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1. INTRODUCTION

Fluorine was first isolated as an element in 1880 by Moisser [1]. Five years later he synthesized the first fluorocarbons through uncontrolled reactions of carbon with elemental fluorine. It was not until the late 1930s that the controlled synthesis of fluorocarbons became possible. In the 1940s, Frigidaire and DuPont developed chlorofluorocarbons, the first commercially available fluorocarbons, for use in refrigeration [1]. During the same period perfluorocarbons, a subclass of perfluorinated organic fluorocarbons with unique properties, were first synthesized to meet the special needs of the Manhattan project [2]. The electrochemical fluorination method for perfluorocarbon production made commercial production of perfluorocarbons possible and opened the door to widespread use of perfluorocarbons [3,4].

Fluorocarbons are wide ranging in their structures and uses. Many commercial applications have been developed for chlorofluorocarbon compounds including refrigeration, degreasing, aerosol dispensing, polymerization, polymer foam blowing, drugs, and reactive intermediates or catalysts. Perfluorocarbons (PFCs) have extensive applications because of their unique physical and chemical properties. These applications include use as artificial blood substitutes, computer coolants, polymers such as teflon, surfactants, lubricants, foaming agents, ski waxes, and in an extensive specialty chemical industry which produces grease and oil repellent coatings for paper and cloth, polymers, insecticides, and a variety of consumer products. Perfluorocarbons are currently being tested as replacements for chlorofluorocarbons in industrial processes and products.

For many years fluorocarbons were generally thought to be nontoxic. Perfluorocarbons were considered to be particularly nontoxic because they were chemically and physically inert and showed low acute toxicity in animals [4]. Recent epidemiological and experimental studies have associated exposure to chlorofluorocarbons, a subclass of fluorocarbons previously classified as nontoxic, with direct and indirect adverse human health effects. Subsequently, researchers and regulators turned their attention to the study of other fluorocarbons. The discovery that one perfluorocarbon, perfluorooctanoic acid

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(PFOA), was present in measurable quantities in residents of several U.S. cities [5-7], the recognition that some perfluorocarbons including PFOA have long half lives in the humans [8] and the observations that PFOA produced toxic effects in animals, including hepatotoxicity, endocrine toxicity, immunotoxicity, and carcinogenesis [9], has led to a re-evaluation of the toxic potential of perfluorocarbons, particularly PFOA, in humans.

Despite widespread exposure to perfluorocarbons, little is known about their effects on human health. It was apparent that additional studies designed to explore their physiologic effects and potential adverse health outcomes and conducted in an occupational cohort with high exposure to PFCs, were necessary. The 3M Chemolite Plant located in Cottage Grove, Minnesota is one of a few PFC production facilities in the world. Biological monitoring data from studies of the Chemolite workforce showed that employees have had high levels and long durations of exposure to PFOA [8, 10]. This occupational cohort provided the opportunity to study the effects of PFOA on humans. The specific goals and objectives of this study were:

GOAL 1) To quantify the human effects of perfluorooctanoic acid on the following physiologic parameters:

a) Hormones: free and bound testosterone, estradiol, lutenizing hormone, thyroid stimulating hormone, prolactin, and follicle stimulating hormone.

b) Serum lipids and lipoproteins: cholesterol, low density lipoprotein, high density lipoprotein, and triglycerides.

c) Hematologic parameters: hemoglobin, mean corpuscular volume, white blood cell count, polymorphonuclear leukocyte count, band count, lymphocyte count, monocyte count, platelet count, eosinophil count, and basophil count.

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d) Hepatic enzymes: serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, gamma glutamyl transferase, and alkaline phosphatase.

OBJECTIVE 1: to conduct a cross-sectional study of production workers to estimate the relationships between total serum fluoride, a surrogate assay for prefluorooctanoic acid, and physiologic parameters.

GOAL 2)To quantify the mortality in an occupational cohort with long term exposure to perfluorooctanoic acid production.

OBJECTIVE 2: to conduct a retrospective cohort occupational study to assess the mortality experience of workers using expected mortality based on Minnesota mortality rates.

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2. REVIEW OF THE LITERATURE

2.1 Introduction

The presence of small amounts of fluoride in human blood was recognized in 1856 [11]. More than 100 years later, Taves [5,6] presented evidence that fluorine exists in two major forms in humans and animals; in a free ionic state and in a covalently bound organic state. Prior to this report, it was assumed that fluorine existed primarily as inorganic ionic fluoride in biological systems. Taves' observations have since been confirmed by several other investigators [12-16]. The discovery that organofluorine compounds constitute the majority of fluorine found in humans focused research on characterizing these undefined compounds. Guy identified a perfluorinated compound, perfluorooctanoic acid (PFOA), as a major constituent of the serum organic fluorine fraction [7,17]. Perfluorooctanoic acid (PFOA) is the only organic fluorine compound to be identified in human serum [18]. The recognition of human and animal toxicities associated with perfluorochemicals [9,19], has renewed interest in understanding the human health effects of perfluorocarbons (PFC), particularly PFOA.

2 Organic Fluorochemicals

Organic fluorochemicals, otherwise referred to as fluorocarbons, are compounds composed of fluorine, carbon and other elements such as oxygen, nitrogen and sulfur. Perfluorocarbons have structures analogous to hydrocarbons, except the hydrogens are exhaustively replaced by fluorine [20]. A limited number of organic fluorochemicals occur in nature [21-23], however no PFCs occur naturally [24,25].

The first report of the synthesis of a fluorocarbon was published in 1890 when Moissan claimed to have purified carbon tetrafluoride. It is likely he isolated fluorographite, however [1]. Pure carbon tetrafluoride was not obtained until 1930 [26]. Work by Ruff and the Belgian chemist, Swarts, in the late 19th and early 20th centuries laid the foundation of organic fluoride chemistry. Midegly and Henne extended Swarts' work and reported the synthesis of dichlorodifluormethane,

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Cl_2F_2, in 1930 [27]. This chlorofluorocarbon with the trade name Freon 12 is an inert, non-toxic refrigerant which was vastly superior to other refrigerants available in the 1930s. After commercial production of Freon 12 began in 1936, it rapidly became a major industrial chemical [2, 26]. A number of cholorfluoromethanes and chlorofluoroethanes have been produced on a commercial scale in many regions of the world. These chlorofluorocarbons have been used in large amounts as aerosol propellants and degreasers, in addition to their use as refrigerants. Currently, their production is being reduced as a result of their ozone depleting properties [28, 29].

