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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

MR 54374

AR226-1093

APR 25 2002

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

Dear Interested Party:

On March 28, 2002, EPA made available copies in PDF file format (389KB) of the *Draft Hazard Assessment Of Perfluorooctanoic Acid (PFOA) And Its Salts*, prepared by the Risk Assessment Division of the EPA Office of Pollution Prevention and Toxics. EPA has become aware that this document included an error in the text appearing on page 8 in section 2.0 of the document, "Production of PFOA and its Salts."

This error has now been rectified, and a corrected copy of the full document is attached. Please replace your earlier version of the draft assessment with this one, or print only the cover page, the Table of Contents, and page 8 from this corrected file, and use them to replace those pages from the original file printout. An errata sheet showing the page 8 deletions in **strikeout** and new revised text in **bold** is also attached for your convenience so that you can see immediately where the corrections were made.

We regret any confusion or inconvenience this may have caused.

If you have any questions or comments concerning the Assessment, please contact Jennifer Seed by phone at 202-564-7634, or by email at [seed.jennifer@epa.gov](mailto:seed.jennifer@epa.gov). If you wish to receive a copy of the Annex to the Assessment, which contains robust summaries of the studies reviewed in the Assessment, or if you have any difficulties opening these files, please contact Mary Dominiak by phone at 202-564-8104, by fax at 202-564-4775, or by email at [dominiak.mary@epa.gov](mailto:dominiak.mary@epa.gov).

Sincerely,

/s/

Charles M. Auer, Director  
Chemical Control Division

Attachments

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**Table 1. Reported Physicochemical Properties**

| Compound                                                                           | CAS REG # | MP    | BP                                 | VP        | Sol.-H2O    | Log P |
|------------------------------------------------------------------------------------|-----------|-------|------------------------------------|-----------|-------------|-------|
| Rf-C(=O)F                                                                          | 335-64-8  |       | 131 C                              |           |             |       |
| Rf-CO2H                                                                            | 335-67-1  | 55 C  | 189 C                              | 10 mm Hg  | 3.4 g/L     |       |
| Rf-CO2-NH4+                                                                        | 3825-26-1 | 130 C | sublimes                           | 1 x 10E-5 | 20 g/L gels | < 5   |
| Rf-C(=O)OMe                                                                        | 376-27-2  |       | 159 C                              |           |             |       |
| pH (1 g free acid /L Water) = 2.6                                                  |           |       | Free acid pKa is approximately 0.6 |           |             |       |
| Sodium or Silver salts of PFOA decompose above 250 C to generate perfluoroolefins. |           |       |                                    |           |             |       |

## 2.0 Production of PFOA and its Salts

PFOA is commercially manufactured by two major alternative processes: 1) the Simons Electro-Chemical Fluorination (ECF) process or 2) the telomerization process.

In the ECF process, an electric current is passed through a solution of anhydrous hydrogen fluoride and an organic feedstock of octanoic acid or a derivative~~1-octanesulfonyl fluoride~~. The ECF process replaces the carbon-hydrogen bonds on molecules of the organic feedstock with carbon-fluorine bonds, in an identical manner used to make PFOS. Perfluorination occurs when all the carbon-hydrogen bonds are replaced with carbon-fluorine bonds. The ECF process yields between 30-45 percent straight chain (normal) perfluorooctanonyl fluoride (PFOF)~~perfluorooctanesulfonyl fluoride (POSF)~~, along with a variable mixture of byproducts and impurities. The output of the ECF process is not a pure chemical, but instead a mixture of isomers and homologues including higher and lower straight-chain homologues; branched-chain perfluoroalkyl fluorides of various chain lengths; straight-chain, branched, and cyclic perfluoroalkanes and ethers; and other byproducts (3M Company, 2000a). After disposal or recovery of some of the byproducts and impurities, the acid fluoride~~POSF~~ is base hydrolyzed in batch reactors to yield PFOA. The PFOA salts are synthesized by base neutralization of the acid to the salt in a separate reactor (3M Company, 2000b).

In the telomerization process, tetrafluoroethylene is reacted with other fluorine-bearing chemicals to yield fluorinated carboxylic acids. This process yields pure straight-chain acids with an even number of carbon atoms. Distillation can be used to obtain pure components (ECT, 1994). Commercial products manufactured through the telomerization process are generally mixtures of perfluorinated compounds with even carbon numbers (Renner, 2001).

3M Company is the largest manufacturer and importer of PFOA and its salts in the United States. 3M has characterized its manufacture of PFOA and its ammonium and sodium salts in 1997 at less than 500,000 kg per year, and its importation at less than 100,000 kg (3M Company, 2000a). These figures may overstate the total production volume of PFOA since the vast majority of

**DRAFT**

**HAZARD ASSESSMENT OF**

**PERFLUOROOCTANOIC ACID**

**AND ITS SALTS**

**U.S. Environmental Protection Agency**  
**Office of Pollution Prevention and Toxics**  
**Risk Assessment Division**

**February 20, 2002**  
**(Corrected April 15, 2002)**

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## **PREFACE**

This is a preliminary assessment of the potential hazards to human health and the environment associated with exposure to perfluorooctanoic acid (PFOA) and its salts. The majority of the toxicology information is for ammonium perfluorooctanoic acid (APFO). This assessment includes a review of the studies that were available as of July 2001.

A two-generation reproductive toxicity study of APFO is currently being conducted and will be available in the spring of 2002. Effects were observed in a two-generation reproductive toxicity study of a related compound, perfluorooctane sulfonate. The results of the APFO study will be important to determine whether similar effects are observed.

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## EXECUTIVE SUMMARY

### Introduction

Perfluorooctanoic acid (PFOA) and its salts are fully fluorinated organic compounds that can be produced synthetically or through the degradation or metabolism of other fluorochemical products. PFOA is primarily used as a reactive intermediate, while its salts are used as processing aids in the production of fluoropolymers and fluoroelastomers and in other surfactant uses. In recent years, less than 600 metric tons per year of PFOA and its salts have been manufactured in the United States or imported. Most of the toxicology studies have been conducted with the ammonium salt of perfluorooctanoic acid, which is referred to as APFO in this report.

### Environmental Fate and Effects

PFOA is persistent in the environment. It has very low volatility and vapor pressure. It does not hydrolyze, photolyze or biodegrade under environmental conditions.

Several wildlife species have been sampled around the world to determine levels of PFOA. PFOA has rarely been found in fish sampled from the U.S., certain European countries, the North Pacific Ocean and Antarctic locations, or in fish-eating bird samples collected from the U.S., including Midway atoll, the Baltic and Mediterranean Seas, and Japanese and Korean coasts. PFOA was found in a few mink livers from Massachusetts at a concentration range of <18 to 108 ng/g, dry wt., but not found in mink from Louisiana, South Carolina and Illinois. PFOA concentrations in river otter livers from Washington and Oregon States were less than the quantification limit of 36 ng/g, wet wt. PFOA was not detected at quantifiable concentrations in oysters collected in the Chesapeake Bay and Gulf of Mexico of the U.S. coast.

The concentrations of PFOA in surface water, sediments, clams, and fish collected from two locations upstream and five locations downstream of the 3M manufacturing facility at Decatur AL have been determined. Of the five downstream sampling locations, the two closest to the facility had PFOA surface water concentrations significantly greater than the two upstream sites (means of 1900ug/L and 1024 ug/L); the nearest three locations had sediment concentrations significantly greater than the upstream sites (wet wt. means 1855 ug/kg, 892 ug/kg, 238 ug/kg). The average fish whole body PFOA concentration for the two upstream locations was 11.7 ug/kg (wet wt.), while that for the five downstream locations was 106.4 ug/kg. The average PFOA concentration in clams at the two upstream locations was 4.38 ug/kg, while the average for the five downstream locations was 8.42 ug/kg.

Based on available data, APFO does not appear to bioaccumulate in fish. In a study of fathead minnows, the calculated BCF for APFO was 1.8.

Several species were tested to assess the acute toxicity of APFO; these included the fathead minnow (*Pimephales promelas*), bluegill sunfish (*Lepomis machrochirus*), water flea (*Daphnia magna*), and a green algae (*Selenastrum capricornutum*). Comparisons of the different studies are problematic for several reasons. The studies were conducted with different test substances. Generally the ammonium salt or the tetrabutylammonium salt was tested. Purity of the test material is a major concern and was not sufficiently characterized in these tests. In some tests it appeared that 100% test chemical was used, for others a chemical of lesser purity (approximately 27 to 85%) was used. Water, a solvent (isopropanol) or a combination of both was used in other tests, for no obvious stated reason. Finally, only nominal test chemical concentrations were reported; the actual concentrations were not reported.

Twelve tests were conducted with fathead minnows; 96-h LC50 values (based on mortality) ranged from 70 to 843 mg/L. It is unclear why this range is so wide. Assuming these studies are valid, and due to the limitations discussed above, these toxicity values indicate low toxicity. The two acute values for bluegill sunfish also indicate low toxicity (96-h LC50s of >420, and 569 mg/L).

Nine acute tests were conducted with daphnids and 48-h EC50 values (based on immobilization) ranged from 39 to >1000 mg/L. The lower values are indicative of moderate toxicity, but the wide range makes interpretation difficult.

Seven tests were conducted with green algae; 96-h EC50 values (based on growth rate, cell density, cell counts, and dry weights) ranged from 1.2 to >666 mg/L (the Er50 cell density value of 1,000 mg/L is excluded from this discussion). The lower value indicates high to moderate toxicity, based on the acute criteria. The lower value would also be indicative of moderate toxicity, based on the chronic moderate criterion ( $0.1 \leq 10$  mg/L). A 14-d EC50 value of 43 mg/L, based on cell counts, for green algae was also calculated in one study. This is indicative of low chronic toxicity, based on the chronic criterion (10 mg/L). Green algae appeared to be the most sensitive test species in the 44% APFO test sample, daphnids were the next most sensitive, and fathead minnows were the least sensitive.

#### Human Health Effects and Biomonitoring

Little information is available concerning the pharmacokinetics of APFO in humans. A preliminary study of retired workers suggests simply that the serum half-life is between 1 and 3.5 years. These data provide evidence of the potential to bioaccumulate PFOA in humans. In addition, this study provides preliminary evidence that the serum half-life may be longer in females than in males.

Animal studies have shown that APFO is well absorbed following oral and inhalation exposure, and to a lesser extent following dermal exposure. In rats and dogs, there are major gender differences in the distribution and elimination of APFO. APFO distributes primarily to the liver,

plasma, and kidney, and to a lesser extent, other tissues of the body including the testis and ovary. It does not partition to the lipid fraction or adipose tissue. APFO binds to macromolecules in the tissues listed above. APFO is not metabolized and there is evidence of enterohepatic circulation of the compound. The urine is the major route of excretion of APFO in the female rat, while the urine and feces are both major routes of excretion of APFO in male rats. In female rats, the half-life is 24 h in the serum and 60 h in the liver; in male rats, the half-life is 105 h in the serum and 210 h in the liver. In beagle dogs, the plasma half-life is 254 h in females and 507 h in males. In rats, the elimination half-life is one day in females and 15 days in males. Female rats appear to have a secretory mechanism that rapidly eliminates APFO; this secretory mechanism is either lacking or relatively inactive in males. Other studies in rats have shown that testosterone exerts an inhibitory effect on renal excretion of APFO. Hormonal changes during pregnancy do not appear to change the rate of elimination in rats. The gender difference observed in rats and dogs has not been observed in primates and humans.

There are limited data on PFOA serum levels in workers and the general population. Occupational data from plants in the U.S. and Belgium that manufacture or use PFOA indicate that mean serum levels in workers range from 0.84 to 6.4 ppm. The highest level reported in a worker in 1997 was 81.3 ppm. In non-occupational populations, serum PFOA levels were much lower. In both pooled blood bank samples and in individual samples in both adults and children, mean PFOA levels ranged from 3 to 17 ppb. The highest serum PFOA level reported was in a sample from a child (56 ppb).

Epidemiological studies on the effects of PFOA in humans have been conducted on workers. Two mortality studies, as well as studies examining effects on the liver, pancreas, endocrine system, and lipid metabolism, have been conducted to date. In addition, a morbidity study was also recently submitted.

A retrospective cohort mortality study demonstrated a weak association with PFOA exposure and prostate cancer. A statistically significant association was observed in prostate cancer mortality as length of employment increased. This result was not observed in a recent update to the study; however, the results cannot be directly compared because the exposure categories were modified in the update. In a morbidity study, workers with the highest PFOA exposures for the longest durations sought care more often for prostate cancer treatment than workers with lower exposures.

Another study reported an increase in estradiol levels in workers with the highest PFOA serum levels; however, none of the other hormone levels analyzed indicated any adverse effects. Some of the same employees who participated in the hormone study also were included in a study of cholecystikinin (CCK) levels in employees. No positive association was noted between CCK values and PFOA. The other available study examined cholesterol and other serum components in workers. There did not appear to be any significant differences among workers of different exposure levels, except among obese workers (aspartate amino transferase and alanine amino transferase). However, PFOA was not measured directly, but indirectly as total serum fluorine.

There are many limitations to these studies, but most notably the small number of workers with PFOA serum levels greater than 10 ppm. Therefore, all of these results must be interpreted carefully.

In acute toxicity studies in animals, the oral LD50 values for CD rats were >500 mg/kg for males and 250-500 mg/kg for females, and <1000 mg/kg for male and female Wistar rats. There was no mortality following inhalation exposure of 18.6 mg/L for one hour in rats. The dermal LD50 in rabbits was determined to be greater than 2000 mg/kg. APFO is a primary ocular irritant in rabbits, while the data regarding potential skin irritancy are conflicting.

APFO is not mutagenic. APFO did not induce mutation in either *S. typhimurium* or *E. coli* when tested either with or without mammalian activation. APFO did not induce chromosomal aberrations *in vitro* in human lymphocytes when tested with and without metabolic activation up to cytotoxic concentrations. APFO was tested twice for its ability to induce chromosomal aberrations in CHO cells *in vitro*. In the first assay, APFO induced both chromosomal aberrations and polyploidy in both the presence and absence of metabolic activation. In the second assay, no significant increases in chromosomal aberrations were observed without activation. However, when tested with metabolic activation, APFO induced significant increases in chromosomal aberrations and in polyploidy. APFO was negative in a cell transformation assay in C<sub>3</sub>H 10T<sub>1/2</sub> mouse embryo fibroblasts and in the *in vivo* mouse micronucleus assay.

Subchronic studies in rats and mice with 28 and 90-days of exposure have demonstrated that the liver is the primary target organ and that males are far more sensitive than females. Dietary exposure to APFO for 90 days resulted in significant increases in liver weight and hepatocellular hypertrophy in female rats at 1000 ppm (76.5 mg/kg/day) and in male rats at doses as low as 100 ppm (5 mg/kg/day). Analyses of serum and liver levels of APFO showed a marked gender difference that accounts for the difference in sensitivity. In a 90-day study with rhesus monkeys, exposure to doses of 30 mg/kg/day or higher resulted in death, lipid depletion in the adrenals, hypocellularity of the bone marrow, and moderate atrophy of the lymphoid follicles in the spleen and lymph nodes. Unlike rodent studies, analyses of the serum and liver levels did not reveal a gender difference in monkeys, but the sample size was very small (N=2). Chronic dietary exposure of rats to 300 ppm APFO (14.2 and 16.1 mg/kg/day for males and females, respectively) for 2 years resulted in increased liver and kidney weights, hematological effects and liver lesions in males and females. In addition, testicular masses were observed in males at 300 ppm and ovarian tubular hyperplasia was observed in females after exposure to 30 ppm (1.6 mg/kg/day), the lowest dose tested.

Prenatal developmental toxicity studies in rats resulted in death and reduced body weight in dams exposed to oral doses of 100 mg/kg/day or by inhalation to 25 mg/m<sup>3</sup> APFO. There was no evidence of developmental toxicity after oral exposure to doses as high as 150 mg/kg/day, while inhalation exposure to 25 mg/m<sup>3</sup> resulted in reduced fetal body weights. In a rabbit oral developmental toxicity study there was a significant increase in skeletal variations after exposure

to 50 mg/kg/day APFO. There was no evidence of maternal toxicity at 50 mg/kg/day, the highest dose tested.

A two-generation reproductive toxicity study is currently being conducted. A two-generation reproductive toxicity study of PFOS showed high mortality of F1 pups at doses as low as 1.6 mg/kg/day. The results of the APFO study will be important to determine whether a similar effect is observed.

Carcinogenicity studies in Sprague-Dawley (CD) rats show that APFO is weakly carcinogenic, inducing Leydig cell adenomas in the male rats and mammary fibroadenomas in the females following dietary exposure to 300 ppm for 2 years (equivalent to 14.2 mg/kg/day in males and 16.1 mg/kg/day in females). The compound (at 300 ppm) has also been reported to be carcinogenic toward the liver and pancreas of male CD rats.

The mechanism(s) of APFO tumorigenesis is not clearly understood. Available data indicate that the induction of tumors by APFO is due to a non-genotoxic mechanism, involving activation of receptors and perturbations of the endocrine system. The liver carcinogenicity/toxicity of APFO appear to be related to induction of peroxisome proliferation following binding to the peroxisome proliferation activation receptor  $\alpha$  (PPAR  $\alpha$ ) in the liver. Available data suggest that the induction of Leydig cell tumors (LCT) and mammary gland neoplasms by APFO may be due to hormonal imbalance resulting from activation of the PPAR $\alpha$  and induction of the cytochrome P450 enzyme, aromatase. Preliminary data suggest that the pancreatic acinar cell tumors are related to an increase in serum level of the growth factor, cholecystokinin.

As the mechanisms of carcinogenic action of APFO have not been fully elucidated, it is assumed that the tumors induced in rats are relevant to humans. Review of available mechanistic data of other drugs and chemicals that induce LCT in animals has led a workshop panel to conclude that all but two modes of induction of the luteinizing hormone (LH), "dopamine agonism" and "GnRH agonism", are considered to be relevant to humans, and that the possibility of induction of Leydig cell adenoma in humans by specific agents with other modes of action cannot be ruled out despite the rarity of LCT in humans. At present, there is no evidence that the induction of LCT by APFO is via the "dopamine agonism" or "GnRH agonism" mode of action. It is recognized that there are quantitative differences in certain biological parameters between rats and humans. However, the principal cell control mechanisms appear similar, and the difference in carcinogenic response is probably quantitative. As binding to the PPAR $\alpha$  appears to be the critical event leading to hormonal imbalance and APFO tumorigenesis, and the level of PPAR $\alpha$  in human livers is lower than that in rodent liver, it appears that humans may be less sensitive than rodents in the development of LCT, mammary gland tumors, or liver neoplasms.

## 1.0 Chemical Identity

Chemical Name: Perfluorooctanoic Acid

Molecular formula: C<sub>8</sub> H F<sub>15</sub> O<sub>2</sub>

Structural formula: F-CF<sub>2</sub>-CF<sub>2</sub>-CF<sub>2</sub>-CF<sub>2</sub>-CF<sub>2</sub>-CF<sub>2</sub>-CF<sub>2</sub>-C(=O)-X,

The free acid and some common derivatives have the following CAS numbers:

The perfluorooctanoate anion does not have a specific CAS number.

|                |                                              |             |
|----------------|----------------------------------------------|-------------|
| Free Acid      | (X = OM <sup>+</sup> ; M = H)                | [335-67-1]  |
| Ammonium Salt  | (X = OM <sup>+</sup> ; M = NH <sub>4</sub> ) | [3825-26-1] |
| Sodium Salt    | (X = OM <sup>+</sup> ; M = Na)               | [335-95-5]  |
| Potassium Salt | (X = OM <sup>+</sup> ; M = K)                | [2395-00-8] |
| Silver Salt    | (X = OM <sup>+</sup> ; M = Ag)               | [335-93-3]  |
| Acid Fluoride  | (X = F)                                      | [335-66-0]  |
| Methyl Ester   | (X = CH <sub>3</sub> )                       | [376-27-2]  |
| Ethyl Ester    | (X = CH <sub>2</sub> -CH <sub>3</sub> )      | [3108-24-5] |

Synonyms: 1-Octanoic acid, 2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-pentadecafluoro-PFOA

## 1.1 Physicochemical Properties

For this report, perfluorooctanoic acid is consistently referred to as PFOA. Most of the toxicology studies have been conducted with the ammonium salt of perfluorooctanoic acid, which will be referred to as APFO in this report. PFOA is a completely fluorinated organic acid. The typical structure has a linear chain of eight carbon atoms produced by the telomerization of tetrafluoroethylene. The physical chemical properties noted below are for the free acid, unless otherwise stated. The data for the free acid, pentadecafluorooctanoic acid [335-67-1], is the most complete. The reported vapor pressure of 10 mm Hg appears high, but is consistent with other perfluorinated compounds with similar boiling points. The free acid is expected to completely dissociate in water.

Determination of the vapor pressure of APFO is problematic. For APFO, the recently reported vapor pressure of  $< 1 \times 10^{-5}$  (3M Environmental Laboratory, 1993) seems too low for a material that sublimates as the ammonium salt. This study measured the water solubility of APFO to be  $> 10\%$ . It was noted in an earlier study that concentrations of 20 g/L “gelled” (3M

Company, 1979). The partition coefficient was reported in these early studies of 5. Another calculated value, -0.9, might not be accurate due to the method used (Hansch and Leo 1979). The formation of an emulsified layer between the octanol and water surface interface would make determination of log P difficult.

