FLUOROCARBONS AND HUMAN HEALTH: STUDIES IN AN OCCUPATIONAL COHORT

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ABSTRACT

Perfluorooctanoic acid (PFOA) has been reported to be a nongenotoxic hepatocarcinogen and reproductive hormonal toxin in rats. Although PFOA is the major component of total fluorine in humans, little information is available concerning human toxicities. The health effects of PFOA were assessed in two studies conducted in occupationally exposed workers. The associations between PFOA and reproductive hormones, hepatic enzymes, lipoproteins, hematology parameters, and leukocyte counts were studied in 115 male employees. Serum PFOA was positively associated with estradiol and negatively associated with free testosterone (TF) but was not significantly associated with luteinizing hormone. The negative association between TF and PFOA was stronger in older men. Thyroid stimulating hormone and PFOA were positively associated. PFOA and prolactin were positively associated in moderate drinkers. The effect of adiposity on serum glutamyl oxaloacetic and glutamyl pyruvic transaminase decreased as PFOA increased. The induction of gamma glutamyl transferase by alcohol was decreased as PFOA increased. The effect of alcohol on HDL was reduced as PFOA increased. A positive association between hemoglobin, mean cellular volume, and leukocyte counts with PFOA was observed. These results suggest that PFOA affects male reproductive hormones and that the liver is not a significant site of toxicity in humans at the PFOA levels observed in this study. However, PFOA appears to modify hepatic and immune responses to xenobiotics. A retrospective cohort mortality study of 2788 male and 749 females workers employed between 1947-1984 at a PFOA production plant was conducted. Overall, there were no significantly increased cause specific SMRs. Among men, ten years of employment in PFOA production was associated with a significant three fold increase in prostate cancer mortality compared to no employment in production. Given the small number of prostate cancer deaths and the natural history of the disease, the association between production work and prostate cancer must be viewed as hypothesis generating and should not be over interpreted. If the prostate cancer mortality excess is related to PFOA, the results of the two studies suggest that PFOA may increase prostate cancer mortality through endocrine alterations.

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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

(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.

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.

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,

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

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, 26, 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.

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

* personal communication from James Johnson, 3M Corporation

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

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

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

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 perfluoroctonate 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.

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 LD_{50} 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,

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

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

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

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

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

* 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

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.

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

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

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 ß-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 ß-oxidation is unclear. Xenobiotic induced changes in one specific peroxisomal enzyme are not necessarily linked to changes in other peroxisomal

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.

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

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

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

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

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 [8]. 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

* 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.

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

3. METHODS

3.1 Introduction

The effects of perfluorooctanoic acid (PFOA) exposure on human health were studied in employees of the 3M Chemolite plant (hereafter referred to as Chemolite) located in Cottage Grove, Minnesota. Two studies were conducted to investigate of the human health effects associated with PFOA exposure. First, mortality associated with occupational PFOA exposure was studied using a retrospective cohort design. Second, a cross sectional study design was used to estimate the relationships between PFOA exposure and selected physiologic parameters.

A retrospective cohort study was designed to examine mortality among workers. All workers ever employed at the Chemolite plant for greater than six months were included in the cohort. All causes and cause-specific mortality were compared to expected mortality. Expected mortality was calculated by applying sex and race specific quinquennial age, calendar period, and cause-specific mortality rates for the United States and Minnesota populations to the distribution of observed person-time [104, 105]. Age adjusted standardized rate ratios were calculated [106]. A relative risk (RR) for PFOA exposed workers compared to unexposed workers was calculated using proportional hazard regression models [107]. The RR were stratified by gender and adjusted for age at first employment, duration of employment and calendar period of first employment. Any significant differences between observed and expected cause-specific mortality were to be explored using nested case control studies. Case studies were completed for causes of death with 5 or more deaths and standardized mortality rates greater than 1.5. Each deceased individual's record was examined for commonalties in job history information including age at first employment, calendar period of employment, years in the Chemical Division, and duration of employment.

Selected physiologic effects of PFOA exposure were studied using a cross sectional study design. The relationships between total serum fluorine and biochemical parameters including reproductive hormones, hepatic biochemical parameters, lipid and lipoprotein parameters, and hematologic parameters, were

explored. A sample of the work force employed on November 1, 1990 was invited to participate. All employees in high exposure jobs were asked to participate. A sample of workers employed in low exposure jobs was frequency matched to the age and sex distribution of the high exposure group. Each participant completed a questionnaire which included medical history and information concerning alcohol, tobacco, and medication use. The questionnaire is provided in Appendix 3-1. Blood was drawn for determination of hematologic and biochemical parameters. Total serum fluorine, free (FT) and bound testosterone (BT), estradiol (E), thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), prolactin (P) and luteinizing hormone (LH) were assayed. The PFOA-hormone dose-response relationship for each hormone was estimated using linear regression techniques to adjust for the effects of age, sex, body mass, alcohol consumption, tobacco use, and other potential confounders. The PFOA-hormone dose-response relationship was further explored by fitting linear multivariate models to hormone ratios. All unique ratios between the seven hormones were defined . Twenty-one hormone ratios were calculated for each participant. The prevalence of hormone values outside the laboratory reference range for men was compared to the expected prevalence assuming a normal distribution for assay values.

3.2 Retrospective Cohort Mortality Study

3.2.1 Definition Of The Cohort

The Chemolite facility opened in 1947. Individuals who were employed at the Chemolite plant between January 1, 1947 and December 31, 1983 were identified from company records. Workers with fewer than six months employment were excluded. In October 1951 large scale commercial PFOA production facilities became operational (Abe 1982). Because large scale PFOA production did not begin until 1951, a second cohort with potentially significant PFOA exposure was defined as those workers employed between October 1, 1951 and December 31, 1983. Subjects with greater than six months employment were included in this second PFOA cohort.

The cohort was initially assembled in 1979. Subsequently, the cohort was updated to include new employees through 1983. Personnel records for employees working prior to 1979 were coded for demographic items and work history by trained abstractors. Computerized corporate personnel databases were utilized to provide information for workers employed in the 1979 to 1983 period. Abstracted work history included year of first employment, year of last employment, years employed at Chemolite, and months worked in the chemical division. Individual job histories were not abstracted because job titles were defined by wage grades and did not correspond to specific jobs or locations within the plant.

3.2.2 Study Databases And Files

A Chemolite cohort database was created on a VAX computer using Ingres software. Data stored on magnetic tape were transferred to the VAX. Duplicate records were identified and removed. Missing data were identified. The Ingress update function was used for data editing. Final analytic files for the Monson program, SAS programs, and custom programs were constructed using the Ingress report writer.

3.2.3 Data Editing

The Ingres relational database allowed extensive internal consistency checks to be made. All dates were checked for plausibility. Those records with implausible, inconsistent, or improperly formatted dates were edited and corrected if information was available. Records of workers with fewer than six months employment were flagged and excluded from the analytic data set. A random check of 50 of the 364 workers with fewer than six month employment found no errors in classification of employment length. Extensive attempts were made to obtain all missing data items. Sources of information included plant personnel records, corporate personnel databases, benefit records, archived corporate records, plant medical records, and death certificates. No individual employees or next-of-kin were contacted. Four employees were excluded from the cohort as a result of missing demographic data items.

3.2.4 Validation Of The Historical Cohort Information

3.2.4.1 Assessment Of Completeness Of Ascertainment

The cohort was initially defined from personnel records stored at the Chemolite plant. Complete records were maintained on all workers ever employed at the plant. Hourly and salaried workers were included in these files, as were all transferred, terminated and retired former employees. Records for workers first employed in the 1947-1978 period were abstracted from documents, coded and computerized. A corporate computerized database was used to update the cohort through December 1, 1983. Since insufficient induction time had lapsed between 1983 and 1989, no new employees or work history information was added to the cohort database for the post 1983 period for this study.

Verifying the ascertainment of all eligible cohort members was problematic. The assumption that the personnel records represented a complete roster was difficult to check because of a lack of independent information. Several sources were used to exclude major errors in the enumeration of the cohort. The historical plant hiring pattern based on seniority dates was compared with the distribution of dates of first employment. Qualitatively, dates of major plant expansion corresponded to peaks in the distribution of dates of first employment and to seniority dates. Large increases in hiring due to new plant openings were reflected in peaks in the distribution of starting dates in the cohort. A sample of 25 annuity beneficiaries retired from the Chemolite plant were obtained from the corporate personnel office. All 25 were found to be included in the enumerated cohort.

Several plant personnel record systems were randomly sampled. Separate files were maintained for active workers, retirees, transferred and terminated workers, and workers whose employment at Chemolite ended prior to 1960. A sample of records for current employees with start dates prior to December 31, 1983 was compared to the cohort. All 12 records from the 1945-1960 period for start dates were found in the cohort database. Of 30 records sampled from the 1961-1969, 28 (93%) were included in the cohort. Fifty two records had starting dates in the 1970-1978 period. Of these 52 records, forty seven (90%) were found in the

database. In the 1979-1980 period 18 of 44 (41%) records were in the database. Lastly, in the 1981 through 1983 period, 36 of 37 records were in the database (97%). The low ascertainment for workers first employed in the 1979-1980 period was further examined. Of the 34 workers not in the cohort database, 16 (47%) were first employed in the 7/79-1/80 period. These omissions occurred in the transition period between document abstracting and electronic updating of the cohort. Using seniority lists, 44 workers currently employed were hired between 1979 and 1980. They represent approximately 1% of the total number of individuals in the workforce and less than 0.5% of the total person time at risk for the cohort. Records for retired workers were sampled from files containing all workers retired from Chemolite. Forty seven of the 48 (98%) sampled records were present in the database. A sample of the files containing the personnel records of employees completing employment before 1960 was randomly drawn. Of the 67 selected records, 65 (97%) were in the database. Finally, files containing records of all transferred, terminated, or disabled employees were randomly sampled. Of the 120 sampled records, 116 (97%) were present in the cohort database.

3.2.4.2 Validation Of Cohort Information

Information in the edited database was compared to information in the personnel records. A random sample of 25 records was drawn from the personnel files. Database names, social security numbers (SSN), dates of birth (DOB), and dates of employment were verified against record information. The sole error occurred in coding the last digit of one SSN. All other information was correctly entered into the database.

The reliability of ICD8 coding of death certificates for underlying cause of death was evaluated by resubmitting a sample of death certificates for coding by the same nosologist. The sample consisted of 25 death certificates from 1970 -1989. No change in the major categories of cause of death was noted. All cancer deaths were coded concordantly. Within cardiovascular causes of death, two certificates were discordant.

3.2.5 Vital Status Ascertainment

The vital status was ascertained from the Social Security Administration (SSA) and the National Death Index (NDI). All individuals with unknown vital status were traced successfully and vital status determined. Vital status determination in the 1979-1989 period was obtained through the NDI. Death certificates were requested from the appropriate state health departments for those individuals identified as, or presumed to be, deceased. A professional nosologist coded the death certificates for underlying cause of death according to International Classification of Diseases, 8th revision (ICD8). Information concerning the date and cause of two deaths which occurred outside the United States was obtained from family members or other available sources. Date of death and the ICD8 code for the underlying cause of death were entered into the database.

3.2.6 Validation of Vital Status Ascertainment

The vital status determination procedures for the cohort was evaluated. Corporate benefit records were utilized as an independent source for vital status among the retirees. Vital status from the database was compared to vital status in corporate records. A list of all retirees in the 1947-1984 cohort was sent to 3M benefits department. These individuals were matched to retirees who had received 3M death benefits. 3M records were not complete for periods prior to 1975. In the pre-1983 period, 4 deaths in retirees were identified by 3M records. Vital status was correctly ascertained by the SSA matching procedurefor only one of these retirees. In the 1983-1989 period, 34 deaths in retirees were identified in 3M records. The NDI matching procedure ascertained all 34 of these deaths. The NDI was not available for 1990. 3M records indicate that 8 retirees died during 1990. The incomplete SSA ascertainment in the period 1975 to 1983 resulted in extending the NDI search to include 1979 to 1983. All 3M identified deaths were also identified in the subsequent NDI search covering the 1979 to 1983 period.

3.2.7 Analysis

Analytic methods employed in this study were appropriate for cohort studies. The relative risk was estimated by calculating an adjusted standardized mortality ratio

(SMR) [105]. This study used both national and Minnesota mortality rates for comparisons. Mortality for men in the Chemolite cohort was compared to expected national and Minnesota mortality, adjusted for age, calendar period, sex and race. The use of mortality rates in the rural counties surrounding the plant were not considered to be stable for many causes of death and were not used. Since less than one percent of plant employees are non-white, white male and female rates were used for comparison. For women, only U.S. rates were used because cause- and calendar period-specific Minnesota rates were not available. SMRs were calculated for all cause, all cancer, and cause-specific mortality. The effects of disease latency, duration of employment, duration of follow-up, and work in the Chemical Division were examined using stratified SMR analyses.

Three additional methods of analysis were used to assess the validity of the SMR contrasts. The three methods were: standardized rate ratios (SRR) [106], Mantel Haenszel adjusted relative rates (RR_{MH}) [108], and proportional hazard regression adjusted RR [107].

Limited exposure data were available from plant records. Exposed workers were defined as all workers who worked for 1 month or more in the chemical division. Exposed and unexposed workers' all cause, all cancer, and cause-specific mortality was compared using stratified SMRs, SRRs [106], and stratified Mantel Haenszel analysis [108, 109]. Additionally, the same summary measures were calculated contrasting the rates for workers with at least ten years duration of employment and those with less than ten years employment.

The relative risk (RR) and 95% CI for the RR for deaths from all causes, cancer, cardiovascular diseases, and selected specific causes were estimated using a proportional hazard model (PH) [107, 109]. The time to event or censoring was defined as time from first employment to event or December 31, 1989. In PH models for specific causes of death, deaths from other causes were censored at the time of death. Exposure was quantified by months of chemical division employment. Covariates included in the models were age at first employment, year of first employment, and duration of employment. The analyses were stratified by gender. The appropriateness of the proportional hazard assumptions were tested using stratified models with graphical analysis of log (-log(survival))

versus follow-up time relationships and models that tested the significance of a product term between exposure and log(follow-up time) [109, 110].

3.3 Cross Sectional Study Of PFOA Exposed Workers

3.3.1 Population Definition And Recruitment

Medical screening of workers employed at the Chemolite plant occurs every two years. The general medical screening program included a medical questionnaire (Appendix 3-1), measurement of height, weight and vital signs, pulmonary function evaluation, urinalysis, serum assays, and hematology indices. This screening program offered an opportunity to assess the physiologic effects of PFOA exposure in workers engaged in commercial production of a limited spectrum of PFCs. Of particular interest were the effects of PFOA, the primary fluorochemical found in the serum of Chemolite workers. (Griffith and Ubel, 1980).

Participation in the Physiologic Effects Study required the subjects' willingness to undergo hormonal and biochemical testing and to have an additional 15 ml of blood drawn for total fluorine assay. In the cross-sectional study, exposure classification was based on the potential for PFOA exposure in a workers job and plant location. All workers engaged in any facet of PFOA production in the previous five years were considered to have potentially high PFOA exposure. The jobs considered to have high exposure potential included all jobs in the production buildings (bldg 6 and 15), all maintenance workers who were assigned to the PFOA production areas, and all management jobs requiring physical presence in the production building. Plant records and job history information was used to assign exposure status to individual workers. A random sample of workers in jobs with low exposure potential was frequency matched to the age and sex distribution of the high exposure workers. Workers with low exposure potential were defined as those assigned to jobs not involved in the production of PFCs for at least five years. A roster of workers meeting the low exposure potential was defined from plant records and knowledge of plant personnel about the location of high exposure jobs. A gender stratified sample from the group of workers in low exposure jobs with an age (5 year strata) distribution similar to the exposed group was identified and invited to participate. If a worker in a job with

low exposure declined to participate, another worker in the same age and sex stratum was randomly selected and invited to participate. In all cases informed consent was obtained. Participation in this study was voluntary.

3.3.2 Data Collection

3.3.2.1 Study Logs And Files

A roster of participants was maintained by the plant occupational health nurses. A log for biological sample information was completed by the laboratory technician. The date and time of ample coolection was recorded. Quality assurance samples were recorded on a separate log. Results reported on paper records were maintained as medical records. Results for other tests were transmitted electronically to a computerized database and coded as SAS datasets. All records were stored with employee medical records or in the corporate medical offices for confidentiality purposes. Printed laboratory results and questionnaire data were entered into a SAS dataset.

3.3.2.2 Questionnaire

Each participant completed a medical questionnaire prior to reporting to the plant medical office. (Appendix 3-1) Items included demographic information, symptoms, illness history and diagnoses, and medication usage. Detailed questions concerning tobacco use and alcohol use were included. Workers were not re-contacted to obtain missing information or to correct inconsistencies. Responses were not validated. Two plant occupational health nurses collected the questionnaires and returned them to the corporate medical department. In the corporate medical office, data were coded and entered into a SAS data base.

3.3.2.3 Laboratory Procedures

3.3.2.3.1 Height and Weight

Upon reporting to the plant medical office, participants had their height and weight determined by an occupational health nurse. Height and weight were measured once on the same calibrated scale.

3.3.2.3.2 Blood

3.3.2.3.2.1 Drawing And Handling

Four vacutainers of blood were drawn from a single venipuncture by a laboratory technologist. Two 15 ml red top vacutainers of blood were drawn and allowed to clot. One 10 ml purple top vacutainer was drawn for hematology studies. A specially prepared fluorine free 15 ml vacutainer was used to collect blood for total serum fluorine determination. Venipunctures were scheduled to occur at the same time of day and on the same shift for each worker. All blood was drawn between 6:30 and 8:00 a.m. Workers in the Chemical Division of the Chemolite plant rotate shifts on a weekly basis. Blood was drawn after a worker was assigned to the day shift for at least 3 days.

All specimens were refrigerated at the plant prior to transport to the appropriate laboratory. Clotted red top vacutainer specimens were centrifuged for 12 minutes to separate serum from cells before transport to the contract laboratory. In order to render the total serum fluorine specimens non-infectious, serum for total fluorine assays was ether extracted in the corporate medical department prior to sending the samples to the 3M Chemical Division analytic laboratories.

3.3.2.3.2.2 Assays

Serum samples were analyzed for total serum fluorine, hepatic biochemical parameters, cholesterol, lipoproteins, and seven hormones. Assayed biochemical parameters included serum glutamic oxaloacetic transaminase (SGOT), serum glutamatic pyruvic transaminase (SGPT), gamma glutamyl transferase (GGT), and alkaline phospatase (AKPH). The following hormones were assayed: bound testosterone, free testosterone, estradiol, prolactin, luteinizing hormone (LH), follicle stimulating hormone(FSH), and thyroid stimulating hormone (TSH). EDTA preserved whole blood samples underwent routine hematologic analysis including

complete blood count with erythrocyte indices and leukocyte differential cell count (CBC). Analyses were done without knowledge of the subject status or purpose of the study.

Total serum fluorine was determined in 3M's Chemical Division analytic laboratory using the sodium biphenyl extraction method (Venkateswarlu, 1982). The accurate determination of total fluorine in the parts per million (ppm) range required specialized equipment, procedures, and personnel. Assays were completed in a dedicated laboratory following tested protocols.

Upon receipt of extracted serum samples divided aliquots were frozen at -70 degrees centigrade. After all samples had been received, batches of 15 samples were assayed on successive working days. Each batch included high and low quality control samples. Each sample was assayed twice. If the difference in assayed values was greater than 1 ppm, the sample was re-assayed. The total serum fluorine value was reported as a mean value and a rounded integer value.

Serum glutamic oxaloacetic transaminase (SGOT), serum glutamatic pyruvic transaminase (SGPT), gamma glutamyl transferase (GGT), and alkaline phospatase (AKPH) were assayed by the United Health Services Laboratory in Apple Valley, Minnesota using clinical colorimetric assays. CBCs were determined using automated Coulter counters. Light microscopy was utilized for differential counts.

Estradiol, prolactin, thyroid stimulating hormone (TSH), luteinizing hormone (LH), and follicle stimulating hormone (FSH) were assayed by the United Health Services laboratory using radioimmunoassay (RIA) and enzyme linked immunosorbent assay (ELISA). FSH, LH, and prolactin were assayed using Abbott laboratories IMX microparticle enzyme linked immunoassays. TSH was assayed using London Diagnostics chemiluminescense immunometric assay. Estradiol was determined using Diagnostic Products Corporation's Coat-a-count assay.

Testosterone was assayed by the Mayo Clinic clinical laboratories. Total testosterone was determined by RIA using proprietary immunoglobulins. Free and bound testosterone was determined using equilibrium dialysis.[111].

3.3.2.3.2.3 Quality Assurance

Two methods were used to assess the accuracy and reliability of the laboratory assays. The laboratories routinely followed quality assurance programs. Three standards were run with each batch. If the control values were outside two standard deviations of the intra assay mean value for each standard, the assay was repeated. If 10 controls were outside 1 standard deviation of the mean, the assay was flagged for review. The reliability of each of these assays was assessed. For each assay, five specimens were randomly selected and split into two aliquots. The aliquots were labeled with different identifiers ensuring that the assays were carried out in a blinded fashion. Both aliquots were submitted on the same day to the laboratory. The coefficient of variation was calculated for each hormone.

