity and purity. No reserve sample will be required.

Compound Administration: Oral intubation of compound suspended in water with 2% Tween 80 on days 6 thru 17 of gestation. Analysis of dosing preparation will not be done, however, records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: On days 0, 4, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation

Food Consumption: On days when body weights are obtained

Cesarean Section: On day 20 of gestation the females will be anesthetized and the uterus and its contents will be removed and weighed. The number of implanations, the number of live and dead fetuses, and the number of early and late resorptions will be determined and recorded. The fetuses will be sexed, weighed and examined for any gross abnormalities. The ovaries will examined for the number of corpora lutea.

January 21, 1999

Rat Teratology Study on N-EtFOSE

Study Outline

Study Objective: To determine maternal and fetal toxicity and teratogenic potential of orally administered N-EtFOSE in pregnant rats

GLP Status: A GLP study with appropriate QA audits; a signed QUA statement to be included in the final report.

Animals: Mated female Charles River CD rats

Dose Groups: Five - control, 0 mg/kg; low, (1 or 1.5 ?) mg/kg; mid-1, (5 ?) mg/kg; mid-2, (10 or 15 ?) mg/kg; high, (20 or 25 ?) mg/kg. Final dose levels will be adjusted after range finder study results are available.

Number per Group: 25 mated females per group

Test Article: N-EtPFOS will be furnished by the Sponsor who is responsible for compound identity and purity. Reserve sample to be retained.

Compound Administration: Oral intubation of compound suspened in water with 2% Tween 80 on days 6 thru 17 of gestation. Samples of dosing preparations will be frozen and retained for possible analysis by the Sponsor. Records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: On days 0, 4, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation

Food Consumption: On days when body weights are obtained

Cesarean Section: On day 20 of gestation the females will be anesthetized and the uterus and its contents will be removed and weighed. The number of implanations, the number of live and dead fetuses, and the number of early and late resorptions will be determined and recorded. The fetuses will be sexed, weighed and examined for any gross abnormalities. The ovaries will examined for the number of corpora lutea.

Fetal Examinations: Approximately one-half of the fetuses from each litter will processed for visceral examination by the Wilson Technique for soft tissue development. The remaining fetuses will be eviscerated and processed for skeletal examination using Alizarin Red S staining method.

Toxicokinetic Satellite Animals: Groups of mated females, five females at the low dose (1 or 1.5 mg/kg) and five females at the high dose (20 or 25 mg/kg) plus three females at other dose levels, will be dosed. On the day after the last dose (day 18 of gestation) the satellite females will be killed and following samples will be collected: serum and liver from the dam and placentas and fetuses from the uterus. (Note placentas from a single litter can be stored together as one sample; likewise for the fetuses) These samples will be frozen and shipped to the sponsor for possible analysis.

January 21, 1999

Rabbit Teratology Rangefinder on N-EtFOSE

Study Outline

Study Objective: Explore possible dose levels for an oral rabbit teratology study

GLP Status: Study should be conducted under GLP principles but since it is a rangefinder study QA audits will not be done

Animals: Mated female New Zealand White rabbits (Note the rabbits are to be mated at the testing laboratory not at the supplier and shipped as mated females to the testing laboratory.)

Dose Groups: Seven - control, 0 mg/kg; low-1, 1 mg/kg; low-2, 5 mg/kg; mid-1, 10 mg/kg; mid-2, 25 mg/kg; high-1, 50 mg/kg; high-2, 75 mg/kg

Number per Group: 5 mated females per group

Test Article: N-EtFOSE will be furnished by the Sponsor who is responsible for compound identity and purity. No reserve sample will be required.