**The available physicochemical properties for the PFOA free acid are:**

MW: 414 (Beilstein, 1975)

MP: 45 - 50 C (Beilstein, 1975)

BP: 189 - 192 C / 736 mm Hg (Beilstein, 1975)

VP: 10 mm Hg @ 25 C (approx.) (Exfluor MSDS)

Sol. - Water: 3.4 g/L (telomeric [mp = 34 C ref. 0.01 - 0.02 mol/L ~4 - 8 g/L] (MSDS from Merck, Fischer, and Chinameilan Internet sites)

pKa: 2.5 (USEPA AR-226 473)

pH (1g/L): 2.6 (MSDS Merck)

Due to the surface-active properties of PFOA, and the test protocol for the OECD method, PFOA is anticipated to form multiple layers in octanol/water, much like those observed for PFOS. Therefore, an n-octanol/water partition coefficient cannot be determined. Water solubility has been reported for PFOA, but it is unclear whether these values are for a microdispersion of micelles, rather than true solubility. Several reports note that PFOA salts self-associate as micelles at higher concentrations. (Simister, 1992; Calfours, 1985; Edwards, 1997). In aqueous solutions, micelles partition between the air / water interface on the surface.

Decomposition of different salts produces perfluoroheptene (loss of metal fluoride and carbon dioxide). This occurs at 320°C for the sodium salt and at 250-290°C (Beilstein 1975). The ammonium salt sublimates at 130°C (USEPA AR-226 473).

The physicochemical properties of PFOA and its derivatives are summarized in Table 1.

**Table 1. Reported Physicochemical Properties**

| Compound                                                                           | CAS REG # | MP    | BP                                 | VP        | Sol.-H2O    | Log P |
|------------------------------------------------------------------------------------|-----------|-------|------------------------------------|-----------|-------------|-------|
| Rf-C(=O)F                                                                          | 335-64-8  |       | 131 C                              |           |             |       |
| Rf-CO2H                                                                            | 335-67-1  | 55 C  | 189 C                              | 10 mm Hg  | 3.4 g/L     |       |
| Rf-CO2-NH4+                                                                        | 3825-26-1 | 130 C | sublimes                           | 1 x 10E-5 | 20 g/L gels | < 5   |
| Rf-C(=O)OMe                                                                        | 376-27-2  |       | 159 C                              |           |             |       |
| pH (1 g free acid /L Water) = 2.6                                                  |           |       | Free acid pKa is approximately 0.6 |           |             |       |
| Sodium or Silver salts of PFOA decompose above 250 C to generate perfluoroolefins. |           |       |                                    |           |             |       |

## 2.0 Production of PFOA and its Salts

PFOA is commercially manufactured by two major alternative processes: 1) the Simons Electro-Chemical Fluorination (ECF) process or 2) the telomerization process.

In the ECF process, an electric current is passed through a solution of anhydrous hydrogen fluoride and an organic feedstock of octanoic acid or a derivative. The ECF process replaces the carbon-hydrogen bonds on molecules of the organic feedstock with carbon-fluorine bonds, in an identical manner used to make PFOS. Perfluorination occurs when all the carbon-hydrogen bonds are replaced with carbon-fluorine bonds. The ECF process yields between 30-45 percent straight chain (normal) perfluorooctanonyl fluoride (PFOF), along with a variable mixture of byproducts and impurities. The output of the ECF process is not a pure chemical, but instead a mixture of isomers and homologues including higher and lower straight-chain homologues; branched-chain perfluoroalkyl fluorides of various chain lengths; straight-chain, branched, and cyclic perfluoroalkanes and ethers; and other byproducts (3M Company, 2000a). After disposal or recovery of some of the byproducts and impurities, the acid fluoride is base hydrolyzed in batch reactors to yield PFOA. The PFOA salts are synthesized by base neutralization of the acid to the salt in a separate reactor (3M Company, 2000b).

In the telomerization process, tetrafluoroethylene is reacted with other fluorine-bearing chemicals to yield fluorinated carboxylic acids. This process yields pure straight-chain acids with an even number of carbon atoms. Distillation can be used to obtain pure components (ECT, 1994). Commercial products manufactured through the telomerization process are generally mixtures of perfluorinated compounds with even carbon numbers (Renner, 2001).

3M Company is the largest manufacturer and importer of PFOA and its salts in the United States. 3M has characterized its manufacture of PFOA and its ammonium and sodium salts in 1997 at less than 500,000 kg per year, and its importation at less than 100,000 kg (3M Company, 2000a). These figures may overstate the total production volume of PFOA since the vast majority of

PFOA is consumed in the manufacture of the ammonium or sodium salts. More precise production volumes of PFOA and the ammonium and sodium salts have been reported to USEPA by 3M, but have been claimed as TSCA confidential business information, preventing disclosure in this report.

Industry participants have characterized 3M as the dominant global producer of PFOA-related chemicals, manufacturing approximately 85 percent or more of total worldwide volumes of the ammonium salt of PFOA (FMG, 2001). USEPA has not located information that would contradict this claim. Current production volume information for manufacturers other than 3M has not been provided by industry, nor is it available in USEPA's Chemical Update System (which contains information on non-polymeric organic chemicals manufactured in the United States or imported in volumes above 4,525 kg). Furthermore, there is no information on the total cumulative production volumes of PFOA since initial commercialization.

Since 1985, USEPA has received a total of approximately 25 notifications for PFOA-related chemicals that were not previously on the TSCA Chemical Inventory. Most of these notifications were from companies other than 3M. In most cases, the notifications qualified for the Low Volume Exemption for new chemicals with a production volume less than 10 metric tons per year.

In terms of on-going production, 3M has not committed publicly to a complete phase-out of PFOA and PFOA-related chemicals as it has for PFOS and PFOS-related chemicals. However, 3M has indicated that it is phasing out certain FLUORAD Brand specialty materials that contain PFOA and its salts such as FC-26, FC-118 and FC-143, FX-1001 and others (3M Company, 2000c).

Aside from the United States, OECD Member countries that reportedly have production capacity include France, Germany, Italy, and Japan. There may also be some production in non-OECD countries such as China. Following are companies that may manufacture PFOA and its salts (3M Company, 2000b; Directory of World Chemical Producers, 1998; Dynax, 2000; Renner, 2001; SEMI, 2001):

#### OECD

- 3M Company (United States)
- DuPont (United States)
- Exfluor Research Corporation (United States)
- PCR Inc. (United States)
- Atofina (France)
- Ciba Specialty Chemicals (Germany)
- Clariant (Germany)
- Dyneon (Germany)
- Hoechst Aktiengesellschaft (Germany)

- EniChem Synthesis S.p.A. (Italy)
- Miteni S.p.A. (Italy)
- Asahi Glass (Japan)
- Daikin (Japan)
- Dainippon (Japan)
- Tohkem Products Corporation (Japan)

#### Non-OECD

- Chenguang Research Institute of the Chemical Industry (China)
- Shanhai 3F New Materials Co., Ltd. (China)

### **2.1 Uses of PFOA and its Salts**

PFOA is used mainly as a chemical intermediate, and its salts are used in emulsifier and surfactant applications.

According to 3M, the vast majority of PFOA is consumed to make the ammonium or sodium salts. 3M also uses PFOA as a reactive intermediate in the industrial synthesis of a fluoroacrylic ester. The fluoroacrylic ester is used in an industrial coating application (3M Company, 2000a).

The salts of PFOA have additional uses, mostly in surfactant and emulsifier applications. These include the following:

- Processing aid in the industrial synthesis of fluoropolymers and fluoroelastomers such as polytetrafluoroethylene and polyvinylidene fluoride with a variety of industrial and consumer uses (3M Company, 2000a; DuPont, 2000; Daikin, 2001).
- Post-polymerization processing aids in the stabilization of suspensions of fluoropolymers and fluoroelastomers prior to further industrial processing (3M Company, 2000a).
- Processing aid for factory-applied fluoropolymer coatings on fabrics, metal surfaces, and fabricated or molded parts (3M Company, 2000a).
- Extraction agent in ion-pair reversed-phased liquid chromatography (Petritis, 1999).

Based on the physicochemical properties of the salts of PFOA, they may also have other related surfactant or emulsifier uses as a photographic chemical or in the manufacture of electronic components such as semiconductors. These same properties may lead industry to explore PFOA as a replacement chemical for PFOS in other applications in which PFOA is not currently used.

## **2.2 Environmental Fate**

### **2.2.1 Photolysis**

Direct photolysis of APFO was examined in two separate studies (Todd, 1979; Hatfield, 2001) and photodegradation was not observed in either study. In the Todd (1979) study, a solution of 50 mg/l APFO in 2.8 liters of distilled water was exposed to simulated sunlight at  $22 \pm 2$  °C. Spectral energy was characterized from 290-600 nm with a max output at ~360 nm. Direct photolysis of the test substance was not detected. However, the author noted that sample purity was not properly characterized which may have contributed to experimental error.

In the Hatfield (2001) study, both direct and indirect photolysis were examined utilizing techniques based on EPA and OECD guidance documents. To determine the potential for direct photolysis, APFO was dissolved in pH 7 buffered water and exposed to simulated sunlight (Scrano, 1999; Nubbe, 1995). For indirect photolysis, APFO was dissolved in 3 separate matrices and exposed to simulated sunlight for periods of time from 69.5 to 164 hours. These exposures tested how each matrix would affect the photodegradation of APFO. One matrix was a pH 7 buffered aqueous solution containing H<sub>2</sub>O<sub>2</sub> as a well-characterized source of OH radicals (Ogata, 1983; Lunak, 1992). This tested the propensity of APFO to undergo indirect photolysis. The second matrix contained Fe<sub>2</sub>O<sub>3</sub> in water that has been shown to generate hydroxyl radicals via a Fenton-type reaction in the presence of natural and artificial sunlight (Kachanova, 1973; Behar, 1966). The third matrix contained a standard solution of humic material. Neither direct nor indirect photolysis of APFO was observed based on loss of starting material. Predicted degradation products were not detected above their limits of quantitation. There was no conclusive evidence of direct or indirect photolysis whose rates of degradation are highly dependent on the experimental conditions. Using the iron oxide (Fe<sub>2</sub>O<sub>3</sub>) photoinitiator matrix model, the APFO half-life was estimated to be greater than 349 days.

### **2.2.2 Volatility**

Impinger studies were performed to examine the volatility of APFO and PFOS. Solutions of APFO or PFOS containing ammonium acetate in water/1-propanol (50:50) or phase transfer agents, e.g., n-alkyldimethylbenzylammonium chloride (3M Environmental Laboratory, 1993) were blown with 280 liters of air at a flow rate of 1 L/min. (3M Environmental Laboratory, 1993). The results indicate there is some loss of APFO and PFOS, but most of the solutions retained over 80% or more of the fluorochemicals. The average retention was 92% for both APFO and PFOS. This indicates that there is loss from the solutions. However, some of the solutions, particularly the n-alkyldimethylbenzylammonium chloride solution, appear to retain all the fluorochemicals. These results were reviewed by Dr. Edwin Tucker of the Chemistry Dept. at the University of Oklahoma (3M Environmental Laboratory, 1993). He concluded that

it is very unlikely that these fluorochemicals were removed by bubbling air through water due to their very low vapor pressures. He suggested that a more plausible mechanism for loss from the solution phase is concentration of the surfactants in foam and loss from the bubbled solutions as foam or micro-droplets.

In the second part of the experiment, air was passed over the fluorochemicals and bubbled through a train of impingers containing the ammonium acetate solution. It was expected that if any fluorochemicals were present in the air they would be transferred and retained by the ammonium acetate solution. However, no fluorochemicals were present in either the first or second impinger. The report concludes that the vapor pressure of both compounds is less than  $10 \text{ E-}07$ .

According to these experiments, APFO and PFOS (potassium salt) have very low volatility and vapor pressure. Quantitative conclusions regarding rates of volatilization from water or Henry's Law constant are not possible. However, APFO and PFOS are capable of transport out of water. Also, the loss of the fluorochemicals may have been as the free acids, not the salt forms. APFO sublimates at  $130 \text{ C}$  (see Physicochemical Properties Section 1.1). There is no information on the validity of the test method for determining volatility of the test substance. The study also lacks characterization of the purity of the test substance.

### **2.2.3 Biodegradation**

Using an acclimated sludge inoculum, the biodegradation of APFO was investigated using a shake culture study modeled after the Soap and Detergent Association's presumptive test for degradation (Reiner, 1978). Both thin-layer and liquid chromatography did not detect the presence of any metabolic products over the course of 2 1/2 months indicating that PFOA does not readily undergo biodegradation. In a related study,  $2.645 \text{ mg/L}$  APFO was not measurably degraded in activated sludge inoculum (Pace Analytical, 2001). Test flasks were prepared using a mineral salts medium,  $1 \text{ mL}$  methanol, and  $50 \text{ mL}$  settled sludge. Analysis was conducted with a HPLC/MSD system. Several other studies conducted between 1977-1987 also did not observe APFO biodegradation using what probably were standard COD and BOD methods, however, the methods used in these studies were either insufficiently described (i.e. no description of experimental protocols) or there were indications of a high degree of experimental error. The results were, therefore, deemed unreliable by the submitter (3M Company, 1977; 3M Company, 1980; 3M Company, 1985b; Pace Analytical, 1997).

### **2.2.4 Hydrolysis**

The 3M Environmental Laboratory (2001a) performed a study of the hydrolysis of PFOA. The study procedures were based on EPA's OPPTS Guideline Document 835.2110 (EPA 1998); although the procedures do not fulfill all the requirements of the guideline, they were more than

adequate for these studies. Results were based on the observed concentrations of PFOA in buffered aqueous solutions as a function of time. The chosen analytical technique was high performance liquid chromatography with mass spectrometry detection (HPLC/MS).

During the study, samples were prepared and examined at six different pH levels from 1.5 to 11.0 over a period of 109 days. Experiments were performed at 50°C and the results extrapolated to 25°C. Data from two of the pH levels (3.0 and 11) failed to meet the data quality objective and were rejected. Also rejected were the data obtained for pH 1.5 because ion pairing led to artificially low concentrations for all the incubation periods. The results for the remaining pH levels (5.0, 7.0, and 9.0) indicated no clear dependence of the degradation rate of PFOA on pH. From the data pooled over the three pH levels, it was estimated that the hydrolytic half-life of PFOA at 25°C is greater than 92 years, with the most likely value of 235 years. From the mean value and precision of PFOA concentrations, it was estimated the hydrolytic half-life of PFOA to be greater than 97 years.

### **2.2.5 Bioaccumulation**

To determine the potential for bioaccumulation, Fathead minnows were exposed to 25 mg/l APFO for 13 days (Howell et al., 1995). After 13 days exposure, the fish were then removed from APFO contaminated water and analyzed for depuration over 15 days. After 192 and 312 hours exposure to APFO contaminated water, the average concentration of APFO in fish tissue was 44.7 and 46.7 µg/g wet weight (ww), respectively. At this point, APFO appeared to reach steady state. Twenty-four hours after being transferred to clean water, the concentration of APFO decreased to 19.9 µg/g ww and by 96 hours post-exposure, the concentration had decreased to approximately 8 µg/g ww and remained relatively constant until test termination at 360 hours. The calculated BCF for APFO was 1.8. It should be noted that questions have been raised about this study regarding the analytical techniques, high-test chemical concentration, and short test duration.

Vraspir (1979) conducted a study to determine if bluegill sunfish bioaccumulate fluorochemicals from the 3M Decatur plant. Two lots of 30 fish were used. One lot was exposed to Decatur plant effluent for 21 days and the other to river water only for 23 days. Exposed fish, both living and dead, as well as the control fish were homogenized and analyzed for fluorochemicals by GC, TLC, and GC/MS. There were no detectable amounts of APFO in the ethyl acetate or toluene extracts of the tissues. No fluorochemicals were detected in the river water exposed fish. However, interpretation of this study is problematic for several reasons. Effluent concentrations of subject fluorochemicals were not characterized and the specific protocol for exposure of the fish was not found. There was also no information on analysis of the Tennessee River water or effluent used in the study. Additionally, it was not known if there was any opportunity for the depuration of the fish prior to sacrifice. No explanation was attempted as to what was the cause

of the twelve dead fish in the effluent-exposed group. The study also did not differentiate between the bioaccumulation of the test compound and the sorption onto the surface of the fish.

### **2.2.6 Soil Adsorption**

The adsorption-desorption of APFO was studied in 25 ml solutions of <sup>14</sup>C-labeled APFO in distilled water with 5 g Brill sandy loam soil for 24 hours at a temperature of 16-19 °C. The study reported a K<sub>d</sub> of 0.21 and a K<sub>oc</sub> of 14 indicating that PFOA has high mobility in Brill sandy loam soil (Welsh 1978). The K<sub>oc</sub> value, however, is questionable due to the lack of accurate information on the purity of the <sup>14</sup>C-labeled test substance (Boyd 1993a,b).

Moody and Field (1999) conducted sampling and analysis of samples taken from groundwater 1 to 3 meters below the soil surface in close proximity to two fire-training areas with a history of aqueous film forming foam use. Perfluorooctanoic acid was detected at maximum concentrations ranging from 116 to 6750 ug/L at the two sites many years after its use at those sites had been discontinued. These results suggest that APFO may have the potential to migrate through soils to relatively shallow groundwater where it persists.

## **2.3 Environmental Exposure**

### **2.3.1 Combustion**

For 1997, 3M estimated 1950 pounds of PFOA-compound (PFOA and related salts) stack releases at its Cottage Grove MN location and another 4500 lbs. from Cottage Grove incinerated offsite (3M Company, 2000a,b). In 1998, 70% of the fluoride-containing wastes at 3M's Decatur location were incinerated off-site; incineration is now the primary disposal method for these materials (3M Company, 2000a,b). For 1999, DuPont estimated stack releases of 24,000 lbs. APFO at its Washington Works WV location, plus another 16,000 lbs. from Washington Works incinerated offsite (DuPont, 2000).

Canadian research has stated that the thermolysis of fluoropolymers, e.g., Teflon, Kel-F, can liberate small quantities of polycarboxylic acids, which include PFOA (Ellis et al., 2001). This information was insufficient to estimate potential yields.

### **2.3.2 Discharge to Water**

By analogy to PFOS, PFOA discharged to water may remain there, become adsorbed to particulate matter and sediment, and/or be assimilated by organisms. For 1999, 3M estimated PFOA-compound water releases of <30,000 lbs. at its Decatur AL location, and <15,000 lbs. at its Cottage Grove MN location (3M Company, 2000a,b). For 1999, DuPont estimated the

following APFO water releases per location: Washington Works WV, 55,000 lbs; Parlin NJ, 300 lbs.; Spruance VA, 150 lbs.; Chambers Works NJ, 9500 lbs. (DuPont, 2000).

DuPont measured and modeled the following APFO concentrations at its sites: Washington Works WV: 0.552 ug/l from a 1999 drinking water sample obtained from GE Plastics immediately downstream on the Ohio River. Modeled 1996 APFO-compound releases indicated an average annual PFOA concentration of 0.423 ug/l, with APFO concentrations likely to exceed 1 ug C-8/l about 50% of the time during the year, and likely to exceed 10 ug APFO/L about 2.2% of the time during the year.

### **2.3.3 Discharge to Land**

3M reported that land treatment of sludge from wastewater treatment at their Decatur AL location ended in mid-1998; less than 500 lbs. were disposed to land at that site in 1997. Sludge from the Decatur site is now transported to an offsite landfill; sludge from 3M's Cottage Grove MN facility is sent to an industrial landfill (3M Company, 2000a,b). DuPont (2000) estimated 3,900 lbs. of APFO sludge landfilled on site in 1999 at their Chambers Works NJ facility. DuPont estimated 2,600 lbs. APFO transferred offsite to a hazardous waste landfill from their Washington Works WV facility.

Prior operations resulted in ground- and surface water concentrations of APFO monitored at three landfills operated by DuPont's Washington Works WV facility. Average surface water concentrations for two landfills were 1392 ug/L and 18.5 ug/L, respectively. A third landfill had a maximum concentration of 33 ug/L in the permitted outfall. Average groundwater concentrations for two landfills were 2537 ug/L and 8.83 ug/L, respectively. A third landfill had a maximum groundwater concentration of 15 ug/L (DuPont, 2000).

DuPont also reported the following APFO concentrations, measured January 2000, in three drinking water wells of the Lubeck Public Service District, downstream of DuPont's Washington Works WV site: 0.8 ug/L, 0.44 ug/L, and 0.313 ug/L (DuPont, 2000). As of August 2000, the Lubeck Public Service District (LPSD) reported APFO concentrations of 0.2 ppb in drinking water at DuPont's Washington Works facility, and 0.2, 0.5, and 0.1 ppb in the three LPSD wells (LPSD, 2000).

### **2.3.4 Environmental Monitoring**

3M's Multi-City Study reported on PFOA concentrations from water, sludge, sediment, POTW effluent, and landfill leachate samples taken in six cities (3M, 2001a). Four of the cities (Decatur AL, Mobile AL, Columbus GA, Pensacola FL) were "supply" cities that have manufacturing or industrial use of fluorochemicals; two of the cities (Cleveland TN, Port St.

Lucie FL) were “control” cities that do not have significant fluorochemical activities. Across all cities, POTW effluent concentrations ranged from 0.040 to 2.42 ppb. The POTW sludge (dry wt.) range was non-detect to 244 ppb; the drinking water range was non-detect to 0.029 ppb; the landfill leachate range was non-detect to 48.1 ppb; the surface water range was non-detect to 0.083; the sediment range was non-detect to 1.75 ppb (dry wt.); and the quiet water range was non-detect to 0.097 ppb. The “control” cities samples generally inhabited the lower end of the above ranges, except for the POTW effluent and sludge findings for Cleveland, which were intermediate in their ranges.