3.3.3 Analysis

There were two analytic strategies. First, assay results were treated as continuous parameters and modeled using regression methods. Models were fit to assess the relationship between assay results and total fluorine, body mass index, alcohol consumption, and smoking. Second, hormonal assay results were dichotomized into those within the reference range and those outside the reference range. The hormone assay categories were based on published sex specific normal reference values for each assay. The purpose of this dichotomization was to evaluate the possibility that highly susceptible individuals may be affected at lower levels of exposure and not follow the adjusted dose-response curve.

The relationships between total serum fluorine and the assayed parameters were estimated by fitting linear multivariate regression models to the data. The clinical parameters and ratios of selected parameters were first modeled as functions of nominally categorized exposure and covariates. Dependent variables that were

not normally distributed were appropriately transformed. Total serum fluorine was categorized into mutually distinct categories. Cutoff values for the categories were chosen to assure adequate numbers in each category while maintaining the fullest range of exposure values possible. Accordingly, total serum fluorine level categories were defined as the following: less than 1 ppm, greater than 1 ppm toless than 4 ppm, 4 ppm to 10 ppm, greater than 10 ppm to 15 ppm, and greater than 15 ppm. If insufficient numbers of events occurred within individual categories, the number of categories was reduced by combining adjacent categories. Additionally, models were fitted with total serum fluorine entered as a continuous variable using linear, square, square root transformations.

Age, body mass index (BMI), alcohol use and tobacco use were included in the model as potential confounders. Age was included in the models as both a categorical variable and a continuous variable. Age was grouped into four ten year age categories. Age was treated as a continuous variable using linear, square, square root, and log transformations. BMI was entered in the models as a categorical variable and as a continuous variable. BMI categories were less than 25 kg/m^2, 25-30 kg/m^2 , and greater than 30 kg/m^2. Additionally, BMI was dichotomized into obese, greater than 28 kg/m^2, and non-obese, less than or equal to 28 kg/m^2. The continuous variable was entered as linear, square, log, and square transformations. Alcohol use was categorized into 3 categories: less than 1 drink per day, greater than one to 3 drinks per day, and non response to the questionnaire item. Smoking was categorized as current nonsmokers and current smokers. A nonresponse category was not included since only two individuals were in this category. These two individuals were excluded from analyses that required smoking history. Smoking was quantified as cigarettes smoked per day. Linear, square and square root transformations of cigarettes per day were used in regression models.

The choice of the final model was somewhat subjective. For each dependent variable, other covariates were included in the final model if they were potential confounders. Other potential confounding hormones and biochemical parameters were included in the models if they produced significant changes in effect estimates.

Total serum fluorine and confounding covariates were entered into models as continuous variables. Significant nonlinear dose-response relationships were evaluated by comparing model fit and parameter estimates using categorical variables and continuous variables. Square, square root, exponential, and logarithmic transformations were used if the transformed variables produced models of superior predictive power as assessed by model fit. All two way interactions between total serum fluorine and the included covariates were evaluated. Interaction terms were included in the final model if the parameter estimate for the interaction term was significant at the alpha =.10 level.

The potential for susceptibility to confound the relationship between PFOA exposure and the assayed parameters was examined by comparing the observed prevalence of assay results outside of the reference range with the expected prevalence. The prevalence of abnormal assays was based on published reference values for the adult male US population. Reference ranges for test parameters were defined as being within 2 standard deviations above or below the mean value for the parameter. The laboratory maintains laboratory and assay specific reference range for each assay. Given that the distribution of values is approximately normal, about 2.5% of individual values are expected to fall above the upper limit and 2.5% below the lower limit. It follows that the prevalence for a high test is .025. The prevalence for a low value is .025. Using these prevalences, an expected number of tests outside of the reference range can be defined. A priori hypotheses based upon animal and in vitro studies defined the expected direction of the effect. The calculation of an observed to expected ratio allowed the estimation of the relative prevalence for a test outside of the normal range in the study subjects as compared to the general population. The 95% CI for the ratio was calculated assuming that the expected number is a constant and the observed number is a random variable with a Poisson distribution.

4. RESULTS

4.1 Cross Sectional Perfluorocarbon Physiologic Effects Study

In October 1990, at the time of the cross sectional study, the workforce at Chemolite consisted of 880 salaried and hourly employees. There were 50 men and 2 women in high exposure potential jobs. Since there were only 2 women in this group, the study was restricted to males. Forty-eight (96%) of the 50 male workers in high exposure potential jobs agreed to participate. The exact number of low exposure workers invited to participate in the study was not recorded. However, few individuals in this group refused to participate. Thus, it is estimated that over 80% of low exposure workers participated.

4.1.1 Participant Characteristics

Since frequency matching for age was used to select study participants, the overall age distribution reflected the age distribution of workers in high exposure potential jobs (Table 4.1.1). Ages ranged from 24 to 59 years, with a median age of 37 years and a mean age of 39.2 years.

Table 4.1.2 presents the alcohol and tobacco use profile of the study participants. The light drinkers category included 22 participants who reported no alcohol use. Consumption of one to three ounces of ethanol per day was reported by 20 (18.7%) participants. No participants reported drinking greater than three ounces of ethanol per day. Eight workers (7.0%) did not complete this item of the questionnaire. There were 28 (24.8%) smokers who smoked an average of 21.7 cigarettes per day. Smoking status was not available for two workers (1.8%). The association between smoking and alcohol consumption is presented in Table 4.1.3. Thirteen (15.3%) of 85 nonsmokers and seven (25.0%) of 28 smokers reported moderate drinking ($p=.24$). Table 4.1.4 displays the age distribution for alcohol and tobacco use categories. There were no significant differences in mean ages among smoking or drinking categories.

Total fluorine was not significantly correlated with age, BMI, alcohol, or tobacco use (Table 4.1.5). BMI and age were correlated (r=.26, p=.005). Alcohol use and tobacco use were not significantly correlated (r=.08; p>.7).

BMI ranged from 18.8 to 40.5 kg/m^2 with a median value of 26.3 kg/m^2 and a mean of 26.9 kg/m^2 (Table 4.1.6). Half of all workers had BMIs between 25 and 30 kg/m^2 . The mean BMI in smokers was not significantly different from that of nonsmokers (Table 4.17). The mean BMI for moderate drinkers was not significantly different from the BMI of light drinkers. Smoking status and BMI were not significantly associated (Table 4.1.8). There was a significant linear relationship between BMI and age (β=.10 SE(β)=.035). This relationship was not substantially altered after adjusting for smoking status, alcohol use, and total serum fluorine level.

4.1.2 Total Serum Fluorine

The total serum fluorine values ranged from zero to 26 with a median value of two ppm, a mean of 3.27 ppm and a standard deviation of 4.68 ppm (Table 4.1.9). The inter-assay coefficient of variation was 66% calculated from repeated assays on different days.

Twenty-three (20.0%) of 115 workers had total serum fluorine values less than one ppm. This group included eight workers values reported as zero ppm (below the limits of detection). Eighty-eight workers (76.5%) had levels less than or equal to three ppm. Six (5.2%) of 115 workers had values between 10 and 15 ppm and five (4.4%) had values greater than 15 ppm. All workers with levels greater than ten had worked in Building 15, the primary PFC production area at the Chemolite Plant.

There were no significant differences in total serum fluoride mean values among the BMI, age, alcohol use and tobacco use categories (Table 4.1.10). No statistically significant differences in mean age between total fluorine categories were observed (Table 4.1.11).

Participants with less than one ppm total fluorine smoked the least (16.3) number of cigarettes per day (Table 4.1.12). Those with one ppm to three ppm total fluorine smoked the greatest number of cigarettes per day (24.5). This difference was statistically significant ($p<.005$). As estimated in a regression model, the linear relationship between total fluorine and smoking status, adjusted for age and BMI, was small in magnitude ($\beta=0.10$, $SE(\beta)=0.062$, $p=.09$). Smokers average total serum fluorine was estimated to be 0.1 ppm higher than nonsmokers. The number of cigarettes smoked per day was weakly correlated with total serum fluorine (Table 4.1.5).

Drinking status was not associated with total fluorine (Table 4.1.13). Overall, eight (7.0%) participants did not respond to this question. Four had less than one ppm total serum fluorine.

Table 4.1.14 presents the distribution of BMI in the total fluorine categories defined previously. BMI mean values were not significant differences among the total serum fluorine categories. The linear relationship between BMI and total fluorine, adjusted for age, smoking, and alcohol use, was weak and not significant ($\beta=-.016$ $SE(\beta)=.069$, $p>.5$).

4.1.3 Hormone Assays

The intra-assay coefficient of variation (CV) for the bound and free testosterone, estradiol, TSH, LH, prolactin, and FSH assays are provided in Table 4.1.15. The estradiol assay had the highest CV, 18.3%. The prolactin assay had the lowest CV of 3.1%.

Table 4.1.16 presents the observed and expected number of hormone assays out of the assay reference range, the observed to expected (O/E) ratio , and the 95% confidence limits. The O/E ratio was significantly greater than one for estradiol, free testosterone, bound testosterone and prolactin. The O/E ratios for LH, FSH, and TSH were not significantly different from one.

The Pearson correlation coefficients among the seven hormones assayed in study participants are presented in Table 4.1.17. As expected, estradiol was

correlated with free testosterone (r=.40, p=.0001) and bound testosterone (r=.32, p=.0006). Bound testosterone was correlated with free testosterone (r=.74 p=.0001), LH (r=.28, p=.003) and FSH (r=.16, p=.04). LH and FSH were significantly correlated (r=.63, p=.0001). FSH and TSH were significantly correlated (r=.23, p=.01).

As shown in Table 4.1.18, total fluorine was significantly correlated with prolactin (r=.19, p=.045) and TSH (r=.26, p=.005). Age was negatively correlated with estradiol (r=-.25, p=.01), free testosterone (r=-.45, p=.0001), bound testosterone (r=-.24, p=.01), and prolactin (r=-.19, p=.01). Age was positively correlated with FSH (r=.33, p=.0003). As expected, BMI was negatively correlated with free and bound testosterone(r=-.26, p=.005 and r=-.36, p=.0001 respectively). BMI was correlated positively with LH (r=.20, p=.03). Alcohol consumption was significantly correlated with FSH (r=-.24 p=.01).

Bound testosterone ranged from 141 to 1192 ng/dl with a mean of 572 ng/dl and a median of 561 ng/dl (Table 4.1.19). The standard deviations were large. The mean bound testosterone values were not significantly different among the total serum fluorine groups. As expected, the mean bound testosterone decreased significantly as BMI increased. The mean bound testosterone values were significantly different among the age categories (p=.016).

There was a significant nonlinear relationship between total serum fluorine and bound testosterone (BT) in the final regression model (Table 4.1.20). Bound testosterone, which was positively associated with both LH and estradiol, decreased as both age and BMI increased. Alcohol and cigarette use were weakly associated with BT. There was a significant interaction between age and total serum fluorine. There was a negative association between bound testosterone and total serum fluorine in young workers than in older workers. In workers greater than 45 years of age, total serum fluorine was associated with a slight increase in BT. The relationship between bound testosterone and total serum fluorine is presented for four different sets of covariate value (Figure 2). Dose-response curves for bound testosterone were plotted for young, lean individuals aged 30 with BMIs of 25, young obese individuals aged 30 with BMIs of 35, middle aged lean individuals aged 50 with BMIs of 25, and middle aged

obese individuals aged 50 with BMIs of 35. Each of the relationships is for nonsmoking, light drinking men with the sample mean LH value (5.4 mU/l) and mean estradiol value (33.4 pg/ml). In 30 year old workers, bound testosterone decreased as total serum fluorine increased in both BMI groups. The dose-response relationship for 40 year old workers was approximately flat (not shown). In workers greater than 50 year of age, BT increased as total serum fluorine increased.

Total serum fluoride was not significantly associated with free testosterone (FT) (Table 4.1.21). Within BMI categories, free testosterone was highest in the less than 25 kg/m^2 group and lowest in the greater than 30 kg/m^2 category. The difference in mean FT among BMI categories was statistically significant (p=.03).

There was a significant nonlinear dose-response relationship between total serum fluorine and FT in the final regression model (Table 4.1.22). As total serum fluorine increased, free testosterone decreased. There was a significant interaction between age and total serum fluorine. Figure 4.2 illustrates the modifying effect of age on the total serum fluorine free testosterone relationship. The covariate vectors (nonsmoker, light drinker, mean LH and estradiol, age=30 and BMI=25 or 25, age=50 and BMI=25 or 35) were the same as used Figure 1. Lean or obese 50 year old men had low free testosterone (less than 9 ng/dl) for all values of total serum fluorine. In 30 year olds, free testosterone decreased asymptotically toward the 50 year old values. In this model, a 50 year old, obese, moderate drinker with any total serum fluorine level (the lower limit of assay sensitivity was approximately 1 ppm total serum fluorine) had free testosterone below nine ng/dl.

As shown in Table 4.1.23, the estradiol means in the three BMI groups were not significantly different (p=.88). As the age of participants increased, mean estradiol levels decreased. In the greater than 30 to 40 year age group, mean estradiol was 36.8 pg/ml compared to 25.9 pg/ml in the greater than 50 to 60 year age group. The age group means were significantly different (p=.018). There was a nonsignificant positive association between mean estradiol and total serum fluorine.

As shown in Table 4.1.24, estradiol and total serum fluorine were positively associated in the final regression model. Total serum fluorine followed a nonlinear relationship with estradiol. No interaction terms were statistically significant. As expected, free testosterone and estradiol were positively associated (ß=.85 p=.0007). The relationship between total serum fluorine and estradiol is illustrated in Figure 3. The plotted curves depict the relationship for lean (25 kg/m^2) and obese (35 kg/m^2) male workers who were 30 years old with sample mean free testosterone and who were nonsmokers and light drinkers. As total serum fluorine increased over the observed range, estradiol increased quadratically. In obese men (BMI=35 kg/m^2) aged 30, estradiol exceeded 44 pg/ml when total serum fluorine was between 15 and 20 ppm. The highest estradiol levels were in young, obese smokers who consumed 1 to 3 ounces of ethanol per day.

LH was not significantly associated with serum fluorine, but was negatively associated with BMI (p=.003) and positively associated with smoking (p=.025), age, and BT . There was no association between total serum fluorine and FT. (Table 4.1.25, Table 4.1.26, and Figure 4).

FSH was not significantly related to total serum fluorine levels but was positively associated with age (p=.014) (Table 4.1.27, Table 4.1.28). The final regression model for FSH is illustrated in Figure 5. The relationship was essentially flat over the total fluorine range.

TSH was positively associated with total serum fluorine in both univariate and multivariate analyses (Table 4.1.29, Table 4.1.30 and Figure 7). TSH was not significantly related to age, BMI, alcohol use, smoking, and other hormones.

Prolactin was positively associated with total serum fluorine and smoking (Table 4.1.31, Table 4.1.32). Moderate drinkers had a different prolactin-total serum fluorine relationship compared to light drinkers and nonrespondents. Figure 6 illustrates the relationship of prolactin with total serum fluorine and the modifying effect of alcohol use. Total serum fluorine was weakly associated with prolactin in light and moderate drinkers. However, in moderate drinkers (1-3 oz/day), there was a positive association between prolactin and total serum fluorine.

4.1.4 Hormone Ratios

The univariate distributions of the 21 ratios are provided in Appendices 4.1 and 4.2. A table is presented for each of the 21 ratios showing the number of participants, mean ratio value with the standard deviation, median ratio value, and the range of ratio values in each of the previously defined categories of BMI, age, alcohol use, tobacco use, and total serum fluorine

Correlations between total serum fluorine (ppm), age (years), BMI (kg/m^2), alcohol use (oz/day), and cigarette consumption (cigarettes/day) and all possible ratios between E, free testosterone TF, TB, and LH are displayed in Table 4.1.33. The estradiol to bound testosterone ratio (E/TB) and estradiol to free testosterone ratio (E/TF) were significantly correlated with BMI (r=.32, p=.001 and r=.27, p=.004 respectively). The estradiol to luteinizing hormone ratio (E/LH) was negatively correlated with age (r=-.26, p=.005), and positively correlated with BMI (r=.18, p=.06). The bound testosterone to luteinizing hormone ratio (TB/LH) followed a different pattern as compared to E/LH. The correlation coefficient between TB/LH and age was -.32 (p=.001) while the coefficient between TB/LH and BMI was -.14, (p=.13). The free testosterone to luteinizing hormone ratio (TF/LH) had the strongest correlation with age (r=-.40, p=.0001) but was not significantly correlated with BMI. The bound testosterone to free testosterone ratio (TB/TF) followed a unique pattern. TB/TF was positively correlated with age (r=.24, p=.01), and negatively correlated with BMI (r=-.16,p=.08).

Prolactin ratios with bound testosterone (TB/P), free testosterone (TF/P), estradiol (E/P), follicle stimulating hormone (FSH/P), luteinizing hormone (P/LH), and thyroid stimulating hormone (P/TSH) are presented in Table 4.1.34. None of the prolactin-hormone ratios were significantly correlated with total serum fluorine or BMI. All except P/TSH were significantly correlated with cigarette consumption.

Table 4.1.35 presents the Pearson correlation coefficients for the bound testosterone to thyroid stimulating hormone (TB/TSH) ratio, the free testosterone to thyroid stimulating hormone (TF/TSH), and the estradiol to thyroid stimulating

hormone (E/TSH). Total serum fluorine and TF/TSH were negatively correlated (r=-.18,p=.05). All three ratios were significantly and negatively correlated with age. TB/TSH and TF/TSH were negatively correlated with BMI, (r=-.24 p=.01 and r=-.23, p=01 respectively).

The Pearson correlation coefficients for the bound testosterone to follicle stimulating hormone (TB/FSH) ratio, the free testosterone to follicle stimulating hormone (TF/FSH), and the estradiol to follicle stimulating hormone (E/FSH) are provided in Table 4.1.36. Age was the only covariate that was significantly correlated with the three ratios.

The correlation coefficients for selected ratios between pituitary glycoprotein hormones, TSH, LH, and LH, are presented in Table 4.1.37. The thyroid stimulating hormone to follicle stimulating hormone (TSH/FSH), the thyroid stimulating hormone to luteinizing hormone (TSH/LH), and the follicle stimulating hormone to luteinizing hormone (FSH/LH) are provided. Age was significantly correlated with the FSH/LH ratio and the TSH/FSH ratio. Alcohol consumption was correlated with both TSH/FSH and TSH/LH.

As shown in the final regression models, the TB/TF ratio increased as total serum fluorine increased (Tables 4.38 and 4.39). Alcohol consumption, cigarette consumption, estradiol, prolactin, and TSH were not significantly related to the TB/TF ratio in either model. These covariates do not substantially alter the estimated relationship between total serum fluorine and TB/TF ratio when included in the regression model. The quadratic increase of the TB/TF ratio over the observed range of total serum fluorine is illustrated in Figure 4.8. The covariates used were: nonsmoker, less than one ounce of alcohol consumed per day, 30 years of age, and a BMI of 30 kg/m^2.

Table 4.1.40 presents the full regression model for the estradiol to bound testosterone ratio (E/TB). Total serum fluorine was not significantly associated with the E/TB ratio. BMI was a determinant of the E/TB ratio. Free testosterone was negatively related to the E/TB ratio.

The full regression model for estradiol to free testosterone ratio (E/TF) is displayed in Table 4.1.41. There was a significant positive dose-response relationship between the E/TF ratio and total serum fluorine. Although the dose-response relationship for free testosterone was modified by age, the dose-response relationship for the ratio was not modified by age.

As shown in Tables 4.1.42, 4.1.43 and 4.1.44, total serum fluorine was not significantly associated with E/LH and TB/LH, but was positively association with the TF/LH ratio (ß=-.05, p=.09). Bound testosterone and FSH were associated with the TF/LH ratio (ß=.003, p=.0001) and (ß=-.33, p=.0001).

Cigarette consumption and free testosterone were strongly and significantly related to the TB/P ratio (ß=1.49, p=.02 and ß=3.93, p=.008 respectively) (Table 4.1.45). Cigarette consumption and bound testosterone were significantly related to the TF/P ratio (ß=.04, p=.03 and ß=.002, p=.03 respectively) (Table 4.1.46). Only cigarette consumption was significantly related to the E/P ratio (ß=.10, p=.005) (Table 4.1.47).

Tables 4.48 through 4.50 present full regression models for the ratios of prolactin to FSH (P/FSH), prolactin to LH (P/LH), and prolactin to TSH (P/TSH). In each of the three regression models total serum fluorine was positively and significantly associated with the prolactin-hormone ratio. Moderate drinkers had a significantly different ratio total serum fluorine dose-response relationship compared to the relationships in light drinker and nonrespondents.

The full regression models for the glycoprotein hormone ratios are presented in Table 4.1.51 to 4.1.59. As shown in table 4.1.52, total serum fluorine was significantly related to TF/TSH (ß=-.28, p=.03) and bound testosterone and FSH were significantly related to the TF/TSH ratio (ß=.01, p=.006 and ß=.68, p=.04 respectively). Total serum fluorine was not significantly associated with the other glycoprotein hormone ratios.

4.1.5 Cholesterol, Low Density Lipoprotein, High Density Lipoprotein, And Triglycerides

Table 4.1.60 provides the correlation coefficients for serum lipids, specifically cholesterol, low density lipoprotein (LDL), and high density lipoprotein (HDL), with total serum fluorine, age, BMI, alcohol consumption, and cigarette consumption. Total serum fluorine was not significantly correlated with cholesterol, LDL, HDL, or triglycerides. Cholesterol and triglycerides were correlated with age (r=.25, p=.008 and r=.19, p=.04, respectively), and BMI (r=.19, p=.04 and r=.27, p=.004, respectively). Cigarette smoking was positively and significantly correlated with cholesterol (r=.35, p=.0001), LDL (r=.28, p=.002), and triglycerides (r=.19, p=.04). HDL was not significantly correlated with any variable, although the correlation with alcohol consumption was suggestive (r=.18, p=.06).