Compound Administration: Oral intubation of compound suspended in water with 2% Tween 80 on days 7 thru 20 of gestation. Analysis of dosing preparation will not be done; however, records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: On days 0, 7, 10, 13, 16, 19, 24 and 29 of gestation

Food Consumption: On days when body weights are obtained

Cesarean Section: On day 29 of gestation the females will be anesthetized and the uterus and its contents will be removed and weighed. The number of implanations, the number of live and dead fetuses, and the number of early and late resorptions will be determined and recorded. The fetuses will be weighed and examined for any gross abnormalities. The ovaries will be examined for the number of corpora lutea

January 21, 1999

Rabbit Teratology Study on N-EtFOSE

Study Outline

Study Objective: To determine maternal and fetal toxicity and teratogenic potential of orally administered N-EtFOSE in pregnant rabbits

GLP Status: A GLP study with appropriate QA audits; a signed QUA statement to be included in the final report.

Animals: Mated female New Zealand White rabbits (Note the rabbits are to be mated at the testing laboratory not at the supplier and shipped as mated females to the testing laboratory.)

Dose Groups: Five - control, 0 mg/kg; low, (1 ?) mg/kg; mid-1, (5 ?) mg/kg; mid-2, (10 ?) mg/kg; high, (25 ?) mg/kg. Final dose levels will be adjusted after range finder study results are available.

Number per Group: 22 mated females per group

Test Article: N-EtFOSE will be furnished by the Sponsor who is responsible for compound identity and purity. Reserve sample to be retained.

Compound Administration: Oral intubation of compound suspended in water with 2% Tween 80 on days 7 thru 20 of gestation. Samples of dosing preparations will be frozen and retained for possible analysis by the Sponsor. Records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: On days 0, 7, 10, 13, 16, 19, 24 and 29 of gestation

Food Consumption: On days when body weights are obtained

Cesarean Section: On day 29 of gestation the females will be anesthetized and the uterus and its contents will be removed and weighed. The number of implantations, the number of live and dead fetuses, and the number of early and late resorptions will be determined and recorded. The fetuses will be weighed and examined for any gross abnormalities. The ovaries will examined for the number of corpora lutea.

Fetal Examinations: A mid-coronal slice will be made in the head of each fetus to evaluate the contents of the cranium. The internal organs of the thoracic and abdominal cavities of all fetuses will be examined in the fresh state using Staples' technique for internal abnormalities. At this time the sex of the each fetus will be determined. After removal of the viscera, the carcasses will processed for skeletal examination.

Toxicokinetic Satellite Animals: Groups of mated females, five females at the low dose (1 ? mg/kg) and five females at the high dose (25 ? mg/kg) plus three females at other dose levels, will be dosed. On the day after the last dose (day 21 of gestation) the satellite females will be killed and following samples will be collected: serum and liver from the dam and placentas and fetuses from the uterus. (Note placentas from a single litter can be stored together as one sample; likewise for the fetuses) These samples will be frozen and shipped to the sponsor for possible analysis.

January 21, 1999

Rat Two Generation Reproduction Study of N-EtFOSE

Study Outline

Study Objective: To evaluate the effect of oral administration of N-EtFOSE on the reproductive function of male and female rats (F_0 generation) and on the development and reproductive capacity of the subsequent F_1 generation which were exposed in utero and via lactation.

GLP Status: A GLP study with appropriate QA audits; a signed QUA statement to be included in the final report.

Animals: Male and female Charles River CD rats

Dose Groups: Five - control, 0 mg/kg; low, 1 mg/kg; mid-1, 5 mg/kg; mid-2, 10 mg/kg; high, 15 mg/kg.

Number per Group: 35 males and 35 females per group

Test Article: N-EtFOSE will be furnished by the Sponsor who is responsible for compound identity and purity. Reserve sample to be retained.

Compound Administration: Daily oral intubation of compound suspended in water with 2% Tween 80. Compound administration will start 4 week prior to mating. Compound administration will continue through mating and in the females through gestation and lactation. The F_1 pups will not receive any doses via gastric intubation. Samples of dosing preparations on week 2 and 8 will be frozen and retained for possible analysis by the Sponsor. Records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: Weekly except when male and females are co-inhabited during mating.