Giesy reported that PFOA was rarely found in fish and fish-eating water birds. Fish were sampled from the U.S., certain European countries, the North Pacific Ocean, and Antarctic locations (Giesy, 2001a). Fish-eating bird samples were collected from the U.S., including Midway atoll, the Baltic and Mediterranean Seas, Japanese and Korean coasts (Giesy, 2001b)

Giesy reported on PFOA in mink and river otter livers from the U.S. (Giesy, 2001c). PFOA was found in a few mink livers from Massachusetts at a concentration range of <18 to 108 ng/g, dry wt., but not found in mink from Louisiana, South Carolina and Illinois. PFOA concentrations in river otter livers from Washington and Oregon States were less than the quantification limit of 36 ng/g, wet wt.

Giesy reported that PFOA was not detected at quantifiable concentrations in oysters collected in the Chesapeake Bay and Gulf of Mexico of the U.S. coast (Giesy, 2001d).

Giesy reported on the concentrations of PFOA in surface water, sediments, clams, and fish collected from locations upstream and downstream of the 3M facility at Decatur AL (Giesy, 2001e). Of the five downstream sampling locations, the two closest to the 3M facility had PFOA surface water concentrations significantly greater than the two upstream sites (means of 1900ug/L and 1024 ug/L, vs. 0.008 (est.) and 0.028 ug/L); the nearest three locations had sediment concentrations significantly greater than the upstream sites (wet wt. means 1855 ug/kg, 892 ug/kg, 238 ug/kg vs. 0.08(est.) and 0.09(est.)). Clam and fish samples were collected at two locations, one upstream and one downstream of the 3M facility. The average fish whole body PFOA concentration for the upstream location was 11.7 ug/kg (wet wt.), while that for the downstream location was 106.4 ug/kg. The average PFOA concentration in clams at the upstream location was 4.38 ug/kg; that for the downstream location was 8.42 ug/kg.

## **2.4 Human Biomonitoring**

Table 1 provides serum PFOA levels in both occupational cohorts and in the general population. The highest levels reported to date in the general population are similar to some of the lowest

levels in workers exposed to PFOA occupationally. The data are currently limited to those discussed below.

3M has offered voluntary medical surveillance to workers at plants that produce or use perfluorinated compounds since 1976. Serum PFOA levels have been measured and reported since 1993. Prior to this time, only total organic fluorine was measured. The results of biomonitoring for PFOA have been reported for 3 plants: Cottage Grove, Minnesota; Decatur, Alabama; and Antwerp, Belgium. Surveillance years include 1993, 1995, 1997, 1998, and 2000, although not all of the plants offered surveillance in all of these years. The 1998 data reported for the Decatur plant consist of a random sample of employees; however, volunteers participated in all of the other sampling periods for all of the plants.

Mean serum PFOA levels have increased slightly at both the Cottage Grove and Decatur plants since 1993. Workers at the Cottage Grove plant, where PFOA exposures are highest, have the highest PFOA serum levels. The latest sample was in 1997, and the mean serum PFOA level was 6.4 ppm (range = 0.1 – 81.3 ppm) (Olsen et al., 1998). Only 74 employees participated in the 1997 surveillance. The total number of employees working at the plant was not reported.

At the Decatur plant, 263 of 500 employees participated in 2000 (Olsen et al., 2001d). The mean serum PFOA level was 1.78 ppm. This was slightly higher than the mean in 1998 (1.54 ppm). In 2000, 5 employees had serum levels greater than 5 ppm, the Biological Limit Value established by the 3M Exposure Guideline Committee. Cell operators had the largest increase in serum PFOA between 1998 and 2000. The highest level was in a chemical operator on the Scotchgard team (12.70 ppm). The mean level for the rest of the members of the team was 5.06 ppm (range 5 - 9 ppm). Other job categories did not exhibit such a large increase. 3M reports that this is due to increased PFOA production at the Decatur plant beginning in 1999.

Serum PFOA levels at the Antwerp plant have been lower than at Decatur or Cottage Grove, and have decreased slightly since 1995 (Olsen et al., 2001e). Participation in medical surveillance at the Antwerp plant was the highest it had ever been in 2000 (258 volunteers out of 340 workers). The mean serum PFOA level was 0.84, and the highest serum level reported was 7.04 ppm. Three employees had levels greater than 5 ppm.

3M's Specialty Materials Manufacturing Division laboratories, where employees perform fluorochemical research (Building 236), conducted voluntary biomonitoring of 45 employees in 2000 (Olsen et al., 2001f). The mean PFOA serum level was 0.106 ppm (range 0.008 – 0.668 ppm).

Data on PFOA levels in the general population are very limited. They are very recent and are only available on small cohorts. The mean serum PFOA levels are much lower in the general population than in workers exposed to PFOA.

Pooled blood samples from U.S. blood banks indicate mean PFOA levels of 3 to 17 ppb (3M Company, 1999a, 1999b). The highest pooled sample reported was 22 ppb. Samples were collected in 1998 and 1999. These data provide a very preliminary view of the PFOA levels that may be present in the U.S. general population. However, it cannot be assumed that these levels are representative of the U.S. population for several reasons: 1) blood donors are not necessarily representative of the U.S. population, 2) many of the blood banks originally contacted for possible inclusion in the study declined to participate, 3) only a small number of samples have actually been analyzed for PFOA, and 4) no other data such as age, sex, or other demographic information are available on the donors.

Preliminary data on individual blood samples have recently been reported (Olsen et al., 2001b, 2001c). Blood samples from 652 U.S. adult blood donors, ages 20-69, were obtained from six American Red Cross blood banks located in: Los Angeles, CA; Minneapolis/St. Paul, MN; Charlotte, NC; Boston, MA; Portland, OR, and Hagerstown, MD. The mean serum PFOA level was 5.6 ppb. The range was <lower limit of quantitation (LLOQ = 1.92 or 2.11) to 52.3 ppb. Blood samples from U.S. children have also been analyzed for serum PFOA. A sample of 599 children, ages 2-12 years old, participating in a study of group A streptococcal infections, revealed a mean PFOA serum level of 5.6 ppb. The range was <LLOQ to 56.1 ppb. The LLOQ was 1.92 or 2.88. The samples were collected from equal numbers of male and female children residing in 23 states. The samples in both of these studies were analyzed using high-pressure liquid chromatography/electrospray tandem mass spectrometry (HPLC/ESMSMS). These data are only preliminary and have not completed quality assurance procedures.

In another study, the PFOA concentration was analyzed in human sera and liver samples (Olsen et al., 2001g). Thirty-one donor samples were obtained from 16 males and 15 females over an 18-month period from the International Institute for the Advancement of Medicine (IIAM). The average age of the male donors was 50 years (SD 15.6, range 5-69) and the average age of the female donors was 45 years (SD 18.5, range 13-74). The causes of death were intracranial hemorrhage (n = 16 or 52%), motor vehicle accident (n = 7 or 23%), head trauma (n = 4 or 13%), brain tumor (n = 2 or 6%), drug overdose (n = 1 or 3%) and respiratory arrest (n = 1 or 3%). Both serum and liver tissue were obtained from 23 donors; 7 donors contributed liver tissue only and 1 donor contributed serum only. Serum samples were obtained from 5 ml of blood; liver samples consisted of 10 g of tissue. Samples were frozen at IIAM and shipped frozen to 3M for analysis. Samples were extracted using an ion-pairing extraction procedure and were quantitatively assayed using HPLC-ESMSMS and evaluated versus an unextracted curve. Extensive matrix spike studies were performed to evaluate the precision and accuracy of the extraction procedure. Serum values for PFOA ranged from < LOQ (<3.0) – 7.0 ng/mL. Assuming the midpoint value between zero and LOQ serum value for samples <LOQ, the mean serum PFOA level was 3.1 ng/mL with a geometric mean of 2.5 ng/mL. No liver to serum ratios were provided because more than 90% of the individual liver samples were <LOQ.

Serum PFOA levels in corporate staff and managers at a 3M plant in St. Paul, Minnesota, where occupational exposure to PFOA should not have occurred, were reported (3M Company, 1999a).

Four of 31 employees had serum PFOA levels greater than the detection limit of 10 ppb. The mean for these employees was 12.5 ppb.

**Table 1. SERUM PFOA LEVELS IN HUMAN POPULATIONS**

| Occupational Exposures (ppm)                            |                 |               |                |                         |
|---------------------------------------------------------|-----------------|---------------|----------------|-------------------------|
| Plant                                                   | Arithmetic Mean | Range         | Geometric Mean | 95% Confidence Interval |
| Cottage Grove Plant                                     |                 |               |                |                         |
| 1997 (n = 74)                                           | 6.4             | 0.1 – 81.3    | *              | *                       |
| 1995 (n = 80)                                           | 6.8             | 0.0 – 114.1   | *              | *                       |
| 1993 (n = 111)                                          | 5.0             | 0.0 – 80.0    | *              | *                       |
| Decatur Plant                                           |                 |               |                |                         |
| 2000 (n = 263)                                          | 1.78            | 0.04 – 12.70  | 1.13           | 0.99 – 1.30             |
| 1998 (n = 126)                                          | 1.54            | 0.02 – 6.76   | 0.90           | 0.72 – 1.12             |
| 1997 (n = 84)                                           | 1.57            | not reported  | *              | *                       |
| 1995 (n = 90)                                           | 1.46            | not reported  | *              | *                       |
| Antwerp Plant                                           |                 |               |                |                         |
| 2000 (n = 258)                                          | 0.84            | 0.01 – 7.04   | 0.33           | 0.27 – 0.40             |
| 1995 (n = 93)                                           | 1.13            | 0.00 – 13.2   | *              | *                       |
| Building 236                                            |                 |               |                |                         |
| 2000 (n = 45)                                           | 0.106           | 0.008 – 0.668 | 0.053          | 0.037 – 0.076           |
| General Population Exposures (ppb)                      |                 |               |                |                         |
| Source                                                  | Arithmetic Mean |               | Range          |                         |
| Pooled samples                                          |                 |               |                |                         |
| Commercial sources of blood, 1999 (n = 35 lots)         | 3               |               | 1 - 13         |                         |
| Blood Banks (n = 18), 1998 ~340-680 donors              | 17**            |               | 12 - 22        |                         |
| Individual samples                                      |                 |               |                |                         |
| American Red Cross blood banks, 2000 (n = 652)          | 5.6             |               | 4.27 - 52.3    |                         |
| Children, 1995 (n= 599)                                 | 5.6             |               | 4.27 - 56.1    |                         |
| 3M Corporate managers/staff St. Paul, MN, 1998 (n = 31) | 12.5***         |               | not reported   |                         |

\*Geometric mean and 95% confidence intervals were not included in the reports.

\*\*PFOA detected in about 1/3 of the pooled samples but quantifiable in only 2

\*\*\*only 4 employees were above the detection limit of 10 ppb

### 3.0 Human Health Hazards

#### 3.1. Metabolism and Pharmacokinetics

##### 3.1.1 Half-life in Humans

In order to determine the half-life of PFOA, a group of retirees (n = 20) volunteered to participate in a 5-year half-life study in which serum samples will be drawn every 6 months (Burris et al., 2000). The only other data available on the half-life of PFOA is from a 1980 study in which it was estimated to be approximately 1 year; however, this analysis was based on total organic fluorine in blood serum.

Twenty-seven retirees, age 55 to 74 years, volunteered to participate in this half-life study. PFOA levels in this group ranged from 0.1 to 3.1 ppm. Most of the retirees were employed at the Decatur, Alabama plant for an average of 28 years. The number of years since retirement varied greatly among the participants. The average length of time between retirement and the start of the study was 30 months (2.5 years) but ranged from 5 to 130 months (~.5 to 10 years). There were 3 collection periods during which serum PFOA samples were collected and analyzed: November 1998, June 1999, and November 1999. Cottage Grove employees, where PFOA exposure was much higher, have only participated in 2 sampling periods; therefore, they were not included in this analysis.

Half-lives were calculated using a one-compartment model. A log-linear relationship (slope =  $-k_{el}(2.303)$ ) was used to estimate the half-life. The half-life was calculated after the elimination constant was determined, using the relationship:  $t_{1/2} = 0.693/k_{el}$ . Only those retirees who fit the linear one compartmental model ( $r^2 \geq 0.6$ ) for PFOA were included in the analyses. If 3 data points were not available for any of the subjects and if there was a lack of fit to the model, that retiree was not included in the analysis. Twenty participants met these requirements. The median serum half-life of PFOA was 344 days, with a range of 109 to 1308 days. The two highest half-life calculations were for the 2 female retirees who participated in this study (654 and 1308 days). It should be noted that the difference in PFOA serum levels between retirees was quite large (0.1 - 3.1 ppm). It was not specifically stated in the report; however, based on a statement in the report, it is assumed that the 2 female retirees did not have the highest PFOA serum levels.

For most of the participants not included in the analysis, the second measurement was higher than the first. Therefore, the data did not fit the model and they were excluded. Although this may justify not including those participants in the analysis, it is an indication of the many limitations of the data. It is stated in the report that neither age nor number of months retired was associated with the serum PFOA half-life calculations; however, this statement is not supported with any data in the report. In addition, no individual data were provided in the report and the relationship between number of years exposed in the workplace and PFOA levels and half-life were excluded. Also, elimination of PFOA occurs via urine and feces; however, these

measurements were not collected. Therefore, it cannot be determined whether the half-life suggested by the preliminary results reported here represents a true elimination half-life from the body. Finally, the effect of continued non-occupational, low-level exposure on the half-life is unknown.

The data presented above provide a very rough estimate of the plasma half-life of PFOA. It does not provide an elimination rate. In addition, these data do not provide any information about the distribution of PFOA in the body. Without the individual data or supporting information, the statement that time between retirement and entry into the study does not affect the half-life calculation is highly suspect. One would expect age, length of exposure, and time elapsed since occupational exposure to affect PFOA serum levels. Since these data were not provided in the report and since only 3 data points have been calculated to date, one can only estimate that the half-life of PFOA is between 1 and 3.5 years. These data provide evidence of the potential to bioaccumulate PFOA in humans. In addition, these preliminary data suggest that gender plays a role in the half-life.

### **3.1.2 Absorption Studies in Animals**

APFO is well absorbed following oral and inhalation exposure, and to a lesser extent following dermal exposure. In rats, an average of 749 ug or 37% of the fluorine in the administered dose was recovered in the urine within 4.5 hr after PFOA dose (by stomach intubation 2 ml of an aqueous solution containing 2 mg PFOA) (Ophaug and Singer, 1980). The quantity of nonionic fluorine recovered in the urine increased to 61% of the dose at 8 hr, 76% at 24 hr, and 89% at 96 hr.

After a single oral dose of  $^{14}\text{C}$ -PFOA (mean dose, 11.0 mg/kg) in solution to groups of three male rats, at least 93% of the total carbon-14 was absorbed at 24 hours (Gibson and Johnson, 1979). The half-life for elimination of total carbon-14 from plasma was 4.8 days.

Following APFO head-only inhalation exposure in male rats (6 hr/day, 5 days/wk for 2 wk to 0, 1, 8 or 84 mg/m<sup>3</sup>) concentrations of organofluoride in the blood showed a dose relationship with initial levels of 108 ppm in rats treated at 84 mg/m<sup>3</sup> (Kennedy et al., 1986). Immediately after the tenth exposure period, the mean organofluoride blood levels were 13 ppm, 47 ppm, and 108 ppm in the 1, 8, and 84 mg/m<sup>3</sup> dose groups.

Subchronic dermal APFO treatment in rats and rabbits (10 applications, 5 doses, 2 rest days, 5 doses) with either 0, 20, 200, or 2000 mg/kg resulted in elevated blood organofluorine levels which increased in a dose-related manner (Kennedy, 1985).

O'Malley and Ebbins (1981) conducted a range finding study which indicates significant dermal absorption of PFOA in male and female rabbits. PFOA (100 mg/kg, 1000 mg/kg, and 2000 mg/kg in saline slurry) was applied to approximately 40% of the shaved trunk of the animals, which were then fitted with a plastic collar, and the trunk was wrapped with impervious plastic

sheeting. The exposure period was 24 hr, 5 days/week over 14 days. Mortality was 100% (4/4) in the 2000 mg/kg group, 75% (3/4) in the 1000 mg/kg group and 0% (0/4) in the 100 mg/kg group.

In the past, Chemolite workers have been exposed to large dermal doses of PFOA. It appears that dermal exposure may have played a significant role in the absorption of PFOA in these workers. Upon recognition that PFOA could be absorbed dermally, work practices were changed and engineering controls were adopted that reduced dermal exposures (Gilliland, 1992).

A t-butyl ammonium salt of perfluorooctanoate in the form of treated fabric and as a liquid formulation was applied dermally to rabbits (Johnson, 1995b). Liver samples were analyzed at 28 days post dose for total organic fluorine. The results from treated animals were the same as control values. All total organic values were below the practical quantitation limit. Serum levels were also below the practical quantitation limits of the analysis for samples collected at day 1 and 2 after administration of the mixture or the treated fabric. From the pharmacokinetic study (Johnson, 1995a), it would be unlikely that any extent of absorption could have been detected in this study.

### **3.1.3 Distribution Studies in Animals**

PFOA distributes primarily to the liver, plasma, and kidney, and to a lesser extent, other tissues of the body. It does not partition to the lipid fraction or adipose tissue, but does bind to macromolecules in the tissues. There is evidence of enterohepatic circulation of the compound. Major sex-related differences in the disposition of PFOA have been observed.

Serum and liver concentrations of PFOA were determined in rhesus monkeys in a 90 day oral toxicity study (Griffith and Long, 1980). In monkeys at the 3 mg/kg/day dose, mean serum PFOA was 50 ppm in males and 58 ppm in females. At the same dose, males had 3 ppm and females 7 ppm in liver samples. At 10 mg/kg/day doses, male monkeys had a mean serum PFOA of 63 ppm and females 75 ppm. Liver levels were 9 and 10 ppm for males and females, respectively.

Ophaug and Singer (1980) measured ionic fluoride and total fluorine in the serum of female rats following the administration of PFOA by stomach intubation (2 ml of an aqueous solution containing 2 mg PFOA). Serum from rats 4.5 hr after the administration of PFOA had a nonionic fluorine level 13.6 ppm and virtually all of this was bound to components in the serum and not ultrafilterable. Despite the large increase in nonionic fluorine in the serum, the ionic fluoride level remained very low (0.03 ppm). Prior to intubation of PFOA, the ionic and nonionic fluorine levels in serum were 0.032 and 0.07 ppm, respectively. The nonionic fluorine level in the serum decreased to 11.2 ppm at 8 hr, 0.35 ppm at 24 hr, and 0.08 ppm at 96 hr. The authors conclude that PFOA is rapidly absorbed from the gastrointestinal tract and rapidly cleared from the serum.

Twenty-four hours after oral administration of APFO (2 mg APFO in 2 ml aqueous solution by stomach intubation), female rats had a mean serum nonionic fluorine level of 0.35 ppm, while male rats had a mean serum nonionic fluorine level of 44.0 ppm (Hanhijarvi et al., 1982). APFO was bound to a similar extent in the plasma of male and female rats (97.5% bound).

In male and female rats administered  $^{14}\text{C}$ -PFOA in propylene glycol/water (9.4  $\mu\text{mol/kg}$ , i.p.), the concentration of  $^{14}\text{C}$ -PFOA-derived radioactivity in the blood was higher and eliminated more slowly in males ( $t_{1/2}=9$  days, males vs 4 hr, females, Vanden Heuvel et al., 1991). In the male rats, the liver had the highest PFOA concentration (21% of dose at 2 hr, 2% of dose at 28 days) followed by the plasma and kidney. Far lower PFOA concentrations were found in the heart, testis, fat, and gastrocnemius muscle. In females at 2 hr post dose, the highest concentrations of PFOA were found in the plasma followed by the kidney, liver and ovaries in that order. The average  $t_{1/2}$  for elimination of PFOA from the liver in male rats was 11 days compared to an average of 9 days for extrahepatic tissues. In females, the average  $t_{1/2}$  for tissue elimination was approximately 3 hr.

Vanden Heuvel et al. (1991) investigated the disposition of PFOA in perfused male rat liver. Approximately 11% of the cumulative dose of  $^{14}\text{C}$ -PFOA infused (0.08  $\mu\text{mol/min} \times 48$  min, 3.84  $\mu\text{mol}$  total) was extracted by the liver during a first pass. In addition, the cumulative percent of PFOA extracted by the liver at 2 min (33%) was substantially greater than that seen after 48 min (11%) indicating that first-pass hepatic uptake of PFOA may be saturable.