Total fluorine was not significantly associated with cholesterol, LDL or triglycerides (Tables 4.1.61, Table 4.1.62, Table 4.1.64). Smoking, age, and GGT were positively and significantly associated with cholesterol. Smoking and prolactin were positively and significantly associated with LDL. Smoking and free testosterone were positively associated and bound testosterone was negatively associated with triglycerides.

The final regression model for HDL, displayed in Table 4.1.63, presents a different picture. HDL decreased as total fluorine increased in moderate drinkers. In light drinkers, there was a negligible change in HDL as total fluorine increased. Self-reported moderate alcohol consumption was positively associated with HDL. Additionally, bound testosterone was positively associated with HDL, while free testosterone was negatively associated.

4.1.6 Hepatic Parameters: Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Alkaline Phosphatase (AKPH), Gamma Glutamyl Transferase (GGT).

Table 4.1.65 presents the correlation coefficients between the hepatic parameters, SGOT, SGPT, GGT, AKPH, and total serum fluorine, age, BMI, alcohol consumption, and cigarette consumption. The hepatic parameters were not significantly correlated with total serum fluorine. SGOT was not significantly correlated with any of the participant characteristics. SGPT and GGT were correlated significantly only with BMI (r=.20, p=.02 and r=.27, p=.004

respectively). AKPH was significantly correlated with age, BMI, alcohol consumption, and cigarette consumption.

The correlation coefficients between the hepatic parameters and cholesterol, LDL, HDL, triglycerides, estradiol, TF, TB, and prolactin are displayed in Table 4.1.66. SGOT and AKPH were significantly correlated with prolactin. SGPT was correlated with cholesterol and triglycerides. GGT was correlated with cholesterol, triglycerides, and free testosterone. As expected, SGOT, SGPT, and GGT were highly correlated (Table 4.1.67). AKPH was only correlated with GGT.

The SGOT, SGPT, GGT, and AKPH mean values were not significantly different among the five total serum fluorine categories (Table 4.1.68). SGOT and SGPT mean values were not significantly different for BMI, age, alcohol use, and smoking (Tables 4.1.69 to 4.1.72) . Mean GGT was significantly higher in the greater than thirty BMI group (p=.03). As shown in Table 4.1.72, mean and median AKPH values were significantly higher in smokers compared to nonsmokers (p=.012).

Tables 4.1.73 A, B, and C present three linear multiple regression models for SGOT. In non-obese workers (BMI=25), SGOT decreased as total fluorine increased. In obese workers (BMI= 35), the association between total serum fluorine and SGOT was in the opposite direction. Model 2 included GGT as a covariate (Table 4.1.73 B). The association between total fluorine and SGOT, as well as the effect modification by BMI, were present after adjusting for GGT. When SGPT was included in the regression model (Table 4.1.73 C), the association between total fluorine and SGOT was weak and nonsignificant . The effect modification by BMI was no longer present. AKPH had little effect on the regression estimates when included in the model.

Three linear multiple regression models for SGPT are provided in Tables 4.1.74 A, B, and C. In non-obese workers (BMI=25), SGPT decreased as total fluorine increased. However, in obese workers (BMI= 35), the association between total serum fluorine and SGPT was in the opposite direction. Little change occurred in the estimates after adjusting for GGT. As seen in Table 4.1.74 C, the association was significant, although weaker in strength, after adjusting for SGOT. The effect

modification by BMI was present. When AKPH was included in the model, effect estimates did not change significantly.

The final regression models for GGT, provided in Tables 4.75 A, B, and C, present a different picture. GGT decreased as total fluorine increased in moderate drinkers. In light drinkers, GGT decreased less steeply as total fluorine increased. Controlling for SGOT and SGPT (model 2 and 3) did not significantly alter the relationship between total fluorine and GGT. Moderate alcohol consumption was positively associated with GGT.

Table 4.1.76 presents the final regression model for AKPH. In nonsmokers, total serum fluorine was negatively associated with AKPH. As the number of cigarettes smoked per day increased to more than five per day, AKPH increased as total serum fluorine increased.

4.1.7 Hematology Parameters: Hemoglobin, White Blood Count, Polymorphonuclear Leukocyte Count, Band Count, Eosinophil Count, Lymphocyte Count, Monocyte Count, Platelet Count, And Basophil Count.

Table 4.1.77 presents the correlation coefficients between the nine hematology parameters and total serum fluorine, age, BMI, alcohol use, and cigarette consumption. The only parameter that was significantly correlated with total serum fluorine was lymphocyte count (r=.19, p=.04). Monocyte count was correlated with BMI (r=-.22, p=.04) and alcohol consumption, (r=-.21, p=.03). All the parameters, except the basophil and band counts, were strongly associated with cigarette consumption. Alcohol consumption was correlated with hemoglobin, (r=-.20, p=.04), and band count (r=.26, p=.005).

The final regression models for hemoglobin and the erythrocyte indices, mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV), are presented in Tables 4.1.78, 4.1.79, and 4.1.80 respectively. Total serum fluorine was significantly associated with hemoglobin.. The association hemoglobin and MCV were modified by smoking. In smokers who smoked seven or more cigarettes per day, hemaglobin and MCV increased significantly as total fluorine increased. In nonsmokers, hemaglobin and MCVdecreased as total fluorine

increased . The association of total fluorine with MCH was modified by smoking and by alcohol use. The increase in MCH as total fluorine increased was enhanced with increased smoking. In light drinkers, total serum fluorine had a weak association with MCH. In moderate drinkers, MCH increased as total fluorine increased. There was a positive association of both MCH and MCV with alcohol consumption. None of the estimated associations are of clinically significant magnitude over the range of total fluorine values.

The white blood cell count (WBC) increased significantly in nonrespondents as total fluorine increased above 2ppm, increased less in moderate drinkers, and increased the least in light drinkers (Table 4.1.81). As expected, cigarette smoking intensity was positively associated with WBC. PMN increased significantly in alcohol use nonrespondents as total fluorine increased and increased less steeply in moderate drinkers (Table 4.1.82). In light drinkers, total serum fluoride above 10 ppm was associated with a decreased in PMN. Cigarette smoking was positively associated with PMN. As shown in Table 4.1.83, the final regression models for band count provides little evidence that total fluorine was associated with band count. Moderate alcohol use was estimated to reduce the band count. Smoking was positively associated with band count.

The negative association between total fluorine and lymphocyte count was modified by adiposity, alcohol consumption, and cigarette smoking (Table 4.1.84). The decrease in lymphocyte count was smaller as BMI increased. The decrease in lymphocyte count associated with total fluorine above 3 ppm was greater in moderate drinkers compared to nonrespondents. As cigarette consumption increased, the decrease in lymphocyte count increased.

The positive association between total fluorine and monocyte count (MONO) was modified by adiposity (Table 4.1.85). As BMI increased, the association with MONO was weaker Cigarette smoking and LH were positively associated with MONO. Alcohol consumption was negatively associated with MONO. The association between total fluorine and eosinophil count (EOS) was negative for nonsmokers, but was positive as more than ten cigarettes per day were smoked

(Table 4.1.86). As smoking increased, the PFOA associated decrease in BASO was smaller (Table 4.1.88).

The association between total fluorine and platelet count (PLAT) was modified by adiposity and cigarette smoking intensity (Table 4.1.87.). In lean participants (BMI=25), PLAT increased as total fluorine increased. In obese participants (BMI=40), the PLAT decreased as total fluorine increased. As smoking increased, the rate of increase in PLAT associated with total fluorine above 10 ppm decreased.

4.1.8 Summary Of Results

The serum fluorine levels in Chemolite workers were 20-100 times higher than expected in workers not directly involved in PFOA production. All workers with levels above 10 ppm fluorine work in PFOA production areas. Smoking was associated with a small increase in serum fluorine. Age was not associated with serum fluorine levels. The two women employed in the PFOA production areas had total serum fluorine levels similar to men.

Alcohol use, smoking, age, BMI, and hormones had the expected associations with peripheral leukocyte counts, hematology parameters, cholesterol, HDL, LDL, and hepatic enzymes.

The main hormone results are:

1. The number of male workers with hormone values outside of the laboratory reference range was greater than expected for estradiol, free testosterone, bound testosterone, and prolactin.
2. Total serum fluorine was negatively associated with free testosterone and positively associated with estradiol. No association was noted between total serum fluorine and LH.
3. E/TF and TB/TF, but not E/TB, were positively associated with total serum fluorine.
4. E/LH and TB/LH were not associated with total serum fluorine. However, the relationship between total serum fluorine and TF/LH was suggestive.

5. TSH was positively associated with total serum fluorine. TF/TSH was negatively associated with total serum fluorine; TB/TSH and E/TSH were not.
6. Prolactin and total serum fluorine were positively associated in moderate drinkers, but not in light drinkers.
7. P/FSH, P/LH, P/TSH were positively associated with total serum fluorine. TB/P, TF/P, and E/P were not associated with total serum fluorine

The main hepatic parameter results are:

1. The increase in SGOT and SGPT levels associated with adiposity was enhanced by total serum fluorine.
2. The induction of GGT by alcohol was decreased as total serum fluorine increased.
3. The induction of AKPH by smoking was increased by increasing levels of total serum fluorine.

The main cholesterol and lipoprotein results are:

1. Cholesterol and triglyceride levels were not associated with total serum fluorine.
2. LDL was not associated with total serum fluorine.
3. The positive association between moderate alcohol use and HDL levels was reduced as total serum fluorine increased.

The main hematology parameter and peripheral leukocyte count results are:

1. The effect of smoking on hemoglobin and MCV was enhanced by total serum fluorine.
2. Total serum fluorine was negatively associated with all peripheral leukocyte counts except PMNs and MONOs, which were positively associated.
3. The associations between cell counts and total serum fluorine were modified by smoking, drinking, and adiposity.

4.2 The 1990 Chemolite Retrospective Cohort Mortality Study

A total of 3,537 individuals who were employed at the Chemolite plant between January 1, 1947 and December 31, 1983 were identified from company records. The cohort consisted of 2,788 (79%) male and 749 (21%) females employees (Tables 4.2.1 and 4.2.2). The majority of women (67.3%) never worked in the Chemical Division. Of the 19,309 person years (PY) observed for women, 68.8% occurred in those who were never employed in the Chemical Division. The mean follow-up for women was 25.8 years in the overall cohort, 24.6 years in the Chemical Division (CD) cohort, and 26.4 years in the non-CD cohort. The distribution of follow-up periods was similar in the women's CD and non-CD cohorts.

The women's mean age at first employment was 27.6 years. Sixty-eight percent were less than 30 years old at employment; 9.7% were older than 40 at first employment at Chemolite. The CD cohort was slightly older than the non-CD cohort. The CD and non-CD distributions of latency times were not statistically different ($p=.66$). The mean duration of employment for women was 8.7 years and ranged from six months to 41.4 years. The distribution of years of employment was significantly different for CD and non-CD women ($p<.0001$). Of non-CD women, 11.9% were employed for more than twenty years. Of 245 women in the CD cohort, 51(21.1%) were employed for more than twenty years.

As shown in Table 4.2.2, the 2,788 men who were ever employed for more than six months at Chemolite contributed a total of 71,117.7 PY which was about equally divided between the CD and non-CD cohorts. The mean follow-up for the overall male cohort was 25.5 years. The distribution of follow-up periods and distribution of year of first employment was similar in the male CD and non-CD cohorts. The average age at death was higher in the male non-CD group, 58.1 years, compared to the CD group, 54.2 years. The duration of employment for men (mean 13.6 years, median 9.8 years) was longer than for women. The distribution of years of employment was significantly different for CD and non-CD men ($p<.0001$). Of non-CD men, 25.5% were employed for longer than twenty

years. Of men in the CD cohort, 38.0% were employed for longer than twenty years.

Vital status was obtained for 100% of the women's cohort (Table 4.2.3). Among the 749 women there were 50 deaths; 11 in the CD cohort and 39 in the non-CD cohort. Vital status was obtained for 100% of the men's cohort. Among the 2788 men there were 348 deaths; 148 deaths in the CD group and 200 in the non-CD group. Six individuals who had employment records that were missing information were excluded from the cohort and their vital status was not ascertained. Death certificates were obtained for 99.5% of deaths. Two deaths occurred outside the U.S. and causes of death were ascertained by other means.

4.2.1 Standardized Mortality Ratios (SMRs)

4.2.1.1 SMRs For Women

The numbers of deaths, the SMRs and 95% confidence intervals (CI) among women in the 1947-1989 follow-up period are shown in Table 4.2.5. The SMRs for all causes of death (SMR=.75, 95% CI .56-.99), and cancer (SMR=.71, 95% CI .42-1.14) were significantly lower than expected in comparison to national rates. No association was found with duration of employment or latency for deaths from all causes, cancer, and cardiovascular diseases (Tables 4.2.6 and 4.2.7). SMRs for CD women and non-CD women are displayed in Table 4.2.8. The estimated SMR for the CD cohort of women were less than expected. In CD women, the all causes SMR was .46 (95% CI .23,.86) and the cancer SMR was .31 (95% CI .07,1.05). The SMRs for the non-CD women were closer to unity.

4.2.1.2 SMRs For Men

The number of male deaths, the expected number of male deaths based on U.S. national white male rates, and age and calendar period adjusted SMRs with associated 95% CIs are presented in Table 4.2.9. The SMR for all causes (.73, 95% CI .66,.81), for cardiovascular diseases (SMR=.71, 95% CI 60,.48), for all gastrointestinal (GI) diseases (.50,95% CI .26,.87) and for all respiratory diseases (.50,95% CI .27,.86) were significantly less than one. None of the

cause-specific SMRs were large nor were the estimates significantly different from one. As shown in Table 4.2.10, the results were similar when the expected numbers of male deaths was based on Minnesota white male rates.

Table 4.2.11, Table 4.2.12, and Table 4.2.13 present adjusted SMRs and 95% CI for males based on Minnesota mortality rates for three latency intervals 10, 15, and 20 years respectively. The three latency intervals the all causes SMR ranged from .75 to .77. For all cancers, SMRs ranged from 1.06 to 1.12 and were nonsignificant. Among men there was no association between any cause of death and duration of employment (Table 4.2.14, Table 4.2.15, and Table 4.2.16).

Table 4.2.17 and 4.2.18 display the SMRs and 95% CI for CD and non-CD male workers. The all causes SMRs were .69 (.59,.79) for the non CD group and .86 (.72,1.01) for the CD group. The SMRs for prostate cancer, based on a comparison with Minnesota population rates, were 2.03 (95% CI .55,4.59) in the CD group and .58 (95% CI .07,2.09) in the non-CD cohort. There were 4 observed deaths from prostate cancer compared to 2 expected in the CD group. The latency analysis for non-CD and CD men are presented in Tables 4.2.19 and 4.2.20. There was no associations between any cause of death and latency in either group.

As shown in Table 4.2.21 and 4.2.22, male CD cohort members with more than 10 or more than 20 years of employment had SMRs that were less than one for all causes of death, all malignancy, cardiovascular diseases and all respiratory diseases. Among male non-CD cohort members with more than ten years of employment or more 20 years of employment, the SMRs for all causes, cardiovascular disease and all respiratory diseases were significantly less than expected (Table 4.2.23 and 4.2.24). There was no association of any cause of death with duration of employment at Chemolite in either CD or non-CD groups.

4.2.2 Standardized Rate Ratios (SRRs)

Age adjusted standardized rate ratios (SRRs) were calculated for all causes, all cancer, and cardiovascular diseases mortality comparing men employed at the

plant for ten years or more to men employed for less than ten years. The SRRs are presented in Table 4.2.25. The 95% CIs for all causes, all cancer, and all cardiovascular diseases were wide and include one. Confounding variables such as year of first employment and length of follow-up were not controlled in this analysis due to small numbers and unstable rates within the large number of strata.

Table 4.2.26 presents the age adjusted SRRs for all causes, all cancers, lung cancer, GI cancer, and all cardiovascular diseases mortality comparing men ever employed in the CD with men never employed in the CD. All SRRs were slightly greater than one, however, none was statistically significant.

4.2.3 Mantel- Relative Risks (RR_{MH})

Age stratified RR_{MH}, contrasting the rates in men ever employed in the CD compared to the rates in men never employed in the CD, were calculated for all causes, all cancer, and all cardiovascular diseases mortality and are displayed in Table 4.2.27. The estimated RR for CD employment versus non-CD employment did not follow a monotonic pattern and the 95% CIs include one for each of the three endpoints.

Table 4.2.28 presents the RR_{MH} for men employed for less than ten years to those employed for more than ten years. The all causes RR_{MH} (2.16, 95% CI 1.52, 2.70) in the 30 to 39 year age at first employment strata was reflected in both the RR_{MH} for all cancers (1.75, 95% CI.95,3.21) and cardiovascular diseases (3.53 , 95% CI 1.68,6.21). The RR_{MH} were not adjusted for important time covariates such as the year of first employment.

4.2.4 Proportional Hazard Regression Model Relative Risk Estimates

4.2.4.1 Proportional Hazard Models For Male Workers

Table 4.2.29 to 4.2.36 show the final proportional hazard (PH) model for death from all causes, cardiovascular diseases, all cancers, lung cancer, GI cancer, prostate cancer, pancreatic cancer, and diabetes among the 2788 male workers

ever employed at Chemolite for greater than six months. There was no evidence for violation of the PH assumptions or for significant nonlinear associations between the independent variables and mortality. As expected, age at first employment was positively associated with all causes of death. The RR for a one year increase in age at first employment was 1.082 (95% CI 1.069,1.094). Year of first employment and duration of employment were negatively associated with all causes mortality. The risk of death associated with months in the Chemical Division was small and nonsignificant.

For cardiovascular diseases mortality, the RR for a one year increase in age at first employment was 1.126 (95% CI 1.069,1.094). Year of first employment was negatively associated with cardiovascular diseases mortality. Time in the CD was not associated with death from cardiovascular diseases.

Age at first employment was positively associated with cancer mortality. The RR for a one year increase in age of employment was 1.08 (95% CI 1.06,1.10). Duration of employment was negatively associated with cancer. The RR was .972 (95% CI.96,.99) for a one year increase in employment. There was no association of cancer mortality with employment time in the CD.

The final prostate cancer mortality proportional hazard model for male cohort members is shown in Table 4.2.34. Time in the Chemical Division was positively and significantly associated with prostate cancer mortality. The relative risk for a one year increase in CD employment time was 1.13 (95% CI 1.01,1.43). Age at first employment was positively associated with prostate cancer mortality risk. A one year increase in age at first employment was associated with a RR of 1.09 (95% CI .99,1.19). The RR for lung cancer mortality was 1.07 (95% CI .03,1.12) for a one year increase in age of employment. Months in the chemical division was not significantly associated with lung cancer mortality. Table 4.2.33 shows the final proportional hazard (PH) model for all GI cancer mortality. The estimated RR for a one year increase in age at first employment was 1.14 (95% CI 1.09,1.19). Year of first employment, duration of employment and time employed in the CD were not associated with GI cancer risk. Age at first employment was positively associated with pancreatic cancer mortality. The other covariates were weakly associated with pancreatic cancer risk and were

not significantly different from one. A one year increase in age at first employment was positively associated with diabetes mortality (RR = 1.10, 95% CI 1.01,1.19).

4.2.4.2 Proportional Hazard Models For Female Workers

Table 4.2.37, 4.2.38 and 4.2.39 show the final PH model for death from all causes, cardiovascular diseases and all cancers among the 749 female cohort members. Age at first employment was positively associated with all causes mortality. The RR for all causes of death among women employed for two to ten years (3.72) and among women employed for greater than ten years (2.33) were significantly greater than the all causes mortality in women employed for less than two years. Time in the CD was not related to mortality. The RR for death from cardiovascular diseases associated with a one year increase in age at first employment was 1.13 (1.07,1.18). The year at first employment, duration of employment, and time in the CD were not significantly associated with female cardiovascular diseases mortality. The RR for death from cancer was associated with age at first employment. A one year increase in age at first employment increase the RR for death from cancer (1.09 (1.04,1.14). The year at first employment, duration of employment, and time in the chemical division were weakly and non-significantly associated with female cancer mortality.