In 1937, Simons and Block developed a method to produce laboratory quantities of perfluorocarbons, such as C_3F_8, C_4F_{10}, cycloC_5F_{10} and cycloC_6F_{12} [2, 3]. The analysis of these compounds led to the understanding that many of the structures of saturated hydrocarbons could be replicated in the form of perfluorocarbons. Research in the area of perfluorocarbons was stimulated by two developments. First, Plunkett discovered the polymer, polytetrafluoroethylene, or Teflon [1]. Second, the development of perfluorocarbon chemistry was stimulated by the U.S. effort to develop atomic weapons during World War II under the Manhattan Project. The ^{235}U isotope of uranium was required for the development of atomic bombs. One method of uranium isotope separation was gaseous diffusion. The only volatile uranium compound available for use in this diffusion process was uranium hexafluoride, UF_6, an extremely reactive gas. Materials were needed for use as coolants, lubricants, sealers and buffer gases in equipment exposed to this highly reactive gas [1, 2, 26]. Perfluorocarbons prepared by Simons were found to be inert to UF_6. This discovery led to a research effort directed toward understanding the properties of a variety of perfluorocarbons and developing commercial methods for preparation of perfluorocarbons. The development by Simons of the electrochemical fluorination (ECF) was a major milestone in the fluorochemical industry. Since World War II there has been much interest and work in this new branch of organic chemistry based on perfluorocarbons.

The use of Simons' ECF method has allowed the production of a wide variety of perfluorocarbons including perfluorinated alkanes, alkenes, ethers, esters, amides, sulfonamides and compounds with cyclic and ring structures [2]. The 'inert' perfluorocarbons are compounds made up of only carbon and fluorine. This class

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of compounds ranges from carbon tetrafluoride to complex multiple ring structures such as perfluorodecalin. Perfluorinated surfactants include carboxylic acids, sulfonic acids, and their derivatives. These compounds form the basis of an extensive fluorochemical industry. A variety of perfluorinated polymers and elastomers exist. The most widely used are polytetrafluoroethylene and Kel-F, a elastomer of vinyldiene fluoride and hexafluoropropylene.

2.3 Physical Properties

Perfluorooctanoic acid is a straight chain eight carbon carboxylic acid with a molecular weight of 414.16. The melting point of POFA is 59-60°C. Its boiling point is 189°C at standard conditions [30]. Perfluorooctanoic acid is produced as a complex mixture of branched chain isomers. In practice, all eight carbon carboxylic acid isomers are refered to as PFOA. The ammonium salt of PFOA (APFOA) is the common industrially used form of PFOA. It is a white crystalline powder that easily becomes airborne and sublimes at 130°C.

Perfluorocarbons have unique chemical and physical properties [20, 28, 31, 32]. The importance of perfluorination in producing these properties cannot be overemphasized. Perfluorocarbons are not just another hydrocarbon-like molecule. Chemically, perfluorocarbons are remarkably inert. They are stable to boiling in strong acids and bases. Very few oxidizing or reducing agents react appreciably with perfluorocarbons. Perfluorocarbons that contain other organic molecules such as nitrogen, oxygen and sulfur will participate in reaction at the site of these molecules. For instance, perfluoroctanoyl sulfonic acid will react and form the sulfonamide derivative. The amide portion of this molecule can then be conjugated with many other organic compounds. The perfluorinated portion of these larger molecules remains non-reactive.

Perfluorocarbons are heat stable. They can be heated to greater than 250°C without breakdown. At high temperatures, greater than 400°C, some compounds will breakdown. For example, PTFE, breaks down to perfluoroisobutylene (PFIB), an extremely toxic gas [1]. Because most perfluorochemicals are heat stable they are used in high temperature applications.

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The inert perfluorocarbons are excellent insulators. Polymers, such as PTFE, and inerts PFCs, such as perfluorohexane, are used in electrical applications because of their superior dielectric properties. Their heat stability and insulation properties make perfluorocarbon materials the insulators of choice [20].

Perfluorinated surfaces are the most non-wettable and non-adhesive surfaces known [20, 26]. Fluorochemical surfactants are some of the most potent surface active agents yet discovered [31]. Very low concentrations of fluorochemical surfactants effectively reduce the surface tension at interphase boundaries.

Most perfluorocarbons are poorly soluble in both aqueous and organic solutions. They form a group of fluorophilic compounds, however some perfluorocarbons with functional groups such as the salts of PFOA, are highly water soluble [31, 32]. Perfluorocarbon liquids dissolve oxygen avidly. This unique property is the basis for the use of perfluorocarbons as blood substitutes [33].

Perfluorinated carboxylic and sulfonic acids are some of the strongest organic acids known [31]. The pK_a of PFOA is 2.5 [34]. Thus, when in physiologic solutions, they exist in primarily anionic forms. The anionic forms have a strong propensity to form complex ion pairs[*].

In the past, some investigators have assumed that the chemical and physical properties of many fluorocarbons is synonymous with lack of activity in biologic systems [35, 36]. However, abundant evidence exists that their chemical and physical inertness does not imply biologic inertness [19, 30, 37, 38].

2.4. Synthesis

Synthesis of fluorocarbons has been accomplished using four major methods; electrochemical fluorination (ECF), direct fluorination, teleomerization, and catalytic methods using high valence heavy metals. The ECF was developed by Simons in 1941 [3]. The Simons process is the oldest commercial technique and remains a commercial method to obtain many perfluorocarbons. A solution of

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[*] personal communication from James Johnson, 3M Corporation

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organic substrate is electrolyzed in anhydrous HF at a low voltage, high current, nickel anode. The products of these electrolysis cell reactions are largely perfluorinated. The spectrum of material produced by the ECF process is defined by the starting material. Commercial products from this process include perfluoroalkanes, perfluoroalkyl ethers, perfluoroalkenes, perfluoroalkyl esters, perfluorotrialkyl amines, perfluorocarboxylic acids and perfluorosulfonic acids [2]. Products of ECF often include a significant proportion of complex isomers and fragmentation products. For example, ECF production of PFOA from straight chain octanoic acid produces 30% complex branch chain isomers [39]. The mixture of products from each ECF run is unpredictably variable. These isomeric mixes are difficult to separate and purify [33]. Workers producing PFCs using ECF may be exposed to a complex mixture that changes composition over time.

Direct fluorination is another method used to produce perfluorocarbons. It is not subjected to the impurity problems associated with the ECF process. Direct fluorination reacts fluorine gas with hydrocarbon substrate. Because fluorine gas is extremely reactive, direct fluorination is a technically difficult process and has only recently been pilot tested for commercial production of fluorocarbons.

World production of fluorocarbons is limited to a handful of commercial plants. The 3M Corporation operates PFC production plants in Minnesota, Illinois, Alabama and Antwerp, Belgium. A plant in Italy owned by a Japanese and Italian consortium produces limited amount of fluorocarbons. Perfluorocarbons are also produced in Germany and have been produced, in the past, in the former Soviet Union.