Ylinen et al. (1990) studied the difference between male and female Wistar rats in the distribution and accumulation of PFOA after a single and subchronic administration. The single dose of PFOA (50 mg/kg in propylene glycol-water mixture, 1:1, vol. 0.25 ml/100g) was administered intraperitoneally to 10 week old rats (20 male, 20 female). Subchronic administration of PFOA consisted of 3, 10, and 30 mg/kg/day by gavage (in 0.9% NaCl, 0.5 ml/100g) to newly weaned rats (18 male, 18 female). After the single dose, samples were collected for PFOA determination 12, 24-168 (at 24 h intervals), 244 and 336 hours after the administration, and in the subchronic test on the 28th day. The serum was collected by cardiac puncture; after decapitation the brain and at necropsy samples from the liver, kidney, lung, spleen, ovary, testis, and adipose tissue were collected and frozen. The biological half-life of PFOA in the serum and tissues was determined from the linear relationship between time and PFOA concentration in the semilogarithmic plot. In the single-dose study, concentration of PFOA in the serum and tissues was higher in males than females at all time periods. Twelve hours after the administration of PFOA about 10% of the dose was found in the serum of females, whereas about 40% was in the serum of males. After 14 days about 3.5% of the dose remained in the serum. In females, PFOA concentration in the serum, liver, and kidney occurred in a discontinuous fashion, indicating distinct phases. The half-life in the serum was 24 and 105 h in the females and males, respectively. In the females, a half-life of 60 h was estimated in the liver during the first week. In the males, the half-life in liver was 210 h. Although PFOA was retained by the liver, it was not found in the lipid fraction. In the kidney, the half-life was 145 h and 130 h in females and males, respectively. In the spleen, the half-life was 73 h and 170 h in

females and males, respectively. PFOA was also found in brain tissue. PFOA was not detectable in adipose tissue. In the subchronic study, samples taken on the 28th day indicated significantly higher PFOA concentrations in the serum and tissues of males versus females in all three dose levels. After subchronic, as well as single-dose administration, PFOA was mainly distributed in the serum of rats. High concentrations of PFOA were also found in the liver, kidney, and lung of males and females. At the high dose level (30 mg/kg/day), females and males exhibited, respectively, serum concentrations of 13.92 and 51.65 ug/ml, liver concentrations of 6.64 and 49.77 ug/g, kidney concentrations of 12.54 and 39.81 ug/g, spleen concentrations of 1.59 and 4.10 ug/g, lung concentrations of 0.75 and 23.71 ug/g, and brain concentrations of 0.044 and 0.710 ug/g. The ovary contained 1.16 ug/g and the testis contained 7.22 ug/g. A significant positive correlation existed between the administered dose and the concentration of PFOA in the liver, kidney, spleen, and lung of females. On the contrary, no significant correlation between the administered dose and the concentration of PFOA was observed in the males, as 10 mg/kg/day produced higher PFOA concentrations in the serum and organs than 30 mg/kg/day. However, in males, the concentration in the spleen, testis, and brain correlated positively with the concentration in the serum.

Vanden Heuvel et al. (1992) demonstrated that PFOA covalently binds to proteins in the liver, plasma, and testes of rats *in vivo*. Carbon-14-labeled PFOA was administered to six-week old male Harlan Sprague-Dawley rats in propylene glycol/water (1:1, v/v; 1 ml/kg) at a dose of 9.4 umol/kg, i.p. No time-dependent changes in either absolute or relative concentrations of covalently bound PFOA-derived <sup>14</sup>C were found at 2 h, 1 and 4 days post-treatment. Covalently bound PFOA was represented by 0.1 to 0.3% of the tissue <sup>14</sup>C content. The absolute concentration of covalently bound PFOA was significantly higher in the plasma than in the liver. The testes had the highest relative concentration of PFOA-derived radioactivity covalently bound. In *in vitro* tests, covalent binding of <sup>14</sup>C-PFOA to a constant concentration of albumin (8 uM) increased in a linear fashion with increasing PFOA concentration. The covalent binding of PFOA to hemoglobin *in vitro* was diminished by the addition of cysteine but not methionine, suggesting that protein sulfhydryl groups may be involved.

Hanhijarvi et al. (1987) compared the disposition of PFOA between male and female Wistar rats during subchronic administration. PFOA was administered by gavage to 48 newly-weaned animals at 0, 3, 10, and 30 mg/kg (in 0.9% NaCl, 0.5ml/100g) for 28 consecutive days. Urine was collected on the 7th and 28th day of the study (discussed below). At the end of the study, blood was collected via cardiac puncture. At each dose level, the mean PFOA concentrations in the plasma of the male rats were significantly higher than those of the female rats. The mean plasma PFOA concentrations for the male rats were 48.6+-26.5 ug/ml (dosed at 3 mg/kg), 83.1+-24.7 ug/ml (10 mg/kg), and 53.4+-11.2 ug/ml (30 mg/kg). The corresponding figures for female rats were 2.43+-5.96 ug/ml, 11.3+-8.59 ug/ml, and 9.06+-8.80 ug/ml in the same order. The PFOA concentrations in the plasma of the male animals suggested that the binding sites of PFOA may become saturated at the chronic daily dose level of 30 mg/kg. Although the plasma PFOA concentrations were significantly higher in the male rats, no significant histopathological differences between the sexes were observed at necropsy.

The disposition of PFOA was studied in male Wistar rats after castration and estradiol administration as well as in intact males and females (Ylinen et al., 1989). The male rats (N=20) were castrated at the age of 28 days and after 5 weeks were used in the tests. Half of the operated and 10 intact males were administered estradiol valerate subcutaneously 500 ug/kg every second day during 14 days before the test. Blood samples were collected by cardiac puncture. At the end of the test (96 hr), the concentration of PFOA in the serum of intact males was considerably higher (17-40 times) than in the serum of other groups. There was no statistically significant difference in the serum concentrations between the other groups. PFOA was similarly bound to the proteins in the serum of males and females.

Johnson et al. (1984) investigated the effect of feeding cholestyramine to rats on the fecal elimination of APFO. Since APFO exists as an anion at physiologic pH, it would be expected to complex with cholestyramine in vivo. Ten Male Charles River CD rats (12 weeks old, 300-342 g) were administered ammonium <sup>14</sup>C-perfluorooctanoate (2.1 mg/ml) dissolved in 0.9% NaCl as a single intravenous dose (2 ml/rat, average APFO dose 13 mg/kg). Five rats were given 4% cholestyramine in feed. Urine and feces samples were collected at intervals for 14 days, at which time the animals were sacrificed and liver samples were collected. At 14 days post dose, the mean percentage of APFO dose eliminated in the feces of cholestyramine-treated rats (43.2±5.5) was 9.8-fold the mean percentage of dose eliminated in feces by untreated rats (4.4±1.0). Excretion in urine was 41% for treated rats and 67% for untreated rats. Carbon-14 present in the liver represented 12.1±2.1 ug eq/g and 22.3±6.2 ug eq/g in treated and untreated rats, respectively (4% and 8% of dose, respectively). In plasma, the levels were 5.1±1.7 ug eq/ml and 14.7±6.8 ug eq/ml in treated and untreated rats, respectively. In red blood cells, the levels were 1.8±0.7 ug eq/ml and 4.2±2.4 ug eq/ml in treated and untreated rats, respectively. The high concentration of <sup>14</sup>C-APFO in liver at 2 weeks after dosing and the fact that cholestyramine treatment enhances fecal elimination of carbon-14 nearly 10-fold suggests that there is enterohepatic circulation of APFO.

The disposition of PFOA (tetrabutyl ammonium salt perfluorooctanoic acid) in female rabbits has been reported (Johnson, 1995a). Individual rabbits were given intravenous doses at 0, 4, 16, and 24 mg/kg and appeared normal throughout the study (the animal treated at the 40 mg/kg dose level died within 5 minutes of dosing). Serum samples were analyzed for total organic fluorine at 2, 4, 6, 8, 12, 24, and 48 hours post dose. At 2 hrs, serum organic fluorine levels in the 0, 4, 16, and 24 mg/kg dosed rabbits were 1.25 ppm, 4.09 ppm, 14.9 ppm, and 41.0 ppm, respectively. There was a rapid decrease in serum level of total organic fluorine with time, non-detectable at 48 hr. The biological half-life was on the order of 4 hours. The total organic fluorine in whole liver at 48 hr post dose for control animals, 4 mg/kg, 16 mg/kg, and 24 mg/kg intravenous doses were 20 ug, 43 ug, 66 ug, and 54 ug.

### 3.1.4 Metabolism Studies in Animals

Vanden Heuvel et al. (1991) investigated the in vivo metabolism of PFOA in rats administered <sup>14</sup>C-PFOA (9.4 umol/kg, i.p.). Pooled daily urine samples (0-4 days post-treatment) and bile

extracts analyzed by HPLC contained a single radioactive peak eluting identically to the parent compound. Tissues were taken from rats treated 4, 14, and 28 days previously with  $^{14}\text{C}$ -PFOA to determine the presence of PFOA-containing lipid conjugates. Only the parent compound was present in rat tissues; no PFOA-containing hybrid lipids were detected. Fluoride concentrations in plasma and urine before and after PFOA treatment were unchanged, indicating that PFOA does not undergo defluorination *in vivo*.

Ophaug and Singer (1980) also found no change in ionic fluoride level in the serum or urine following oral administration of PFOA to female rats. Ylinen et al. (1989) found no evidence of phase II metabolism of PFOA following a single intraperitoneal PFOA dose (50 mg/kg) in male and female rats.

### 3.1.5 Elimination Studies in Animals

The urine is the major route of excretion of PFOA in the female rat, while the urine and feces are both major routes of excretion of PFOA in male rats (Vanden Heuvel et al., 1991). Male and female rats were administered  $^{14}\text{C}$ -PFOA in propylene glycol/water (9.4  $\mu\text{mol/kg}$ , i.p.). Female rats eliminated PFOA-derived radioactivity rapidly in the urine with 91% of the dose being excreted in the first 24 hr, while male rats excreted only 6% of the dose in that time period. Negligible radioactivity was recovered in the feces of female rats. In male rats during the 28-day collection period the cumulative excretion of PFOA-derived  $^{14}\text{C}$  in urine and feces was 36.4% and 35.1%, respectively. The female rat retained less than 10% of the administered dose after 24 hr, while the male rats retained 30% of the administered dose after 28 days. The whole-body elimination half-life in females was less than one day, and in males it was 15 days. In renal-ligated rats injected i.p. with  $^{14}\text{C}$ -PFOA, approximately 0.3% of the PFOA-derived radioactivity was excreted in the bile after 6 hr (Vanden Heuvel et al., 1991). No sex-related difference in the biliary excretion of PFOA was observed when the kidneys were ligated.

Johnson and Gibson (1980) observed a sex difference in extent and rate of excretion of total carbon-14 between male and female rats after a single iv dose (mean dose: female, 16.7 mg/kg; male 13.1 mg/kg) of  $^{14}\text{C}$ -PFOA. Female rats excreted essentially all of the dose via urine in 24 hours while at the same time period male rats excreted only 20 percent of the dose; male rats excreted 83% via urine and 5.4% via feces by 36 days post dose. No radioactivity was detected in tissues of female rats at 17 days post dose; male rats had 2.8% of the dose in liver and 1.1% in plasma at 36 days post dose with lower levels (< 0.5% of the dose) in other organs.

Ophaug and Singer (1980) investigated the metabolic fate of PFOA in female Holtzman rats. Animals weighing approximately 250 g were administered by stomach intubation 2 ml of an aqueous solution containing 2 mg PFOA. The animals were then placed in metabolism cages and provided rat chow and tap water for 4.5, 8, 24, or 52.5 hr. In addition, four rats were placed in metabolism cages and fed a low fluoride (<0.5 ppm) diet and distilled water for a period of 96 hr. At the end of the experimental period the urine, feces and serum were collected. Within 4.5 hr after PFOA dose, an average of 749  $\mu\text{g}$  or 37% of the fluorine in the administered dose was

recovered in the urine. The quantity of nonionic fluorine recovered in the urine increased to 61% of the dose at 8 hr, 76% at 24 hr, and 89% at 96 hr. Urinary excretion of ionic fluoride in the PFOA dosed animals was not significantly different than that of the control animals. Fecal excretion of nonionic fluorine was 4.5% of the administered dose at 52.5 hr and 14.3% at 96 hr. The urine from undosed animals contained no detectable nonionic fluorine.

The urinary excretion of APFO in rats was investigated by Hanhijarvi et al. (1982). Four male and six female Holtzman rats were administered 2 mg APFO in 2 ml aqueous solution by stomach intubation. Seven female rats were administered 2 ml distilled water as controls. The animals were then placed in metabolism cages with rat chow and tap water. Urine was collected until animals were sacrificed at 24 h by cardiac puncture. Serum was collected. Ionic fluoride and total fluorine content of serum and urine was determined, and nonionic fluorine was calculated as the difference. For clearance studies of APFO and inulin, the rats were anesthetized with Inactin. The femoral artery was cannulated for continuous infusion of 5% mannitol in isotonic saline and the femoral artery was cannulated for drawing blood samples. The urinary bladder was also cannulated for serial collections of urine. Intravenous priming doses of 5.2-5.6 mg [1-14C] ammonium perfluorooctanoate (sp act 0.5 uCi/mg) and 8.8 ug tritiated inulin (methoxy-3H, sp act 114 uCi/mg) were given to each animal. The radiolabeled inulin and APFO in 5% mannitol in isotonic saline was then infused at a rate of 0.21 ml/min. An additional 0.42-0.63 mg/hr 14C-APFO and 9.6 ug/hr tritiated inulin was infused during the experiments. When the urine and serum collections for the clearance study were complete, probenecid was administered (65-68 mg/kg, ip) and additional clearance tests were performed. In the cumulative excretion study, rats were dosed iv with a mixture of radiolabeled APFO (10-20%) and unlabeled APFO (80-90%). Five percent mannitol in isotonic saline was infused at a rate of 0.081 ml/min and urine specimens were collected over 30-min intervals. The effect of probenecid was assessed by administering 65-68 mg/kg ip at least 30 min prior to the administration of APFO. Twenty-four hours after oral administration of APFO, female rats had excreted 76 $\pm$ 2.7% of the dose in the urine and had a mean serum nonionic fluorine level of 0.35 $\pm$ 0.11 ppm, while male rats had excreted only 9.2 $\pm$ 3.5% of the dose and had a mean serum nonionic fluorine level of 44.0 $\pm$ 1.7 ppm. APFO was bound to a similar extent in the plasma of male and female rats (97.5 $\pm$ 0.25% bound). The clearance studies demonstrated major differences between the sexes in rats. The APFO clearance in female rats was several times greater than the inulin clearance. Administration of probenecid, which strongly inhibits the renal active secretion of organic acids, reduced APFO/inulin clearance ratio in females from 14.5 to 0.46. APFO clearance was reduced from 5.8 to 0.11 ml/min/100g. Net APFO excretion was reduced from 4.6 to 0.13 ug/min/100g. In male rats, however, the APFO/inulin clearance ratio and the net excretion of APFO were virtually unaffected by probenecid. In the males, APFO clearance was 0.17 ml/min/100g, APFO/inulin clearance ratio was 0.22, and net APFO excretion was 0.17 ug/min/mg. In the cumulative excretion studies, female rats excreted 76% of the APFO dose, while males excreted only 7.8% of the dose over a 7-hr period. Probenecid administration modified the cumulative excretion curve for males only slightly. However, in females probenecid markedly reduced PFO elimination to 11.8%. It is concluded that the female rat possesses an active secretory mechanism which rapidly eliminates APFO from the body. This

secretory mechanism is lacking or is relatively inactive in male rats and accounts for the greater toxicity of APFO in male rats.

Hanhijarvi et al. (1987) compared the urinary elimination of PFOA between male and female Wistar rats during subchronic administration. PFOA was administered by gavage to 48 newly-weaned animals at 0, 3, 10, and 30 mg/kg (in 0.9% NaCl, 0.5ml/100g) for 28 consecutive days. Urine was collected on the 7th and 28th day of the study. At the end of the study, blood was collected via cardiac puncture. At necropsy, tissue specimens for histopathologic examination were collected from the controls and from the group receiving 30 mg/kg/day PFOA. On the seventh day of the study period, the female rats in lowest dose group (3 mg/kg/day) exhibited significantly greater urinary PFOA excretion than the males ( $3.12 \pm 0.30$  vs  $1.50 \pm 0.57$  mg/24hr/kg). Unlike the female rats, on the 7th day of the study all three groups of male rats excreted significantly less PFOA than their daily dose of PFOA, which suggested that the males had not reached a steady state by seven days. On the 28th day, the males excreted an amount of PFOA equal to their daily dose.

Hanhijarvi et al. (1988) investigated the excretion kinetics of PFOA in the beagle dog. Six laboratory bred beagle dogs (3 male, 3 female) were anesthetized with methoxyflurane and catheters were placed in both ureters after laparotomy and cystotomy. The animals were given an intravenous dose of 30 mg/kg of PFOA followed by continuous infusion with 5% mannitol solution at 1.7 ml/min. Urine was collected at 10 minute intervals for 60 min. A 5 ml blood sample was collected in the middle of each urine sampling period. Probenecid (30 mg/kg i.v.) was then administered, and urine and blood samples were again collected as before. Renal clearance of PFOA was calculated for the before and after probenecid injection periods. Four additional dogs (2male, 2 female) were given 30 mg/kg PFOA intravenously. These dogs were kept in metabolism cages, and blood samples were collected intermittently for 30 days. From these dogs, plasma PFOA half-lives were determined. There was no difference between the renal clearances of the male and female dogs either before or after probenecid. Renal clearance rate was approximately 0.03 ml/min/kg. Probenecid significantly reduced the PFOA clearance in both sexes, indicating an active secretion mechanism for PFOA. The plasma half-lives of PFOA were longer in the male dogs (473 h and 541 h) than in the female dogs (202 h and 305 h).

The urinary excretion of PFOA was studied in male Wistar rats after castration and estradiol administration as well as in intact males and females (Ylinen et al., 1989). The male rats (N=20) were castrated at the age of 28 days and after 5 weeks were used in the tests. Half of the operated and 10 intact males were administered estradiol valerate subcutaneously 500 ug/kg every second day during 14 days before the test. Urine was collected in metabolism cages during 96 hr after a single intraperitoneal PFOA dose (50 mg/kg). Blood samples were collected by cardiac puncture. Castration and administration of estradiol to the male rats had a significant stimulatory effect on the urinary excretion of PFOA. During the first 24 hours, female rats excreted  $72 \pm 5\%$  (N=6) of the dose, whereas the intact males excreted only  $9 \pm 4\%$  (N=6). After the estradiol treatment, both the intact and castrated males excreted PFOA in amounts similar to females ( $61 \pm 19\%$  and  $68 \pm 14\%$ , respectively). The castrated males without

estradiol treatment excreted PFOA in urine faster than the intact males (50+-13%), but less than the females and the estrogen treated males. At the end of the test (96 hr), the concentration of PFOA in the serum of intact males was considerably higher (17-40 times) than in the serum of other groups. There was no statistically significant difference in the serum concentrations between the other groups. PFOA was similarly bound by the proteins in the serum of males and females.

Vanden Heuvel et al. (1992a) investigated whether androgens or estrogens are involved in the marked sex-differences in the urinary excretion of PFOA. Castration of males greatly increased (> 1-fold) the elimination of <sup>14</sup>C-PFOA (9.4 umol/kg, i.p.) in urine, demonstrating that a factor produced by the testis is responsible for the slow elimination of PFOA in male rats. Castration plus 17B-estradiol had no further effect on PFOA elimination whereas castration plus testosterone replacement at the physiological level reduced PFOA elimination to the same level as rats with intact testis. Thus, in male rats, testosterone exerts an inhibitory effect on renal excretion of PFOA. In female rats, neither ovariectomy nor ovariectomy plus testosterone affected the urinary excretion of PFOA, demonstrating that the inhibitory effect of testosterone on PFOA renal excretion is a male-specific response. Probenecid, which inhibits the renal transport system, decreased the high rate of PFOA renal excretion in castrated males but had no effect on male rats with intact testis.

Hormonal changes during pregnancy do not appear to cause a change in the rate of elimination of carbon-14 after oral administration of a single dose of ammonium <sup>14</sup>C-PFOA (Gibson and Johnson, 1983). At 8 or 9 days after conception, four pregnant rats and 2 nonpregnant female rats were dosed (mean dose, 15 mg/kg) and individual urine samples were collected at 12, 24, 36, and 48 hours post dose and analyzed for carbon-14 content. Essentially all of the carbon-14 was eliminated via urine within 24 hours for both groups of rats.

Feeding of cholestyramine to rats enhanced the fecal elimination of APFO (Johnson et al. (1984). Male rats were administered ammonium [14]perfluorooctanoate (2.1 mg/ml) dissolved in 0.9% NaCl as a single intravenous dose (2 ml/rat, average APFO dose 13 mg/kg). At 14 days post dose, the mean percentage of APFO dose eliminated in the feces of cholestyramine-treated rats (43.2+-5.5) was 9.8-fold the mean percentage of dose eliminated in feces by untreated rats (4.4+-1.0). Excretion in urine was 41% for treated rats and 67% for untreated rats.

## **3.2 Epidemiology Studies**

### **3.2.1 Mortality Study**

A retrospective cohort mortality study was performed on employees at the Cottage Grove, Minnesota plant which produces APFO (Gilliland and Mandel, 1993). At this plant, APFO production was limited to the Chemical Division. The cohort consisted of workers who had been employed at the plant for at least 6 months between January 1947 and December 1983. Death certificates of all of the workers were obtained to determine cause of death. There was almost

complete follow-up (99.5%) of all of the study participants. The exposure status of the workers was categorized based on their job histories. If they had been employed for at least 1 month in the Chemical Division, they were considered exposed. All others were considered to be not exposed to PFOA. The number of months employed in the Chemical Division provided the cumulative exposure measurements. Of the 3537 (2788 men and 749 women) employees who participated in this study, 398 (348 men and 50 women) were deceased. Eleven of the 50 women and 148 of the 348 men worked in the Chemical Division, and therefore, were considered exposed to PFOA.