4.3 Physiologic Effects Tables

TABLE 4.1.1 AGE DISTRIBUTION IN FIVE YEAR AGE GROUPS
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

AGE	NUMBER	PERCENT
21-25	3	2.6
26-30	18	15.7
31-35	26	22.6
36-40	22	19.1
41-45	18	15.7
46-50	9	7.8
51-55	13	11.3
56-60	6	5.2
TOTAL	115	100.0
MEAN	39.2	
SD	8.91	
MEDIAN	37	
RANGE	24-59	

TABLE 4.1.2 DISTRIBUTION OF ALCOHOL AND TOBACCO USE
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

USE STATUS	NUMBER	PERCENT
TOBACCO USE		
CURRENT SMOKER	28	24.3
NONSMOKER	85	73.9
MISSING VALUES	2	1.8
TOTAL	115	100.0
ALCOHOL USE		
<1oz ETHANOL/DAY*	87	75.6
1-3oz ETHANOL/DAY	20	17.4
MISSING VALUES	8	7.0
TOTAL	115	100.0

*Includes 22 nondrinkers

TABLE 4.1.3 THE JOINT DISTRIBUTION OF TOBACCO AND ALCOHOL USE
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOBACCO USE			
	SMOKER	NONSMOKER	MISSING	TOTAL
ALCOHOL USE				
<1oz/day	19 (67.9%)	67 (78.8%)	1 (50.0%)	87 (75.6%)
1-3oz/day	7 (25.0%)	13 (15.3%)	0 (0%)	20 (17.4%)
missing	2 (7.1%)	5 (5.9%)	1 (50.0%)	8 (7.0%)
TOTAL	28 (100%)	85 (100%)	2 (100%)	115 (100%)

TABLE 4.1.4 DISTRIBUTION OF AGE BY SMOKING AND DRINKING STATUS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	AGE(years)					
	N	MEAN	SD	MEDIAN	RANGE	TEST#
Alcohol						
<1oz/d	87	39.9	9.31	37	24-59	
1-3oz/d	20	37.5	6.95	37	27-51	*p=.29
missing	8	36.6	8.70	35	27-54	*p=.17
Tobacco						
smoker	28	40.4	7.59	39	28-54	
nonsmoker	85	39.0	9.35	37	24-59	*p=.47
missing	2	32.5	3.53	32	30-35	
TOTAL	115	39.2	8.91	37	24-59	

*Student t test, Prob>T, reference groups <1oz/day, smoker

TABLE 4.1.5 PEARSON CORRELATION COEFFICIENTS BETWEEN TOTAL SERUM FLUORINE, AGE, BODY MASS INDEX (BMI), DAILY ALCOHOL USE, AND DAILY TOBACCO CONSUMPTION. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m^2)	ALCOHOL (oz/day)	TOBACCO (cigs/day)
TOTAL FLUORINE	1	.004	.0002	-.007	.006
AGE	-	1	.26 p=.005	-.14	.15
BMI	-	-	1	.08	-.04
ALCOHOL	-	-	-	1	.08
TOBACCO	-	-	-	-	1

TABLE 4.1.6 BODY MASS INDEX DISTRIBUTION
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

BMI (kg/m^2)	NUMBER	PERCENT
>15-20	1	0.9
>20-25	40	34.8
>25-30	57	49.5
>30-35	15	13.0
>35-45	2	1.8
TOTAL	115	100.0
MEAN BMI	26.9	
SD	3.4	
MEDIAN BMI	26.3	
RANGE	18.8-40.5	

TABLE 4.1.7 BODY MASS INDEX BY SMOKING AND DRINKING STATUS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	BMI(kg/m^2)					
	N	MEAN	SD	MEDIAN	RANGE	TEST#
Alcohol						
<1oz/d	87	26.9	3.54	26.1	18.8-40.5	
1-3oz/d	20	27.2	3.10	27.0	22.8-33.7	*p=.71
missing	8	25.9	3.64	26.1	21.3-30.4	
Tobacco						
smoker	28	26.6	3.63	26.3	18.8-28.2	
nonsmoker	85	27.0	2.99	26.6	21.4-33.7	*p=.57
missing	2	26.2	2.87	26.2	24.1-28.2	
Total	115	26.9	3.45	26.3	18.8-40.5	

*Student t test, t test p-value, reference groups <1oz/day, smoker

TABLE 4.1.8 THE DISTRIBUTION OF AGE, ALCOHOL AND TOBACCO USE BY BODY MASS INDEX
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	BMI mg/kg^2		
	<25	25-30	>30
TOBACCO USE			
SMOKER	11 (26.8%)	15 (26.3%)	2 (11.8%)
NONSMOKER	29 (70.7%)	41 (71.9%)	15 (88.2%)
MISSING	1 (2.5%)	1 (1.8%)	0 (0%)
TOTAL	41 (100%)	57 (100%)	17 (100%)
ALCOHOL USE			
<1 oz/day	31 (75.6%)	43 (75.4%)	13 (76.4%)
1-3 oz/day	6 (14.6%)	11 (19.3%)	3 (17.7%)
MISSING	4 (9.8%)	3 (5.3%)	1 (5.9%)
TOTAL	41 (100%)	57 (100%)	17 (100%)
AGE			
<40 years	31 (75.6%)	28 (49.1%)	6 (35.3%)*
>40 years	10 (24.4%)	29 (50.9%)	11 (64.7%)
TOTAL	41 (100%)	57 (100%)	17 (100%)

*t test p=.005

TABLE 4.1.9 TOTAL SERUM FLUORIDE DISTRIBUTION
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

TOTAL FLUORINE (PPM)	NUMBER	PERCENT
<1	23	20.0
1-3	65	56.5
>3-10	16	13.9
>10-15	6	5.2
>15-26	5	4.4
TOTAL	115	100.0
MEAN TF	3.3	
SD	4.7	
MEDIAN TF	2	
RANGE	0-26	

TABLE 4.1.10 TOTAL SERUM FLUORIDE BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		FLUORINE (ppm)				
	N(%)	MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	41(35.7)	2.8	3.74	2	0-19	F=1.47#
25-30	57(49.6)	4.0	5.47	2	0-26	P=.24
>30	17(14.8)	2.1	3.51	1	0-14	
AGE						
<31	21(18.3)	3.7	4.95	2	0-20	F=.10#
31-40	48(41.7)	3.2	4.08	2	0-14	P=.96
41-50	27(23.5)	3.3	4.26	2	0-19	
51-60	19(16.5)	3.0	6.42	1	0-26	
Alcohol						
<1oz/d	87(75.6)	3.4	5.15	2	0-26	p=.83*
1-3oz/d	20(17.4)	3.2	2.87	2	0-12	
missing	8(7.0)	2.1	2.53	1	0-6	
Tobacco						
smoker	28(24.8)	3.6	4.36	2	0-20	p=.66*
nonsmoker	85(75.2)	3.2	4.13	2	0-26	
missing	2(1.7)	3.0	4.24	3	0-6	
TOTAL	115	3.3	4.67	2	0-26	

#univariate Anova
*Student t test, Prob>T

TABLE 4.1.11 AGE DISTRIBUTION BY TOTAL SERUM FLUORINE CATEGORY.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL SERUM FLUORINE (ppm)				
	<1	1-3	>3-10	>10-15	>15-26
	NUMBER (PERCENT)				
AGE					
20-25	1 (4.4)	1 (1.5)	0 (0)	1 (16.7)	0 (0)
26-30	3 (13.0)	10 (15.4)	4 (25.0)	0 (0)	1 (20.0)
31-35	6 (26.1)	13 (20.0)	4 (25.0)	2 (33.2)	1 (20.0)
36-40	4 (17.4)	12 (18.5)	5 (31.2)	0 (0)	1 (20.0)
41-45	2 (8.7)	13 (20.0)	2 (12.5)	1 (16.7)	0 (0)
46-50	0 (0)	7 (10.7)	0 (0)	1 (16.7)	1 (20.0)
51-55	6 (26.1)	6 (9.3)	0 (0)	1 (16.7)	0 (0)
56-60	1 (4.3)	3 (4.6)	1 (6.3)	0 (0)	1 (20.0)
TOTAL	23 (100)	65 (100)	16 (100)	6 (100)	5 (100)
MEAN AGE SD	39.9	39.6	36.0	39.3	41.6
	10.2	8.5	7.5	11.1	10.5
MEDIAN AGE	37	38	35.5	37.5	40
AGE RANGE	25-59	24-56	27-57	25-54	30-57

TABLE 4.1.12 DISTRIBUTION OF TOBACCO USE BY TOTAL SERUM FLUORIDE CATEGORY.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL SERUM FLUORINE (ppm)					
	<1	1-3	>3-10	>10-15	>15-26	TOTAL
Tobacco use			NUMBER(%)			
Smoker	3 (13.0)	16 (24.6)	6 (37.5)	2 (33.3)	1 (20.0)	28 (24.3)
Nonsmoker	19 (82.7)	49 (75.4)	9 (56.2)	4 (66.7)	4 (80.0)	85 (73.9)
Missing	1 (4.3)	0 (0)	1 (6.3)	0 (0)	0 (0)	2 (1.7)
Total	23 (100)	65 (100)	16 (100)	6 (100)	5 (100)	115 (100)
Cigarettes/day (among smokers)						
MEAN	16.3	24.5*	18.0	20	20	21.5
SD	14.0	8.8	9.9	0	-	10.1
MEDIAN	17	20	20	20	20	20
RANGE	2-30	7-40	3-30	20	20	2-40

*significantly different from <1 ppm mean ($p<.005$)

TABLE 4.1.13 DISTRIBUTION OF ALCOHOL USE BY TOTAL SERUM FLUORIDE CATEGORY.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL SERUM FLUORINE (ppm)				
	<1	1-3	>3-10	>10-15	>15-26
ALCOHOL USE	NUMBER (PERCENT)				
<1 oz/day	17 (73.9)	51 (78.5)	9 (56.3)	5 (83.3)	5 (100)
1-3 oz/day	2 (8.7)	13 (20.0)	4 (25.0)	1 (16.7)	0 (0)
MISSING	4 (17.4)	1 (1.5)	3 (18.7)	0 (0)	0 (0)
TOTAL	23 (100)	65 (100)	16 (100)	6 (100)	5 (100)

TABLE 4.1.14 BODY MASS INDEX DISTRIBUTION BY TOTAL SERUM FLUORINE CATEGORY.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL SERUM FLUORINE (ppm)				
	<1	1-3	>3-10	>10-15	>15-26
BMI(kg/m^2)		NUMBER (PERCENT)			
>15-20	1 (4.4)	0 (0)	0 (0)	0 (0)	0 (0)
>20-25	9 (39.1)	21 (32.3)	8 (50.0)	1 (16.7)	1 (20.0)
>25-30	5 (21.7)	39 (60.0)	5 (31.2)	4 (66.6)	4 (80.0)
>30-35	7 (30.4)	5 (7.7)	3 (18.8)	0 (0)	0 (0)
>35-40	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)
>40-45	1 (4.4)	0 (0)	0 (0)	0 (0)	0 (0)
TOTAL	23 (100)	65 (100)	16 (100)	6 (100)	5 (100)
MEAN BMI	27.6	26.6	26.3	29.4	26.0
SD	5.3	2.6	3.3	3.7	1.4
MEDIAN	27	26.8	25.7	29.8	25.6
BMI RANGE	18.8-40.5	22.5-33.7	21.4-32.5	24.5-35.5	24.1-27.6

TABLE 4.1.15 COEFFICIENT OF VARIATION FOR SEVEN HORMONE ASSAYS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

HORMONE	CV
BOUND TESTOSTERONE	10.6%
FREE TESTOSTERONE	12.1%
ESTRADIOL	18.3%
TSH	10.0%
LH	8.6%
PROLACTIN	3.1%
FSH	5.6%

TABLE 4.1.16 THE OBSERVED VERSUS EXPECTED NUMBER OF WORKERS WITH HORMONE ASSAYS OUTSIDE THE ASSAY REFERENCE RANGE. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	OBSERVED	EXPECTED	O/E*	95% CI**
Estradiol >=44 pg/ml	17	2.8	6.0	(3.6,9.8)
Testosterone bound <=300 ng/dl	13	2.8	4.5	(2.6,8.1)
Testosterone free <=9 ng/dl	11	2.8	3.9	(2.0,7.1)
Prolactin >=15 ng/ml	10	2.8	3.5	(1.8,6.7)
LH 2-12 mU/ml	3	2.8	1.1	(0.3,3.3)
FSH 1-12 mU/ml	1	2.8	.4	(0.1,2.0)
TSH >=4.6 mU/ml	1	2.8	.4	(0.1,2.0)

*O/E - OBSERVED TO EXPECTED RATIO
**CI -95% CONFIDENCE INTERVAL

TABLE 4.1.17 PEARSON CORRELATION COEFFICIENTS BETWEEN SERUM HORMONES.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA.

	ESTRADIOL	FREE TEST.	BOUND TEST.	PROLACTIN	LnLH++	FSH+	LnTSH#
ESTRADIOL@	1	.40 p=.0001	.32 p=.0006	.16 p=.08	.06	-.14 p=.15	.05
FREE TESTOSTERONE*	-	1	.74 p=.0001	.13	.10	-.05	.07
BOUND TESTOSTERONE*	-	-	1	.21 p=.03	.28 p=.003	.16 p=.04	-.02
PROLACTIN#	-	-	-	1	.15	.004	.11
LnLH++	-	-	-	-	1	.63 p=.0001	-.15 p=.11
FSH+	-	-	-	-	-	-	-.23 p=.01

@pg/ml
*ng/dl
#ng/ml
++LOG LUTENIZING HORMONE (mU/ml)
+ FOLLICLE STIMULATING HORMONE (mU/ml)
#LOG THYROID STIOMULATING HORMONE (mU/ml)

TABLE 4.1.18 PEARSON CORRELATION COEFFICIENTS BETWEEN TOTAL SERUM FLUORIDE, AGE, BODY MASS INDEX (BMI), DAILY ALCOHOL USE, DAILY TOBACCO CONSUMPTION, AND SERUM HORMONES.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m^2)	ALCOHOL (oz/day)	TOBACCO (cigs/day)
ESTRADIOL@	.13 p=.16	-.25 p=.01	-.01	.05	.12 p=.2
FREE TESTOSTERONE*	.03	-.45 p=.0001	-.26 p=.005	-.08	.05
BOUND TESTOSTERONE*	.08	-.24 p=.01	-.36 p=.0001	-.16 p=.11	.11
PROLACTIN#	.19 p=.045	-.19 p=.01	-.06	.03	-.16 p=.09
LnLH++	.04	.11	.20 p=.03	-.14	.18 p=.06
FSH+	-.03	.33 p=.0003	-.08	-.24 p=.01	.17 p=.06
LnTSH#	.26 p=.005	.09	.04	.15 p=.15	-.03

@pg/ml
*ng/dl
#ng/ml
++LOG LUTENIZING HORMONE (mU/ml)
+ FOLLICLE STIMULATING HORMONE (mU/ml)
#LOG THYROID STIOMULATING HORMONE (mU/ml)

TABLE 4.1.19 BOUND TESTOSTERONE (TB) BY BODY MASS INDEX, AGE, SMOKING, DRINKING STATUS AND TOTAL SERUM FLUORIDE 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA.

		TB(ng/dl)				
	N(%)	MEAN	SD	MEDIAN	RANGE	TEST#
BMI (kg/m²)						
<25	40(35.4)	641	242.9	592	275-1192	F=5.64
25-30	56(49.6)	565	196.8	560	141-954	p=.005
>30	17(15.0)	436	172.7	438	210-803	
Age						
<31	20(17.7)	598	232.8	673	278-1192	F=3.60
31-40	48(42.5)	634	214.1	605	275-1189	p=.016
41-50	26(23.0)	512	185.8	498	141-947	
51-60	19(16.8)	470	226.1	409	210-954	
Alcohol						
<1oz/d	86(76.1)	581	212.5	574	210-1192	F=1.23
1-3oz/d	19(16.8)	484	215.1	417	141-1039	p=.27
missing	8(7.1)	690	272.8	602	409-1101	
Tobacco						
smoker	27(23.9)	622	177.7	617	379-1039	F=1.69
nonsmoker	84(74.3)	559	233.0	556	141-1192	p=.20
missing	2(1.8)	432	97.6	432	363-501	
Total Fluorine						
<1 ppm	23(20.4)	584	295.4	438	275-1192	F=0.39
1-3	64(56.6)	567	202.9	572	141-1039	p=.82
>3-10	15(13.3)	530	189.3	574	210-819	
>10-15	6(5.3)	600	234.6	563	244-947	
>15-26	5(4.4)	662	149.8	659	517-880	
Total	113(100)	572	220.7	561	141-1192	

#univariate Anova

TABLE 4.1.20 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE BOUND TESTOSTERONE (ng/dl) AMONG 112 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	1027	190.7	.0001
Total Fluorine (ppm)*	-148	67.2	.05
Age (years)	-9	3.3	.009
Age X Total Fluoride*	3	1.6	.04
BMI (kg/m^2)	-16	5.4	.003
Smoker**	74	45.0	.28
Alcohol (<1oz/day)#	89	47.5	.11
Estradiol (pg/ml)	2	1.0	.02
LH (mU/ml)	116	6.1	.004
Prolactin (ng/ml)	8	4.1	.04

R^2= .39
*Square root transformation of total serum fluoride measured in ppm.
**Reference category is nonsmokers.
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.

TABLE 4.1.21 FREE TESTOSTERONE (TF) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS AND TOTAL SERUM FLUORIDE. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		TF(ng/dl)				
	N(%)	MEAN	SD	MEDIAN	RANGE	TEST#
BMI kg/m^2						
<25	40(35.4)	17.4	6.22	16.7	7.4-45.3	F=3.58
25-30	56(49.6)	15.1	4.13	15.8	3.2-23.8	p=.03
>30	17(15.0)	13.7	6.08	13.5	5.6-30.5	
Age years						
<30	20(17.7)	18.7	7.64	16.7	9.3-45.3	F=9.14
31-40	48(42.5)	17.0	3.75	17.1	7.4-2937	p=.0001
41-50	26(23.0)	14.1	4.73	14.3	3.2-23.8	
51-60	19(16.8)	11.5	3.78	11.5	5.6-19.0	
Alcohol						
<1 oz/d	86(76.1)	15.8	5.36	15.8	5.6-45.3	F=1.45
1-3 oz/d	19(16.8)	14.2	4.79	15.3	3.2-23.9	p=.23
missing	8(7.1)	18.1	6.40	17.2	11.0-29.7	
Tobacco						
smoker	27(23.9)	16.6	3.71	17.1	8.4-24.3	F=.95
nonsmoker	84(74.3)	15.4	5.84	15.3	3.2-45.3	p=.33
missing	2(1.8)	15.9	4.45	15.9	12.7-19.0	
Total Fluorine						
<1 ppm	23(20.4)	16.4	8.4	13.9	6.4-45.3	F=0.13
1-3	64(56.6)	15.6	4.5	15.8	3.2-30.5	p=.97
>3-10	15(13.3)	15.2	3.8	15.3	7.1-19.7	
>10-15	6(5.3)	15.9	5.2	17.6	5.6-19.9	
>15-26	5(4.4)	15.3	2.2	14.1	13.3-18.2	
Total	113(100)	15.7	5.4	16	3.2-45.3	

#univariate Anova

TABLE 4.1.22 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE FREE TESTOSTERONE VALUE (ng/dl) AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	ß	SE(ß)	p-value
Intercept	29.72	4.57	.0001
Total Fluorine (ppm)*	-3.56	1.62	.03
Age (years)	-.34	.08	.0001
Age X Total Fluoride*	.07	.04	.05
BMI (kg/m^2)	-.21	.13	.11
Smoker**	1.46	1.03	.16
Alcohol (<1oz/day)#	1.65	1.14	.15
Estradiol (pg/ml)	.10	.03	.003
LH (mU/ml)	.18	.15	.20

R^2= .39
*Square root transformation of total serum fluoride measured in ppm.
**Reference category is nonsmokers.
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.

TABLE 4.1.23 PARTICIPANT ESTRADIOL BY BODY MASS INDEX, AGE, SMOKING DRINKING STATUS AND TOTAL SERUM FLUORIDE. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		ESTRADIOL (pg/ml)				
	N(%)	MEAN	SD	MEDIAN	RANGE	TEST#
BMI (kg/m^2)						
<25	40(35.4)	34.1	12.91	40	8-69	F=.13
25-30	56(49.6)	33.2	13.89	33	8-83	p=.88
>30	17(15.0)	32.2	12.36	27	18-57	
AGE						
<30	20(17.7)	34.4	10.15	34	19-56	F=3.50
31-40	48(42.5)	36.8	11.54	36	12-69	p=.018
41-50	26(23.0)	31.6	18.48	28	8-83	
51-60	19(16.8)	25.9	7.93	24	15-47	
Alcohol						
<1 oz/d	86(76.1)	33.0	11.78	33	8-66	F=.14
1-3 oz/d	19(16.8)	31.8	16.61	30	8-69	p=.71
missing	8(7.1)	41.1	18.20	40	23-83	
Tobacco						
smoker	27(23.9)	36.3	17.40	34	14-83	F=.13
nonsmoker	84(74.3)	32.5	11.63	32	8-66	p=.88
missing	2(1.8)	30.5	13.44	30	21-40	
Total Fluorine						
<1 ppm	23(20.4)	36.2	13.1	34	14-60	F=1.27
>=1-3	64(56.6)	31.4	13.6	30	8-83	p=.29
>3-10	15(13.3)	32.8	10.6	34	10-58	
>10-15	6(5.3)	38.2	15.2	35.5	22-66	
>15-26	5(4.4)	41.2	11.4	42	26-56	
Total	113(100)	33.4	13.2	33	8-83	

#univariate Anova

TABLE 4.1.24 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE ESTRADIOL VALUE (pg/dl) AMONG 113 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	12.89	12.13	.29
Total Fluorine (ppm)*	.03	.01	.03
Age (years)	-.22	.15	.14
BMI (kg/m^2)	.51	.34	.14
Cigarettes/day	.16	.11	.15
Alcohol (<1oz/day)#	.09	.11	.98
Free Testosterone (ng/dl)	.85	.24	.0007

R^2= .24
*Square transformation of total serum fluoride measured in ppm.
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.