2.5 Sources Of Organic Fluoride Exposure

Guy [17] presented possible candidates for the organic fluorine constituents of human blood based on observation made during the isolation of PFOA from serum. The organic fluorine was not likely to be a macromolecule such as a protein or nucleic acid, because of its solubility in organic solvents such as ether or chloroform/methanol. It was not covalently bound to albumin since it was removed on charcoal at pH 3 at room temperature. The solubility characteristics suggested that multiple compounds existed with different polarities. The major

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compound was a polar lipid like molecule that was identified as PFOA. Other less polar compounds appeared to be present. This data suggests that fluorocompounds other than PFOA were bound to albumin. These compounds were not esters of C_{13} - $_{18}$ fatty acids and were less polar than PFOA. Perfluorooctanyl sulfonamide (PFOS) and its derivative compounds fit this description and may be constituents of the organic fluorine fraction. Although exposure is probably low, the properties of PFOS suggest that it may accumulate to measurable levels.

In contrast to ionic fluoride, little has been reported concerning the organic fluorine content of water and beverages. The fluorine content of ground water is essentially all in ionic form. Some fluorochemicals, such as the perfluorinated carboxylic acid surfactants and their salts, are soluble in water. Such water soluble compounds may locally contaminate surface and ground water near industrial plants that use these compounds. Other perfluorinated compounds such as the alkanes, alkenes, and ethers are fluorophilic and are insoluble in aqueous solutions. Although data on the oral organic fluorine intake is limited, it is unlikely that water and beverages are significant sources of organic fluorine in humans.

The diet as a source of the organic fluorine found in human serum has been the subject of speculation [5, 6, 18, 40]. Non-perfluorinated fluorocompounds have found in biological systems. Marais showed that fluoroacetate was the compound responsible for toxicity from the poisonous plant *Dichapetalum cymosum* [41]. Other investigators have found plant species that synthesize fluoroacetate, fluorocitrate, and monofluorinated fatty acids. Peters reported that a few toxic plants produce fluoroacetate [42]. Fluoroacetate and fluorocitrate have been found in beans grown in high fluoride soil [23]. Peters [21] and Lovelace et al. [22] have reported the occurrence of fluorocitrate in a few plants and foods. In animals, the metabolic activation of fluoroacetate into (-)-erythro-fluorocitrate blocks the transport of citrate into the mitochondria and citrate breakdown by aconitase [42, 43]. Other omega-fatty acids with even numbers of carbon atoms are highly toxic as a result of oxidation that produces fluoroacetate. Fluorocitrate also undergoes rapid defluorination in rat liver in the presence of glutathione (GSH) [44]. Given the low environmental levels, the infrequent occurrence, the toxicity, and the rapid

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metabolism of these compounds in mammalian species, it is unlikely that these monofluorinated compounds contribute substantially to the organic fluorine content in humans.

Taves measured the organic and inorganic fluorine in 93 food items [45]. No significant organic fluorine was found in the tested foods. Ophaug and Singer tested a market basket of food. They concluded that there was no significant organic fluorine content in food. Although food and beverages generally do not contain PFCs, it is possible that they may be contaminated by fluorochemical packaging materials. Water and grease repellent coatings in packaging material could leach into food items in small quantities. This could occur when materials that are not designed for microwave use are used in microwave ovens. Studies have not been reported that quantify human exposures from food packaging sources.

Perfluorocarbons are contained in many consumer products. Fluorocarbon surfactants such as PFOA, PFOS, and it's derivatives are present in window cleaning products, floor waxes and polishes, fabric and leather coatings and carpet and upholstery treatments [20]. Additionally these compounds are used to coat food wraps and are incorporated into plastic food storage bags. Fluorocarbons are the basis for a new generation of cross country ski waxes. Teflon and Teflon related products are widely used as lubricants, electrical insulators, heat and chemical stable gaskets and linings and in non-stick cookware. Fluoroalkanes such as perfluorohexane are being evaluated as CFC replacements. If perfluorohexane or other fluorocarbons are used as replacements for CFC's, consumer exposure from aerosols and other products will increase dramatically. PFC's have several experimental medical uses including use as blood substitutes, x-ray and magnetic resonance imaging contrast agents [46], vitreous replacement and in liquid ventilation therapeutic methods [47]. Recently, a potent fluorocarbon insecticide has been marketed to control fire ants [48].

Perfluorocarbons have a variety of industrial uses. Teflon and other polymers are used where heat stable and chemically inert liners, gaskets and lubricants are necessary. In addition, they are used as electrical insulators both in solid and

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liquid form and used as inert non-conductive liquid coolants in electrical devices such as Cray supercomputers. Perfluorinated surfactants are important fire suppression materials. Perfluorocarbons have been used to control the metal vapors in electroplating processes and to prevent the release of toxic gases\ from landfills[20]. Perfluorocarbons are being considered to replace CFC's in many processes such as refrigeration, polymer foam blowing and building insulation. New applications are being continually developed for these unique compounds, making increased exposure to workers probable.

2.6 Toxicokinetics of PFOA

Since Taves and Guy's observations, perfluorocarboxylic acids, perfluorosulfonic acids and their derivatives have been the subject of numerous toxicokinetic and toxicodynamic studies in animals. These studies have focused primarily on two compounds, PFOA, and perfluorodecanoic acid (PFDA).

Perfluorooctanoic acid or its salts are well absorbed by ingestion, inhalation or dermal exposure. Absorption has been studied primarily in rats, although a number of other species have been studied.

Five male and five female rats were exposed to airborne APFOA for one hour. In this experiment the nominal air concentration of ammonium perfluorooctanoate was 18.6 mg/l. No animals died during the inhalation exposure or the 14 day post exposure observation period. Pooled serum samples contained 42 ppm of organic fluorine for males and 2 ppm for females. Inorganic fluoride content was 0.02 ppm for males and 0.01 ppm for females [9]. Kennedy and Hall [38] studied the inhalation toxicity in male rats of ammonium perfluorooctonate using both single dose and repeated dose schedules. They found a LC_{50} of 980 mg/m^3 for a 4 hour exposure placing PFOA in the moderately toxic by inhalation category. Following ten repeated doses at levels of 1.0, 7.6, and 84 mg/m^3 blood ammonium PFOA levels were obtained. At the 1.0 mg/m^3 level PFOA levels were 13 ppm, at the 7.6 mg/m^3 level PFOA levels were 47 ppm and at 84 mg/m3 level PFOA levels were 108 ppm. Therefore it appears that PFOA is well absorbed by inhalation. It should be noted that the exposures were to APFOA dust, the likely form for occupational exposure.

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Ammonium perfluorooctonoate in food and PFOA administered by gavage in propylene glycol or corn oil vehicles are well absorbed in rats. In an acute oral LD50 study [9], rats displayed a dose dependent spectrum of toxicities indicating that PFOA was absorbed after ingestion. PFOA levels were not measured in this study. In a subacute oral toxicity study, rats were fed PFOA for 90 days [9]. Serum concentration of organic fluorine showed a dose response relationship in both sexes. A marked gender difference in organic fluorine levels was observed. Males had organic fluorine 50 times higher than females at each dose level.