Food Consumption: Weekly except when male and females are co-inhabited during mating.

Estrus Cycles: Daily vaginal smears will be taken from females two weeks prior to mating to determine estrus cycle information.

Mating: After 4 weeks of compound administration, within a dose group one male will be co-inhabited with one female. A female will be determined as mated upon the

presence of sperm positive vaginal smear. The day of mating shall be considered as day 0 of gestation.

Early Gestation Evaluation: 10 F_0 females per group will killed on day 10 of gestation. Pregnancy will be determined as well as the number of implanations and the number of early and late resorptions. At necropsy serum and liver samples will be taken from five of the females in each dose group. These samples will be frozen and shipped to the sponsor for possible analysis.

Male Sacrifice: One week after mating all F_0 male rats will be killed after a terminal body weight has been obtained. The animals will be necropsied and any gross lesions will be described and recorded. The testes, epididymis, prostate and seminal vesicles will be weighed and fixed for possible histologic examination. (Note: testis will be fixed in Bouin's solution; other tissues in 10% buffered formalin). At necropsy serum and liver samples will be taken from five of the males in each dose group. These samples will be frozen and shipped to the sponsor for possible analysis.

Female Parturition: The remaining 25 females will continue until term and be allow to deliver their litters. The offspring (F_1 generation) will be counted, sexed and weighed on postnatal days 0, 4, 7,14 and 21. Offspring will be randomly culled to four males and four females on day 4 postnatal.

Milk Samples: On day 4 postnatal when litters are culled to eight pups, milk curds will be collected from the stomach of the discarded pups from five litters of each dose groups. The milk curds from the pups in a litter are to be combined as a single sample.

Pup Examinations: Pups will be observed daily for moribundity/mortality. Developmental landmarks consisting of eye opening, pinna detachment, surface righting, testes descent, and vaginal opening will be recorded for each litter.

F_1 Dosing: After weaning at day 21 of lactation, the F_1 pups will receive compound by daily oral (gastric intubation) dosing. Dose level on a mg/kg basis will be the same as the pup's dam dose level.

Female Sacrifice: One or two days after weaning of the litter on day 21 of lactation, all F_0 female rats will be killed after a terminal body weight has been obtained. The animals will be necropsied and any gross lesions will be described and recorded. The ovaries will be weighed and fixed for possible histologic examination. (Note: ovaries will be fixed in 10% buffered formalin). At necropsy serum and liver samples will be taken from five of the females in each dose group. These samples will be frozen and shipped to the sponsor for possible analysis.

F1 Pup Neurological Testing: During the 4th week postpartum, the F_1 pups will be evaluated in a passive avoidance test for learning and short term memory retention. During the 10th week postpartum, the F_1 pups will be evaluated in a water-filled maze for neuromuscular coordination, learning, and longer term more complex memory.

F_1 Growth and Reproduction: The F_1 pups will be weaned after 21 days of lactation and litters will be culled to one of each sex. The pups will then allow to grow and body weights and food consumption will be recorded weekly. The F_1 pups will undergo behavioral/functional testing. At sexual maturity, within a dose group, one male will be mated with one female (sibling mating to be avoided). The females will be allowed to litter and raise the F_2 pup through 21 days of lactation.

January 21, 1999

Rat Two Generation Reproduction Study of PFOS

Study Outline

Study Objective: To evaluate the effect of oral administration of PFOS on the reproductive function of male and female rats (F_0 generation) and on the development and reproductive capacity of the subsequent F_1 generation which were exposed in utero and via lactation.

GLP Status: A GLP study with appropriate QA audits; a signed QUA statement to be included in the final report.

Animals: Male and female Charles River CD rats

Dose Groups: Five - control, 0 mg/kg; low, 0.1 or 0.2 mg/kg; mid-1, 0.5 or 1.0 mg/kg; mid-2, 2 or 3 mg/kg; high, 5 - 8 mg/kg.