TABLE 4.25 LUTENIZING HORMONE (LH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS, AND TOTAL SERUM FLUORINE 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		LH (mU/ml)				
	N(%)	MEAN	SD	MEDIAN	RANGE	TEST#
BMI mg/kg^2						
<25	40(35.4)	5.49	3.06	4.60	2.6-21.7	F=6.19
25-30	56(49.6)	5.84	3.25	5.15	1.7-23.0	p=.003
>30	17(15.0)	3.72	1.21	3.60	2.0-7.2	
Age years						
<30	20(17.7)	4.81	2.26	4.45	1.7-10.1	F=.69
31-40	48(42.5)	5.49	3.14	4.75	2.4-21.7	p=.56
41-50	26(23.0)	5.33	1.64	5.15	2.5-9.6	
51-60	19(16.8)	5.90	4.73	4.10	2.0-23.0	
Alcohol						
<1oz/d	86(76.1)	5.60	3.34	4.70	1.7-23.0	F=1.24
1-3oz/d	19(16.8)	4.69	1.80	4.21	2.3-10.1	p=.27
missing	8(7.1)	4.86	1.00	4.05	3.4-6.2	
Tobacco						
smoker	27(23.9)	6.30	3.78	5.30	2.6-21.7	F=5.16
nonsmoker	84(74.3)	5.05	2.71	4.52	1.7-23.0	p=.025
missing	2(1.8)	7.45	2.47	7.45	5.7-9.2	
Total Fluorine						
<1 ppm	23(20.4)	5.0	2.1	4.4	2.5-9.3	F=0.16
>=1-3	64(56.6)	5.6	3.6	4.8	1.7-23.0	p=.98
>3-10	15(13.3)	5.1	2.7	4.9	2.0-13.9	
>10-15	6(5.3)	5.4	0.3	4.9	3.7-7.5	
>15-26	5(4.4)	5.3	1.3	5.5	3.7-7.2	
Total	113(100)	5.4	3.0	4.7	1.7-23.0	

#univariate Anova

TABLE 4.1.26 LINEAR MULTIVARIATE REGRESSION MODEL #1 OF FACTORS PREDICTING THE LUTENIZING HORMONE* VALUE (mU/ml) AMONG 113 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	1.26	.40	.002
Total Fluorine (ppm)*	.001	.008	.93
Age (years)	.01	.005	.03
BMI (kg/m^2)	-.02	.01	.15
Smokers**	.24	.23	.29
Alcohol (<1oz/day)#	.06	.10	.60
Bound Testosterone (ng/dl)	.001	.0002	.008

R^2= .28
*logarithmic transformation of lutenizing hormone (LH).
** Reference category is nonsmokers.
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.

TABLE 4.1.27 FOLLICLE STIMULATING HORMONE (FSH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS, AND TOTAL SERUM FLUORINE
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	FSH(mU/ml)					
	N(%)	MEAN	SD	MEDIAN	RANGE	TEST#
BMI$_{mg/kg^2}$						
<25	40(35.4)	5.02	2.39	4.6	1.8-10.3	F=1.27
25-30	56(49.6)	5.39	2.71	4.5	1.4-14.8	p=.29
>30	17(15.0)	4.31	1.75	3.9	1.6-8.3	
Age years						
<30	20(17.7)	3.88	1.86	3.6	1.4-9.8	F=3.72
31-40	48(42.5)	4.86	2.24	4.6	1.6-10.3	p=.014
41-50	26(23.0)	5.65	2.55	4.6	2.1-14.8	
51-60	19(16.8)	6.22	3.01	5.0	2.7-14.8	
Alcohol						
<1oz/d	86(76.1)	5.37	2.62	4.6	1.4-14.8	F=3.47
1-3oz/d	19(16.8)	4.18	1.92	3.9	2.0-9.8	p=.065
missing	8(7.1)	4.38	1.49	4.8	2.6-6.4	
Tobacco						
smoker	27(23.9)	5.77	2.46	4.9	2.6-11.9	F=2.80
nonsmoker	84(74.3)	4.85	4.49	4.2	1.4-14.8	p=.09
missing	2(1.8)	6.10	0.42	6.1	5.8-6.4	
Total Fluorine						
<1 ppm	23(20.4)	4.4	1.95	4.4	1.6-10.3	F=0.75
1-3	64(56.6)	5.4	2.75	4.6	1.4-14.8	p=.56
>3-10	15(13.3)	4.8	2.23	4.9	2.1-9.7	
>10-15	6(5.3)	5.4	2.14	4.4	3.5-8.9	
>15-26	5(4.4)	4.9	2.36	3.7	2.6-7.7	
Total	113(100)	5.1	2.49	4.5	1.4-14.8	

#univariate Anova

TABLE 4.1.28 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE FOLLICLE STIMULATING HORMONE VALUE (mU/ml) AMONG 113 MALE WORKERS.
13M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	1.20	1.62	.46
Total Fluorine(ppm)*	.004	.04	.91
Age (years)	.08	.02	.0006
BMI (kg/m^2)	-.04	.05	.41
Cigarettes/day	.02	.02	.29
Alcohol(<1oz/day)#	.45	.48	.34
TSH (mU/ml)@	-.43	.22	.05
LH (mU/ml)##	.44	.06	.0001

R^2= 48
*logarithmic transformation of follicle stimulating hormone (FSH).
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
@Thyroid Stimulating Hormone
##Lutienizing Hormone

TABLE 4.1.29 THYROID STIMULATING HORMONE (TSH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS, AND TOTAL SERUM FLUORINE.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		TSH(mU/ml)				
	N(%)	MEAN	SD	MEDIAN	RANGE	TEST#
BMI mg/kg²						
<25	40(35.4)	1.55	0.66	1.04	0.37-3.14	F=.35
25-30	56(49.6)	1.64	1.01	1038	0.45-6.80	p=.70
>30	17(15.0)	1.72	0.71	1.55	0.62-3.23	
Age years						
<30	20(17.7)	1.43	0.56	1.42	0.38-2.33	F=.47
31-40	48(42.5)	1.66	1.04	1.46	0.37-6.80	p=.70
41-50	26(23.0)	1.64	0.75	1.34	0.75-3.56	
51-60	19(16.8)	1.70	0.74	1.53	0.62-3.09	
Alcohol						
<1oz/d	86(76.1)	1.57	0.70	1.40	0.38-3.56	F=1.23
1-3oz/d	19(16.8)	1.93	1.39	1.55	0.60-6.80	p=.27
missing	8(7.1)	1.49	0.63	1.61	0.37-2.22	
Tobacco						
smoker	27(23.9)	1.53	0.61	1.37	0.61-3.03	F=.09
nonsmoker	84(74.3)	1.66	0.92	1.49	0.37-6.80	p=.76
missing	2(1.8)	1.28	0.42	1.28	0.98-1.57	
Total fluorine						
<1 ppm	23(20.4)	1.5	0.64	1.5	0.3-3.3	F=2.30
>=1-3	64(56.6)	1.6	0.94	1.3	0.4-6.8	p=.08
>3-10	15(13.3)	1.6	0.67	1.4	0.6-3.0	
>10-15	6(5.3)	2.4	0.87	2.5	0.83-3.5	
>15-26	5(4.4)	2.2	1.66	2.1	1.7-3.5	
Total	113(100)	1.6	0.85	1.4	0.3-6.8	

#univariate Anova

TABLE 4.1.30 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE THYROID STIMULATING HORMONE* VALUE (mU/ml) AMONG 113 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	ß	SE(ß)	p-value
Intercept	-.190	.465	.68
Total Fluorine (ppm)*	.027	.009	.004
Age (years)	.006	.005	.29
BMI (kg/m^2)	-.002	.013	.89
Cigarettes/day	-.001	.004	.74
Alcohol (<3oz/day)#	-.140	.194	.26
Free Testosterone**	.020	.009	.04
FSH##	.060	.019	.003

R^2= .30
*logarithmic transformation of thyroid stimulating hormone (TSH).
#Reference category is moderate drinkers who consume 3 oz ethanol/day.
** ng/dl
##Follicle stimulating hormone mU/ml

TABLE 4.1.31 PROLACTIN BY BODY MASS INDEX, AGE, SMOKING, DRINKING STATUS, AND TOTAL SERUM FLUORINE 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		PROLACTIN (ng/ml)				
	N(%)	MEAN	SD	MEDIAN	RANGE	TEST#
BMI (kg/m^2)						
<25	40(35.4)	9.10	5.13	8.4	2.7-24.3	F=.69
25-30	56(49.6)	8.71	5.18	7.8	1.2-33.7	p=.51
>30	17(15.0)	7.45	3.08	7.2	2.5-13.6	
Age						
<30	20(17.7)	9.63	4.20	8.5	3.9-18.3	F=.96
31-40	48(42.5)	9.01	5.30	8.7	12-33.7	p=.51
41-50	26(23.0)	8.38	5.52	6.2	2.9-23.5	
51-60	19(16.8)	7.16	3.37	6.8	2.5-15.1	
Alcohol						
<1oz/d	86(76.1)	8.61	4.57	7.8	1.2-24.3	F=.44
1-3oz/d	19(16.8)	9.46	6.87	8.7	2.9-33.7	p=.50
missing	8(7.1)	7.25	2.33	6.9	43.-10.5	
Tobacco						
smoker	27(23.9)	6.97	3.14	6.6	1.2-12.8	F=4.18
nonsmoker	84(74.3)	9.13	5.18	8.5	2.5-33.7	p=.043
missing	2(1.8)	11.85	9.40	11.7	5.0-18.3	
Total Fluorine						
<1 ppm	23(20.4)	7.9	3.19	7.5	2.5-18.3	F=3.02
>=1-3	64(56.6)	8.5	4.34	8.1	1.2-24.3	p=.02
>3-10	15(13.3)	4.9	1.15	6.6	1.4-18.1	
>10-15	6(5.3)	15.1	11.01	9.4	6.8-33.7	
>15-26	5(4.4)	8.3	4.16	7.7	3.8-15.1	
Total	113(100)	8.7	4.90	7.7	1.2-33.7	

#univariate Anova

TABLE 4.1.32 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE PROLACTIN VALUE (ng/ml) AMONG 113 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	ß	SE(ß)	p-value
Intercept	7.41	4.14	.07
Total Fluorine (ppm)	1.43	.36	.0002
Age (years)	-.04	.05	.41
BMI (kg/m^2)	-.08	.13	.53
Cigarettes/day#	-.08	.04	.08
Estradiol (pg/ml)	.06	.03	.07
Alcohol Use##			
Light (<1oz/day)	3.21	1.65	.05
Nonresponse (NR)	2.14	2.69	.43
Light X total fluoride	-1.67	.77	.03
NR X total fluoride	-1.34	.37	.0006

R^2= .22

##Reference category is moderate drinkers who consume 1-3 oz ethanol/day. Nonrespondants (NR) failed to complete the alcohol use questionnaire items. Light X total fluoride and NR X total fluoride are interaction terms for alcohol categories and total serum fluoride.

TABLE 4.1.33 PEARSON CORRELATION COEFFICIENTS BETWEEN HORMONE RATIOS AND TOTAL FLUORIDE, AGE, BODY MASS INDEX, ALCOHOL AND TOBACCO CONSUMPTION
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m^2)	ALCOHOL (oz/day)	TOBACCO (cigs/day)
E/TB@	-.01	.004	.32 p=.001	.05	.05
E/TF*	.11	.15 p=.15	.27 p=.004	.01	.01
E/LH**	.001	-.26 p=.005	.18 p=.06	.05	.04
TB/LH+	.002	-.32 p=.001	-.14 p=.13	-.01	-.01
TF/LH++	-.09	-.40 p=.0001	-.02	.03	.03
TB/TF"	.16 p=.09	.24 p=.01	-.16 p=.08	-.12	.09

@ESTRADIOL TO BOUND TESTOSTERONE RATIO
*ESTRADIOL TO FREE TESTOSTERONE
**ESTRADIOL TO LUTENIZING HORMONE RATIO
+BOUND TESTOSTERONE TO LUTENIZING HORMONE RATIO
++FREE TESTOSTERONE TO LUTENIZING HORMONE RATIO
"BOUND TESTOSTERONE TO FREE TESTOSTERONE RATIO

TABLE 4.1.34 PEARSON CORRELATION COEFFICIENTS BETWEEN PROLACTIN HORMONE RATIOS AND TOTAL FLUORIDE, AGE, BODY MASS INDEX, ALCOHOL AND TOBACCO CONSUMPTION 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m^2)	ALCOHOL (oz/day)	TOBACCO (cigs/day)
TB/P@	-.05	-.04	-.13	-.03	.24 p=.01
TF/P*	-.08	-.11	-.08	.06	.22 p=.02
E/P+	-.03	-.05	.03	.007	.25 p=.008
FSH/P"	-.09	.37 p=.0001	.004	-.13 p=.16	.21 p=.02
P/LH**	.11	-.24 p=.003	.09	.15 p=.11	-.22 p=.02
P/TSH++	.07	.17 p=.07	.07	.17 p=.07	.09

@Free testosterone to prolactin ratio
*Free testosterone to prolactin ratio
+Estradiol to prolactin ratio
"Follicle stimulating hormone to prolactin ratio
**Prolactin to lutenizing hormone ratio
++Prolactin to thyroid stimulating hormone ratio

TABLE 4.1.35 PEARSON CORRELATION COEFFICIENTS BETWEEN THYROID STIMULATING HORMONE RATIOS AND TOTAL FLUORIDE, AGE, BODY MASS INDEX, ALCOHOL AND TOBACCO CONSUMPTION 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m^2)	ALCOHOL (oz/day)	TOBACCO (cigs/day)
TB/TSH#	-.13	-.23 p=.01	-.24 p=.01	-.16 p=.09	.03
TF/TSH*	-.18 p=.05	-.34 p=.0002	-.23 p=.01	-.13	.01
E/TSH+	-.13	-.24 p=.01	-.05	-.05	.04

#Bound testosterone to thyroid stimulating hormone ratio
*Free testosterone to thyroid stimulating hormone ratio
+Estradiol to thyroid stimulating hormone ratio

TABLE 4.1.36 PEARSON CORRELATION COEFFICIENTS BETWEEN FOLLICLE STIMULATING HORMONE RATIOS AND TOTAL FLUORIDE,AGE, BODY MASS INDEX, ALCOHOL AND TOBACCO CONSUMPTION 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m^2)	ALCOHOL (oz/day)	TOBACCO (cigs/day)
TB/FSH#	.07	-.43 p=.0001	-.16 p=.08	.06	-.06
TF/FSH*	-.01	-.47 p=.0001	.04	.08	-.12
E/FSH+	.04	-.36 p=.0001	.07	.04	-.02

#Bound testosterone to follicle stimulating hormone ratio
*Free testosterone to follicle stimulating hormone ratio
+Estradiol to follicle stimulating hormone ratio

TABLE 4.1.37 PEARSON CORRELATION COEFFICIENTS BETWEEN PITUITARY GLYCOPROTIEN HORMONE RATIOS AND TOTAL FLUORIDE, AGE, BODY MASS INDEX, ALCOHOL AND TOBACCO CONSUMPTION 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m^2)	ALCOHOL (oz/day)	TOBACCO (cigs/day)
TSH/FSH@	.12	-.16 p=.08	.04	.24 p=.01	-.14
TSH/LH*	.09	-.02	.15	.21 p=.03	-.14
F/LH+	-.05	.28 p=.003	.13	-.14	.05

@Thyroid stimulating hormone to follicle stimulating hormone ratio
*Thyroid stimulating hormone to lutenizing hormone ratio
+Follicle stimulating hormone to lutenizing hormone ratio

TABLE 4.1.38 LINEAR MULTIVARIATE REGRESSION MODEL1 OF FACTORS PREDICTING THE BOUND-FREE TESTOSTERONE RATIO AMONG 112 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	36.60	6.87	.0001
Total Fluorine (ppm)*	.02	.008	.02
Age (years)	.19	.101	.07
BMI (kg/m^2)	-.48	.244	.05
LH+	.12	.337	.73
FSH@	.92	.440	.04

R^2= .21
*square transformation of total serum fluoride
+lutienizing hormone mU/ml
@ follicle stimulating hormone mU/ml

TABLE 4.1.39 LINEAR MULTIVARIATE REGRESSION MODEL2 OF FACTORS PREDICTING THE BOUND-FREE TESTOSTERONE RATIO AMONG 112 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	37.3	6.97	.0001
Total Fluorine (ppm)*	.02	.009	.03
Age (years)	.25	.097	.009
BMI (kg/m^2)	-.52	.250	.03
LH+	.55	.271	.05

R^2= .17
*square transformation of total serum fluoride
+luteinizing hormone mU/ml
@ follicle stimulating hormone mU/ml

TABLE 4.1.40 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE ESTRADIOL-BOUND TESTOSTERONE RATIO AMONG 112 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	ß	SE(ß)	p-value
Intercept	.05	.027	.05
Total Fluorine (ppm)	.00001	.00001	.74
Age (years)	-.0004	.0004	.29
BMI (kg/m^2)	.002	.0007	.006
Cigarettes/day	-.00001	.00002	.96
Alcohol (<1oz/day)#	.003	.007	.63
Free Testosterone*	-.001	.0006	.008
LH+	.0001	.0006	.94
FSH@	-.002	.001	.12
TSH++	-.003	.003	.30
Prolactin**	.0001	.0005	.78

R^2= .21
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
+luteinizing hormone mU/ml
@ follicle stimulating hormone mU/ml
++ Thyroid stimulating hormone (mU/ml)
** prolactin ng/ml

TABLE 4.1.41 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE ESTRADIOL-FREE TESTOSTERONE RATIO AMONG 112 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	ß	SE(ß)	p-value
Intercept	1.31	.880	.15
Total Fluorine (ppm)	.002	.001	.03
Age (years)	.012	.011	.34
BMI (kg/m^2)	.048	.026	.07
Cigarettes/day	.005	.008	.51
Alcohol (<1oz/day)#	.090	.730	.70
Bound Testosterone*	-.001	.0004	.01
LH+	.012	.035	.73
FSH@	-.059	.046	.21
TSH++	-.204	.110	.05
Prolactin**	.027	.018	.15

R^2= .22
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
+lutienizing hormone mU/ml
@ follicle stimulating hormone mU/ml
++ Thyroid stimulating hormone (mU/ml)
** prolactin ng/ml

TABLE 4.1.42 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE ESTRADIOL-LH+ RATIO AMONG 112 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	3.07	3.58	.39
Total Fluorine (ppm)	.02	.07	.80
Age (years)	-.03	.05	.39
BMI (kg/m^2)	.27	.10	.008
Cigarettes/day	.009	.03	.77
Alcohol (<1oz/day)#	.37	.90	.68
Free Testosterone*	.13	.10	.21
Bound Testosterone*	.001	.002	.71
FSH@	-.75	.15	.0001
TSH++	-.39	.42	.35
Prolactin**	-.03	.07	.72

R^2= .34
+estradiol to lutenizing hormone (mU/ml) ratio
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
@follicle stimulating hormone mU/ml
++ Thyroid stimulating hormone (mU/ml)
** prolactin ng/ml

TABLE 4.1.43 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE BOUND TESTOSTERONE-LH+ RATIO AMONG 112 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	74.46	48.53	.13
Total Fluorine (ppm)	.27	.98	.79
Age (years)	.29	.62	.64
BMI (kg/m^2)	-.43	1.35	.75
Cigarettes/day	-.15	.44	.73
Alcohol (<1 oz/day)#	7.55	12.1	.54
Free Testosterone*	5.96	1.01	.0001
estradiol@	-.28	.38	.45
FSH@@	-8.65	2.03	.0001
TSH++	-.23	5.69	.97
Prolactin**	.11	.95	.90

R^2= .43
+bound testosterone to lutenizing hormone (mU/ml) ratio
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
@ pg/ml
@@follicle stimulating hormone mU/ml
++ Thyroid stimulating hormone (mU/ml)
** prolactin ng/ml

TABLE 4.1.44 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE FREE TESTOSTERONE-LH+ RATIO AMONG 112 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	ß	SE(ß)	p-value
Intercept	3.00	1.38	.03
Total Fluorine (ppm)	-.05	.03	.09
Age (years)	.001	.01	.91
BMI (kg/m^2)	.07	.04	.08
Cigarettes/day	-.007	.01	.58
Alcohol (<1oz/day)#	.30	.36	.41
Bound Testosterone*	.003	.0007	.0001
Estradiol@	.001	.01	.91
FSH@@	-.33	.06	.0001
TSH++	.18	.17	.30
Prolactin**	-.05	.03	.08

R^2= .46
+free testosterone to lutenizing hormone (mU/ml) ratio
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
@ pg/ml
@@follicle stimulating hormone mU/ml
++ Thyroid stimulating hormone (mU/ml)
** prolactin ng/ml

TABLE 4.1.45 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE BOUND TESTOSTERONE-PROLACTIN RATIO AMONG 111 MALE WORKERS.
13M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	60.05	68.04	.38
Total Fluorine (ppm)	-.15	1.38	.91
Age (years)	.84	.88	.34
BMI (kg/m^2)	-1.54	1.92	.42
Cigarettes/day	1.49	.62	.02
Alcohol (<1oz/day)#	13.9	17.2	.42
Estradiol++	-.22	.53	.68
Free Testosterone *	3.93	1.45	.008
LH**	-2.23	2.63	.40
FSH@	-2.55	3.50	.47
TSH+	-.95	.80	.24