Standardized Mortality Ratios (SMRs), adjusted for age, sex, and race were calculated and compared to U.S. and Minnesota white death rates for men. For women, only state rates were available. The SMRs for males were stratified for 3 latency periods (10, 15, and 20 years) and 3 periods of duration of employment (5, 10, and 20 years).

For all female employees, the SMRs for all causes and for all cancers were less than 1. The only elevated (although not significant) SMR was for lymphopoietic cancer, and was based on only 3 deaths. When exposure status was considered, SMRs for all causes of death and for all cancers were significantly lower than expected, based on the U.S. rates, for both the Chemical Division workers and the other employees of the plant.

In all male workers at the plant, the SMRs were close to 1 for most of the causes of death when compared to both the U.S. and the Minnesota death rates. When latency and duration of employment were considered, there were no elevated SMRs. When employee deaths in the Chemical Division were compared to Minnesota death rates, the SMR for prostate cancer for workers in the Chemical Division was 2.03 (95% CI .55 - 4.59). This was based on 4 deaths (1.97 expected). There was also a statistically significant association with length of employment in the Chemical Division and prostate cancer mortality. Based on the results of proportional hazard models, the relative risk for a 1-year increase in employment in the Chemical Division was 1.13 (95% CI 1.01 to 1.27). It rose to 3.3 (95% CI 1.02 -10.6) for workers employed in the Chemical Division for 10 years when compared to the other employees in the plant. The SMR for workers not employed in the Chemical Division was less than expected for prostate cancer (.58).

An update of this study was conducted to include the death experience of employees through 1997 (Alexander, 2001a). The cohort consisted of 3992 workers. The eligibility requirement was increased to 1 year of employment at the Cottage Grove plant, and the exposure categories were changed to be more specific. Workers were placed into 3 exposure groups based on job history information: definite PFOA exposure (n = 492, jobs where cell generation, drying, shipping and packaging of PFOA occurred throughout the history of the plant); probable PFOA exposure (n = 1685, other chemical division jobs where exposure to PFOA was possible but with lower or transient exposures); and not exposed to fluorochemicals (n = 1815, primarily non-chemical division jobs).

In this new cohort, 607 deaths were identified: 46 of these deaths were in the PFOA exposure group, 267 in the probable exposure group, and 294 in the non-exposed group. When all employees were compared to the state mortality rates, SMRs were less than 1 or only slightly higher for all of the causes of death analyzed. None of the SMRs were statistically significant at  $p = .05$ . The highest SMR reported was for bladder cancer (SMR = 1.31, 95% CI = 0.42 – 3.05). Five deaths were observed (3.83 expected).

A few SMRs were elevated for employees in the definite PFOA exposure group: 2 deaths from cancer of the large intestine (SMR = 1.67), 1 from pancreatic cancer (SMR = 1.34), and 1 from prostate cancer (SMR = 1.30). In addition, employees in the definite PFOA exposure group were 2.5 times more likely to die from cerebrovascular disease (5 deaths observed, 1.94 expected; 95% CI = 0.84 – 6.03).

In the probable exposure group, 3 SMRs should be noted: cancer of the testis and other male genital organs (SMR = 2.75, 95% CI = 0.07 – 15.3); pancreatic cancer (SMR = 1.24, 95% CI = 0.45 – 2.70); and malignant melanoma of the skin (SMR = 1.42, 95% CI = 0.17 – 5.11). Only 1, 6, and 2 cases were observed, respectively. The SMR for prostate cancer in this group was 0.86 ( $n = 5$ ).

There were no notable excesses in SMRs in the non-exposed group, except for cancer of the bladder and other urinary organs. Four cases were observed and only 1.89 were expected (95% CI = 0.58 – 5.40).

It is difficult to interpret the results of the prostate cancer deaths between the first study and the update because the exposure categories were modified in the update. Only 1 death was reported in the definite exposure group and 5 were observed in the probable exposure group. All of these deaths would have been placed in the chemical plant employees exposure group in the first study. The number of years that these employees worked at the plant and/or were exposed to PFOA was not reported. This is important because even 1 prostate cancer death in the definite PFOA exposure group resulted in an elevated SMR for the group. Therefore, if any of the employees' exposures were misclassified, the results of the analysis could be altered significantly.

The excess mortality in cerebrovascular disease noted in employees in the definite exposure group was further analyzed based on number of years of employment at the plant. Three of the 5 deaths occurred in workers who were employed in jobs with definite PFOA exposure for more than 5 years but < 10 years (SMR = 15.03, 95% CI = 3.02 – 43.91). The other 2 occurred in employees with less than 1 year of definite exposure. The SMR was 6.9 (95% CI = 1.39 – 20.24) for employees with greater than 5 years of definite PFOA exposure. In order to confirm that the results regarding cerebrovascular disease were not an artifact of death certificate coding, regional mortality rates were used for the reference population. The results did not change. When these deaths were further analyzed by cumulative exposure (time-weighted according to exposure category), workers with 27 years of exposure in probable PFOA exposed jobs or those with 9 years of definite PFOA exposure were 3.3 times more likely to die of cerebrovascular

disease than the general population. A dose-response relationship was not observed with years of exposure.

The slight excess in bladder cancer in the cohort as a whole should be noted, especially given the bladder cancer mortality experience at 3M's Decatur plant, which produces mostly PFOS. Bladder cancer mortality was 4 times higher in workers with high PFOS exposure jobs at 3M's Decatur, Alabama plant than the general population (SMR = 4.81, 95% CI = 0.99 – 14.06) (Alexander, 2001b). Three deaths were reported, and all of them occurred in the high exposure group. Serum PFOA levels in workers are lower at the Decatur plant, where PFOA is used as an elastomer in fluoropolymer production or is produced as a by-product, than at Cottage Grove; however, the manufacture of PFOA began at Decatur in 1999. Therefore, PFOA exposures will likely increase at this plant. It is not clear whether PFOA, PFOS or some other chemical may be responsible for the bladder cancer deaths observed at these plants; however, follow up should continue in an effort to shed some light on this observation.

It is difficult to compare the results of the first and second mortality studies at the Cottage Grove plant since the exposure categories were modified. Although the authors claim that the newer exposure categories are more accurate, it is still likely that exposure misclassification occurred. Without measured exposures (serum PFOA levels), it is difficult to judge the reliability of the exposure categories that were defined. In the second study, the chemical plant employees were sub-divided into PFOA-exposed groups, and the film plant employees essentially remained in the "non-exposed" group. This was an effort to more accurately classify exposures; however, these new categories do not take into account duration of exposure or length of employment. Another limitation to this study is that 17 death certificates were not located for deceased employees and therefore were not included in the study. The inclusion or exclusion of these deaths could greatly change the analyses for the causes of death that had a small number of cases. Follow up of worker mortality at Cottage Grove (and Decatur) needs to continue. Although there were more than 200 additional deaths included in this analysis, it is a small number and the cohort is still relatively young. Given the results of studies on fluorochemicals in both animals and humans, further analysis is warranted.

### **3.2.2 Hormone Study**

Endocrine effects have been associated with PFOA exposure in animals; therefore, 2 cross-sectional studies were conducted on employees of a plant producing PFOA (Olsen, et al., 1998a). Medical surveillance, hormone testing and PFOA serum levels were obtained for volunteer workers in 1993 (n = 111) and 1995 (n = 80). Sixty-eight employees were common to both sampling periods. In 1993, the range of PFOA was 0-80 ppm (although 80 ppm was the limit of detection that year, so it could have been higher) and 0-115 ppm in 1995 using thermospray mass spectrophotometry assay. Eleven hormones were assayed from the serum samples. They were: cortisol, dehydroepiandrosterone sulfate (DHEAS), estradiol, FSH, 17 gamma-hydroxyprogesterone (17-HP), free testosterone, total testosterone, LH, prolactin, thyroid-stimulating hormone (TSH) and sex hormone-binding globulin (SHBG).

Employees were placed into 4 exposure categories based on their serum PFOA levels: 0-1 ppm, 1- < 10 ppm, 10- < 30 ppm, and >30 ppm. Statistical methods used to compare PFOA levels and hormone values included: multivariable regression analysis, ANOVA, and Pearson correlation coefficients.

PFOA was not highly correlated with any of the hormones or with the following covariates: age, alcohol consumption, BMI, or cigarettes. Most of the employees had PFOA serum levels less than 10 ppm. In 1993, only 12 employees had serum levels > 10 ppm, and 15 in 1995. However, these levels ranged from approximately 10 ppm to over 114 ppm. There were only 4 employees in the >30 ppm PFOA group in 1993 and only 5 in 1995. Therefore, it is likely that there was not enough power to detect differences in either of the highest categories. The mean age of the employees in the highest exposure category was the lowest in both 1993 and 1995 (33.3 years and 38.2 years, respectively). Although not significantly different from the other categories, BMI was slightly higher in the highest PFOA category.

Estradiol was highly correlated with BMI ( $r = .41$ ,  $p < .001$  in 1993, and  $r = .30$ ,  $p < .01$  in 1995). In 1995, all 5 employees with PFOA levels > 30 ppm had BMIs > 28, although this effect was not observed in 1993. Estradiol levels in the >30 ppm group in both years were 10% higher than the other PFOA groups; however, the difference was not statistically significant. The authors postulate that the study may not have been sensitive enough to detect an association between PFOA and estradiol because measured serum PFOA levels were likely below the observable effect levels suggested in animal studies (55 ppm PFOA in the CD rat). Only 3 employees in this study had PFOA serum levels this high. They also suggest that the higher estradiol levels in the highest exposure category could suggest a threshold relationship between PFOA and estradiol.

Free testosterone was highly correlated with age in both 1993 and 1995. The authors did not report a negative association between PFOA serum levels and testosterone. There were no statistically significant trends noted for PFOA and either bound or free testosterone. However, 17-HP, a precursor of testosterone, was highest in the >30 ppm PFOA group in both 1993 and 1995. In 1995, PFOA was significantly associated with 17-HP in regression models adjusted for possible confounders. However, the authors state that this association was based on the results of one employee (data were not provided in the report). There were no significant associations between PFOA and cortisol, DHEAS, FSH, LH, and SHBG.

There are several design issues that should be noted when evaluating the results of this study. First, although there were 2 study years (1993 and 1995), the populations were not independent. Sixty-eight employees participated in both years. Second, there were 31 fewer employees who participated in the study in 1995, thus reducing the power of the study. There were also very few employees in either year with serum PFOA levels greater than 10 ppm. Third, the cross-sectional design of the study does not allow for analysis of temporality of an association. Since the half-life of PFOA is at least 1 year, the authors suggest that it is possible that there may be some biological accommodation to the effects of PFOA. Fourth, only one sample was taken for

each hormone for each of the study years. In order to get more accurate measurements for some of the hormones, pooled blood taken in a short time period should have been used for each participant. Fifth, some of the associations that were measured in this study were done based on the results of an earlier paper that linked PFOA with increased estradiol and decreased testosterone levels. However, total serum organic fluorine was measured in that study instead of PFOA, making it difficult to compare the results. Finally, there may have been some measurement error of some of the confounding variables.

In 1997, voluntary medical surveillance was again offered to employees (Olsen, et al., 1998b). In this sampling period, cholecystokinin (CCK) levels were analyzed in 74 employees to determine if they were positively associated with serum PFOA levels. CCK levels were observed because research has suggested that pancreas acinar cell adenomas seen in rats exposed to PFOA may be the result of increased CCK levels. Seventeen of the subjects were common to all three sampling periods (1993, 1995, and 1997). The same statistical methods were used in this study period as used in 1993 and 1995, and the four PFOA exposure categories were also the same.

The mean PFOA serum level in employees participating in the 1997 study period was 6.4 ppm (range 0.1 - 81.3 ppm). The mean CCK value was 28.5 pg/ml (range 8.8 - 86.7 pg/ml). The highest CCK values were reported in the 2 exposure categories less than 10 ppm. The means were 50% higher in these 2 categories than in the categories greater than 10 ppm ( $p = .06$ ). When adjusted for potential confounders, multivariable regression models indicated a weak negative association between CCK and PFOA; however, the data were not included in the report.

The following explanations may indicate why this study failed to find a positive association between PFOA and CCK values:

- It is possible that the hepatocarcinogenic effects of peroxisome proliferators in rodents do not act the same biochemically in humans.
- The serum PFOA levels observed in workers may have been too low to detect an effect. Effects in animals were observed at higher doses than most of the serum levels found in workers.
- CCK receptors may be different between rats and humans. Therefore, the monkey may be a more appropriate animal model to study the pancreatic effects of PFOA in humans.
- The involvement of CCK in the initiation or promotion of pancreatic cancer is controversial.
- The rat may not be an appropriate model in the study of pancreatic cancer in humans, since acinar cell malignancies, induced by carcinogens in rats, are rare in humans.

The same methodological issues that applied to the study in 1993 and 1995 apply to this portion of the study as well.

### 3.2.3 Cholesterol Study

Based on animal testing which reported that animals exposed to PFOA develop hepatomegaly and alterations in lipid metabolism, a cross-sectional, occupational study was performed to determine if similar effects are present in workers exposed to PFOA. In a PFOA production facility, 115 workers were studied to determine whether serum PFOA affected their cholesterol, lipoproteins, and hepatic enzymes (Gilliland and Mandel, 1996). Forty-eight workers who were exposed to PFOA from 1985-1989 were included in the study (96% participation rate). Sixty-five employees who either volunteered or were asked to participate, were included in the unexposed group. These employees were assumed to have little or no PFOA exposure based on their job description. However, when serum levels were analyzed, it was noted that this group of workers had PFOA levels much greater than the general population. Therefore, instead of job categories, total serum fluorine was used to classify workers into exposure groups.

Total serum fluorine was used as a surrogate measure for PFOA. Serum PFOA was not measured, due to the cost of analyzing the samples. Blood samples were analyzed for total serum fluorine, serum glutamyl oxaloacetic transaminase (SGOT), serum glutamyl pyruvic transaminase (SGPT), gamma glutamyl transferase (GGT), cholesterol, low-density lipoproteins (LDL), and high-density lipoproteins (HDL). All of the participants were placed into five categories of total serum fluorine levels: <1 ppm, 1-3 ppm, >3 - 10 ppm, >10 - 15 ppm, and > 15 ppm. The range of the serum fluorine values was 0 to 26 ppm (mean 3.3 ppm). Approximately half of the workers fell into the > 1 - 3 ppm category, while 23 had serum levels < 1 ppm and 11 had levels > 10 ppm.

There were no significant differences between exposure categories when analyzed using univariate analyses for cholesterol, LDL, and HDL. In the multivariate analysis, there was not a significant association between total serum fluorine and cholesterol or LDL after adjusting for alcohol consumption, age, BMI, and cigarette smoking. There were no statistically significant differences among the exposure categories of total serum fluorine for SGOT, SGPT, and GGT. However, increases in SGOT and SGPT occurred with increasing total serum fluorine levels in obese workers (BMI = 35 kg/m<sup>2</sup>).

Since PFOA was not measured directly and there is no exposure information provided on the employees (eg. length of employment/exposure), the results of the study provide limited information. The authors state that no adverse clinical outcomes related to PFOA exposure have been observed in these employees; however, it is not clear that there has been follow-up of former employees. In addition, the range of results reported for the liver enzymes were fairly wide for many of the exposure categories, indicating variability in the results. Given that only one sample was taken from each employee, this is not surprising. It would be much more helpful to have several samples taken over time to ensure their reliability. It also would have been interesting to compare the results of the workers who were known to be exposed to PFOA to those who were originally thought not to be exposed to see if there were any differences among

the employees in these groups. There were more of the “unexposed” employees (n = 65) participating in the study than those who worked in PFOA production (n = 48).

### **3.2.4 Study on Episodes of Care (Morbidity)**

In order to gain additional insight into the effects of fluorochemical exposure on workers’ health, an “episode of care” analysis was undertaken at the Decatur plant to screen for morbidity outcomes that may be associated with long-term, high exposure to fluorochemicals. An “episode of care” is a series of health care services provided from the start of a particular disease or condition until solution or resolution of that problem. Episodes of care were identified in employees’ health claims records using Clinical Care Groups (CCG) software. All inpatient and outpatient visits to health care providers, procedures, ancillary services and prescription drugs used in the diagnosis, treatment, and management of over 400 diseases or conditions were tracked.

Episodes of care were analyzed for 652 chemical employees and 659 film plant employees who worked at the Decatur plant for at least 1 year between January 1, 1993 and December 31, 1998.

Based on work history records, employees were placed into different comparison groups: Group A consisted of all film and chemical plant workers; Group B had employees who only worked in either the film or chemical plant; Group C consisted of employees who worked in jobs with high POSF exposures; and Group D had employees who worked in high exposures in the chemical plant for 10 years or more prior to the onset of the study. Film plant employees were considered to have little or no fluorochemical exposure, while chemical plant employees were assumed to have the highest exposures.

Ratios of observed to expected episodes of care were calculated for each plant. Expected numbers were based on 3M’s employee population experience using indirect standardization techniques. A ratio of the chemical plant’s observed to expected experience divided by the film plant’s observed to expected experience was calculated to provide a relative risk ratio for each episode of care (RREpC). 95% confidence intervals were calculated for each RREpC. Episodes of care that were of greatest interest were those which had been reported in animal or epidemiologic literature on PFOS and PFOA: liver and bladder cancer, endocrine disorders involving the thyroid gland and lipid metabolism, disorders of the liver and biliary tract, and reproductive disorders.

The only increased risk of episodes for these conditions of a priori interest were for neoplasms of the male reproductive system and for the overall category of cancers and benign growths (which included cancer of the male reproductive system). There was an increased risk of episodes for the overall cancer category for all 4 comparison groups. The risk ratio was greatest in the group of employees with the highest and longest exposures to fluorochemicals (RREpC = 1.6, 95% CI = 1.2 – 2.1). Increased risk of episodes in long-time, high-exposure employees also was reported for male reproductive cancers (RREpC = 9.7, 95% CI = 1.1 - 458). It should be noted that the confidence interval is very wide for male reproductive cancers and the sub-category of prostate

cancer. Five episodes of care were observed for reproductive cancers in chemical plant employees (1.8 expected), of which 4 were prostate cancers. One episode of prostate cancer was observed in film plant employees (3.4 expected). This finding should be noted because an excess in prostate cancer mortality was observed in the Cottage Grove plant mortality study when there were only 2 exposure categories (chemical plant employees and film plant employees). The update of the study sub-divided the chemical plant employees and did not confirm this finding when exposures were divided into definitely exposed and probably exposed employees.

There was an increased risk of episodes for neoplasms of the gastrointestinal tract in the high exposure group (RREpC = 1.8, 95% CI = 1.2- 3.0) and the long-term employment, high exposure group (RREpC = 2.9, 95% CI = 1.7 – 5.2). Most of the episodes were attributable to benign colonic polyps. Similar numbers of episodes were reported in film and chemical plant employees.

In the entire cohort, only 1 episode of care was reported for liver cancer (0.6 expected) and 1 for bladder cancer (1.5 expected). Both occurred in film plant employees. Only 2 cases of cirrhosis of the liver were observed (0.9 expected), both in the chemical plant. There was a greater risk of lower urinary tract infections in chemical plant employees, but they were mostly due to recurring episodes of care by the same employees. It is difficult to draw any conclusions about these observations, given the small number of episodes reported.

Chemical plant employees in the high exposure, long-term employment group were 2 ½ times more likely to seek care for disorders of the biliary tract than their counterparts in the film plant (RREpC = 2.6, 95% CI = 1.2 - 5.5). Eighteen episodes of care were observed in chemical plant employees and 14 in film plant workers. The sub-categories that influenced this observation were episodes of cholelithiasis with acute cholecystitis and cholelithiasis with chronic or unspecified cholecystitis. Most of the observed cases occurred in chemical plant employees.

Risk ratios of episodes of care for endocrine disorders, which included sub-categories of thyroid disease, diabetes, hyperlipidemia, and other endocrine or nutritional disorders, were not elevated in the comparison groups. Conditions which were not identified a priori but which excluded the null hypothesis in the 95% confidence interval for the high exposure, long-term employment group included: disorders of the pancreas, cystitis, and lower urinary tract infections.

The results of this study only should be used for hypothesis generation. Although the episode of care design allowed for a direct comparison of workers with similar demographics but different exposures, there are many limitations to this design. The limitations include: 1) episodes of care are reported, not disease incidence, 2) the data are difficult to interpret because a large RREpC may not necessarily indicate high risk of incidence of disease, 3) many of the risk ratios for episodes of care had very wide confidence intervals, thereby providing unstable results, 4) the analysis was limited to 6 years, 5) the utilization of health care services may reflect local medical practice patterns, 6) individuals may be counted more than once in the database because they can

be categorized under larger or smaller disease classifications, 7) episodes of care may include the same individual several times, 8) not all employees were included in the database, such as those on long-term disability 9) the analysis may be limited by the software used, which may misclassify episodes of care, 10) the software may assign 2 different diagnoses to the same episode, and 11) certain services, such as lab procedures may not have been reported in the database.

### **3.3 Acute Toxicity Studies in Animals**

#### **3.3.1 Oral Studies**

The acute oral toxicity of APFO was tested in male and female rats in three studies. Death occurred at concentrations  $\geq 464$  mg/kg (Internat'l Res and Dev Corp., 1978). Abnormal findings upon necropsy (kidney, stomach, uterus) were observed (Glaza, 1997) at 500 mg/kg (higher concentrations were not tested). Clinical signs of toxicity observed in these three studies included the following: red-stained face, stained urogenital area, wet urogenital area, hypoactivity, hunched posture, staggered gait, excessive salivation, ptosis, piloerection, decreased limb tone, ataxia, corneal opacity, and hypothermic to touch.