Studies have since demonstrated excellent oral absorption of PFOA in a variety of species including rats, mice, guinea pigs, dogs, hamsters and monkeys [9, 19]. Of most immediate relevance to humans have been studies in a small number of rhesus monkeys [9]. In a 90 day oral toxicity study, monkeys were given 3, 10, and 30 mg/kg/day doses of APFOA. 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. Because all but 1 monkey died at the 30 and 100 mg/kg/day dose levels, only 1 serum sample from a male monkey in the 30 mg/kg/day dose group was available. In this monkey the serum level of PFOA was 145 ppm. In the 30 and 100 mg/kg/day dose group mean liver levels were greater than 100 ppm. Thus, the oral route of absorption may be a significant contributor to the body burden of PFOA in exposed workers.

Dermal absorption of PFOA has been studied in rats and rabbits. Ammonium perfluorooctanoate is a fine white powder that may come into contact with skin and be absorbed. In rats dermally exposed to ammonium perfluoroctonate at 4 dose levels, PFOA was absorbed in a dose dependent fashion [37]. In single dose dermal exposure experiments using rabbits, PFOA appeared to be absorbed. Levels of fluorine were not measured, but dose dependent toxic changes were noted [9]. In a multi-dose experiment, ten male and ten female rabbits were injected dermally with a 100 mg/kg dose of PFOA on a five day a week schedule for two weeks. Total serum fluorine levels were increased in a dose-dependent fashion. Dose-dependent changes in weight were noted [49]. From these studies,

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it appears that dermal exposure to the salts of PFOA are absorbed in animals. In the past, Chemolite workers have been exposed to large dermal doses of ammonium perfluoroctonate. 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. The role that dermal exposures currently play in PFOA absorption at Chemolite has not been well studied.

Once absorbed, PFOA enters the plasma probably by diffusing as a neutral ion pair. In plasma, PFOA is strongly bound to proteins in the serum with more than 97.5 percent in bound form [50]. It is likely that albumin is the major site for high affinity binding [5-7, 50-54]. There does not appear to be a sex difference in protein binding [50, 54]. Hanhijarvi et al. have suggested that protein binding is saturable in rats [55]. Using human serum, Ophaug and Singer [39] found that PFOA was 99% protein bound at PFOA levels up to 16 ppm total fluorine, however. Guy suggested that perfluorocarboxylic acids bind to albumin in a similar fashion to fatty acids [24]. This hypothesis is consistent with the results of several studies. Taves observed that the organic fraction of serum co-migrated with albumin during electrophoresis [6]. Dialysis and ultrafiltration studies observed the retention of organic fluorine during dialysis and ultrafiltration [7, 17, 56]. Belisle and Hagen reported that PFOA appeared to be strongly protein bound in human serum [51]. Extraction of PFOA from acidified water is quantitatively complete using hexane. When PFOA is extracted from plasma, recovery is only 35 percent. Plasma appeared to complex PFOA and PFDA. The partitioning of the bound into organic phase during extraction was more difficult and necessitated the use of more polar solvents. Klevens [53] suggested that CF_2 and CF_3 groups complex with polar groups that are present in the amino acids in proteins such as albumin. In protein precipitation studies using bovine serum albumin, PFOA bound to albumin at an estimated 28 binding sites per molecule [52]. Nordby and Luck studied the precipitation of human albumin by PFOA. Under acidic pH conditions, PFOA produced reversible precipitation of albumin [57] by binding to high affinity sites. These studies do not rule out significant binding to other plasma proteins or erythrocyte components. In studies using serum protein electrophoresis, the protein bound organic fluorine was distributed in a diffuse

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pattern [6, 17] suggesting that PFOA protein binding may be nonspecific. The large amount bound to albumin may reflect the abundance of albumin in plasma and serum.

In rats, PFOA is distributed to all tissues studied except adipose tissue. The highest concentrations of PFOA are in the serum, liver, and kidneys. Ylinen et al. [34] studied the disposition of PFOA in male and female rats after single and 28 day oral dosing. After a single dose of 50 mg/kg, PFOA was concentrated in the serum. Twelve hours after dosing 40% of the PFOA dose was found in the serum of males and 10% in females. Males retained 3.5% of the dose in serum after 14 days. PFOA was retained in the liver for much longer than in serum. In females, the half-life of PFOA in liver was 60 hours compared to 24 hours in serum. In males the half-life was 210 hours in liver and 105 hours in serum. It is noteworthy that PFOA was not found in adipose tissue in detectable quantities. After 28 days of PFOA treatment, PFOA was distributed to the following sites in decending amounts: serum, liver, lung, spleen, brain, and testis. Again, no PFOA was found in adipose tissue. The distribution of PFO from serum to the tissues occurred in a dose dependent manner for females. In male rats, the concentrations of PFOA in testis and spleen followed a dose dependent trend. The levels in male rat serum and liver was the same for the 10 mg/kg and 30 mg/kg dose group. Johnson and Gibson [58, 59] studied the distribution of ^{14}C labeled ammonium perfluorooctonoate after a single iv dose in rats. Their findings were similar to those of Ylinen et al. The primary sites of distribution were the liver, kidneys, and plasma. Other sites, including adipose tissue, had less than 1% of the administered dose. The level of PFOA in the testis of male rats was not reported. As discussed previously, the 90 day oral toxicity study in rhesus monkeys showed that the relative amounts of PFOA in serum and liver was different in monkeys compared to rats. In the low dose group of monkeys (3 and 10 mg/kg/day) serum had 5 to 10 times the PFOA levels found in liver. However, at higher dose levels, the PFOA levels were equally distributed. Additionally, no sex differences were noted in the monkeys liver and serum PFOA levels.

There is no evidence that perfluorinated compounds including PFOA are biotransformed by living organisms. Several studies have examined whether

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PFOA is conjugated or incorporated into tissue constituents such as triglycerides or lipids. Ylinen et al. found no evidence in Wistar rats for metabolism or incorporation of PFOA into lipids [34]. Although the lipid content in PFOA treated rats was different than that in untreated rats, Pastoor et al. did not find evidence for PFOA incorporation into lipids or of metabolism [60] Vander Heuval et al. showed that PFOA was not incorporated into triacylglycerols, phospholipids, or cholesterol esters in the liver, kidney, heart, fat pat, or testis of male or female rats [61]. No evidence has been found that PFOA is conjugated in phase II metabolism [61]. Kuslikis et al. studied the formation of activated coenzyme A (CoA) derivatives of PFOA using rat liver microsomes. They found no evidence for the formation of a CoA derivative.