Number per Group: 35 males and 35 females per group

Test Article: PFOS will be furnished by the Sponsor who is responsible for compound identity and purity. Reserve sample to be retained.

Compound Administration: Daily oral intubation of compound suspended in water with 2% Tween 80. Compound administration will start 4 week prior to mating. Compound administration will continue through mating and in the females through gestation and lactation. The F_1 pups will not receive any doses via gastric intubation. Samples of dosing preparations on week 2 and 8 will be frozen and retained for possible analysis by the Sponsor. Records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: Weekly except when male and females are co-inhabited during mating.

Food Consumption: Weekly except when male and females are co-inhabited during mating.

Estrus Cycles: Daily vaginal smears will be taken from females two weeks prior to mating to determine estrus cycle information.

Mating: After 4 weeks of compound administration, within a dose group one male will be co-inhabited with one female. A female will be determined as mated upon the

presence of sperm positive vaginal smear. The day of mating shall be considered as day 0 of gestation.

Early Gestation Evaluation: 10 females per group will killed on day 7 of gestation. Pregnancy will be determined as well as the number of implanations and the number of early and late resorptions.

Male Sacrifice: One week after mating all F_0 male rats will be killed after a terminal body weight has been obtained. The animals will be necropsied and any gross lesions will be described and recorded. The testes, epididymis, prostate and seminal vesicles will be weighed and fixed for possible histologic examination. (Note: testis will be fixed in Bouin's solution; other tissues in 10% buffered formalin). At necropsy serum and liver samples will be taken from five of the males in each dose group. These samples will be frozen and shipped to the sponsor for possible analysis.

Female Parturition: The remaining 25 females will continue until term and be allow to deliver their litters. The offspring (F_1 generation) will be counted, sexed and weighed on postnatal days 0, 4, 7,14 and 21. Offspring will be randomly culled to four males and four females on day 4 postnatal.

Milk Samples: On day 4 postnatal when litters are culled to eight pups, milk curds will be collected from the stomach of the discarded pups from five litters of each dose groups. The milk curds from the pups in a litter are to be combined as a single sample.

Pup Examinations: Pups will be observed daily for moribundity/mortality. Developmental landmarks consisting of eye opening, pinna detachment, surface righting, testes descent, and vaginal opening will be recorded for each litter.

F_1 Dosing: After weaning at day 21 of lactation, the F_1 pups will receive compound by daily oral (gastric intubation) dosing. Dose level on a mg/kg basis will be the same as the pup's dam dose level.

Female Sacrifice: One or two days after weaning of the litter on day 21 of lactation, all F_0 female rats will be killed after a terminal body weight has been obtained. The animals will be necropsied and any gross lesions will be described and recorded. The ovaries will be weighed and fixed for possible histologic examination. (Note: ovaries will be fixed in 10% buffered formalin). At necropsy serum and liver samples will be taken from five of the females in each dose group. These samples will be frozen and shipped to the sponsor for possible analysis.

F1 Pup Neurological Testing: During the 4th week postpartum, the F_1 pups will be evaluated in a passive avoidance test for learning and short term memory retention. During the 10th week postpartum, the F_1 pups will be evaluated in a water-filled maze for neuromuscular coordination, learning, and longer term more complex memory.

F_1 Growth and Reproduction: The F_1 pups will be weaned after 21 days of lactation. The pups will then allow to grow and body weights and food consumption will be recorded weekly. The F_1 pups will undergo behavioral/functional testing. At sexual maturity, within a dose group, one male will be mated with one female (sibling mating to be avoided). The females will be allowed to litter and raise the F_2 pup through 21 days of lactation.

Rabbit Teratology Rangefinder on PFOS

Study Outline

Study Objective: Explore possible dose levels for an oral rabbit teratology study

GLP Status: Study should be conducted under GLP principles but since it is a rangefinder study QA audits will not be done

Animals: Mated female New Zealand White rabbits (Note the rabbits are to be mated at the testing laboratory not at the supplier and shipped as mated females to the testing laboratory.)