In one study (Internat'l Res and Dev Corp., 1978), the oral LD50 values for Charles River CD rats were 680 mg/kg (399 – 1157 mg/kg 95% confidence limit) for males; 430 mg/kg (295 – 626 mg/kg 95% confidence limit) for females; and 540 mg/kg (389 – 749 mg/kg 95% confidence limit) for males and females. The remaining two studies provided LD50 values of (1)  $>500$  mg/kg for male Crl:CD(SD)BR rats, and 250-500 mg/kg for female Crl:CD(SD)BR rats (Glaza, 1997); and (2)  $<1000$  mg/kg for male and female Sherman-Wistar rats (3M Company, 1976b).

#### **3.3.2 Inhalation Studies**

The acute inhalation toxicity of APFO was tested in male and female Sprague-Dawley rats, at a dose level of 18.6 mg/L (nominal concentration), and exposure duration of one hour. Signs of toxicity, during, and up to 14 days after the exposure period, included the following: excessive salivation, excessive lacrimation, decreased activity, labored breathing, gasping, closed eyes, mucoid nasal discharge, irregular breathing, red nasal discharge, yellow staining of the anogenital fur, dry and moist rales, red material around the eyes, and body tremors. Upon necropsy, lung discoloration was observed in a higher than normal incidence of rats (8/10). Based on the study results, the test substance was not fatal to rats at a nominal exposure concentration of 18.6 mg/L and exposure duration of one hour (Bio/dynamics, Inc. 1979).

### 3.3.3 Dermal Studies

The acute dermal toxicity of APFO was tested in male and female Hra(NZW)SPF rabbits, at a dose level of 2000 mg/kg, and a 24-hour exposure period. All animals appeared normal and exhibited body weight gain throughout the study, with the exception of one male that lost weight during the first week. Dermal irritation consisted of slight to moderate erythema, edema, and atonia; slight desquamation; coriaceousness; and fissuring. No visible lesions were observed upon necropsy. The dermal LD50 in rabbits was determined to be greater than 2000 mg/kg (Glaza, 1995).

### 3.3.4 Eye Irritation Studies

The eye irritation potential of APFO was tested in albino rabbits, at a dose level of 0.1 gram. In two of three studies, APFO was determined to be a primary ocular irritant. In the studies in which APFO was found to be a primary ocular irritant, APFO was left in contact with the eye for 7 days, then rinsed, or not rinsed. Irritation scores varied during the observation period. Irritation scores of the conjunctivae, iris, and cornea ranged from 2 – 4 in one study (Biosearch, Inc. 1976) and from 2 – 10 in the other study (3M Company, 1976a). In both studies, irritation remained evident for the duration of the observation period (7-days post-exposure). In the study in which APFO was determined to be a non-irritant (Gabriel), the test substance was left in contact with the eye for 5 or 30 seconds, and then the eyes were rinsed. In this study, positive scores were reported for conjunctivae irritation for up to 7-days post-exposure, so the author's negative conclusion for ocular irritancy is problematic.

### 3.3.5 Skin Irritation Studies

The skin irritation potential of APFO was tested in albino rabbits in two studies, at a dose level of 0.5 grams, under occluded test conditions. In one study (Riker Laboratories, Inc. 1983), APFO produced irreversible tissue damage in female rabbits, following a 3-minute, 1-hour, and 4-hour contact period. Moderate erythema and edema, as well as chemical burn, eschar, and necrosis, were observed following all three contact periods. An endpoint was not achieved in this study due to extreme irritation following each contact period. In the second study (Gabriel), APFO was reported as a non-irritant of skin after an exposure period of 24 or 72 hours, based on primary irritation scores of zero.

## 3.4 Mutagenicity Studies

APFO was tested twice (Lawlor, 1995; 1996) for its ability to induce mutation in the *Salmonella* – *E. coli*/mammalian-microsome reverse mutation assay. The tests were performed both with and without metabolic activation. A single positive response seen at one dose level in *S. typhimurium* TA1537 when tested without metabolic activation was not reproducible. APFO did not induce mutation in either *S. typhimurium* or *E. coli* when tested either with or without mammalian activation.

APFO did not induce chromosomal aberrations *in vitro* in human lymphocytes when tested with and without metabolic activation up to cytotoxic concentrations (Murli, 1996c; NOTOX, 2000).

APFO was tested twice for its ability to induce chromosomal aberrations in CHO cells *in vitro*. In the first assay, APFO induced both chromosomal aberrations and polyploidy in both the presence and absence of metabolic activation. In the second assay, no significant increases in chromosomal aberrations were observed without activation. However, when tested with metabolic activation, APFO induced significant increases in chromosomal aberrations and in polyploidy (Murli, 1996b).

APFO was tested in a cell transformation and cytotoxicity assay conducted in C<sub>3</sub>H 10T<sub>1/2</sub> mouse embryo fibroblasts. The cell transformation was determined as both colony transformation and foci transformation potential. There was no evidence of transformation at any of the dose levels tested in either the colony or foci assay methods (Garry & Nelson, 1981).

APFO was tested twice in the *in vivo* mouse micronucleus assay. APFO did not induce any significant increases in micronuclei and was considered negative under the conditions of this assay (Murli, 1996a).

### **3.5 Subchronic Toxicity Studies in Animals**

Two unpublished 28-day feeding studies were performed at Industrial Bio-Test Laboratories, Inc. (Metrick and Marias, 1977 and Christopher and Marias, 1977). In both rats and mice the liver was the target organ. In rats, males had more pronounced hepatotoxicity and histopathologic effects than females.

In a 28-day study of ChR-CD albino rats, eight randomly assigned groups of five males and five females were studied (Metrick and Marias, 1977). After rats were allowed to acclimate for a week in individual cages they then received similar feed containing 0, 30, 100, 300, 1000, 3000, 10,000, or 30,000 ppm APFO for 28 days. At the beginning of the study the animals averaged 88 grams for males and 76 grams for females. The animals were observed daily and body weights and food consumption were recorded weekly. Animals that died during the study were examined for gross pathology, as were surviving animals at 28 days. It is stated that the study included a complete examination of gross pathology and a complete set of tissues and organs were examined, but the specific list is not supplied. Livers were weighed to determine relative organ weight then stained for histopathologic examination.

All animals in the 10,000 and 30,000-ppm groups died before the end of the first week. There were no premature deaths or other clinical signs of toxicity in the other groups. Body weight gains were reduced in the groups receiving 1000 or more ppm. Slight reductions in body weight gain were also observed in males exposed to 300 ppm and males and females fed 100 ppm. Reduced food intake was observed in rats fed 1000 ppm or higher in a dose-related manner.

Relative liver weights were increased in males fed 30 ppm or more and females fed 300 ppm or more. Gross pathological exam did not reveal treatment-related effects in kidneys or other organs besides livers. Focal to multifocal cytoplasmic enlargement of hepatocytes was noted in animals fed 300 ppm, and multifocal to diffuse enlargement of hepatocytes was noted in animals fed 1000 ppm or higher. These effects were more pronounced in males (Metrick and Marias, 1977).

In a 28-day study of Charles River-CD albino mice, eight randomly assigned groups of five males and five females were studied (Christopher and Marisa, 1977). After mice were allowed to acclimate for 8 days in individual cages they then received similar feed containing 0, 30, 100, 300, 1000, 3000, 10,000, or 30,000 ppm of APFO for 28 days. At the beginning of the study the animals averaged 88 grams for males and 76 grams for females. The animals were observed daily and body weights and food consumption were recorded weekly. Animals that died during the study were examined for gross pathology, as were surviving animals at 28 days. It is stated the study included a complete examination of gross pathology and a representative set of tissues and organs were examined, but the specific list is not supplied. Livers were weighed to determine relative organ weight then stained for histopathologic examination.

All animals in the 1000-ppm and higher groups died before the end of day 9. The entire 300-ppm group died within 26 days except 1 male. One animal in each of the 30 and 100-ppm groups died prematurely. Clinical signs were observed in mice exposed to 100 ppm and higher doses of PFOA. At 100 ppm some animals exhibited cyanosis on days 10 and 11 of testing, but appeared normal throughout the rest of the study. Animals fed 300 ppm exhibited roughed fur and muscular weakness as well as signs of cyanosis after 9 days of treatment. Animals fed 1000 ppm exhibited similar effects after 6 days and those receiving 3000 ppm or greater doses exhibited effects after 4 days.

All mice fed APFO lost weight. Reductions in body weight gain were followed by weight losses in mice fed 30, 100, or 300 ppm. A dose-related pattern was seen in the depressed body weights.

Relative and absolute liver weights were increased in mice fed 30 ppm or more APFO. Gross pathological examination of kidneys or other organs besides livers is not discussed. Treatment-related changes were observed in the livers among all APFO treated animals including enlargement and/or discoloration of 1 or more liver lobes. Histopathologic examination of all APFO treated mice revealed diffuse cytoplasmic enlargement of hepatocytes throughout the liver (pan lobular hypertrophy) accompanied by focal to multifocal cytoplasmic vacuoles. Degeneration and /or necrosis of hepatocytes and focal bile duct proliferation were also noted in mice within all groups (Christopher and Marias, 1977).

Three 90-day subchronic toxicity studies have been conducted. One was conducted in rats (Goldenthal, 1978a), one was conducted in rhesus monkeys (Goldenthal, 1978b) and the third was conducted in male rats (Palazzolo, 1993).

In the monkey study, Goldenthal (1978b) administered rhesus monkeys (2/sex/group) doses of 0, 3, 10, 30 or 100 mg/kg/day perfluorooctanoic acid (FC-143) in 0.5% Methocel<sup>7</sup> by gavage for 7 days/week for 90 days. All doses were given in a constant volume; individual daily doses were based upon the weekly body weights. Animals were observed twice daily for general physical appearance and behavior and pharmacotoxic signs. General physical examinations were performed during the control period and monthly during the study period. Individual body weights were recorded weekly. Blood and urine samples were collected once during the control period and at 1 and 3 months of the study for hematology, clinical chemistry and urinalysis. Monkeys were fasted overnight prior to the collection of blood and urine samples. Organs and tissues from animals that were sacrificed at the end of the study and from animals that died during the treatment period were weighed, examined for gross pathology and samples taken for histopathology. Histopathology was performed on the following organs from all monkeys in the control and treatment groups: adrenals, aorta, bone, brain, esophagus, eyes, gallbladder, heart (with coronary vessels), duodenum, ileum, jejunum, cecum, colon, rectum, kidneys, liver, lung, skin, mesenteric lymph node, retropharyngeal lymph node, mammary gland, nerve (with muscle), spleen, pancreas, prostate/uterus, rib junction (bone marrow), salivary gland, lumbar spinal cord, pituitary, stomach, testes/ovaries, thyroid, parathyroid, thymus, trachea, tonsil, tongue, urinary bladder, vagina, identifying tattoo, and any tissues(s) with lesions.

All monkeys in the 100-mg/kg/day groups died during the study. The first death occurred during week 2; all animals were dead by week 5. Signs and symptoms which first appeared during week 1 included anorexia, frothy emesis which was sometimes brown in color, pale face and gums, swollen face and eyes, slight to severe decreased activity, prostration and body trembling. Three monkeys from the 30-mg/kg/day group died during the study; one male died during week 7 and the two females died during weeks 12 and 13. Beginning in week 4, all four animals showed slight to moderate and sometimes-severe decreased activity. One monkey had emesis and ataxia, swollen face, eyes and vulva, as well as pallor of the face and gums. Beginning in week 6, two monkeys had black stools and one monkey had slight to moderate dehydration and ptosis of the eyelids.

No monkeys in the 3 or 10 mg/kg/day groups died during the study. Animals in the 3-mg/kg/day-dose group occasionally had soft stools or moderate to marked diarrhea; frothy emesis was also occasionally noted in this group. One monkey in the 10 mg/kg/day group was anorexic during week 4, had a pale and swollen face in week 7 and had black stools for several days in week 12. The other animals in the 10-mg/kg/day groups did not show any unusual signs or symptoms.

Changes in body weight were similar to the controls for animals from the 3 and 10 mg/kg/day dose groups. Monkeys from the 30 and 100 mg/kg/day groups lost body weight after week 1. At the end of the study, this loss was statistically significant for the one surviving male in the 30-mg/kg/day group (2.30 kg vs 3.78 kg for the control).

Hematology values at the end of the 1 and 3 months of treatment were similar for the control and the 3 and 10 mg/kg/day groups. At 30 mg/kg/day, the surviving male had decreased numbers of erythrocytes, decreased hemoglobin, decreased hematocrit, and increased platelets. Prothrombin time and activated prothrombin time were also increased. These increases were apparent at 1 month but were much more marked at three months.

Following one month of treatment, glucose was significantly elevated in the 3-mg/kg/day group (117 vs 89 mg/100 ml in the control). The authors of the report attribute this to a single high value for male #7366 who had a value of 131. The other three monkeys in the 3-mg/kg/day groups had levels of 112, 105, and 120-mg/100 ml. Glucose levels in the 10 and 30 mg/kg/day groups were 104 and 122-mg/100 ml, respectively, after one month of treatment. At three months of treatment, glucose levels were 81, 96, 88, and 66-mg/100 ml in the control, 3, 10 and 30 mg/kg/day groups respectively.

There was a decrease in alkaline phosphatase levels in the 30-mg/kg/day group (365 vs 597 IU/l in the control) at one month, which persisted in the one surviving male (360 vs 851 IU/l in the control) at 3 months. Alkaline phosphatase levels in the 3- and 10 mg/kg/day groups at three months were 783 and 743 IU/l showing a dose-related trend toward decreased levels.

SGOT levels were reduced in the 30-mg/kg/day groups at one month (59 vs 29 IU/l in the control) and in the one surviving male at 3 months (88 vs 45 IU/l in the control). SGPT was elevated in both the 10 and 30 mg/kg/day dose groups at 1 month; the levels were 15, 34, and 44 IU/l in the control, 10 and 30 mg/kg/day groups, respectively. SGOT levels in the 10-mg/kg/day group were comparable to the controls at 3 months (34 vs 31 IU/l in the control) but were still elevated in the one surviving male in the 30-mg/kg/day dose group (46 IU/l).

Cholesterol in the one surviving male in the 30 mg/kg/day group was elevated (240 vs 165 mg/100ml) and total protein and albumin in this animal were reduced. Total protein was 5.52 vs a control level of 8.21 g/100 ml and total albumin was 2.00 vs a control level of 4.82 g/100 ml.

There were no treatment related changes in urinalysis studies at any time period studied.

There were no macroscopic lesions noted at gross necropsy of any animals which died during the study or which were sacrificed at the end of the treatment period.

The following changes in absolute and relative organ weight changes were noted: absolute and relative weight of the hearts in females from the 10 mg/kg/day group were decreased; absolute brain weight of females from this same group were also decreased and relative group mean weight of the pituitary in males from the 3 mg/kg/day group was increased. The significance of these weight changes is difficult to assess, as they were not accompanied by morphologic changes.

One male and two females from the 30 mg/kg/day group and all animals from the 100 mg/kg/day group had marked diffuse lipid depletion in the adrenals. All males and females from the 30 and 100 mg/kg/day groups also had slight to moderate hypocellularity of the bone marrow and moderate atrophy of lymphoid follicles in the spleen. One female from the 30-mg/kg/day group and all animals in the 100-mg/kg/day group had moderate atrophy of the lymphoid follicles in the lymph nodes. No other compound related lesions were seen in at the 30 and 100 mg/kg/day groups. No treatment related lesions were seen in the organs of animals from the 3 and 10 mg/kg/day groups.

The levels of PFOA in the serum and liver are presented below.

| Dose | Serum (ppm)  |                | Liver (ppm)  |                | Liver total (ug) |                |
|------|--------------|----------------|--------------|----------------|------------------|----------------|
|      | <u>Males</u> | <u>Females</u> | <u>Males</u> | <u>Females</u> | <u>Males</u>     | <u>Females</u> |
| 0    | ND           | 1              | 0.05         | 0.07           | 3                | 5              |
| 3    | 53           | 65             | 3            | 7              | 250              | 350            |
| 3    | 48           | 50             | ND           | ND             | ND               | ND             |
| 10   | 45           | 79             | 9            | ND             | 600              | ND             |
| 10   | 71           | 71             | ND           | 10             | ND               | 750            |
| 30   | ND           | ND             | 125          | 80             | 8000             | 7500           |
| 30   | 145          | ND             | 60           | 125            | 4000             | 9000           |
| 100  | ND           | ND             | 100          | 325            | 6000             | 20000          |

In the first rat study, Goldenthal (1978a) administered CD rats (5/sex/group) dietary levels of 0, 10, 30, 100, 300, and 1000 ppm perfluorooctanoic acid. These dose levels are approximately equivalent to 0.56, 1.72, 5.64, 17.9, and 63.5 mg/kg/day in males, and 0.74, 2.3, 7.7, 22.36 and 76.47 mg/kg/day in females. Animals were housed individually in wire mesh cages and had free access to food and water. Animals were observed twice daily for signs of toxicity and for mortality. Detailed examinations were performed once a week. Individual body weight and food consumption were recorded weekly during the pretest and treatment periods. Blood and urine samples were collected during the pretest period and at 1 and 3 months of the study for hematology and clinical chemistry and urinalysis. At week 13, sex and group, frozen and shipped to the sponsor for analysis, pooled serum samples. Organs and tissues from animals that were sacrificed at the end of the study and from two females that died during the treatment period were weighed, examined for gross pathology and samples taken for histopathology. Histopathology was performed on the following organs from rats from the control, 100, 300, and 1000 ppm dose groups: brain with cervical cord, lumbar spinal cord, peripheral nerve, eyes, pituitary, thyroid with parathyroid, adrenals, lung, heart with coronary vessels, aorta, spleen, mesenteric lymph node, thymus, bone with marrow (sternum), salivary gland, small intestines (duodenum, jejunum, ileum) colon, pancreas, liver, kidneys, urinary bladder, testes, ovaries, prostate, uterus, skin (mammary gland), any tissue(s) with gross lesions. Livers from rats from the 10 and 30-ppm dose groups were also examined microscopically and liver samples from all dose groups were frozen and sent to the sponsor for analysis.

One female in the 100 and one female in the 300-ppm group died during collection of blood. These deaths were not considered to be treatment related. All other animals survived until scheduled sacrifice.

There was a significant reduction in mean body weight in males in the 1000-ppm group (362 g vs 466 g in the control group). Food consumption was reduced in males in the 100, 300 and 1000-ppm groups, but the differences were not statistically significant.

Males in the 30, 100, 300 and 1000-ppm groups had significantly reduced numbers of erythrocytes at the end of the treatment period. The values were 7.95, 7.05, 7.16, 6.72, and 6.94 in the control, 30, 100, 300 and 1000-ppm groups, respectively. Males had reduced leukocyte values compared to the controls in all dose groups, but were statistically significant at the 300 ppm group only; leukocyte values were 10.64, 8.88, 9.33, 9.35, 7.63, and 8.06 in the control, 10, 30, 100, 300 and 1000 ppm groups, respectively. A similar phenomenon was seen with hemoglobin values, which were reduced at all dose levels but were significant at the 10-ppm dose level only. Hemoglobin values were 16.2, 14.7, 15.0, 15.4, 14.9, 13.1 in the control, 10, 30, 100, 300 and 1000 ppm groups, respectively. There was no similar effect upon the hematological parameters of female rats in the study.

Males at the 30, 100, 300, and 1000-ppm dose levels had increased glucose levels (mg/100 ml), which were statistically significant at all but the 100-ppm dose level. Reported glucose levels were 121, 120, 136, 134, 143 and 135 mg/100 ml for the 0, 10, 30, 100, 300 and 1000 ppm groups, respectively. B.U.N. levels were elevated in males at the 100, 300, and 1000 ppm dose levels; mean values at 90 days were 20.4, 23.9 and 35.1 mg/100 ml for the three dose groups, respectively, compared to 16.2 mg/100 ml for the controls. Alkaline phosphatase was elevated in males in the 100, 300, and 1000-ppm groups; the levels were 147, 204 and 212 IU/l for the three groups, respectively, compared to 104 IU/l for the controls. Females showed no similar changes in biochemical measurements.

Neither males nor females showed any treatment related changes in urinalysis parameters although females from all groups showed a higher frequency of occult blood in the urine than did males.

The only gross necropsy observation was noted in males at the 1000-ppm dose level. These animals had enlarged livers that showed varying degrees of surface discoloration. Neither females from the 1000-ppm dose level nor males or females from the lower dose levels showed such effects.

Both absolute and relative liver weights were significantly increased in males in the 30, 300 and 1000-ppm groups and in one female in the 1000-ppm group. Compound-related liver lesions occurred in all male rats in the 100, 300 and 1000-ppm groups. These lesions consisted of focal to multifocal, very slight-to-slight hypertrophy of hepatocytes in centrilobular to midzonal regions of the affected liver lobules. In some instances these lesions were accompanied by an

increased amount of yellowish-brown pigment resembling lipofuscin in the cytoplasm of hepatocytes and occasionally in sinusoidal lining cells. The incidence and severity of the lesions was more pronounced among male rats at the 1000-ppm dietary level.

A comparison of the serum levels of PFOA is shown below. The greater toxicity observed in the males than in the females is due to the gender difference in elimination as demonstrated by the differences in serum PFOA levels.

| Dose | PFOA in Serum (ppm) |                |
|------|---------------------|----------------|
|      | <u>Males</u>        | <u>Females</u> |
| 0    | 0                   | 0              |
| 10   | 21                  | ND             |
| 30   | 34                  | 0.15           |
| 100  | 36                  | ND             |
| 300  | 38                  | 0.25           |
| 1000 | 49                  | 0.65           |

ND = Not Determined.