Sex related differences in the toxicokinetics of PFOA have been reported for rats. The mechanism of PFOA excretion appears to be species-dependent since these gender differences are not seen in mice, monkeys, rabbits, or dogs [9, 62]. The half-life of PFOA in female rats has been estimated to be less than one day [39], whereas the half-life of PFOA in males is five to seven days [34, 38]. It is of note that PFDA does not exhibit this gender difference [63]. It is hypothesized that the sex differences in sensitivity to the toxicities of PFOA are as a result of the slower excretion of PFOA in male rats compared to female rats. Investigators have reported that rats have an estrogen-dependent active renal excretion mechanism for PFOA which can be inhibited by probenecid [50, 54]. As noted previously, females have a much shorter half-life than male rats. The half-life in males can be reduced by castration or estrogen administration. It can be reduced to the female half-life by a combination of castration and estrogen treatment. Estrogen administration alone is almost as effective as the combination of castration and estradiol treatment in reducing the PFOA half-life. This treatment increased the renal excretion of PFOA in male rats to those observed in female rats. Other investigators have reported that the gender difference in half-life depends on a testosterone mediated increase in PFOA tissue binding [64]. This hypothesis is consistent with the gender difference in tissue half-life discussed previously [34]. Johnson has suggested that the primary method of excretion in intact males is via the hepatobiliary route [58, 59]. He reported that cholestyramine enhanced the fecal elimination of carbon 14 labeled PFOA in male rats. These data suggest there was biliary excretion with enterohepatic circulation of PFOA, particularly in

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male rats. However, in a male worker with high serum PFOA levels who was treated with cholestyramine, little if any change in excretion of PFOA was noted. In this study PFOA was excreted slowly in the urine.

In humans, the half-life of PFOA appears to be extremely long and is not sex dependent. Ubel and Griffith [8] reported kinetic data for one highly exposed worker. At the time he was removed from exposure his serum organic fluorine was 66 ppm, 80 percent of which was PFOA. Over the next 18 months his organic fluorine level decreased to 39 ppm. Urinary excretion of PFOA fell from 387 micrograms/24 hours to 80 micrograms/24 hours. The decline in organic fluorine levels was consistent with two compartment kinetics, with a calculated half-life of 2 to 5 years. Additional unpublished biological monitoring data from three Chemolite workers is consistent with the 2 to 5 year half-life. In the Chemolite workforce, male and female workers employed in jobs with similar PFOA exposure have increased PFOA levels. Since men and women with similar exposures have similar levels, a large gender difference in PFOA toxicokinetics is unlikely. Therefore, the relevance of the rat data in assessing the effects of PFOA in humans is questionable.

2.7 Toxicodynamics of PFOA

2.7.1 Male Reproductive Toxicities

Both PFOA and PFDA have been found to produce significant toxicities in the reproductive systems of male rodents [19, 63, 65]. The testis has been reported as the target organ of toxicity for both PFOA and PFDA [19, 66]. Additional evidence exists suggesting that these compounds affect the function of the hypothalamic-pituitary-gonad axis (HPG) [19, 65].

Perfluorodecanoic acid, but not PFOA, has been shown to produce degenerative changes in rat seminiferous tubules that could progress to tubular necrosis. Van Rafelghem et al. reported that a single ip dose of 50 mg/kg of PFDA, produced degenerative changes in rat seminiferous tubules 8 days after injection [66]. Similar but lesser changes were noted in the seminiferous tubules of hamsters and guinea pigs treated in the same manner. They reported no such change in

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treated mice. Bookstaff and Moore [65] did not observe similar changes in rats treated with 20-80 mg/kg of PFDA. They used a different strain of rats in their experiments which is less susceptible to testicular toxicants than those used by Van Rafelghem et al. Thus, the effects of perfluorocarboxylic acids on seminiferous tubules may be limited to a specific compound, PFDA, in a specific strain of rats. The effects observed by Van Rafelghem et al. in other species were not consistent and did not demonstrate a dose-response relationship. In monkeys treated orally with PFOA, no compound related histopathologic changes in the seminiferous tubules were noted [8].

In a two year rat feeding study, PFOA treated animals were observed to have increased numbers of Leydig cell tumors[*]. Male and female rats were fed PFOA containing diets resulting in a mean intake of 1.5 and 15 mg/kg/day. A statistically significant increase in Leydig cell adenomas of 0%, 7%, and 14% in the control, low dose, and high dose groups, respectively, was observed at the end of the two year study. The result was statistically significant as a result of the unexpectedly low number of adenomas in control animals. Historically, CD rats experience a lifetime mean Leydig cell incidence of 6.3 percent with a range of 2 to 12 percent. The high dose group incidence is outside the expected range and may represent a compound related effect. Although the evidence was not definitive, it suggested that PFOA may alter the histology as well as the function of Leydig cells in rats. Perfluorooctanoic acid was not mutagenic in the standard tests including the Ames assay using five species of *Salmonella typhimurium* and in *Saccharomyces cerevisiae* [9]. Mammalian cell transformation assays using C3H 10T 1/2 cells were also negative [67]. These data suggest that PFOA is not a genotoxic xenobiotic. The increase in Leydig cell tumors may be the result of an epigenetic mechanism.

The observation that rats fed PFOA for 2 years had an increased incidence of Leydig cell adenomas prompted researchers to examine the hormonal effects of PFOA in male rats [19]. Adult male CD rats were treated orally with PFOA in doses of 1 to 50 mg/kg. Serum estradiol levels were elevated in the rats treated with more than 10 mg/kg of PFOA. In the highest dose group estradiol was 2.7 times

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[*] Report: 3M Riker Laboratories. Two Year Oral Toxicity/Carcinogenicity Study of FC143 in Rats #281CR0012,1983

greater than the estradiol levels in pair fed control group rats. Serum testosterone levels were significantly decreased in a dose dependent manner when compared with *ad libitum* feed control animals. No significant differences were observed between the high dose rats and their pair fed controls, however. No significant differences were noted in serum luteinizing hormone (LH) levels. Additionally, the accessory sex organ relative weights of the highest group were significantly less than those of their pair-fed controls.

In order to clarify the site of PFOA action, Cook [19] conducted a set of challenge experiments in PFOA treated rats. The results of these experiments demonstrate that the altered testosterone levels were PFOA related. Human chorionic gonadotropin (hCG) challenge can be used to identify abnormalities in the steriodogenic pathway. Human chorionic gonadotropin binds to the LH receptors on Leydig cells and stimulates sex steroid hormone synthesis [68]. Abnormalities in Leydig cell function can be detected by challenging Leydig cells with hCG and measuring steroid hormone production. Similarly, abnormalities in pituitary secretion of gonadotropins can be identified using a gonadotropin releasing hormone (GnRH) challenge that stimulates LH release [69]. Hypothalamic dysfunction can be identified using a naloxone challenge to stimulate GnRH release [70]. In rats treated with PFOA for 14 days at the same dose level as the initial experiment, the Leydig cell production of testosterone was significantly blunted after hCG challenge in the highest dose group compared to ad libitum fed controls. A small, non-significant blunting of the testosterone production in response to GnRH and naloxone was observed. Following GnRH and naloxone stimulation, LH levels were not significantly different in the treatment and control animals. The hCG challenge showed that the decrease in testosterone in PFOA treated rats resulted from altered steroidogenesis in the Leydig cell. The results from the GnRH and naloxone stimulation were not definitive. The results were compatible with an effect at the pituitary level as well as at the Leydig cell level. Cook et al. examined the site at which testosterone steroidogenesis was affected by PFOA. Progesterone, 17 alpha-hydroxyprogesterone and androstenedione were measured after hCG challenge. Progesterone and 17 alpha-hydroxyprogesterone were unaffected. Androstenedione levels were significantly decreased in PFOA treated rats compared to controls. Given that the conversion of 17 alpha-hydroxyprogesterone to androstenedione by C17/20 lyase is