Dose Groups: Seven - control, 0 mg/kg; low-1, 0.1 mg/kg; low-2, 1 mg/kg; mid-1, 2.5 mg/kg; mid-2, 5 mg/kg; high-1, 10 mg/kg; high-2, 20 mg/kg

Number per Group: 5 mated females per group

Test Article: N-EtPFOS will be furnished by the Sponsor who is responsible for compound identity and purity. No reserve sample will be required.

Compound Administration: Oral intubation of compound suspended in water with 2% Tween 80 on days 7 thru 20 of gestation. Analysis of dosing preparation will not be done; however, records will be maintained on how dosing preperations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: On days 0, 7, 10, 13, 16, 19, 24 and 29 of gestation

Food Consumption: On days when body weights are obtained

Cesarean Section: On day 29 of gestation the females will be anesthetized and the uterus and its contents will be removed and weighed. The number of implanations, the number of live and dead fetuses, and the number of early and late resorptions will be determined and recorded. The fetuses will be weighed and examined for any gross abnormalities. The ovaries will examined for the number of corpora lutea.

January 21, 1999

Rabbit Teratology Study on PFOS

Study Outline

Study Objective: To determine maternal and fetal toxicity and teratogenic potential of orally administered PFOS in pregnant rabbits

GLP Status: A GLP study with appropriate QA audits; a signed QUA statement to be included in the final report.

Animals: Mated female New Zealand White rabbits (Note the rabbits are to be mated at the testing laboratory not at the supplier and shipped as mated females to the testing laboratory.)

Dose Groups: Five - control, 0 mg/kg; low, 0.1 mg/kg; mid-1, 1 mg/kg; mid-2, 5 mg/kg, high, 10 mg/kg. Final dose levels will be adjusted after range finder study results are available.

Number per Group: 22 mated females per group

Test Article: FOS will be furnished by the Sponsor who is responsible for compound identity and purity. Reserve sample to be retained.

Compound Administration: Oral intubation of compound suspended in water with 2% Tween 80 on days 7 thru 20 of gestation. Samples of dosing preparations will be frozen and retained for possible analysis by the Sponsor. Records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: On days 0, 7, 10, 13, 16, 19, 24 and 29 of gestation

Food Consumption: On days when body weights are obtained

Cesarean Section: On day 29 of gestation the females will be anesthetized and the uterus and its contents will be removed and weighed. The number of implantations, the number of live and dead fetuses, and the number of early and late resorptions will be determined and recorded. The fetuses will be weighed and examined for any gross abnormalities. The ovaries will examined for the number of corpora lutea.

Fetal Examinations: A mid-coronal slice will be made in the head of each fetus to evaluate the contents of the cranium. The internal organs of the thoracic and abdominal cavities of all fetuses will be examined in the fresh state using Staples' technique for internal abnormalities. At this time the sex of the each fetus will be determined. After removal of the viscera, the carcasses will processed for skeletal examination.

Toxicokinetic Satellite Animals: Groups of mated females, five females at the low dose (0.1 mg/kg) and five females at the high dose 10 mg/kg) plus three females at other dose levels, will be dosed. On the day after the last dose (day 21 of gestation) the satellite females will be killed and following samples will be collected: serum and liver from the dam and placentas and fetuses from the uterus. (Note placentas from a single litter can be stored together as one sample; likewise for the fetuses) These samples will be frozen and shipped to the sponsor for possible analysis.

January 21, 1999

Rabbit Teratology Rangefinder on N-EtFOSE

Study Outline

Study Objective: Explore possible dose levels for an oral rabbit teratology study

GLP Status: Study should be conducted under GLP principles but since it is a rangefinder study QA audits will not be done

Animals: Mated female New Zealand White rabbits (Note the rabbits are to be mated at the testing laboratory not at the supplier and shipped as mated females to the testing laboratory.)