R^2= .17
++pg/ml
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
** lutenizing hormone mU/ml
@ follicle stimulating hormone mU/ml
+ Thyroid stimulating hormone mU/ml

TABLE 4.1.46 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE FREE TESTOSTERONE-PROLACTIN RATIO AMONG 111 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	2.41	1.76	.17
Total Fluorine (ppm)	-.03	.04	.35
Age (years)	-.004	.02	.95
BMI (kg/m^2)	-.004	.05	.93
Cigarettes/day	.04	.02	.03
Alcohol (<1oz/day)#	-.03	.76	.97
Estradiol++	-.0001	.01	.99
Bound Testosterone *	.002	.0001	.03
LH**	-.08	.07	.24
FSH@	-.12	.09	.21
TSH+	-.18	.21	.40

R^2= .15
++pg/ml
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
** lutenizing hormone mU/ml
@ follicle stimulating hormone mU/ml
+ Thyroid stimulating hormone mU/ml

TABLE 4.1.47 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE ESTRADIOL-PROLACTIN RATIO AMONG 111 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	2.65	4.01	.51
Total Fluorine (ppm)	.005	.081	.95
Age (years)	.01	.05	.80
BMI (kg/m^2)	.07	.116	.53
Cigarettes/day	.10	.036	.005
Alcohol (<1oz/day)#	.86	1.01	.40
Bound Testosterone*	-.001	.003	.95
Free Testosterone *	.12	.12	.31
LH**	-.13	.15	.39
FSH@	-.29	.21	.17
TSH+	-.67	.47	.16

R^2= .16
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
** lutenizing hormone mU/ml
@ follicle stimulating hormone mU/ml
+ Thyroid stimulating hormone mU/ml

TABLE 4.1.48 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE PROLACTIN-FSH@ RATIO AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	2.56	1.52	.09
Total Fluorine (ppm)	.31	.11	.008
Alcohol #			
low (<1oz/day)	.81	.52	.13
nonresponse (NR)	.19	.85	.82
low X Fluoride	-.31	.12	.01
NR X Fluoride	-.08	.29	.78
Age (years)	-.05	.02	.01
BMI (kg/m^2)	.01	.04	.86
Cigarettes/day	-.03	.01	.03
Estradiol++	.02	.01	.06
Bound Testosterone*	-.001	.001	.92
Free Testosterone *	.01	.04	.75
LH**	-.07	.05	.15
TSH+	.31	.17	.07

R^2= .31
++ pg/ml
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
** lutenizing hormone mU/ml
@ follicle stimulating hormone mU/ml
+ Thyroid stimulating hormone mU/ml

TABLE 4.1.49 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE PROLACTIN-LH** RATIO AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	1.07	1.27	.38
Total Fluorine (ppm)	.34	.09	.0003
Alcohol #			
low (<1oz/day)	.68	.43	.11
nonresponse (NR)	.41	.69	.55
low X Fluoride	-.35	.09	.0004
NR X Fluoride	-.39	.20	.05
Age (years)	-.02	.01	.12
BMI (kg/m^2)	.05	.04	.17
Cigarettes/day	-.02	.01	.09
Estradiol++	.003	.009	.76
Bound Testosterone*	.001	.0008	.17
Free Testosterone *	-.04	.03	.30
FSH@	-.11	.05	.03
TSH+	.15	.14	.29

R^2= .31
**lutenizing hormone
++ pg/ml
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
@ follicle stimulating hormone mU/ml
+ Thyroid stimulating hormone mU/ml

TABLE 4.1.50 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE PROLACTIN-TSH+ RATIO AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	5.06	4.83	.30
Total Fluorine (ppm)	1.61	.37	.0001
Alcohol #			
low (<1oz/day)	4.16	1.67	.01
nonresponse (NR)	3.85	2.72	.16
low X Fluoride	-1.76	.38	.0001
NR X Fluoride	-2.11	.77	.008
Age (years)	-.19	.06	.003
BMI (kg/m^2)	.11	.14	.43
Cigarettes/day	-.06	.04	.14
Estradiol++	.03	.04	.46
Bound Testosterone*	.008	.003	.02
Free Testosterone *	-.35	.14	.01
FSH@	.51	.20	.01

R^2= .34
++ pg/ml
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
@ follicle stimulating hormone mU/ml
+ Thyroid stimulating hormone mU/ml

TABLE 4.1.51 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE BOUND TESTOSTERONE-TSH+ RATIO AMONG 112 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	ß	SE(ß)	p-value
Intercept	559.5	360.7	.12
Total Fluorine (ppm)	37.7	109.8	.73
Age (years)	-1.1	6.2	.85
BMI (kg/m^2)	-9.8	8.9	.27
Cigarettes/day	-.66	2.9	.82
Alcohol (<1oz/day)#	74.9	78.3	.34
Free Testosterone*	12.5	6.6	.06
Estradiol@	-1.6	2.5	.51
FSH@@	47.1	15.9	.004
LH++	5.7	12.2	.64
Prolactin**	-1.1	6.2	.85

R^2= .29
+bound testosterone to thyroid stimulating hormone (mU/ml) ratio
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
@ pg/ml
@@follicle stimulating hormone mU/ml
++ Thyroid stimulating hormone (mU/ml)
** prolactin ng/ml

TABLE 4.1.52 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE FREE TESTOSTERONE-TSH+ RATIO AMONG 112 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	15.65	6.34	.02
Total Fluorine (ppm)	-.28	.13	.03
Age (years)	-.29	.08	.003
BMI (kg/m^2)	-.01	.19	.94
Cigarettes/day	-.03	.06	.65
Alcohol (<1oz/day)#	1.50	1.64	.36
Bound Testosterone*	.01	.003	.006
Estradiol@	-.01	.05	.80
FSH@@	.68	.33	.04
LH++	-.001	.25	.99
Prolactin**	-.18	.13	.17

R^2= .37
+free testosterone to thyroid stimulating hormone (mU/ml) ratio
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
@ pg/ml
@@follicle stimulating hormone mU/m++ Thyroid stimulating hormone (mU/ml)
** prolactin ng/ml

TABLE 4.1.53 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE ESTRADIOL-TSH+ RATIO AMONG 112 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	30.80	16.10	.06
Total Fluorine (ppm)	-.425	.31	.18
Age (years)	-.53	.20	.01
BMI (kg/m^2)	.32	.46	.50
Cigarettes/day	.06	.14	.70
Alcohol (<1oz/day)#	2.36	4.00	.55
Free Testosterone*	-.28	.46	.55
Bound Testosterone*	.009	.01	.42
FSH@	.81	.81	.31
LH++	.20	.62	.75
Prolactin**	-.07	.32	.83

R^2= .15
+estradiol to thyroid stimulating hormone (mU/ml) ratio
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
@follicle stimulating hormone mU/ml
++ thyroid stimulating hormone (mU/ml)
** prolactin ng/ml

TABLE 4.1.54 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE BOUND TESTOSTERONE-FSH+ RATIO AMONG 112 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	101.89	61.25	.10
Total Fluorine (ppm)	.66	1.24	.60
Age (years)	-.13	.75	.14
BMI (kg/m^2)	-1.08	1.70	.53
Cigarettes/day	-.37	.55	.50
Alcohol (<1oz/day)#	.29	15.30	.98
Free Testosterone*	6.87	1.28	.0001
LH@@	-7.61	1.93	.0002
Estradiol@	.77	.47	.11
TSH++	8.90	7.03	.21
Prolactin**	-.03	1.20	.97

R^2= .50
+bound testosterone to follicle stimulating hormone (mU/ml) ratio
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
@luteinizing hormone mU/ml
@@estradiol pg/ml
++ Thyroid stimulating hormone (mU/ml)
** prolactin ng/ml

TABLE 4.1.55 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE FREE TESTOSTERONE-FSH+ RATIO AMONG 112 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	4.31	2.01	.03
Total Fluorine (ppm)	-.04	.04	.27
Age (years)	-.10	.02	.0001
BMI (kg/m^2)	.06	.06	.28
Cigarettes/day	-.02	.02	.31
Alcohol (<1oz/day)#	.18	.52	.74
Bound Testosterone*	.003	.001	.02
LH@@	-.25	.07	.0003
Estradiol@	.03	.04	.27
TSH++	.49	.24	.04
Prolactin**	-.05	.04	.23

R^2= .43
+free testosterone to follicle stimulating hormone (mU/ml) ratio
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
@luteinizing hormone mU/ml
@@estradiol pg/ml
++ Thyroid stimulating hormone (mU/ml)
** prolactin ng/ml

TABLE 4.1.56 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE ESTRADIOL-FSH+ RATIO AMONG 112 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	ß	SE(ß)	p-value
Intercept	6.91	5.69	.23
Total Fluorine (ppm)	.006	.006	.34
Age (years)	-.19	.07	.008
BMI (kg/m^2)	.27	.16	.10
Cigarettes/day	.03	.05	.57
Alcohol (<1oz/day)#	.52	1.42	.71
Free Testosterone*	.26	.16	.11
Bound Testosterone*	-.002	.004	.62
LH@	-.49	.18	.009
TSH++	.08	.65	.90
Prolactin**	.04	.11	.70

R^2= .26
+estradiol to follicle stimulating hormone (mU/ml) ratio
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
@luteinizing hormone mU/ml
++ Thyroid stimulating hormone (mU/ml)
** prolactin ng/ml

TABLE 4.1.57 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE BOUND TSH-FSH+ RATIO AMONG 112 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	.72	.33	.03
Total Fluorine (ppm)	.01	.006	.14
Age (years)	.002	.004	.59
BMI (kg/m^2)	.00007	.01	.94
Cigarettes/day	-.003	.003	.37
Alcohol (<1oz/day)#	-.16	.08	.05
Estradiol++	-.001	.003	.56
Bound Testosterone*	-.0002	.0002	.28
Free Testosterone*	-.01	.009	.15
Prolactin**	.002	.007	.73
LH@	-.03	.01	.005

R^2= .26
++pg/ml
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
** prolactin ng/ml
@ lutenizing hormone mU/ml
+ thyroid stimulating hormone (mU/ml) to follicle stimulating hormone (mU/ml) ratio

TABLE 4.1.58 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE TSH-LH+ RATIO AMONG 112 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	ß	SE(ß)	p-value
Intercept	.32	.25	.21
Total Fluorine (ppm)	.006	.005	.21
Age (years)	.004	.003	.26
BMI (kg/m^2)	.008	.007	.27
Cigarettes/day	-.001	.002	.53
Alcohol (<1 oz/day)#	-.07	.06	.26
Estradiol++	-.004	.002	.07
Bound Testosterone*	-.0001	.001	.84
Free Testosterone*	.007	.007	.32
Prolactin**	.001	.005	.91
FSH@	-.05	.01	.0001

R^2= .26
++pg/ml
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
** prolactin ng/ml
@ follicle stimulating hormone mU/ml
+ Thyroid stimulating hormone (mU/ml) to lutenizing hormone (mU/ml) ratio

TABLE 4.1.59 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE BOUND LH-FSH+ RATIO AMONG 112 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	.60	.43	.17
Total Fluorine (ppm)	-.0001	.009	.98
Age (years)	.009	.005	.09
BMI (kg/m^2)	.01	.01	.40
Cigarettes/day	.0001	.004	.82
Alcohol (<1oz/day)#	.04	.11	.71
Estradiol++	.004	.003	.18
Bound Testosterone*	.0001	.0002	.18
Free Testosterone*	-.004	.01	.78
Prolactin**	-.005	.009	.57
TSH@	-.05	.05	.29

R^2= .12
++pg/ml
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl
** prolactin ng/ml
@ thyroid stimulating hormone mU/ml
+ lutenizing hormone (mU/ml) to follicle stimulating hormone (mU/ml) ratio

TABLE 4.1.60 PEARSON CORRELATION COEFFICIENTS BETWEEN TOTAL SERUM FLUORIDE, AGE, BODY MASS INDEX (BMI), DAILY ALCOHOL USE, DAILY TOBACCO CONSUMPTION, AND LIPOPROTEINS
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL FLUORIDE (ppm)	AGE (years)	BMI (kg/m^2)	ALCOHOL (oz/day)	TOBACCO (cigs/day)
CHOLESTEROL*	.07	.25 p=.008	.19 p=.05	.09	.35 p=.0001
LDL**	.02	.13	.06	-.008	.28 p=.002
HDL#	-.01	.03	-.13	.18 P=.06	-.09
TRIGLYCERIDES*	.09	.19 p=.04	.27 p=.004	.07	.19 p=.04

*mg/dl
**low density lipoprotein
#high density lipoprotein

TABLE 4.1.61 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE CHOLESTEROL AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	ß	SE(ß)	p-value
Intercept	107.30	33.00	.002
Total Fluoride (ppm)	.52	.67	.44
Cigarettes/day	1.12	.31	.0005
BMI (kg/m^2)	1.44	1.01	.16
Age (years)	.77	.38	.05
Alcohol #			
low (<1oz/day)	-5.50	8.71	.53
nonresponse (NR)	-13.53	14.75	.35
GGT (IU/dl)*	.41	.12	.001
Bound Testosterone**	.03	.02	.07

R^2= .29
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
*gamma glutamyl transferase
**ng/dl

TABLE 4.1.62 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE LOW DENSITY LIPOPROTIEN AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	73.93	32.00	.03
Total Fluoride (ppm)	.22	.65	.73
Cigarettes/day	.69	.30	.02
BMI (kg/m^2)	1.26	.95	.19
Age (years)	.37	.37	.32
Alcohol #			
low (<1oz/day)	-3.02	8.33	.71
nonresponse (NR)	-10.85	13.93	.43
Prolactin (ng/ml)	-1.59	.66	.02
Bound Testosterone(ng/dl)	.04	.02	.0071

R^2= .19
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.

TABLE 4.1.63 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE HIGH DENSITY LIPOPROTIEN (HDL) AMONG 111 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	65.00	10.07	.0001
Total Fluoride (ppm)	-1.61	.77	.04
Alcohol #			
low (<1oz/day)	-9.92	3.51	.006
nonresponse (NR)	-6.77	5.73	.24
low X Fluoride	1.62	.80	.04
NR X Fluoride*	2.05	1.63	.21
Age (years)	-.004	.12	.97
BMI (kg/m^2)	-.31	.29	.28
Cigarettes/day	-.12	.09	.18
Bound Testosterone**	.018	.007	.009
Free Testosterone**	-.77	.28	.008

R^2= .17
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
*interaction terms between total fluoride and alcohol category
** ng/dl

TABLE 4.1.64 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE TRIGLYCERIDES AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	-114.50	117.20	.33
Total Fluoride (ppm)	2.38	2.31	.15
Cigarettes/day	2.28	1.05	.03
BMI (kg/m^2)	6.07	3.39	.08
Age (years)	2.32	1.44	.11
Alcohol #			
low (<1oz/day)	-11.48	29.4	.70
nonresponse (NR)	-19.94	49.03	.69
Free Testosterone*	7.34	3.37	.03
Bound Testosterone*	-.21	.08	.009

R^2= .19
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
*ng/dl

TABLE 4.1.65 PEARSON CORRELATION COEFFICIENTS BETWEEN TOTAL SERUM FLUORIDE, AGE, BODY MASS INDEX (BMI), DAILY ALCOHOL USE, DAILY TOBACCO CONSUMPTION, AND HEPATIC PARAMETERS
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m^2)	ALCOHOL (oz/day)	TOBACCO (cigs/day)
SGOT*	.01	-.10	.09	.12	-.11
SGPT**	.01	.01	.20 p=.02	.03	-.11
GGT#	-.04	.12	.27 p=.004	.15	.03
AKPH##	-.03	.27 p=.004	.19 p=.04	-.19 p=.05	.26 p=.006

*SERUM GLUTAMIC OXALOACETIC TRANSAMINASE IU/dl
**SERUM GLUTAMIC PYRUVIC TRANSAMINASE IU/dl
#GAMMA GLUTAMYL TRANSFERASE IU/dl
##ALKALINE PHOSPHATASE IU/dl

TABLE 4.1.66 PEARSON CORRELATION COEFFICIENTS BETWEEN HEPATIC ENZYMES, SERUM HORMONES, AND LIPOPROTEINS
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	SGOT	SGPT	GGT	AKPH
CHOLESTEROL*	.07	.25 p=.008	.19 p=.05	.09
LDL**	.02	13	.06	-.008
HDL#	-.01	.03	-.13	.18 p=.06
TRIGLYCERIDES*	.09	.19 p=.04	.27 p=.004	.07
ESTRADIOL+	-.16 p=.09	-.04	.03	-.003
FREE TESTOSTERONE"	-.12	-.14	-.23 p=.01	-.03
BOUND TESTOSTERONE"	-.16 p=.09	-.10	-.12	-.12
PROLACTIN+	-.20 p=.03	-.15	-.16 p=.09	-.20 p=.03

*mg/dl
**low density lipoprotein
#high density lipoprotein
+pg/ml
"ng/dl

TABLE 4.1.67 PEARSON CORRELATION COEFFICIENTS BETWEEN HEPATIC PARAMETERS
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	SGOT	SGPT	GGT	AKPH
SGOT*	1	.68 P=.0001	.43 P=.0001	.04
SGPT**	-	1	.60 P=.0001	.09
GGT#	-	-	1	.21 P=.02
AKPH##	-	-	-	1

*SERUM GLUTAMIC OXALOACETIC TRANSAMINASE IU/dl
**SERUM GLUTAMIC PYRUVIC TRANSAMINASE IU/dl
#GAMMA GLUTAMYL TRANSFERASE IU/dl
##ALKALINE PHOSPHATASE IU/dl

TABLE 4.1.68 SERUM GLUTAMIC OXALOACETIC TRANSAMINASE (SGOT), GLUTAMIC PYRUVIC TRANSAMINASE (SGPT),GAMMA GLUTAMYL TRANSFERASE (GGT), AND ALKALINE PHOSPHATASE (AKPH) BY TOTAL SERUM FLUORINE
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	SD	MEDIAN	RANGE	TEST#
TOTAL FLUORINE			SGOT (IU/dl)			
<1 ppm	23	22.5	4.1	22	13-29	F=0.41
>=1-3	65	24.1	8.6	23	10-74	p=.80
>3-10	16	25.8	14.5	22.5	17-77	
>10-15	6	25.7	11.3	22.5	17-47	
>15-26	5	22.2	5.1	22	14-27	
TOTAL	115	24.0	8.9	23	10-77	
			SGPT (IU/dl)			
<1	23	47.7	10.7	46	30-69	F=1.19
>=1-3	65	51.3	30.2	45	4-263	p=.32
>3-10	16	53.0	14.0	50.5	29-40	
>10-15	6	73.2	53.2	52.5	38-177	
>15-26	5	44.6	8.6	42	34-54	
TOTAL	115	51.7	26.8	47	4-263	
			Alkaline Phosphatase (IU/dl)			
<1 ppm	23	86.1	25.6	85	43-153	F=0.43
>=1-3	65	85.9	19.9	80	38-137	p=.78
>3-10	16	77.9	20.3	71.5	54-123	
>10-15	6	87.2	34.0	75.5	61-153	
>15-26	5	89.0	42.1	84	41-153	
TOTAL	115	83.3	22.9	80	38-153	
			GGT (IU/dl)			
<1 ppm	23	37.2	29.4	27	6-117	F=0.39
>=1-3	65	32.4	26.7	25	5-174	p=.81
>3-10	16	35.4	35.4	26	10-158	
>10-15	6	38.3	16.7	36.5	19-60	
>15-26	5	22.2	11.5	20	11-37	
TOTAL	115	33.7	27.6	26	5-174	

#univariate Anova

TABLE 4.1.69 SERUM GLUTAMIC OXALOACETIC TRANSAMINASE (SGOT) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	SGOT (IU/dl)					
	N(%)	MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	41(35.7)	24	12.4	22	13-77	F=.92
25-30	57(49.6)	23	5.8	23	10-42	p=.40
>30	17(14.8)	27	8.1	26	17-47	
AGE						
<30	21(18.3)	25	12.7	23	17-77	F=.78
31-40	48(41.7)	24	9.1	23	10-74	p=.51
41-50	27(23.5)	22	5.4	23	13-40	
51-60	19(16.5)	26	7.8	23	14-47	
Alcohol						
<1oz/d	87(81.3)	26	13.5	22	16-77	F=.61
1-3oz/d	20(18.7)	24	8.0	23	10-74	p=.44
missing	8	23	4.3	21	19-31	
Tobacco						
smoker	28(24.8)	24	8.4	23	13-77	F=.02
nonsmoker	85(75.2)	24	11.0	22	10-42	p=.89
missing	2	20	3.5	20	17-47	
TOTAL	115					

#univariate Anova

TABLE 4.1.70 SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		SGPT(IU/dl)				
	N(%)	MEAN	SD	MEDIAN	RANGE	TEST
BMI						
<25	41(35.7)	49	35.4	41	29-263	F=2.1
25-30	57(49.6)	50	14.2	49	4-95	p=.12
>30	17(14.8)	64	32.8	55	38-177	
AGE						
<30	21(18.3)	49	11.5	45	31-80	F=.61
31-40	48(41.7)	53	33.6	47	29-263	p=..61
41-50	27(23.5)	47	15.2	46	4-99	
51-60	19(16.5)	57	32.0	50	34-177	
Alcohol						
<1oz/d	87(81.3)	53	2935	47	29-263	F=.68
1-3oz/d	20(18.7)	47	16.9	46	4-99	p=..41
missing	8	51	10.9	52	35-67	
Tobacco						
smoker	28(24.8)	48	15.2	47	4-90	F=.76
nonsmoker	85(75.2)	53	29.6	48	30-263	p=..39
missing	2	49	25.5	49	31-67	
TOTAL	115					

#univariate Anova

TABLE 4.1.71 GAMMA GLUTAMYL TRANSFERASE (GGT) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		GGT (IU/dl)				
	N(%)	MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	41(35.7)	28	31.1	17	5-174	F=3.54
25-30	57(49.6)	34	23.1	19	8-158	p=.03
>30	17(14.8)	48	28.6	44	19-117	
AGE						
<30	21(18.3)	32	23.4	25	11-111	F=1.58
31-40	48(41.7)	31	32.7	22	5-174	p=.36
41-50	27(23.5)	33	17.2	29	8-72	
51-60	19(16.5)	44	29.3	35	11-117	
Alcohol						
<1oz/d	87(81.3)	40	25.5	35	8-89	F=1.64
1-3oz/d	20(18.7)	32	25.3	26	6-174	p=.36
missing	8	41	50.4	23	12-158	
Tobacco						
smoker	28(24.8)	36	21.3	33	5-89	F=.55
nonsmoker	85(75.2)	32	26.3	25	6-174	p=.46
missing	2	85	103.2	85	12-158	
TOTAL	115					

#univariate Anova

TABLE 4.1.72 ALKALINE PHOSPHATASE (AKPH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		AKPH (IU/dl)				
	N(%)	MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	41(35.7)	79	22.1	75	38-153	F=1.53
25-30	57(49.6)	84	21.9	81	41-153	p=.22
>30	17(14.8)	90	27.1	90	43-153	
AGE						
<30	21(18.3)	78	22.2	76	38-153	F=2.78
31-40	48(41.7)	80	20.3	76	50-15.	p=.45
41-50	27(23.5)	86	24.1	83	43-153	
51-60	19(16.5)	95	24.1	94	41-130	
Alcohol						
<1oz/d	87(81.3)	85	24.0	2	38-153	F=2.05
1-3oz/d	20(18.7)	77	16.9	75	51-124	p=.16
missing	8	82	22.5	70	60-115	
Tobacco						
smoker	28(24.8)	85	23.8	85	61-153	F=6.48
nonsmoker	85(75.2)	77	22.0	77	38-153	p=.012
missing	2	86	24.8	86	68-103	
TOTAL	115					

#univariate Anova

TABLE 4.1.73A LINEAR MULTIVARIATE REGRESSION MODEL 1 OF FACTORS PREDICTING THE SERUM GLUTAMIC OXALOACETIC TRANSAMINASE (SGOT) AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	26.71	7.1	.0003
Total Fluorine (ppm)	-3.23	1.31	.02
BMI (kg/m^2)	-.0004	.23	.99
BMI X T. Fluorine*	.12	.05	.015
Age (years)	-.003	.08	.97
Alcohol #			
low (<1oz/day)	.70	1.85	.71
nonresponse (NR)	-1.10	3.10	.72
Cigarettes/day	-.09	.07	.16
Prolactin (ng/ml)	-.37	.15	.01

R^2= .17
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* interaction term between total serum fluoride and BMI.