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necessary for testosterone synthesis, these results suggest that decreased testosterone is the result of a block in this conversion step. In hCG stimulated rat Leydig cells, the 17 alpha hydroxylase/C-17/20 lyase is inhibited by estradiol. Taken together, these data are consistent with the hypothesis that the elevated estradiol levels associated with PFOA treatment inhibit the C-17/20 lyase enzyme and thereby depress testosterone levels. Cook et al. suggested that the blunted response of LH to low testosterone may be mediated, in part, by elevated estradiol levels. A subtle hypothalamic or pituitary effect may also be present, however. The mechanism for the estradiol elevation was not studied.

Perfluorodecanoic acid alters reproductive hormones in male rats in a fashion similar to PFOA. In male rats treated with doses of PFDA ranging from 20 to 80 mg/kg, given as a single ip dose, PFDA decreased plasma androgen levels in a dose dependent fashion [65]. Both plasma testosterone and 5-alpha dihydrotestosterone were significantly reduced. Compared to *ad libitum* fed control rat values, mean plasma testosterone was decreased by 88 percent in PFDA treated animals and DHT was decreased by 82 percent. These changes were reflected in accessory sex organ weight and histology. The changes in accessory sex organs after PFDA administration were found to be reversed by testosterone replacement. The PFDA decrease in androgens was the result of decreased responsiveness of Leydig cells to LH. There was no evidence for altered metabolism of testosterone. Additionally, plasma LH concentrations did not increase appropriately in the face of low plasma testosterone concentrations. This suggests that PFDA may alter the normal feedback mechanisms of the HPG axis.

It is of interest to note that 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD), which, like PFOA, is a nongenotoxic rat carcinogen, a peroxisome proliferators, and an inducer of P-450 system, has been shown to produce hormonal effects in male rats similar to those observed for PFOA and PFDA. Moore et al. [71] studied the effect of TCDD on steroidogenesis in rat Leydig cells. Exposure of cell to TCDD resulted in depression testosterone and 5-alpha-DHT concentrations without altering LH concentration or testosterone metabolism. Moore concluded that TCDD treatment inhibits the early phase of the synthetic pathway and the mobilization of cholesterol to cytochrome $P450_{scc}$. However, Moore et al.

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observed decreased estradiol. TCDD has been shown to increase the estrogen mediated feedback inhibition of LH secretion [72] Additionally, in studies using MCF-7 breast tumor cells, the antiestrogenic effect of TCDD was mediated by alterations in the cytochrome P450 metabolism of estradiol [73]. The decreased testosterone in rats could be mediated by the effect of TCDD on Leydig cells directly, by alterations in testosterone metabolism, or through increased negative feedback at the pituitary or hypothalamic level. Recently, reports from occupational studies of TCDD exposed workers have associated TCDD exposure with hormonal alterations in human males. Egeland et al. [74] reported that men with high TCDD levels had significantly depressed serum testosterone levels. The changes in testosterone were not associated with altered LH values. Estradiol values were not reported. They concluded that dioxin has a similar effects in men and male rodents. The obvservations that PFOA, PFDA, and TCDD have overlapping spectrums of rodent toxicities suggests that peroxisome proliferators, inducers of the P-450 system and non-genotoxic carcinogens may also alter the hypothalamic -pituitary-gonad function in male animals.

2.7.2 Female Reproductive Toxicities

In the two year rat feeding study, female rats treated with PFOA were observed to have an increased number of mammary fibroadenomas compared to control animals. All mammary carcinomas occurred in control animals. Hyperplasia of the ovarian stroma was observed, but specific histopathological studies were not reported* . No information is available concerning the effect of PFOA and PFDA on HPG axis in women or female animals.

2.7.3 Thyroid Toxicities

Altered thyroid hormone dynamics have been observed in rats exposed to PFDA [75-78]. A single ip dose of PFDA in rats results in a rapid and persistent decrease in thyroxin (T4) and T_3 [78]. Gutshall reported that the decrease in thyroid hormones occurred as early as eight hours after treatment and persistent for at least 90 days [79]. These changes were associated with a hypothyroid-like state in

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* Report: 3M Riker Laboratories. Two Year Oral Toxicity/Carcinogenicity Study of FC143 in Rats #281CR0012,1983.

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the treated rats. The alterations in serum thyroid levels occurred at dose levels that did not produce a hypothyroid syndrome [78]. Animals with depressed T4 levels were found to be metabolically euthyroid [77]. Replacement of T4 resulted in normal food intake, but did not reverse the hypothyroid-like syndrome of hypothermia and bradycardia [76]. This suggests that PFDA has a marked effect on cellular metabolism that is independent of its effect on thyroid homeostasis. The low T4 was thought to be a result of two mechanisms. First, PFDA readily displaces T4 from albumin which results in increased metabolic turn over of the hormone. Second, the response of the hypothalamic-pituitary-thyroid (HPT) axis appeared to be depressed as assessed by thyrotropin releasing hormone simulation testing [75]. In these studies, the animals had increased levels of thyroid responsive hepatic enzyme activities suggesting that the PFDA treated rats were not functionally hypothyroid. The histological appearance of the thyroid glands were unremarkable, although treated rats had significantly lower thyroid weights. TSH levels were not studied. No similar studies are available for PFOA. PFOA has been noted to produce a transient weight loss in treated rats [30]. The hypothyroid-like syndrome observed in PFDA treated rats has not been studied in PFOA treated rats, however. Since the thyroid hormone effects of PFDA do not cause the hypothyroid-like state in rats, PFOA may alter the HPT axis without producing this syndrome.

2.7.4 Hepatic Toxicities

The primary site of PFOA toxicity in rodents is the liver. Peroxisome proliferation (PP), induction of enzymes involved in β-oxidation of fatty acids, and induction of cytochrome P450 occur after a single PFOA dose. Marked hepatomegaly has been noted coincident to the PP and enzyme induction. Increased liver size was the result of a combination of both hypertrophy and hyperplasia. Cell hypertrophy predominated after an initial burst of cell proliferation. The initial hyperplasia is evidenced by large hepatocytes and markers of DNA synthesis [80]. Areas of increased necrosis in the periportal regions have been observed [81].