Dose Groups: Seven - control, 0 mg/kg; low-1, 1 mg/kg; low-2, 5 mg/kg; mid-1, 10 mg/kg; mid-2, 25 mg/kg; high-1, 50 mg/kg; high-2, 75 mg/kg

Number per Group: 5 mated females per group

Test Article: N-EtPFOS will be furnished by the Sponsor who is responsible for compound identity and purity. No reserve sample will be required.

Compound Administration: Oral intubation of compound suspended in water with 2% Tween 80 on days 7 thru 20 of gestation. Analysis of dosing preparation will not be done; however, records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: On days 0, 7, 10, 13, 16, 19, 24 and 29 of gestation

Food Consumption: On days when body weights are obtained

Cesarean Section: On day 29 of gestation the females will be anesthetized and the uterus and its contents will be removed and weighed. The number of implantations, the number of live and dead fetuses, and the number of early and late resorptions will be determined and recorded. The fetuses will be weighed and examined for any gross abnormalities. The ovaries will examined for the number of corpora lutea.

January 21, 1999

Rabbit Teratology Study on N-EtFOSE

Study Outline

Study Objective: To determine maternal and fetal toxicity and teratogenic potential of orally administered N-EtPFOS in pregnant rabbits

GLP Status: A GLP study with appropriate QA audits; a signed QUA statement to be included in the final report.

Animals: Mated female New Zealand White rabbits. (Note the rabbits are to be mated at the testing laboratory not at the supplier and shipped as mated females to the testing laboratory.)

Dose Groups: Five - control, 0 mg/kg; low, (1 ?) mg/kg; mid-1, (5 ?) mg/kg; mid-2, (10 ?) mg/kg; high, (25 ?) mg/kg. Final dose levels will be adjusted after range finder study results are available.

Number per Group: 22 mated females per group

Test Article: N-EtPFOS will be furnished by the Sponsor who is responsible for compound identity and purity. Reserve sample to be retained.

Compound Administration: Oral intubation of compound suspended in water with 2% Tween 80 on days 7 thru 20 of gestation. Samples of dosing preparations will be frozen and retained for possible analysis by the Sponsor. Records will be maintained on how dosing preparations were made.

Clinical Observations: Twice daily; once approximately one hour after dosing and then 4 to 6 hours later.

Body Weights: On days 0, 7, 10, 13, 16, 19, 24 and 29 of gestation

Food Consumption: On days when body weights are obtained

Cesarean Section: On day 29 of gestation the females will be anesthetized and the uterus and its contents will be removed and weighed. The number of implantations, the number of live and dead fetuses, and the number of early and late resorptions will be determined and recorded. The fetuses will be weighed and examined for any gross abnormalities. The ovaries will examined for the number of corpora lutea.

Fetal Examinations: A mid-coronal slice will be made in the head of each fetus to evaluate the contents of the cranium. The internal organs of the thoracic and abdominal cavities of all fetuses will be examined in the fresh state using Staples' technique for internal abnormalities. At this time the sex of the each fetus will be determined. After removal of the viscera, the carcasses will processed for skeletal examination.

Toxicokinetic Satellite Animals: Groups of mated females, five females at the low dose (1 ? mg/kg) and five females at the high dose (25 ? mg/kg) plus three females at other dose levels, will be dosed. On the day after the last dose (day 21 of gestation) the satellite females will be killed and following samples will be collected: serum and liver from the dam and placentas and fetuses from the uterus. (Note placentas from a single litter can be stored together as one sample; likewise for the fetuses) These samples will be frozen and shipped to the sponsor for possible analysis.