In the second rat study, Palazzolo (1993) administered 45-55 male Sprague-Dawley rats per group, doses of 1, 10, 30, or 100 ppm (approximate mean compound consumption at week 13 of 0.05, 0.47, 1.44, and 4.97 mg/kg/day) APFO *ad libitum* in the diet for 13 weeks. Two control groups (a nonpair-fed control group and a control group pair-fed to the 100 ppm dose group) were also exposed during that period. Following the 13-week exposure period, 10 animals per group were fed basal diet for an additional 8-weeks post-treatment and observed for any signs of recovery. All test diets were assayed and evaluated for test material homogeneity and stability. All animals were observed twice daily for mortality, moribundity, and general clinical signs of toxicity. Body weights were recorded once before exposures began, weekly during the treatment period, and then on the day of necropsy. Food consumption was recorded weekly for all dosed-groups, including the nonpair-fed control group; daily for the pair-fed animals, and then weekly for all of the animals retained for the recovery phase of the study. A total of 15 animals per dosed-group were sacrificed following 4, 7, or 13 weeks of treatment; 10 animals per dosed-group were sacrificed after 13 weeks of treatment and following 8 weeks of non-treatment. Serum samples collected from 10 animals per dosed-group at each scheduled sacrifice during treatment and from 5 animals per dosed-group during recovery were analyzed for estradiol, total testosterone, luteinizing hormones, and for test material residue. The level of palmitoyl CoA oxidase, an indicator of peroxisome proliferation, was analyzed from a section of liver that was obtained from 5 animals per dosed-group at each scheduled sacrifice. The following organs from all animals at each scheduled sacrifice were weighed: brain, liver, lungs, testis (one), seminal vesicle, prostate, coagulating gland, and urethra. The following tissues in these same animals were preserved in 10% phosphate-buffered formalin and examined macroscopically: external surface of the body, all orifices, the cranial cavity, the external surfaces of the brain and spinal cord, the nasal cavity and paranasal sinuses; the thoracic, abdominal, and pelvic cavities

and viscera; and also examined microscopically: any observed lesions, brain, liver, lungs, testes (one), seminal vesicle, prostate, coagulating gland, and urethra. In addition, the following tissues were preserved in glutaraldehyde for electron microscopic examination: brain, liver, lungs, testes (one), seminal vesicle, and prostate.

In the analysis of the data, animals in groups exposed to 1, 10, 30, and 100 ppm APFO were compared to the control animals in the nonpair-fed group, while the data from the pair-fed control animals were compared to animals exposed to 100 ppm APFO. All test diets were considered to be homogeneous and stable under the experimental conditions. All animals survived to scheduled sacrifice, with the exception of one animal in the 100-ppm dosed-group that was sacrificed on week 4 due to severe neck sores unrelated to treatment. Twice-daily examinations of all animals were unremarkable. At 100 ppm, significant reductions in body weights were seen compared to the pair-fed control group during week 1 and the nonpair-fed control group during weeks 1-13 (i.e., throughout treatment). During recovery, however, no reductions in body weights were apparent. Body weight data in the other dosed-groups were comparable to controls. At 100 ppm, mean body weight gains were significantly higher than the pair-fed control group during week 1 and significantly lower than the nonpair-fed control group during weeks 1-13. At 10 and 30 ppm, mean body weight gains were significantly lower than the nonpair-fed control group at week 2. These differences in body weight gains were not observed during the recovery period. Significant differences in food consumption were observed at 100 ppm during weeks 1 and 2 only, when compared to the nonpair-fed control group; no other significant differences in food consumption were noted. There were no significant differences among the groups for any of the hormones evaluated in the serum. Likewise, serum analysis of test material residue showed no increase in serum APFO levels over the course of treatment. Statistically significant higher hepatic palmitoyl CoA oxidase activity was observed at 30 and 100 ppm; however, this effect returned to control levels by the end of the recovery period. At 10 ppm, statistically significant higher levels of hepatic palmitoyl CoA oxidase activity were observed at week 5 only. Mean enzyme activities were highest during week 8 for animals exposed to 10, 30, and 100 ppm. All dosed groups exhibited significant increases in absolute and relative liver weights and hepatocellular hypertrophy were observed at weeks 4, 7, and 13, compared to the pair-fed control group. The authors suggested that these changes might be associated with peroxisome proliferation, especially since increases in hepatic palmitoyl CoA oxidase activity were also observed at this dose level during treatment. During recovery, however, none of the liver effects were observed, indicating that these treatment-related liver effects were reversible.

Therefore, under the conditions of this study, a NOAEL of 1.0 ppm (0.05 mg/kg/day) and a LOAEL of 10 ppm (0.47 mg/kg/day) are indicated based on reductions in body weight and body weight gain, and on increases in absolute and relative liver weights with hepatocellular hypertrophy.

### 3.6 Developmental Toxicity Studies in Animals

Three prenatal developmental toxicity studies of APFO have been conducted, one inhalation and two oral studies.

The first of these studies was an oral developmental toxicity study in rats (Gortner, 1981). Based on the results of a range-finding study, an upper dose level of 150 mg/kg/day was set for the definitive study in which five groups of 22 time-mated Sprague-Dawley rats were administered 0, 0.05, 1.5, 5, and 150 mg/kg/day APFO in distilled water by gavage on gestation days (GD) 6-15. Doses were adjusted according to body weight. Dams were monitored on GD 3-20 for clinical signs of toxicity. Individual body weights were recorded on GD 3, 6, 9, 12, 15, and 20. Animals were sacrificed on GD 20 by cervical dislocation and the ovaries, uteri, and contents were examined for the number of corpora lutea, number of viable and non-viable fetuses, number of resorption sites, and number of implantation sites. Fetuses were weighed and sexed and subjected to external gross necropsy. Approximately one-third of the fetuses were fixed in Bouin's solution and examined for visceral abnormalities by free-hand sectioning. The remaining fetuses were subjected to skeletal examination using alizarin red.

Signs of maternal toxicity consisted of statistically significant reductions in mean maternal body weights on GD 9, 12, and 15 at the high-dose group of 150 mg/kg/day. Mean maternal body weight on GD 20 continued to remain lower than controls, although the difference was not statistically significant. Other signs of maternal toxicity that occurred only at the high-dose group included ataxia and death in three rat dams. No other effects were reported. Administration of APFO during gestation did not appear to affect the ovaries or reproductive tract of the dams. Under the conditions of the study, a NOAEL of 5 mg/kg/day and a LOAEL of 150 mg/kg/day for maternal toxicity were indicated.

A significantly higher incidence in fetuses with one missing sternebrae was observed at the high-dose group of 150 mg/kg/day; however this skeletal variation also occurred in the controls and the other three dose groups (at similar incidence but lower than the high-dose group) and therefore was not considered to be treatment-related. No significant differences between treated and control groups were noted for other developmental parameters that included the mean number of males and females, total and dead fetuses, the mean number of resorption sites, implantation sites, corpora lutea and mean fetus weights. Likewise, a fetal lens finding initially described as a variety of abnormal morphological changes localized to the area of the embryonal nucleus, was later determined to be an artifact of the free-hand sectioning technique and therefore not considered to be treatment-related. Under the conditions of the study, a NOAEL for developmental toxicity of 150 mg/kg/day (highest dose group) was indicated.

A second oral prenatal developmental toxicity study was conducted in rabbits (Gortner, 1982). Based on the results of a range-finding study, an upper dose level of 50 mg/kg/day was set for the definitive study in which four groups of 18 pregnant New Zealand White rabbits were administered 0, 1.5, 5, and 50 mg/kg/day APFO in distilled water by gavage on gestation days

(GD) 6-18. Pregnancy was established in each sexually mature female by i.v. injection of pituitary lutenizing hormone in order to induce ovulation, followed by artificial insemination with 0.5 ml of pooled semen collected from male rabbits; the day of insemination was designated as day 0 of gestation. A constant dose volume of 1 ml/kg was administered. Individual body weights were measured on GD 3, 6, 9, 12, 15, 18, and 29. The does were observed daily on GD 3-29 for abnormal clinical signs. On GD 29, the does were euthanized and the ovaries, uterus and contents examined for the number of corpora lutea, live and dead fetuses, resorptions and implantation sites. Fetuses were examined for gross abnormalities and placed in a 37°C incubator for a 24-hour survival check. Pups were subsequently euthanized and examined for visceral and skeletal abnormalities. A blood sample was taken from six does prior to dosing and then on GD 18 and 29; a liver sample was taken from the same animals on GD 29. All samples were sent to the sponsor for analysis. This information was unavailable at the time of this review.

Signs of maternal toxicity consisted of statistically significant transient reductions in body weight gain on GD 6-9 when compared to controls; body weight gains returned to control levels on GD 12-29. Administration of APFO during gestation did not appear to affect the ovaries or reproductive tract contents of the does. No clinical or other treatment-related signs were reported. Under the conditions of the study, a NOAEL of 50 mg/kg/day, the highest dose tested, for maternal toxicity was indicated.

No significant differences were noted between controls and treated groups for the number of males and females, dead or live fetuses, and fetal weights. Likewise, there were no significant differences reported for the number of resorption and implantation sites, corpora lutea, the conception incidence, abortion rate, or the 24-hour mortality incidence of the fetuses. Gross necropsy and skeletal/visceral examinations were unremarkable. The only sign of developmental toxicity consisted of a dose-related increase in a skeletal variation, extra ribs or 13<sup>th</sup> rib, with statistical significance at the high-dose group (38% at 50 mg/kg/day, 30% at 5 mg/kg/day, 20% at 1.5 mg/kg/day, and 16 % at 0 mg/kg/day). A statistically significant increase in 13<sup>th</sup> ribs-spurred occurred in the mid-dose group of 5 mg/kg/day; however, the biological significance of this effect is uncertain since in both the high- and low-dose groups, this effect occurred at the same rate and was not statistically significantly different from controls. Therefore, under the conditions of the study, a LOAEL for developmental toxicity of 50 mg/kg/day (highest dose group) was indicated.

Staples et al. (1984) also conducted a developmental toxicity study of APFO. The study design consisted of an inhalation and an oral portion, each with two trials or experiments. The first trial was the teratology portion of the study, in which the dams were sacrificed on GD 21; while in the second trial, the dams were allowed to litter and the pups were sacrificed on day 35-post partum.

For the inhalation portion of the study, the two trials consisted of 12 pregnant Sprague-Dawley rats per group exposed to APFO by whole-body vapor inhalation to 0, 0.1, 1, 10, and 25 mg/m<sup>3</sup> 6 hours/day, on GD 6-15. In the oral portion of the study, 25 and 12 Sprague-Dawley rats for the first and second trials, respectively, were administered 0 and 100 mg/kg/day APFO in corn oil by gavage on GD 6-15. For both routes of administration, females were mated on an as-needed basis and when the number of mated females was bred, they were ranked within breeding days by body weight and assigned to groups by rotation in order of rank. Finally, two additional groups (six dams per group) were added to each trial that was pair-fed to the 10 and 25 mg/m<sup>3</sup> groups.

For the teratology portion of the study (trial one), dams were weighed on GD 1, 6, 9, 13, 16, and 21 and observed daily for abnormal clinical signs. On GD 21, the dams were sacrificed by cervical dislocation and examined for any gross abnormalities, liver weights were recorded and the reproductive status of each animal was evaluated. The ovaries, uterus and contents were examined for the number of corpora lutea, live and dead fetuses, resorptions and implantation sites. Pups (live and dead) were counted, weighed and sexed and examined for external, visceral, and skeletal alterations. The heads of all control and high-dosed group fetuses were examined for visceral alterations as well as macro- and microscopic evaluation of the eyes.

For trial two, in which the dams were allowed to litter, the procedure was the same as that for trial one up to GD 21. Two days before the expected day of parturition, each dam was housed in an individual cage. The date of parturition was noted and designated Day 1 PP. Dams were weighed and examined for clinical signs on Days 1, 7, 14, and 22 PP. On Day 23 PP all dams were sacrificed. Pups were counted, weighed, and examined for external alterations. Each pup was subsequently weighed and inspected for adverse clinical signs on Days 4, 7, 14, and 22 PP. The eyes of the pups were also examined on Days 15 and 17 PP for the inhalation portion and on Days 27 and 31 PP for the gavage portion of the study. Pups were sacrificed on Day 35 PP and examined for visceral and skeletal alterations.

### Inhalation Exposure

#### Trial One:

Treatment-related clinical signs of maternal toxicity for trial one (teratology) occurred at 10 and 25 mg/m<sup>3</sup> and consisted of wet abdomens, chromodacryorrhea, chromorhinorrhea, a general unkempt appearance, and lethargy in four dams at the end of the exposure period (high-concentration group only). Three out of 12 dams died during treatment at 25 mg/m<sup>3</sup> (on GD 12, 13, and 17). Food consumption was significantly reduced at both 10 and 25 mg/m<sup>3</sup>; however, no significant differences were noted between treated and pair-fed groups. Significant reductions in body weight were also observed at these concentrations, with statistical significance at the high-concentration only. Likewise, statistically significant increases in mean liver weights were seen at the high-concentration group. Under the conditions of the study, a NOAEL and LOAEL for maternal toxicity of 1 and 10 mg/m<sup>3</sup>, respectively, was indicated.

No effects were observed on the maintenance of pregnancy or the incidence of resorptions. Mean fetal body weights were significantly decreased in the 25-mg/m<sup>3</sup> groups and in the control group pair-fed 25 mg/m<sup>3</sup>. A detailed microscopic visceral and eye examination of the fetuses did not reveal any treatment-related effects; however in the control group that was pair-fed 25 mg/m<sup>3</sup>, a statistically significant increased incidence of fetuses with partially ossified sternebrae was observed. Under the conditions of the study, a NOAEL and LOAEL for developmental toxicity of 10 and 25 mg/m<sup>3</sup>, respectively, was indicated.

#### Trial Two:

Clinical signs of maternal toxicity seen at 10 and 25 mg/m<sup>3</sup> were similar in type and incidence as those described for trial one. Maternal body weight gain during treatment at 25 mg/m<sup>3</sup> was less than controls, although the difference was not statistically significant. In addition, 2 out of 12 dams died during treatment at 25 mg/m<sup>3</sup>. No other treatment-related effects were reported, nor were any adverse effects noted for any of the measurements of reproductive performance. Under the conditions of the study, a NOAEL and LOAEL for maternal toxicity of 1 and 10 mg/m<sup>3</sup>, respectively, were indicated.

Signs of developmental toxicity in this group consisted of statistically significant reductions in pup body weight on Day 1 PP (6.1 g at 25 mg/m<sup>3</sup> vs. 6.8 g in controls). On Days 4 and 22 PP, pup body weights continued to remain lower than controls, although the difference was not statistically significant (Day 4 PP: 9.7 g at 25 mg/m<sup>3</sup> vs. 10.3 in controls; Day 22 PP: 49.0 g at 25 mg/m<sup>3</sup> vs. 50.1 in controls). No significant effects were reported following external examination of the pups or with ophthalmoscopic examination of the eyes. Under the conditions of the study, a NOAEL and LOAEL for developmental toxicity of 10 and 25 mg/m<sup>3</sup>, respectively, were indicated.

#### Oral Exposure

##### Trial One:

Three out of 25 dams died during treatment of 100 mg/kg APFO during gestation (one death on GD 11; two on GD 12). Clinical signs of maternal toxicity in the dams that died were similar to those seen with inhalation exposure. Food consumption and body weights were reduced in treated animals compared to controls. No adverse signs of toxicity were noted for any of the reproductive parameters such as maintenance of pregnancy or incidence of resorptions. Likewise, no significant differences between treated and control groups were noted for fetal weights, or in the incidences of malformations and variations; nor were there any effects noted following microscopic examination of the eyes.

Trial Two:

Similar observations for clinical signs were noted for the dams as in trial one. Likewise, no adverse effects on reproductive performance or in any of the fetal observations were noted.

### **3.7 Carcinogenicity Studies in Animals**

#### **3.7.1 Cancer Bioassays**

The carcinogenic potential of APFO has been investigated in a two-year feeding study in rats (3M, 1987). In this study, groups of 50 male and 50 female Sprague-Dawley (CrI:CD BR) rats were fed diets containing 0, 30 or 300 ppm FC-143 for two years. Groups of 15 additional rats per sex were fed 0, or 300 ppm FC-143 and evaluated at the one-year interim sacrifice. The mean actual test article consumption was: males, 1.3 and 14.2 mg/kg/day; females, 1.6 and 16.1 mg/kg/day for the low and high-dose groups, respectively.

There was a dose-related decrease in body weight gain in the male rats and to a lesser extent, in the female rats as compared to the controls; the decreases were statistically significant in the high-dose groups of both sexes. The body weight changes are treatment related since feed consumption was actually increased (rather than decreased). There were no differences in mortality between the treated and untreated groups; the survival rates at the end of 104 weeks for the control, low-, and high-dose groups were: male, 70%, 72% and 88%; females, 50%, 48% and 58%. The only clinical sign observed was a dose-related increase in ataxia in the female rats; the incidences in the control, low- and high-dose groups were: 4%, 18% and 30%. Significant decreases in red blood cell counts, hemoglobin concentrations and hematocrit values were observed in the high-dose male and female rats as compared to control values. Clinical chemistry changes indicative of liver toxicity included increases in alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP) in both treated male groups from 3-18 months, but only in the high-dose males at 24 months. Increases in relative liver and kidney weights were noted in both high-dose male and female rats. Significant non-neoplastic lesions were seen primarily in the liver and testis; there were increases in the incidence of liver masses, hyperplastic nodules and foci, and in testicular masses in the high-dose male group. Other liver toxic effects include dose-related increases in the incidence of diffuse hepatomegalocytosis, cystoid degeneration, and portal mononuclear cell infiltration in both male and female treated groups; these increases were statistically significant in the high-dose males. A statistically significant, dose-related increase in the incidence of ovarian tubular hyperplasia was found in female rats; the incidence of this lesion in the control, low-, and high-dose groups was 0%, 14%, and 32%, respectively. Based on these toxic effects, the high dose selected in this study appears to have reached the Maximum Tolerated Dose (MTD). Based on decreased body weight gain, increased liver and kidney weights and toxicity in the hematological and hepatic systems, the LOAEL for male and female rats is 300 ppm. [Based on increases in the incidence of ataxia (a clinical sign) and ovarian tubular hyperplasia (which is reversible), the LOAEL for female rats is 30 ppm.]

At the termination of the study, a slight increase in the incidence of various neoplasms (tumors of the liver, testis, thyroid, adrenal and mammary glands, etc.) was seen in the treated animals. Among them, the increased incidences of testicular (Leydig) cell adenomas in the high-dose male rats, and of mammary fibroadenoma in both groups of female rats were statistically significant ( $P < 0.05$ ) as compared to the concurrent controls. The incidence of the Leydig cell tumors (LCT) in the control, low- and high-dose males was 0%, 4% and 14%, respectively; the respective incidences of mammary fibroadenoma in the female groups were 22%, 42% and 48%.

The increases are also statistically significant as compared to the historical control incidences (LCT, 0.82%; mammary fibroadenoma, 19.0%) observed in 1,340 male and 1,329 female Sprague-Dawley control rats used in 17 carcinogenicity studies (Chandra et al., 1992). The spontaneous incidence of LCT in 2-year old Sprague-Dawley rats in other studies was reported to be approximately 5% (*cited in*: Clegg et al., 1997). Therefore, under the conditions of this study, APFO is carcinogenic in Sprague-Dawley rats, inducing Leydig cell tumors in the male rats and mammary fibroadenomas in the female rats.

In a follow-up 2-year dietary study (300 ppm) in male Sprague-Dawley (CD) rats, APFO was found to induce liver tumors and pancreatic acinar cell tumors in addition to Leydig cell tumors; however, details on the study design and tumor incidence were not reported (Cook et al., 1994). APFO has also been shown to promote liver carcinogenesis in rodents (Abdellatif et al., 1991; Nilsson et al., 1991).

### **3.7.2 Mode of Action Studies**

The mechanisms of toxicological/carcinogenic action of APFO are not clearly understood. Short-term genotoxicity assays suggest that APFO is not a DNA-reactive compound; it is non-mutagenic in the Ames test using five strains of *Salmonella typhimurium*, or in an assay with *Saccharomyces cerevisiae* (Griffith and Long, 1980). Available data indicate that the induction of tumors by APFO is due to a non-genotoxic mechanism, involving activation of receptors and perturbations of the endocrine system.

#### **3.7.2.1 Liver Tumors**

It has been well documented that APFO is a potent peroxisome proliferator, inducing peroxisome proliferation in the liver of rats and mice (*e.g.*, Ikeda et al., 1985; Pastoor et al., 1987; Sohlenius et al., 1992). A sex-related difference in the induction of liver peroxisome proliferation exists in rats (Kawashima et al., 1989), but not in mice (Sohlenius et al., 1992). The higher induction of liver peroxisome proliferation in male rats was shown to be strongly dependent on the sex hormone testosterone (Kawashima et al., 1989). Like many other peroxisome proliferators, APFO has also been shown to cause hepatomegaly (an early biomarker of peroxisome proliferator hepatocarcinogenesis) in rats (Takagi, et al., 1992; Cook, 1994) and mice (Kennedy, 1987), and induce oxidative DNA damage in liver of rats (Takagi et al., 1991). The totality of these data appears to suggest that the liver toxicity and carcinogenicity of APFO may be related to induction of peroxisome proliferation. Meanwhile, estrogen has been shown to

promote hepatocarcinogenesis in rats (Yager and Yager, 1980; Cameron et al., 1982); an increase in estrogen levels after APFO exposure (discussed below) may also play a role in hepatocarcinogenesis in rats.