The relationship between hepatic enlargement, peroxisome proliferation, and increased β-oxidation is unclear. Xenobiotic induced changes in one specific peroxisomal enzyme are not necessarily linked to changes in other peroxisomal

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enzymes or hepatic enlargement [82]. Studies have suggested that xenobiotic induced hepatomegaly and PP may be related to the endocrine status of experimental animals or to oxidative stress [80, 83-86]. Adrenal and thyroid hormones may play a role in peroxisomal proliferation. [80, 85]. Studies of clofibrate, a PP, have shown that endocrine manipulation can modify its hepatic effects. In adrenalectomized and thryoidectomized rats, clofibrate-induced hepatomegaly was reduced compared to the effect in control rats [83, 84]. Conversely, in thyroidectomized or hypophysectomized rats, clofibrate induced peroxisomal ß-oxidation enzymes were increased compared to normal rats [83]. Thottassery et al. compared the PFOA-induced hepatomegaly in normal rats, adrenalectomized rats and adrenalectomized rats with cortisol replacement [80]. They found that hepatomegaly was cortisol dependent and was primarily a result of hepatocyte hypertrophy. Hyperplastic responses were also cortisol dependent and were noted in periportal regions of the liver. Peroxisomal proliferation did not depend on cortisol and was observed in centrilobular regions. They concluded that PFOA-induced hepatomegaly and peroxisome proliferation were separate processes.

In oral feeding studies, PFOA and other PP were reported to cause increased hepatomegaly in males compared to females. This difference could be reduced by exogenous estradiol administration or castration and eliminated by castration and estradiol administration [54]. These observations may be explained by an estrogen dependent renal excretion mechanism or a testosterone mediated increase in tissue binding [85, 87, 88]

Issemann and Green have cloned a mouse PP activated receptor, mPPAR, a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors that is activated by peroxisome proliferators [89]. This receptor directly mediates the effects of peroxisome proliferators (PPs). Tugwood has shown that PPs activated PPAR recognizes a specific response unit on the Acyl-CoA oxidase gene promoter in a manner similar to the steroid hormone receptor [90]. The action of PFOA and other PPs may be mediated by a family of cytosolic receptors that regulate gene transcription in a manner similar to other nuclear hormone receptors.

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2.7.5 Nongenotoxic Carcinogenesis

In initiation, selection, and promotion experiments in rats, PFOA produced an increased number of hepatocellular carcinomas [91, 92] Several mechanisms for PFOA associated nongenotoxic carcinogenesis have been suggested.

Perfluorooctanoic acid is an archetypal member of a unique sub class of PPs that are not metabolized. Reddy has argued that the structurally diverse peroxisome proliferators (PP) are a distinct class of nongenotoxic carcinogens [86]. Reddy proposed that PPs induce oxidative stress which results in increased tumor formation. According to this theory, the observed increase in hydrogen peroxide formation associated with increased ß-oxidation is not associated with an increase of similar magnitude in detoxifying catalase activity [86]. Oxidative attack by hydrogen peroxide and other reactive oxygen species on cell constituents and membranes leads to DNA damage and increased cell proliferation. Increased proliferation in concert with DNA damage produces increased cell transformation and malignancies.

Studies testing the theory that PFOA induces HCC by increasing oxidative stress have lead to conflicting results. Takagi et al. observed an increase in 8-hydroxydeoxyguanosine in liver DNA from rats exposed to PFOA. They concluded that rat hepatocytes were under increased oxidative stress [93]. Handler et al. found no increase in hydrogen peroxide production in intact livers exposed to PFOA [94]. Lake et al. failed to find an association between hepatic tumor formation and peroxisome proliferation [95]. Thottassery et al. observed that the PFOA induction of ß-oxidation was independent of adrenal hormone status. A PFOA associated increase in catalase activity depended on cortisol [80]. Therefore, the hormonal status in animals used in experiments could confound studies of oxidative stress and account for the conflicting results.

2.7.6 Immunotoxicity

In the 90 day monkey feeding study, bone marrow and lymphoid tissue were a site of histopathology [9]. Treated monkeys in the highest two dose groups were observed to have moderate hypocellularity of the bone marrow. Specific

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histopathological findings were not reported. Atrophy of lymphoid follicles in lymph nodes and the spleen were noted in the same treatment groups. No follow-up studies of these observations have been reported. Studies in PFOA treated rats have not shown histological changes in the immune system [9].

2.7.7 Mechanisms of Action

The mechanism of toxicity of perfluorinated surfactants may be mediated by their effect on cell membranes. Olson and Andersen [30] suggested that PFOA may alter membrane function through changes in fatty acid composition and oxidation status. Levitt and Liss hypothesized that the effect of perfluorinated surfactants is mediated by their alteration of membrane organization or fluidity [96, 97].

Shindo [32] reported that miscibility of fluorocarbon and hydrocarbon surfactants depends strongly on carbon chain length. A carbon chain length greater than eight carbons is necessary for immiscibility. Perfluorocarbon surfactants with eight or fewer carbon atoms are miscible with hydrocarbon surfactants with carbon chain lengths up to nine. These observations could have important implications for biological systems that contain fluorocarbon surfactants. Cellular membranes are a phase boundary, usually between a lipid phase and an aqueous phase. Surfactants will segregate to this phase boundary. Two immiscible surfactants may form two coexistent monolayers on the inside and outside of the membrane whereas miscible surfactants will form only one such monolayer. The presence of two monolayers will maximally reduce the surface tension at the boundary, whereas a single monolayer will affect surface tension to a lesser degree. Changes in surface tension may alter membrane fluidity and affect its function in such processes as signal recognition and transduction. It is interesting to note that the change in miscibility in Shindo's experimental system occurred for fluorocarbon surfactants with carbon chain lengths greater than eight. This change in miscibility depended on hydrocarbon surfactant chain length as well.

The effects of PFOA and PFDA on experimental membrane systems and cellular membranes have been investigated. Inoue studied the differential effects of octanoic acid and perfluorooctanoic acid on experimental cell membrane

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properties [98]. The phase transition temperature of dipalmitoylphosphatidylcholine vesicles decreased linearly as PFOA increased in concentration up to one mM and then reach a plateau. This suggested that PFOA may form aggregates in the membrane above a critical concentration. Such a phase separation is observed to occur in micelles [32]. The partition coefficient between water and the membranes for PFOA, K = 8910, was larger than the coefficient for ionized octanoic acid, K = 135, possibly because of the difference in hydrophobicity between hydrocarbon and fluorocarbon chains in aqueous solution. The differences between the toxicokinetics and toxicodynamics of PFOA and PFDA may be the result of their differing miscibilities with cell membrane surfactants.