January 21, 1999

Corporate Toxicology Study Outline

Title:

1) Effect of Perfluorochemicals on Bioenergetic Metabolism: Phase II Research Plan (Restricted Grant)
2) Mitochondrial Interactions of Peroxisome Proliferators (Unrestricted Grant)

Purpose:

1) Compare mitochondrial bioenergetics between mitochondria from rat, guinea pig and human (Ca^{++} loading capacity, uncoupling potential, et al.) to help set appropriate safety factors for human risk characterization based on animal data;
2) Compare the metabolic response ([ATP], respiration rate, mitochondrial membrane potential, markers of peroxisome proliferation, et al.) of rat, guinea pig and primate hepatocytes to this class of compounds with particular emphasis on mitochondrial and peroxisomal metabolism to:
   a) Further test the proposed mechanism of toxicity;
   b) Identify potentially useful metabolic biomarkers of exposure;
   c) Help validate relative differences between species (assess markers of peroxisome proliferation across species);
3) Compare molecular response (gene expression → mRNA) of rat, guinea pig and primate hepatocytes exposed to these chemicals (genes indicative of cell and peroxisome proliferation) to:
   a) Identify molecular biomarkers of exposure;
   b) Facilitate species extrapolation;
   c) Discriminate between proliferation of peroxisomes and cell proliferation (ongogenic response) to test dogma;
   d) Assess the relevance of rat tumorigenic response to potential human cancer response;
   e) Provide biomarkers for potential use in monitoring and interpreting traditional toxicity studies.

Significance:

1) Comparing sensitivity between species will allow for judging the most appropriate species for predicting human health outcomes following exposures to compounds and has relevance to establishing safety factors and putting human cancer risk in perspective;
2) Species differences in metabolic and molecular response will reveal important insight into mechanisms responsible for compound-induced peroxisome proliferation and/or tumorigenesis which is also vital to identifying valid biomarkers to assess exposures and potential risks;
3) Comparing the enhanced transcription and expression of genes associated with peroxisome versus cell proliferation will allow opportunities to evaluate the

relationship between metabolic and oncogenic effects of these compounds in different species.

Objective(s):

1. Establish hepatocyte cell cultures from different species as models for assessing the metabolic and mitogenic effects test compounds;
2. Develop molecular probes for assessing compound-induced transcription of genes related to peroxisome metabolism and cell proliferation;
3. Establish benchmarks for assessing the effects of test compounds on hepatocyte bioenergetics and cell proliferation in culture.

Protocol:

1) Establish stable primary hepatocyte cultures from several species including rat, guinea pig and primate;
2) Develop molecular probes for compound-initiated transcription of specific genes related to mechanistic endpoints:
   a) ID genes of interest
   b) ID sequences of high homology between species using Gene Bank®
   c) Design primers to 1.5 – 3.0 kbp sequences (Oligo®)
   d) PCR amplify sequences from rat-liver cDNA template
   e) Separate, bands on low melting point gels
   f) Purify bands (band-stab)
   g) Restriction digest to confirm identity (MacDNAsis® to obtain restriction site map for each probe)
   h) Label probe with ^{32}P-dCTP by random priming (High Prime®)
3) Treat sample (cells or flash frozen tissue)
   a) Isolate total RNA by triazole method
   b) Separate on denaturing HCOH agarose gels
   c) Transfer to nylon
   d) Hybridize with ^{32}P probe
   e) Wash and expose to X-ray film to detect complementary mRNA
4) MRNA probes suggested for peroxisome proliferation
   a) CPT1
   b) ACoAO
   c) PPARα
   d) FABP
   e) Also, possibly Catalase, LPL, Aromatase, HMG-CoA synthetase
5) Probes for cell proliferation
   a) PCNA
   b) CDK
6) Establish benchmarks for assessing the effects of PF compounds on hepatocyte bioenergetics and cell proliferation in culture
   a) Effect of exposing cells in culture on induction of peroxisomal metabolism and stimulation of cell proliferation using biochemical markers and molecular markers (e.g., mitochondrial enzyme activities and cytochrome content, Adenine

nucleotides, AcoAO activity, PCNA, apoptosis (morphometric and TUNEL), CyQuant cell proliferation analysis, CPT1, AcoAO, PCNA, CDK)

Timeline:
31 months beginning December 1, 1998 and ending June 30, 2001

Report:
Quarterly progress reports