TABLE 4.1.73B LINEAR MULTIVARIATE REGRESSION MODEL 2 OF FACTORS PREDICTING THE SERUM GLUTAMIC OXALOACETIC TRANSAMINASE (SGOT) AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	27.71	6.22	.0001
Total Fluorine (ppm)	-2.70	1.23	.02
BMI (kg/m^2)	-.09	.06	.11
BMI X T. Fluorine*	.10	.04	.02
Age (years)	-.02	.07	.74
Cigarettes/day	-.11	.06	.11
Alcohol #			
low (<1oz/day)	1.84	1.61	.28
nonresponse (NR)	-1.3	2.7	.64
Prolactin (ng/ml)	-.27	.13	.04
GGT (IU/dl)**	.13	.02	.0001

R^2= .35
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* Interaction term between total serum fluoride and BMI
** Gamma glutamyl transferase

TABLE 4.1.73C LINEAR MULTIVARIATE REGRESSION MODEL 3 OF FACTORS PREDICTING THE SERUM GLUTAMIC OXALOACETIC TRANSAMINASE (SGOT) AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	ß	SE(ß)	p-value
Intercept	120.60	4.0	.002
Total Fluorine (ppm)	.63	.78	.42
BMI (kg/m^2)	-.07	.13	.58
BMI X T. Fluorine*	-.03	.03	.34
Age (years)	.06	.05	.23
Cigarettes/day	-.02	.04	.45
Alcohol #			
low (<1oz/day)	-.65	1.03	.53
nonresponse (NR)	-1.40	1.72	.42
Prolactin (ng/ml)	-.09	.08	.29
SGPT (IU/dl)**	.24	.01	.0001

R^2= .74
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* Interaction term between total serum fluoride and BMI
** Serum glutamic pyruvic transaminase

TABLE 4.1.74A LINEAR MULTIVARIATE REGRESSION MODEL 1 OF FACTORS PREDICTING THE SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT) AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	58.13	24.26	.02
Total Fluorine (ppm)	-15.80	4.58	.0008
BMI (kg/m^2)	.30	.82	.72
BMI X T. Fluorine*	.62	.17	.0004
Age (years)	-.24	.28	.39
Alcohol #			
low (<1oz/day)	5.54	6.36	.39
nonresponse (NR)	1.31	10.63	.90
Cigarettes/day	-.27	.23	.24
Prolactin (ng/ml)	-1.18	.51	.02

R^2= .21
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* interaction term between total serum fluoride and BMI.

TABLE 4.1.74B LINEAR MULTIVARIATE REGRESSION MODEL 2 OF FACTORS PREDICTING THE SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT) AMONG 111 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	62.09	19.63	.002
Total Fluoride (ppm)	-13.70	3.64	.0003
BMI (kg/m^2)	-.70	.66	.30
BMI X T. Fluorine*	.54	.14	.0001
Age (years)	-.33	.22	.14
Cigarettes/day	-.027	.18	.14
Alcohol #			
low (<1oz/day)	10.02	5.09	.05
nonresponse (NR)	.48	8.44	.95
Prolactin (ng/ml)	-.74	.41	.07
GGT (IU/dl)**	.56	.07	.0001

R^2= .51
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* Interaction term between total serum fluoride and BMI
** Gamma glutamyl transferase

TABLE 4.1.74C LINEAR MULTIVARIATE REGRESSION MODEL 3 OF FACTORS PREDICTING THE SERUM GLUTAMIC PYRUVIC TRANSAMINASE (SGPT) AMONG 111 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	ß	SE(ß)	p-value
Intercept	-18.25	14.36	.21
Total Fluorine (ppm)	-6.65	2.61	.01
BMI (kg/m^2)	.30	.45	.51
BMI X T. Fluorine*	.27	.10	.007
Age (years)	-.23	.16	.14
Cigarettes/day	-.001	.13	.99
Alcohol #			
low (<1oz/day)	3.55	3.53	.32
nonresponse (NR)	4.39	5.91	.46
Prolactin (ng/ml)	-.11	.29	.72
SGOT (IU/dl)**	2.85	.19	.0001

R^2= .76
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* Interaction term between total serum fluoride and BMI
** serum glutamic oxaloacetic transaminase

TABLE 4.1.75A LINEAR MULTIVARIATE REGRESSION MODEL 1 OF FACTORS PREDICTING THE GAMMA GLUTAMYL TRANSFERASE (GGT) AMONG 111 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	-12.59	22.62	.58
Total Fluorine (ppm)	-1.93	2.11	.36
Alcohol #			
low (<1oz/day)	-12.37	9.50	.20
nonresponse (NR)	-28.13	15.46	.07
low X Fluorine*	1.59	2.18	.47
NR X Fluorine*	13.90	4.48	.003
Age (years)	.29	.30	.33
BMI (kg/m^2)	1.71	.76	.03
Cigarettes/day	.09	.24	.72

R^2= .18
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
*Interaction terms between total fluoride and alcohol category

TABLE 4.1.75B LINEAR MULTIVARIATE REGRESSION MODEL 2 OF FACTORS PREDICTING THE GAMMA GLUTAMYL TRANSFERASE (GGT) AMONG 111 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	-58.78	21.55	.008
n			
Total Fluoride (ppm)	-1.79	1.83	.33
Alcohol #			
low (<1 oz/day)	-9.04	8.25	.28
nonresponse (NR)	-20.08	13.49	.14
low X Fluorine*	1.39	1.90	.47
NR X Fluorine*	12.18	3.91	.002
Age (years)	.15	.26	.57
BMI (kg/m^2)	1.30	.66	.05
Cigarettes/day	.01	.23	.96
Cholesterol (mg/dl)	.15	.06	.02
SGOT (IU/dl)	1.18	.24	.0001

R^2= .38
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
*interaction terms between total fluoride and alcohol category

TABLE 4.1.75C LINEAR MULTIVARIATE REGRESSION MODEL 3 OF FACTORS PREDICTING THE GAMMA GLUTAMYL TRANSFERASE (GGT) AMONG 111 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	-32.39	18.47	.08
Total Fluorine (ppm)	-1.63	1.60	.31
Alcohol #			
low (<1oz/day)	-13.58	7.17	.06
nonresponse (NR)	-26.68	11.75	.025
low X Fluorine*	.92	1.66	.58
NR X Fluorine*	12.04	3.41	.0006
Age (years)	.25	.23	.27
BMI (kg/m^2)	.51	.59	.38
Cigarettes/day	.09	.20	.65
Cholesterol (mg/dl)	.12	.06	.04
SGPT (IU/dl)**	.59	.07	.0001

R^2= .53
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
*Interaction terms between total fluoride and alcohol category
** serum glutamic pyruvic transaminase

TABLE 4.1.76 LINEAR MULTIVARIATE REGRESSION MODEL 1 OF FACTORS PREDICTING THE ALKALINE PHOSPHATASE (AKPH) AMONG 111 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	24.50	15.69	.09
Total Fluorine (ppm)	-1.03	.43	.02
Cigarettes/day	-.06	.22	.79
Cigarettes/day X Fluorine*	.22	.05	.0001
BMI (kg/m^2)	1.10	.55	.05
Age (years)	.54	.22	.02
Alcohol #			
low (<1 oz/day)	5.78	4.90	.24
nonresponse (NR)	8.12	8.13	.32

R^2= .31
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* interaction term between total serum fluoride and cigarettes/day.

TABLE 4.1.77 PEARSON CORRELATION COEFFICIENTS BETWEEN TOTAL SERUM FLUORIDE, AGE, BODY MASS INDEX (BMI), DAILY ALCOHOL USE, DAILY TOBACCO CONSUMPTION, AND HEMATOLOGY PARAMETERS 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TOTAL FLUORINE (ppm)	AGE (years)	BMI (kg/m^2)	ALCOHOL (oz/day)	TOBACCO (cigs/day)
HEMAGLOBIN*	-.07	-.03	.04	-.20 p=.04	.20 p=.008
WBC**	.10	.07	.07	-.07	.70 p=.0001
PMN COUNT+	.05	.08	.09	-.10	.64 p=.0001
EOSINOPHILS	-.10	.13	.05	.02	.23 p=.003
LYMPHOCYTES	.19 p=.04	-.05	.04	.15	.28 p=.002
MONOCYTES	.05	.04	-.22 p=.02	-.21 p=.03	.32 p=.0004
PLATLETS	.10	-.13	-.11	.05	.29 P=.002
BASOPHILS	.04	-.08	-.02	-.14	-.05
BANDS				.26 p=.005	-.14

* g/dl
**white blood cell count
+ polymorphonuclear leukocyte count

TABLE 4.1.78 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE HEMAGLOBIN AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	14.51	.67	.0001
Total Fluorine (ppm)*	-.002	.0009	.02
Alcohol #			
low (<1oz/day)	.22	.20	.27
nonresponse (NR)	.56	.33	.09
Age (years)	.001	.009	.88
BMI (kg/m^2)	.01	.02	.65
Cigarettes/day	.01	.007	.20
Cigs/day X Fluorine^{2}**	.0003	.0001	.0005
Estradiol (pg/ml)	.01	.006	.07

R^2=.23
*square transformation of total fluoride
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
** interaction term between cigarettes per day and square transformation of total fluoride

TABLE 4.1.79 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE MEAN CORPUSCULAR HEMOBLOBIN (MCH) AMONG 111 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	31.65	.95	.0001
Total Fluorine (ppm)	.15	.09	.10
Alcohol #			
low (<1oz/day)	-.29	.65	.65
nonresponse (NR)	.03	.01	.02
low X Fluorine	-.16	.09	.08
NR X Fluorine	-.04	.19	.80
Age (years)	.03	.01	.02
BMI (kg/m^2)	-.07	.03	.01
Cigarettes/day	.02	.01	.13
Cigs/day X Fluorine*	.006	.003	.03

R^2= .24
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* Interaction terms; alcohol category by total fluoride, cigarettes per day by total fluoride

TABLE 4.1.1.80 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE MEAN CORPUSCULAR VOLUME (MCV) AMONG 111 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	8.74	2.50	.0001
Total Fluorine (ppm)	-.04	.07	.52
Alcohol #			
low (<1 oz/day)	-.61	.78	.43
nonresponse (NR)	-.95	1.27	.46
Age (years)	.11	.03	.002
BMI (kg/m^2)	-.06	.08	.05
Cigarettes/day	.04	.03	.21
Cigs/day X Fluorine*	.02	.007	.004
TSH (mU/ml)	.38	.35	.29

R^2= .28
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* interaction term; cigarettes per day by total fluoride

TABLE 4.1.81 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE WHITE BLOOD CELL COUNT (WBC)* AMONG 111 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	ß	SE(ß)	p-value
Intercept	2.87	1.32	.03
Total Fluorine (ppm)	.07	.10	.49
Alcohol #			
low (<1oz/day)	.44	.46	.33
nonresponse (NR)	-1.08	.74	.15
low X Fluorine	-.04	.10	.68
NR X Fluorine	.59	.21	.006
Age (years)	-.007	.02	.64
BMI (kg/m^2)	.07	.04	.05
Cigarettes/day	.13	.01	.0001
Free Testosterone(ng/dl)	.04	.03	.13
LH@	.10	.04	.02

R^2= .67
*WBC/1000
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
@ lutenizing hormone mU/ml

TABLE 4.1.82 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE POLYMORPHONUCLEAR LEUKOCUTE COUNT (POLY) AMONG 111 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	ß	SE(ß)	p-value
Intercept	368	1151	.75
Total Fluorine (ppm)	165	88	.06
Alcohol #			
low (<1oz/day)	746	399	.06
nonresponse (NR)	-49	651	.94
low X Fluorine	-161	90	.08
NR X Fluorine	370	185	.05
Age (years)	6	14	.66
BMI (kg/m^2)	45	33	.17
Cigarettes/day	95	10	.0001
LH (mU/ml)++	79	36	.03
Bound Testosterone*	-1.62	.8	.04
Free Testosterone *	84	32	.01

R^2= .55
++ Lutenizing hormone
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* ng/dl

TABLE 4.1.83 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE BAND COUNT (BAND) AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	-11.4	129.6	.93
Total Fluorine (ppm)	-3.4	3.2	.30
Alcohol #			
low (<1oz/day)	78.2	40.3	.05
nonresponse (NR)	14.9	67.8	.83
Age (years)	1.0	1.8	.56
BMI (kg/m^2)	2.2	4.6	.63
Cigarettes/day	4.2	1.5	.005

R^2= .12
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.

TABLE 4.1.84 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE LYMPHOCYTE COUNT (LYMPH) AMONG 111 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	ß	SE(ß)	p-value
Intercept	2205.6	611.1	.0005
Total Fluorine (ppm)	-342.7	125.3	.007
Alcohol #			
low (<1oz/day)	-526.6	222.7	.02
nonresponse (NR)	-977.1	355.7	.007
low X Fluorine	189.0	52.3	.0005
NR X Fluorine	247.9	103.9	.02
Cigarettes/day	34.0	6.9	.0001
Cigs/day X Fluorine*	-3.3	1.45	.02
BMI (kg/m^2)	1.58	19.6	.94
BMI X Fluorine*	7.15	4.1	.08
Age (years)	-16.1	8.6	.06
Prolactin (ng/ml)	38.5	14.2	.008
TSH (mU/ml)+	170.4	77.2	.03

R^2= .35
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
*interaction terms alcohol category by total fluoride; cigarettes/day by total fluoride, BMI by total fluoride.
+ thyroid stimulating hormone mU/ml

TABLE 4.1.85 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE MONOCYTE COUNT (MONO) AMONG 111 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	397.4	198.9	.05
Total Fluorine (ppm)	110.4	38.6	.005
Alcohol #			
low (<1oz/day)	132.1	53.8	.02
nonresponse (NR)	40.1	89.1	.66
Age (years)	-.37	2.4	.88
BMI (kg/m^2)	-2.66	7.0	.70
BMI X Fluorine*	-4.0	1.42	.006
Cigarettes/day	7.0	1.9	.0004
LH@	13.9	6.8	.04

R^2= .30
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
*Interaction term, BMI by total fluoride.
@ lutenizing hormone mU/ml

TABLE 4.1.86 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE EOSINOPHIL COUNT (EOS) AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	ß	SE(ß)	p-value
Intercept	50.45	122.30	.68
Total Fluorine (ppm)	-7.31	3.35	.03
Alcohol #			
low (<1 oz/day)	-12.10	37.91	.75
nonresponse (NR)	21.79	62.25	.73
Age (years)	1.56	1.67	.35
BMI (kg/m^2)	2.10	4.13	.61
Cigarettes/day	3.04	1.69	.08
Cigs/day X Fluorine*	.62	.35	.08
TSH@	30.1	17.1	.08

R^2= .18

#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
* interaction term, cigarettes per day by total fluoride
@ Thyroid stimulating hormone mU/ml

TABLE 4.1.87 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE PLATELET COUNT (PLATE) AMONG 111 MALE WORKERS.
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	264.7	54.8	.0001
Total Fluorine (ppm)	29.8	9.5	.002
Alcohol #			
low (<1oz/day)	8.2	13.3	.54
nonresponse (NR)	.9	22.5	.97
Age (years)	-1.3	.6	.04
BMI (kg/m^2)	1.1	1.7	.53
BMI X Fluorine*	-1.0	.4	.004
Cigarettes/day	2.7	.6	.0001
Cigs/day X Fluorine*	-.3	.1	.04
Prolactin (ng/ml)	2.6	.03	.09
Bound Testosterone**	-.04	.03	.10

R^2=.28

#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
*interaction terms, BMI by total fluoride, cigarettes per day by total fluoride.
** ng/dl

TABLE 4.1.88 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE BASOPHIL COUNT (BASO) AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	β	SE(β)	p-value
Intercept	44.35	54.19	.42
Total Fluorine (ppm)*	-.03	.06	.61
Alcohol #			
low (<1oz/day)	-1.73	13.61	.90
nonresponse (NR)	-.56	22.54	.81
Age (years)	-.07	.68	.92
BMI (kg/m^2)	-.61	1.58	.70
Cigarettes/day	-.80	.52	.12
Cigs/day X Fluorine^{2**}	.02	.007	.007
Bound Testosterone##	-.07	.04	.06
Free Testosterone##	2.5	1.6	.11
LH@	5.5	1.8	.002

R^2= .17
*square transformation of total serum fluoride
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
*Interaction term, cigarettes per day by total fluoride.
##ng/dl
@ lutenizing hormone mU/ml

4.4 Mortality Tables

TABLE 4.2.1 CHARACTERISTICS OF 749 FEMALE EMPLOYEES, 1947-1989.

	Chemical Division	Non chemical Division	Total
number of workers	245	504	749
person years of observation	6029.0	13280.4	19309.4
mean follow-up (years)	24.6	26.4	25.8
mean age at employment (years)	28.8	26.9	27.6
mean year of employment (years)	1965.0	1962.8	1963.5
mean year of death (years)	1981.3	1979.2	1979.6
mean age at death (years)	58.7	54.4	55.4

TABLE 4.2.2 CHARACTERISTICS OF 2788 MALE EMPLOYEES, 1947-1990.

	Chemical Division	Non chemical Division	Total
number of workers	1339	1449	2788
person years of observation	33385.3	37732.4	71117.7
mean follow-up (years)	24.8	26.0	25.5
mean age at employment (years)	25.6	28.9	27.3
mean year of employment (years)	1963.8	1962.3	1963.0
mean year of death (years)	1978.3	1978.1	1978.2
mean age at death (years)	54.2	58.1	56.4

TABLE 4.2.3 VITAL STATUS AND CAUSE OF DEATH ASCERTAINMENT AMONG 749 FEMALE EMPLOYEES, 1947-1990.

Vital status	Chemical Division		Non chemical Division		Total	
	No.	%	No.	%	No.	%
Alive	234	95.3	465	91.6	699	93.3
Dead*	11	4.7	39	8.4	50	6.7
Total	245	100.0	504	100.0	749	100

*two deaths occurred outside the U.S. with cause of death ascertained from sources other than death certificates.

TABLE 4.2.4 VITAL STATUS AND CAUSE OF DEATH ASCERTAINMENT AMONG 2788 MALE EMPLOYEES, 1947-1989.