### 3.7.2.2 Leydig Cell Tumors

A large number of non-genotoxic compounds of diverse chemical structures have been reported to induce Leydig cell tumors (LCT) in rats, mice, or dogs. A review of the available information on LCT induction in animals led a workshop panel to classify these compounds into seven groups based on their modes of action (Clegg et al., 1997). The common theme in the mode of action for most compounds is that these compounds affect the hormonal control of Leydig cell growth by disrupting the hypothalamic-pituitary-testicular axis at various points that result in increasing the serum levels of luteinizing hormone (LH). It has been postulated that in addition to stimulating the production of testosterone, LH may also play a mitogenic role in the Leydig cells; a sustained increase in circulating LH levels and chronic stimulation of Leydig cells by growth-stimulating mediators such as IGF-1, TGF- $\beta$ , leukotrienes and various free radicals can lead to LCT development (*rev. in*: Clegg et al., 1997).

A series of studies have been conducted to investigate the mechanism of tumor formation in male Sprague-Dawley (CD) rats exposed to APFO (Cook et al., 1992; Biegel et al., 1995; Liu et al., 1996). No significant increases in LH were seen in the rats after treatment of APFO at various dose levels for 14 days. However, serum and testicular levels of estradiol were significantly increased and testosterone levels were significantly decreased. It was postulated that the elevated estradiol levels may cause Leydig cell hyperplasia and tumor formation by acting as a mitogen and/or enhancing growth factor secretion; the transforming growth factor  $\alpha$  (TGF  $\alpha$ ), which binds to the epidermal growth factor (EGF) receptor and stimulated cell proliferation, for instance, has been detected in Leydig cells (Teerds et al., 1990). Subsequent experiments have shown that APFO increased the levels of estradiol by inducing cytochrome P450 XIX (aromatase), which converts testosterone to estradiol. Peroxisome proliferators are known to induce  $\beta$ -oxidation and cytochrome P-450 monooxygenases by binding to the peroxisome proliferation activation receptor  $\alpha$  (PPAR  $\alpha$ ; a subfamily of steroid hormone receptors). It is believed that APFO induces cytochrome P450 XIX (aromatase) by binding to and activating the PPAR $\alpha$ .

Although significant increases in LH were not seen in Sprague-Dawley rats after treatment of APFO in the 14 day-studies, it appears that increase in LH levels cannot be ruled out to be involved (in addition to increased estradiol level) in the induction of LCT by APFO. In these studies, significant increase in hepatic aromatase (which converts testosterone to estradiol) activities associated with decreased serum testosterone levels and increased estradiol levels were observed in the treated rats. Testosterone, which is synthesized and secreted by the Leydig cells, is regulated by LH; testosterone and LH form a closed-loop feedback system in the HPT axis. In order to maintain adequate testosterone plasma levels, reduced testosterone levels (caused by increased aromatase activity) are expected to lead to increased LH levels through the negative

feedback mechanism. It has been pointed out that increases in LH may not always be seen in all studies of chemicals for which the proposed mode of action calls for elevated LH, and that compensation may have occurred to restore homeostasis and inappropriate timing of sampling are some of the explanations for failing to detect changes in LH levels (Clegg et al., 1997).

### **3.7.2.3 Mammary Gland Tumors**

Estradiol has also been shown to stimulate the secretion of TGF  $\alpha$  by mammary epithelial cells and the overexpression of TGF  $\alpha$  has been suggested as one possible factor in producing sustained cell proliferation of mammary tumor cells and the subsequent development of neoplasia (Liu et al., 1987). Hence, it is possible that the APFO-induced elevation of estradiol levels may also be responsible for the development of mammary fibroadenomas in Sprague Dawley rats in addition to LCT (discussed above). In fact, this is consistent with the mechanism by which spontaneous mammary neoplasms were developed in aging female Sprague Dawley rats. It has been demonstrated that the early appearance and high spontaneous incidence of mammary gland tumors in untreated, aging female Sprague-Dawley rats is due to increased exposure to endogenous estrogen and prolactin as a result of an accelerating effect on normal, age-related perturbations of the estrous cycle in this strain of rat (Cutts and Noble, 1964; Chapin et al., 1996).

### **3.7.2.4 Pancreatic Tumors**

The mechanism by which APFO induced pancreatic acinar cell tumors is unknown. A number of other peroxisome proliferators also produce pancreatic acinar cell tumors in rats. Available data suggest that the pancreatic acinar cell tumors are related to an increase in serum cholecystokinin (CCK) level secondary to hepatic cholestasis (Cook et al., 1994; Obourn et al., 1997). CCK is a growth factor that has been shown to stimulate normal, adaptive, and neoplastic growth of pancreatic acinar cells in rats (Longnecker, 1987). However, data on the role of CCK in pancreatic tumor formation are conflicting.

## **4.0 Hazards to the Environment**

### **4.1 Introduction**

The aquatic toxicity and hazard of APFO to aquatic organisms was assessed. This task was made more difficult by several problems discussed below. These problems complicated the task of determining if the ecotoxicity tests were valid and could be used in the assessment. Furthermore, these problems limited the confidence that could be placed on the toxicity test values, and thus in turn lowered the confidence of conclusions that could be drawn in assessing the inherent toxicity and hazard of APFO to aquatic organisms.

- 1) A variety of different APFOs with varying designations and lot numbers were tested. Generally, the ammonium salt or the tetrabutylammonium salt was tested. The exact composition and identification of impurities, which may affect toxicity, in each lot number used is not known.
- 2) A variety of testing laboratories conducted the APFO toxicity studies over a period of time from approximately 1974-1996. This situation served to increase overall test variability and thus made inter-laboratory comparisons more difficult.
- 3) Purity of the tested material, or percent test material and percent other material(s), was a major concern. Purity was not sufficiently characterized in these tests. In some tests it appeared that 100% test chemical was used; in others a chemical of lesser purity (approximately 85%) was used. Purity of test material does affect toxicity and should be taken into account when possible, by expressing toxicity on the same purity basis.
- 4) Water, an isopropanol solvent, or a combination of both were used with the test material in many of the toxicity tests, for no obvious indicated reason. Solvents are mixed with the test material to make it miscible with the test dilution water before the test is begun. Solvents are used in tests where the concentrations of the test material are extremely low and a very small amount of test material must be added to the test chambers. It was not clear from the summaries of these studies why a solvent was used or was even found to be necessary. In fact, 3M summarized each test and stated "Data may not accurately relate toxicity of the test sample with that of the test substance." Thus, in those tests where 100% test material was not used, the toxicity values had to be adjusted to take into account the percent solvent(s), and to express the values on a 100% test chemical basis, so that the tests could be compared.
- 5) In all these toxicity tests only nominal test chemical concentrations were used. Measured test chemical concentrations are instead always recommended so that one can accurately determine the actual test chemical concentration to which the test organisms are exposed. If it is determined that the nominal concentrations are only, for example 50% of the measured concentrations, the toxicity values will have to accordingly be adjusted by 50%. Analytical measurements of chemical concentration should have been taken or made available. Then, recovery rates could have been determined, and physicochemical processes (e.g., hydrolysis, volatility) that might lower the actual concentrations to which the test organisms were exposed could have been taken into account. Nominals may be used when measured concentrations are taken and the relationship of both is known.

In order to proceed with any sort of environmental hazard review it was necessary to ignore these test limitations and to assume that the nominal concentrations were an "adequate" expression of the measured test chemical concentrations. Criteria for assessing degree of acute toxicity are based on well-established values (low is  $>100$  mg/L; medium or moderate is  $>1 \leq 100$  mg/L; high is  $\leq 1$  mg/L).

## 4.2 Acute Toxicity to Freshwater Species

Several species were tested to assess the acute toxicity of APFO; these included the fathead minnow (*Pimephales promelas*), bluegill sunfish (*Lepomis machrochirus*), water flea (*Daphnia magna*), and a green alga (*Selenastrum capricornutum*). The toxicity test endpoints have been adjusted to 100% test chemical and test results are presented in Tables 2 (organized by test substance) and 3 (organized by test species). Each value is related to a testing facility and reference.

Twelve tests were conducted with fathead minnows; 96-h LC50 values (based on mortality) ranged from 70 to 843 mg/L. It is unclear why this range is so wide. Assuming these studies are valid, and due to the limitations discussed above, these toxicity values indicate low toxicity. The two acute values for bluegill sunfish also indicate low toxicity (96-h LC50s of >420, and 569 mg/L).

Nine acute tests were conducted with daphnids and 48-h EC50 values (based on immobilization) ranged from 39 to >1000 mg/L. The lower values are indicative of moderate toxicity, but the wide range makes interpretation difficult.

Seven tests were conducted with green algae; 96-h EC50 values (based on growth rate, cell density, cell counts, and dry weights) ranged from 1.2 to >666 mg/L (the Er50 cell density value of 1,000 mg/L is excluded from this discussion). The lower value indicates high to moderate toxicity, based on the acute criteria. The lower value would also be indicative of moderate toxicity, based on the chronic moderate criterion ( $0.1 \leq 10$  mg/L). A 14-d EC50 value of 43 mg/L, based on cell counts, for green algae was also calculated in one study. This is indicative of low chronic toxicity, based on the chronic criterion (10 mg/L). Green algae appeared to be the most sensitive test species in the 44% APFO test sample, daphnids were the next most sensitive, and fathead minnows were the least sensitive.

| Table 2 Summary of Acute Ecological Toxicity Data for APFO (grouped by test substance) |             |               |                                    |
|----------------------------------------------------------------------------------------|-------------|---------------|------------------------------------|
| Test Organism                                                                          | Duration    | Value (mg/L)* | Reference                          |
| <b>Test Sample: APFO ammonium salt</b>                                                 |             |               |                                    |
| Fathead minnow ( <i>Pimephales promelas</i> )                                          | 96-h LC50   | 70            | 3M Company, 1974a                  |
|                                                                                        | 96-h LC50   | 766           | 3M Company, 1980a                  |
|                                                                                        | 96-h LC50   | 301           | 3M Company, 1987c                  |
|                                                                                        | 96-h LC50   | 740           | Ward et al., 1995                  |
| Bluegill sunfish ( <i>Lepomis machrochirus</i> )                                       | 96-h LC50   | > 420         | 3M Company, 1978                   |
|                                                                                        | 96-h LC50   | 569           | 3M Company, 1978                   |
| Water flea ( <i>Daphnia magna</i> )                                                    | 48-h EC50   | 126           | 3M Environmental Laboratory, 1982  |
|                                                                                        | 48-h EC50   | > 1000        | 3M Environmental Laboratory, 1982  |
|                                                                                        | 48-h EC50   | 221           | 3M Company, 1987b                  |
|                                                                                        | 48-h EC50   | 720           | Ward et al., 1995                  |
| Green algae ( <i>Selenastrum capricornutum</i> )                                       | 96-h EC50   | 310           | Ward et al., 1995                  |
|                                                                                        | 96-h EC50   | 1000          | Ward et al., 1995                  |
| Bacteria ( <i>Photobacterium phosphoreum</i> )                                         | 30-min EC50 | 870           | 3M Company, 1987a                  |
|                                                                                        | 30-min EC50 | 730           | 3M Environmental Laboratory, 1996a |
| Activated sludge                                                                       | 7-min NOEC  | 1000          | 3M Company, 1980b                  |
|                                                                                        | 30-min EC50 | > 1000        | 3M Company, 1987d                  |
| <b>Test Sample: APFO</b>                                                               |             |               |                                    |
| Fathead minnow ( <i>Pimephales promelas</i> )                                          | 96-h LC50   | 440           | 3M Company, 1974b                  |
|                                                                                        | 96-h LC50   | 843           | 3M Company, 1985                   |
| <b>Test Sample: APFO ammonium salt in 50% water</b>                                    |             |               |                                    |
| Fathead minnow ( <i>Pimephales promelas</i> )                                          | 96-h LC50   | >500          | EnviroSystems, Inc., 1990a         |
|                                                                                        | 96-h NOEC   | 500           | EnviroSystems, Inc., 1990a         |
| Water flea ( <i>Daphnia magna</i> )                                                    | 48-h EC50   | 292           | EnviroSystems, Inc., 1990b         |
| Bacteria ( <i>Photobacterium phosphoreum</i> )                                         | 30-min EC50 | > 500         | 3M Environmental Laboratory, 1990a |
| <b>Test Sample: APFO ammonium salt in 50% water, continued</b>                         |             |               |                                    |
| Activated sludge                                                                       | 30-min EC50 | > 500         | 3M Environmental Laboratory, 1990b |

|                                                                     |             |       |                                        |
|---------------------------------------------------------------------|-------------|-------|----------------------------------------|
| <b>Test Sample: APFO ammonium salt in 80% water</b>                 |             |       |                                        |
| Fathead minnow ( <i>Pimephales promelas</i> )                       | 96-h LC50   | 494   | Ward et al., 1996a                     |
| Water flea ( <i>Daphnia magna</i> )                                 | 48-h EC50   | 240   | Ward et al., 1996c                     |
| Green algae ( <i>Selenastrum capricornutum</i> )                    | 96-h EC50   | 396   | Ward et al., 1996b                     |
|                                                                     | 96-h EC50   | > 666 | Ward et al., 1996b                     |
| Bacteria ( <i>Photobacterium phosphoreum</i> )                      | 30 min EC50 | 630   | 3M Environmental Laboratory, 1996b     |
|                                                                     | 30 min EC50 | 390   | 3M Environmental Laboratory, 1996c     |
| Activated sludge                                                    | 30-min EC50 | >664  | 3M Environmental Laboratory, 1996d     |
| <b>Test Sample: APFO in 50% isopropanol</b>                         |             |       |                                        |
| Fathead minnow ( <i>Pimephales promelas</i> )                       | 96h LC50    | 140   | T.R. Wilbury Laboratories, Inc., 1996a |
| Water flea ( <i>Daphnia magna</i> )                                 | 48-h EC50   | 360   | T.R. Wilbury Laboratories, Inc., 1996b |
| Green algae ( <i>Selenastrum capricornutum</i> )                    | 96-h EC50   | 90    | T.R. Wilbury Laboratories, Inc., 1995  |
| <b>Test Sample: APFO (44%) in 27.9% water and 27.2% isopropanol</b> |             |       |                                        |
| Fathead minnow ( <i>Pimephales promelas</i> )                       | 96-h EC50   | 391   | T.R. Wilbury Laboratories, Inc., 1995  |
| Fathead minnow ( <i>Pimephales promelas</i> )                       | 96-h EC50   | 422   | T.R. Wilbury Laboratories, Inc., 1995  |
| <b>Test Sample: APFO (44%) in 27.9% water and 27.2% isopropanol</b> |             |       |                                        |
| Water flea ( <i>Daphnia magna</i> )                                 | 48-h EC50   | 41    | Ward et al., 1995                      |
| Water flea ( <i>Daphnia magna</i> )                                 | 48-h EC50   | 39    | Ward et al., 1995                      |
| Green algae ( <i>Selenastrum capricornutum</i> )                    | 96-h EC50   | 2.1   | Ward et al., 1995                      |
| Green algae ( <i>Selenastrum capricornutum</i> )                    | 96-h EC50   | 3.6   | Ward et al., 1995                      |
| Green algae ( <i>Selenastrum capricornutum</i> )                    | 96-h EC50   | 1.2   | Ward et al., 1995                      |

\*Values were adjusted to represent 100% active ingredient.

<sup>A</sup>These values may be inconsistent due to different diets tested.

| Table 3 Summary of Ecological Toxicity Data for APFO (grouped by species) |              |                      |                                                    |
|---------------------------------------------------------------------------|--------------|----------------------|----------------------------------------------------|
| Test Organism                                                             | Duration     | Value (mg/L)         | Reference                                          |
| Fathead minnow ( <i>Pimephales promelas</i> )                             | 96-h LC50    | 70 <sup>B</sup>      | 3M Company, 1974a                                  |
|                                                                           | 96-h LC50    | 766 <sup>B</sup>     | 3M Company, 1980a                                  |
|                                                                           | 96-h LC50    | 301 <sup>B</sup>     | 3M Company, 1987c                                  |
|                                                                           | 96-h LC50    | 440 <sup>C</sup>     | 3M Company, 1974b                                  |
|                                                                           | 96-h LC50    | 843 <sup>C</sup>     | 3M Company, 1985                                   |
|                                                                           | 96-h LC50    | > 500 <sup>D</sup>   | EnviroSystems, Inc., 1990a                         |
|                                                                           | 96-h NOEC    | 500 <sup>D</sup>     | EnviroSystems, Inc., 1990a                         |
|                                                                           | 96-h LC50    | 494                  | Ward et al., 1996a                                 |
|                                                                           | 96h LC50     | 140 <sup>F</sup>     | T.R. Wilbury Laboratories, Inc., 1996a             |
|                                                                           | 30-day NOAEL | > 100 <sup>B</sup>   | EG&G Bionomics Aquatic Toxicology Laboratory, 1978 |
|                                                                           | 96-h EC50    | 391 <sup>G</sup>     | T.R. Wilbury Laboratories, Inc., 1995              |
|                                                                           | 96-h EC50    | 422 <sup>G</sup>     | T.R. Wilbury Laboratories, Inc., 1995              |
| Bluegill sunfish ( <i>Lepomis machrochirus</i> )                          | 96-h LC50    | > 420 <sup>B</sup>   | 3M Company, 1978                                   |
|                                                                           | 96-h LC50    | 569 <sup>B</sup>     | 3M Company, 1978                                   |
| Water flea ( <i>Daphnia magna</i> )                                       | 48-h EC50    | 126 <sup>AB</sup>    | 3M Environmental Laboratory, 1982                  |
|                                                                           | 48-h EC50    | > 1000 <sup>AB</sup> | 3M Environmental Laboratory, 1982                  |
|                                                                           | 48-h EC50    | 221 <sup>B</sup>     | 3M Company, 1987b                                  |
|                                                                           | 48-h EC50    | 292 <sup>D</sup>     | EnviroSystems, Inc., 1990b                         |
|                                                                           | 48-h EC50    | 240                  | Ward et al., 1996c                                 |
|                                                                           | 48-h EC50    | 360 <sup>F</sup>     | T.R. Wilbury Laboratories, Inc., 1996b             |
|                                                                           | 21-day IC50  | 43 <sup>B</sup>      | 3M Company, 1984                                   |
|                                                                           | 21-day NOEC  | 22 <sup>B</sup>      | 3M Company, 1984                                   |
|                                                                           | 21-day NOEC  | 22 <sup>B</sup>      | 3M Company, 1984                                   |
|                                                                           | 48-h EC50    | 41 <sup>G</sup>      | Ward et al., 1995                                  |
|                                                                           | 48-h EC50    | 39 <sup>G</sup>      | Ward et al., 1995                                  |
| Green algae ( <i>Selenastrum capricornutum</i> )                          | 96-h EC50    | 396                  | Ward et al., 1996b                                 |
|                                                                           | 96-h EC50    | >666 <sup>E</sup>    | Ward et al., 1996b                                 |
|                                                                           | 96-h EC50    | 90 <sup>F</sup>      | T.R. Wilbury Laboratories, Inc., 1995              |
|                                                                           | 14-day EC50  | 43 <sup>B</sup>      | Elnabarawy, 1981                                   |
|                                                                           | 96-h EC50    | 2.1 <sup>G</sup>     | Ward et al., 1995                                  |
|                                                                           | 96-h EC50    | 3.6 <sup>G</sup>     | Ward et al., 1995                                  |
|                                                                           | 96-h EC50    | 1.2 <sup>G</sup>     | Ward et al., 1995                                  |

|                                                |             |                    |                                    |
|------------------------------------------------|-------------|--------------------|------------------------------------|
| Bacteria ( <i>Photobacterium phosphoreum</i> ) | 30-min EC50 | 870 <sup>B</sup>   | 3M Company, 1987a                  |
|                                                | 30-min EC50 | 730 <sup>B</sup>   | 3M Environmental Laboratory, 1996a |
|                                                | 30-min EC50 | >500 <sup>D</sup>  | 3M Environmental Laboratory, 1990a |
|                                                | 30 min EC50 | 630                | 3M Environmental Laboratory, 1996b |
|                                                | 30 min EC50 | 390                | 3M Environmental Laboratory, 1996c |
|                                                |             |                    |                                    |
| Activated sludge                               | 7-min NOEC  | 1000 <sup>B</sup>  | 3M Company, 1980b                  |
|                                                | 30-min EC50 | >1000 <sup>B</sup> | 3M Company, 1987d                  |
|                                                | 30-min EC50 | > 500 <sup>D</sup> | 3M Environmental Laboratory, 1990b |
|                                                | 30-min EC50 | > 664 <sup>E</sup> | 3M Environmental Laboratory, 1996d |

\*Values were adjusted to represent 100% active ingredient.

<sup>A</sup>These values may be inconsistent due to different diets tested.

<sup>B</sup>Tested substance was APFO ammonium salt.

<sup>C</sup>Tested substance was APFO

<sup>D</sup>Tested substance was APFO ammonium salt in 50% water.

<sup>E</sup>Tested substance was APFO ammonium salt in 80% water.

<sup>F</sup>Tested substance was APFO in 50% isopropanol.

<sup>G</sup>Test Sample: APFO (44%) in 27.9% water and 27.2% isopropanol

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