Levitt and Liss investigated the effect of PFOA and PFDA on the plasma membranes of cells from F4 human B-lymphoblastoid cell line using the dye merocyanine 540 (MC540) [97]. The dye binds to phospholipids that are loosely packed on the outer cell membrane, but does not bind to highly organized lipids and does not penetrate the membrane of healthy cells [99]. A large decrease in MC540 cell surface binding was observed after treatment with sub-lethal concentrations of PFOA and PFDA but not other non-perfluorinated fatty acids. Albumin or serum reduced the change in MC540 binding. This effect may be a result of the strong protein binding of PFOA and PFDA by albumin [50]. These observations suggest that PFOA and PFDA either interact directly with MC540 lipid binding sites or alter the structure of the lipids in the membranes.

In experiments examining functional changes in the lymphoblastoid cell lines, Levitt and Liss observed that PFOA and PFDA could cause direct damage to cells resulting in the release of membrane bound cell proteins and immunoglobulins in soluble form [96]. PFDA was significantly more potent than PFOA in solublizing proteins and killing cells. This may be the result of different miscibilities in the cell membrane of these compounds. However, neither PFOA nor PFDA reduces the ability of surface immunoglobulins to migrate and undergo capping after antigen recognition [97]. In the PFOA concentration ranges that decreased MC540 binding, PFOA did not affect immunoglobulin migration and capping. Capping involves the cytoskeletal mediated polar migration of immunoglobulins within the plane of the membrane [100]. Apparently, the PFOA

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and PFDA associated membrane changes do not affect membrane characteristics that are important for receptor migration.

The membrane effects of PFDA have been studied in greater detail. Pilcher et al. reported that a single injection of PFDA in rats significantly reduced the apparent number of ß adrenergic receptors in cardiac cells [101]. This change in number of receptors was reflected in the diminished response of adenylyl cyclase (AC) to epinephrine in PFDA treated rat cardiac cells. The intrinsic properties of AC were not altered. The action of PFOA was on the epinephrine receptor. The fatty acid composition of the treated rat cardiac cell membranes was significantly altered [101]. Palmitic (16:0) acid was elevated 13 percent, eicosotrienoic (20:3 w6) was elevated 71 percent, and docosahexaenoic acid (22:6 w3) was elevated 18 percent. Arachidonic acid (20:4) was reduced by 18 percent. Several other investigators have reported changes in membrane function following PFDA exposure. Wigler and Shaw [102] demonstrated that PFDA inactivated a membrane transport channel for 2-aminopurine in L 5178 Y mouse lymphoma cells. *In vitro* experiments reported by Olson et al. [103] showed that erythrocytes exposed to PFDA exhibited decreased osmotic fragility and increased fluidity. Taken together, these studies indicate that perfluorinated surfactants exert their effects on cell membranes. The effects appear to be limited to the outer portion of the membranes as the result of differential partitioning within the membrane or binding to specific membrane constituents. Although PFOA and PFDA can be cytotoxic as a result of their detergent action on membranes, their membrane effects at lower doses are not related to their detergent action. From available data, it appears that functional membrane changes may be limited to specific receptor mediated functions.

2.8 Occupational Fluorine Exposures At Chemolite

In workers employed in fluorochemical production plants, blood organic fluorine has far outweighed ionic fluoride [8, 12, 14, 51, 56]. More than 98 percent of the total fluorine in these groups has been reported to be organic fluorine. Therefore, the use of total fluoride levels, which consist predominantly of organic fluorine compounds, is a valid surrogate for organic fluorine in occupationally exposed groups. In workers at the Chemolite plant, PFOA has been identified in the serum

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of these workers and was estimated to account for 90 percent of organic fluorine found in the serum samples [8]. In this cohort of workers, total fluorine is a good surrogate measure for PFOA.

Industrial hygiene measurement of fluorochemicals have been conducted at the Chemolite plant since the 1970s [8]. These measurements include area samples, personal breathing samples and surface wipe samples. In 1977, a comprehensive effort at evaluating fluorochemical exposures was conducted at the Chemolite plant. During certain operations breathing zone PFOA concentrations were as high as 165 ppm. After extensive engineering control alterations, the plant was serially re-surveyed. In general, airborne exposures were below the recommended limit of 0.1mg/m3. However, there was evidence of surface contamination in production buildings [8]. In 1986, airborne PFOA, as well as breathing zone samples were less than 0.1 mg/m^3 based on 8 hour time weighted averages. Levels as high as 1.5 mg/m^3 were measured in breathing zone samples during certain clean-up and maintenance zone samples. Perfluorobutyric acid was also found, but in much lower concentrations. Spray dryer operators had consistently higher exposures, even following extensive equipment improvements. *

It appears that airborne exposure to PFOA was low for most workers. Spray dry operators and workers involved in clean up and maintenance activities have higher intermittent exposures. Although personal protection devices are required in high exposure jobs, worker compliance has not been evaluated. The role that surface contamination plays in worker exposure has not been defined* . The route of PFOA exposure in worker has not been clearly identified.

2.9 Epidemiological Studies

A retrospective cohort mortality study of employees at the Chemolite Plant in the period of 1948-1978 was conducted by Mandel and Schuman [6]. Of the 3,688 male employees who were employed for at least 6 months, 159 deaths were identified. There was no excess mortality in the employees as compared to all

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* personal communication from Stan Sorenson, 3M Corporate Medical Department
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cause or cause specific mortality in the U.S. white male population. The subcohort of all chemical division workers did not show any all cause or cause-specific excess in mortality.

Starting in 1976 medical surveillance examinations were offered to Chemolite employees in the Chemical division [8]. Approximately 90 percent of the workers participated in the program. No health problems related to the exposure to fluorocarbons were encountered in participants. Serially conducted surveillance examinations have failed to reveal any relationship between blood levels of organic fluorine and clinical pathology [*].

2.10 Summary

Animal studies have suggested that there are five areas of toxicity associated with PFOA exposure. These include hepatotoxicity, immune system alterations, reproductive hormone alterations, Leydig cell adenomas, and non-genotoxic hepatocarcinogenicity. Toxicity studies have primarily used rodents. There is considerable variability between strains of rats for some of the toxic endpoints such as Leydig cell adenomas. Additionally, some of the effects seen in rats have not been seen in other rodent species such as mice, hamsters or guinea pigs. The limited data available on PFOA exposed rhesus monkeys and occupationally exposed workers suggests that any extrapolation of the results from rodent experiments to humans requires more information about the mechanism of PFOA toxicity. From this data it does not appear that the liver is a major site for PFOA toxicity in humans. Of greater human health concern are the potential effects on the immune system and the reproductive hormones.

In the past, workers have been found to have significant blood levels of PFOA. Many workers have levels above one ppm. These blood levels are 50-1000 times background levels in the general population. These levels may be high enough to produce toxicities in occupationally exposed humans. A confident estimate of risk cannot be made until further information on the adverse health effects of PFOA exposure in humans is obtained.

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[*] personal communication from Larry Zobel; 3M Corporation Medical Department

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