Vital status	Chemical Division		Non chemical Division		Total	
	No.	%	No.	%	No.	%
Alive	1191	88.9	1249	86.2	2440	87.5
Dead*	148	11.1	200	13.8	348	12.5
Total	1339	100.0	1449	100.0	2788	100.0

*two deaths occurred outside the U.S. with cause of death ascertained from sources other than death certificates.

TABLE 4.2.5 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) AMONG 749 FEMALE EMPLOYEES, 1947-1989.

Cause of Death	Obs	Exp	SMR	95% CI
All causes	50	66.74	.75	.56-.99
Cancer	17	23.04	.71	.42-1.14
Gastrointestinal	2	4.54	.44	.05-1.59
Respiratory	4	4.72	.95	.26-2.43
Breast	3	5.87	.51	.10-1.49
Genital	2	3.37	.59	.07-2.14
Lymphopoietic	3	2.04	1.47	.30-4.29
Heart disease	10	12.39	.81	.49-1.29
Cerebrovascular	3	3.51	.86	.01-4.80
Gastrointestinal	3	3.41	.88	.18-2.57
Injuries	4	6.23	.64	.17-1.64
Suicide	1	1.78	.56	.01-3.13

TABLE 4.2.6 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY DURATION OF EMPLOYMENT AMONG FEMALE EMPLOYEES, 1947-1989.

Cause of Death	Obs	Exp	SMR	95% CI
Duration ≤10 years				
All causes	50	66.74	.75	.56-.99
Cancer	17	23.04	.71	.42-1.14
Cardiovascular	18	22.00	.82	.48-1.29
Duration >10 years				
All causes	20	26.62	.75	.46-1.16
Cancer	6	9.42	.64	.23-1.39
Cardiovascular	8	10.27	.78	.34-1.54

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval.

TABLE 4.2.7 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY LATENCY AMONG FEMALE EMPLOYEES, 1947-1989.

Cause of Death	Obs	Exp	SMR	95% CI
Latency>10 years				
All causes	41	56.94	.72	.52-.98
Cancer	16	20.93	.76	.44-1.24
Cardiovascular	13	19.86	.65	.35-1.12
Latency>15 years				
All causes	37	49.37	.75	.53-1.03
Cancer	14	18.25	.77	.42-1.29
Cardiovascular	13	17.79	.73	.39-1.25
Latency>20 years				
All causes	29	39.20	.74	.49-1.06
Cancer	11	14.47	.76	.38-1.36
Cardiovascular	10	14.67	.68	.33-1.25

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval.

TABLE 4.2.8 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY ANY EMPLOYMENT IN THE CHEMICAL DIVISION AMONG FEMALE EMPLOYEES, 1947-1989.

Cause of Death	Obs	Exp	SMR	95% CI
Not employed in CD				
All causes	39	43.05	.91	.64-1.24
Cancer	14	15.46	.91	.49-1.52
Cardiovascular	13	13.82	.94	.50-1.61
Heart disease	8	7.69	1.04	.45-2.05
All GI	2	2.23	.90	.10-3.23
All respiratory	2	2.23	.90	.10-3.23
Injuries	3	1.48	2.02	.41-5.90
employed in CD				
All causes	11	23.69	.46	.23-.83
Cancer	3	8.38	.36	.07-1.05
Cardiovascular	5	8.19	.61	.20-1.43
Heart disease	2	4.69	.43	.05-1.54
All GI	1	1.18	.85	.01-4.73
All respiratory	1	1.28	.78	.01-4.81
Injuries	1	1.98	.51	.51-2.81

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CD, Chemical Division.

TABLE 4.2.9 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs), BASED ON U.S. WHITE MALE RATES, AMONG 2788 MALE EMPLOYEES, 1947-1989.

Cause of Death	Obs	Exp	SMR	95% CI
All causes	347	473.56	.73	.66- .81
Cancer	103	107.80	.95	.77-1.15
Gastrointestinal	24	25.94	.93	.59-1.38
Colon	9	9.11	.99	.45-1.88
Pancreas	8	5.33	1.50	.65-2.96
Respiratory	31	40.53	.76	.52-1.09
Lung	29	38.72	.75	.50-1.08
Prostate	6	5.10	1.18	.43-2.56
Testis	1	.82	1.22	.02-6.80
Bladder	3	2.20	1.36	.27-3.98
Lymphopoietic	13	11.42	1.14	.54-1.84
Cardiovascular	145	203.31	.71	.60-.84
CHD	110	147.04	.75	.61-.90
Cerebrovascular	10	19.92	.50	.24-.92
All Gastrointestinal	12	23.99	.50	.26-.87
All respiratory	13	25.89	.50	.27-.86
Diabetes	8	6.53	1.23	.53-2.42
Injuries	38	46.56	.82	.58-1.12
Suicide	12	17.10	.70	.32-1.23

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.10 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs), BASED ON MINNESOTA WHITE MALE RATES, AMONG 2788 MALE EMPLOYEES, 1947-1989.

Cause of Death	Obs	Exp	SMR	95% CI
All causes	347	450.79	77	.69-.86
Cancer	103	97.29	1.05	.86-1.27
Gastrointestinal	24	26.78	.90	.57-1.33
Colon	9	9.42	.96	.44-1.81
Pancreas	8	5.58	1.43	.62-2.83
Respiratory	31	30.42	1.02	.69-1.45
Lung	29	28.94	1.00	.67-1.44
Prostate	6	6.07	.99	.36-2.15
Testis	1	.92	1.09	.01-6.05
Bladder	3	2.18	1.37	.28-4.01
Lymphopoietic	13	12.07	1.09	.57-1.84
Cardiovascular	145	212.19	.68	.58-.80
CHD	110	159.09	.69	.57-.83
Cerebrovascular	10	24.66	.60	.32-1.02
All Gastrointestinal	12	21.13	.57	.29-.99
All respiratory	13	21.75	.60	.32-1.06
Diabetes	8	6.52	1.23	.53-2.42
Injuries	38	47.74	.80	.56-1.08
Suicide	12	15.09	.79	.41-1.39

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.11 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY LATENCY, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES, 1947-1989.

	LATENCY ≥ 10 YEARS			
Cause of Death	Obs	Exp	SMR	95% CI
All causes	299	398.27	.77	.68-.86
Cancer	98	88.71	1.10	.90-1.35
Gastrointestinal	24	24.78	.97	.62-1.44
Pancreas	8	5.20	1.54	.66-3.03
Respiratory	29	28.81	1.01	.67-1.45
Lung	27	27.44	.98	.65-1.43
Skin	3	1.53	1.96	.39-5.73
Prostate	6	5.94	1.01	.37-2.20
Bladder	3	1.75	1.72	.34-5.01
Lymphopoietic	11	10.03	1.10	.55-1.96
Cardiovascular	130	195.91	.66	.55-.79
All Gastrointestinal	8	18.58	.43	.19-.86
All respiratory	11	20.16	.55	.27-.98
Diabetes	8	5.37	1.49	.64-2.94
Injuries	21	27.61	.76	.47-1.16
Suicide	11	10.19	1.08	.54-1.93

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.12 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY LATENCY, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES, 1947-1989.

	LATENCY ≥ 15 YEARS			
Cause of Death	Obs	Exp	SMR	95% CI
All causes	266	344	.77	.68-87
Cancer	90	80.64	1.12	.90-1.37
Gastrointestinal	24	22.63	1.06	.68-1.51
Pancreas	8	4.72	1.69	.73-3.32
Respiratory	27	26.71	1.01	.67-1.47
Lung	25	25.45	.98	.64-1.45
Skin	3	1.29	2.33	.47-6.80
Prostate	5	5.73	.87	.28-2.04
Bladder	3	1.96	1.53	.37-4.47
Lymphopoietic	9	8.68	1.04	.47-1.97
Cardiovascular	119	178.25	.67	.55-.80
All Gastrointestinal	8	16.17	.49	.21-.97
All respiratory	9	18.60	.48	.22-.92
Diabetes	7	4.54	1.54	.62-3.18
Injuries	23	29.21	.79	.50-1.16
Suicide	9	7.47	1.21	.55-2.29

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.13 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY LATENCY, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES, 1947-1989.

	LATENCY ≥ 20 YEARS			
Cause of Death	Obs	Exp	SMR	95% CI
All causes	216	286.9	.75	.66-.86
Cancer	73	68.74	1.06	.83-1.34
Gastrointestinal	15	19.33	.77	.43-1.28
Pancreas	4	4.06	.99	.27-2.52
Respiratory	25	23.06	1.08	.70-1.60
Lung	23	21.06	1.05	.66-1.57
Skin	2	.98	2.02	.23-7.34
Prostate	5	5.29	.95	.30-2.21
Bladder	3	1.75	1.72	.34-5.01
Lymphopoietic	7	7.01	.99	.39-2.03
Cardiovascular	99	151.80	1.06	.83-1.34
All Gastrointestinal	8	12.90	.62	.27-1.21
All respiratory	9	16.3	.55	.25-1.05
Diabetes	7	3.65	1.92	.77-3.95
Injuries	13	19.47	.67	.36-1.14
Suicide	7	5.01	1.40	.56-2.80

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.14 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY DURATION OF EMPLOYMENT, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES, 1947-1989.

	DURATION ≥ 5 YEARS			
Cause of Death	Obs	Exp	SMR	95% CI
All causes	256	321.20	.80	.70-.90
Cancer	80	72.21	1.11	.88-1.38
Gastrointestinal	22	20.21	1.09	.68-1.65
Colon	8	7.10	1.13	.49-2.22
Pancreas	7	4.22	1.66	.66-3.42
Respiratory	25	23.72	1.08	.70-1.59
Lung	23	22.10	1.04	.66-1.56
Prostate	4	4.47	.84	.23-2.15
Bladder	2	1.68	1.19	.13-4.29
Brain	3	2.51	1.20	.24-1.50
Lymphopoietic	6	8.41	.71	.26-1.55
Cardiovascular	114	159.50	.71	.59-.86
CHD	90	120.20	.75	.60-.92
Cerebrovascular	6	18.44	.33	.12-.71
All Gastrointestinal	7	15.20	.46	.18-.95
All respiratory	9	16.30	.55	.25-1.05
Diabetes	8	4.53	1.77	.76-3.48
Injuries	29	36.60	.79	.53-1.14
Suicide	9	8.81	1.02	.47-1.94

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.15 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY DURATION OF EMPLOYMENT, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES, 1947-1989.

	DURATION ≥ 10 YEARS			
Cause of Death	Obs	Exp	SMR	95% CI
All causes	203	257.30	.79	.68-.91
Cancer	67	59.36	1.13	.87-1.43
Gastrointestinal	20	16.75	1.19	.73-1.84
Colon	7	5.92	1.18	.47-2.44
Pancreas	6	3.50	1.71	.63-3.71
Respiratory	22	19.38	1.13	.71-1.72
Lung	20	18.47	1.08	.66-1.67
Prostate	4	4.20	.95	.26-2.44
Bladder	1	1.44	.69	.01-3.85
Brain	3	1.89	1.59	.32-4.64
Lymphopoietic	5	6.58	.76	.24-1.77
Cardiovascular	92	132.13	.70	.56-.85
CHD	75	99.75	.73	.57-.92
Cerebrovascular	5	15.49	.32	.10-.75
All Gastrointestinal	4	11.96	.33	.09-.86
All respiratory	7	13.80	.51	.20-1.05
Diabetes	8	3.49	2.29	.99-4.51
Injuries	19	23.46	.68	.34-1.22
Suicide	8	5.88	1.36	.59-2.68

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.16 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY DURATION OF EMPLOYMENT, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES, 1947-1989.

	DURATION ≥ 20 YEARS			
Cause of Death	Obs	Exp	SMR	95% CI
All causes	104	152.36	.68	.56-.83
Cancer	35	37.31	.94	.65-1.30
Gastrointestinal	10	10.52	.95	.46-1.75
Colon	5	3.77	1.33	.43-3.09
Pancreas	1	2.21	.45	.01-2.52
Respiratory	11	12.69	.87	.43-1.55
Lung	10	12.10	.83	.40-1.52
Prostate	2	2.83	.71	.08-2.55
Bladder	1	.94	1.06	.01-5.91
Brain	1	1.03	.97	.01-5.40
Lymphopoietic	4	3.82	1.05	.28-6.02
Cardiovascular	48	80.6	.58	.19-1.36
CHD	39	61.25	.64	.45-.87
Cerebrovascular	1	9.13	.11	.00-.61
All Gastrointestinal	2	6.87	.29	.03-1.05
All respiratory	5	8.61	.58	.19-1.36
Diabetes	5	1.94	2.58	.83-6.02
Injuries	2	6.61	.30	.03-1.09
Suicide	3	2.54	1.18	.24-3.45

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.17 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs), BASED ON MINNESOTA WHITE MALE RATES, AMONG 1339 MALE EMPLOYEES EVER EMPLOYED IN THE CHEMICAL DIVISION, 1947-1989.

Cause of Death	Obs	Exp	SMR	95% CI
All causes	148	172.96	.86	.72-1.01
Cancer	40	36.31	1.10	.79-1.50
Gastrointestinal	9	9.77	.92	.42-1.75
Colon	4	3.46	1.15	.31-4.01
Pancreas	4	2.04	1.96	.53-5.01
Respiratory	12	11.26	1.07	.55-1.86
Lung	11	10.70	1.03	.51-1.84
Prostate	4	1.97	2.03	.55-4.59
Testis	1	.44	2.28	.03-12.66
Bladder	1	.75	1.33	.02-7.40
Lymphopoietic	5	4.76	1.05	.34-2.45
Cardiovascular	54	76.65	.70	.53-.92
CHD	43	57.74	.74	.54-1.00
Cerebrovascular	4	8.53	.47	.13-1.20
All Gastrointestinal	8	8.27	.97	.42-1.91
All respiratory	7	7.770	.91	.36-1.87
Diabetes	3	2.55	1.18	.24-3.44
Injuries	31	31.72	.98	.66-1.39
Suicide	10	6.99	1.43	.68-2.63

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.18 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs), BASED ON MINNESOTA WHITE MALE RATES, AMONG 1449 MALE EMPLOYEES NEVER EMPLOYED IN THE CHEMICAL DIVISION, 1947-1989.

Cause of Death	Obs	Exp	SMR	95% CI
All causes	200	291.25	.69	.59-.79
Cancer	63	67.56	.93	.72-1.19
Gastrointestinal	15	16.46	.91	.51-1.50
Colon	5	5.79	.89	.28-2.01
Pancreas	4	3.37	1.19	.32-3.04
Respiratory	19	25.58	.74	.45-1.16
Lung	18	24.44	.74	.44-1.16
Prostate	2	3.45	.58	.07-2.09
Testis	0	.43	.00	.00-8.45
Bladder	2	1.45	1.38	.16-4.99
Lymphopoietic	8	6.89	1.16	.50-2.29
Cardiovascular	91	129.77	.70	.56-.86
CHD	67	93.84	.71	.55-.91
Cerebrovascular	6	12.93	.46	.17-1.01
All Gastrointestinal	4	14.56	.27	.07-.70
All respiratory	6	16.77	.36	.13-.78
Diabetes	5	4.05	1.24	.40-2.88
Injuries	23	38.28	.60	.38-.98
Suicide	2	9.26	.60	.02-.78

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease.

TABLE 4.2.19 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY LATENCY, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES NEVER EMPLOYED IN THE CHEMICAL DIVISION, 1947-1989.

	LATENCY ≥ 15 YEARS			
Cause of Death	Obs	Exp	SMR	95% CI
All causes	161	216.10	.75	.63-.87
Cancer	56	50.70	1.10	.83-1.43
Gastrointestinal	15	14.37	1.05	.59-1.73
Colon	5	5.13	.98	.31-2.28
Pancreas	4	2.99	1.34	.36-3.43
Respiratory	17	16.73	1.02	.59-1.67
Lung	16	15.94	1.00	.57-1.63
Prostate	2	3.86	.52	.06-1.87
Lymphopoietic	5	5.40	.93	.30-2.16
Cardiovascular	75	113.60	.66	.52-.83
All Gastrointestinal	4	9.80	.41	.11-1.05
All respiratory	4	12.14	.33	.09-.84
Diabetes	5	2.82	1.77	.57-4.14
Injuries	7	11.17	.63	.25-1.29

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease; CD, Chemical Division.

TABLE 4.2.20 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY LATENCY, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES EVER EMPLOYED IN THE CHEMICAL DIVISION, 1947-1989.

	LATENCY ≥ 15 YEARS			
Cause of Death	Obs	Exp	SMR	95% CI
All causes	105	128.4	.82	.67-.99
Cancer	34	29.95	1.14	.79-1.59
Gastrointestinal	9	8.30	1.08	.49-2.06
Colon	7	3.00	1.33	.36-3.42
Pancreas	4	1.75	2.28	.61-5.85
Respiratory	10	9.98	1.00	.48-1.94
Lung	9	9.50	.95	.43-1.80
Prostate	3	1.87	1.61	.32-4.70
Lymphopoietic	4	3.28	1.72	.33-3.12
Cardiovascular	44	64.67	.68	.49-.91
All Gastrointestinal	4	6.37	.63	.17-1.61
All respiratory	5	6.49	.77	.25-1.80
Diabetes	2	1.72	1.17	.43-4.21
Injuries	6	8.54	.70	.26-1.53

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease; CD, Chemical Division.

TABLE 4.2.21 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY DURATION OF EMPLOYMENT, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES EVER EMPLOYED IN THE CHEMICAL DIVISION, 1947-1989.

	DURATION ≥ 10YEARS			
Cause of Death	Obs	Exp	SMR	95% CI
All causes	90	108.7	.83	.67-1.02
Cancer	27	24.4	1.08	.71-1.58
Gastrointestinal	6	6.92	.87	.22-1.89
Colon	3	2.47	1.22	.24-3.55
Pancreas	2	1.46	1.37	.75-4.86
Respiratory	8	8.16	.98	.42-1.93
Lung	7	7.78	.90	.36-1.86
Prostate	3	1.55	1.94	.39-5.66
Lymphopoietic	4	2.84	1.41	.38-3.61
Cardiovascular	38	54.60	.70	.50-.97
All respiratory	3	5.42	.55	.11-1.62
Diabetes	3	1.51	1.99	.40-5.80
Injuries	7	8.11	.86	.35-1.78

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease; CD, Chemical Division.

TABLE 4.2.22 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY DURATION OF EMPLOYMENT, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES EVER EMPLOYED IN THE CHEMICAL DIVISION, 1947-1989.

	DURATION ≥ 20YEARS			
Cause of Death	Obs	Exp	SMR	95% CI
All causes	45	66.29	.68	.50-.91
Cancer	16	16.21	.99	.56-1.20
Gastrointestinal	3	4.53	.66	.13-1.94
Colon	3	1.61	1.84	.37-5.38
Pancreas	0	.96	.00	0-3.84
Respiratory	5	5.57	.90	.29-2.09
Lung	4	5.31	.75	.20-1.93
Prostate	2	1.10	1.82	.20-6.58
Lymphopoietic	3	1.67	1.79	.36-5.24
Cardiovascular	18	34.48	.52	.31-.83
All respiratory	2	3.52	.57	.06-2.52
Diabetes	2	.84	2.37	.27-8.56
Injuries	2	3.27	.61	.07-2.21

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease; CD, Chemical Division.

TABLE 4.2.23 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY DURATION OF EMPLOYMENT, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES NEVER EMPLOYED IN THE CHEMICAL DIVISION, 1947-1989.

	DURATION ≥ 10YEARS			
Cause of Death	Obs	Exp	SMR	95% CI
All causes	113	148.60	.76	.63-.91
Cancer	40	34.43	1.16	.83-1.58
Gastrointestinal	14	9.82	1.43	.78-2.39
Colon	4	3.45	1.16	.31-2.97
Pancreas	4	2.04	1.96	.53-5.01
Respiratory	14	11.22	1.25	.68-2.09
Lung	13	10.69	1.22	.65-2.08
Prostate	1	2.65	.38	.01-2.10
Lymphopoietic	1	3.47	.27	.01-1.49
Cardiovascular	54	78.31	.69	.52-.90
All respiratory	4	8.35	.48	.13-1.27
Diabetes	5	1.98	2.52	.81-3.87
Injuries	4	7.96	.50	0.14-1.29

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease; CD, Chemical Division.

TABLE 4.2.24 NUMBERS OF DEATHS AND STANDARDIZED MORTALITY RATIOS (SMRs) BY DURATION OF EMPLOYMENT, BASED ON MINNESOTA WHITE MALE RATES, AMONG MALE EMPLOYEES NEVER EMPLOYED IN THE CHEMICAL DIVISION, 1947-1989.

	DURATION ≥ 20YEARS			
Cause of Death	Obs	Exp	SMR	95% CI
All causes	59	86.1	.69	.52-.88
Cancer	19	21.09	.90	.54-1.41
Gastrointestinal	7	5.99	1.17	.47-2.41
Colon	2	2.15	.93	.10-3.32
Pancreas	1	1.25	.80	.01-4.45
Respiratory	6	7.12	.84	.31-1.83
Lung	6	6.79	.88	.32-1.92
Prostate	0	1.73	.00	.0-2.12
Lymphopoietic	1	2.15	.46	.01-2.58
Cardiovascular	30	46.14	.65	.44-.93
All respiratory	3	5.43	.59	.12-1.72
Diabetes	3	1.10	2.74	.55-8.00
Injuries	0	3.34	.00	.00-1.14

Abbreviations used are: Obs, observed; Exp, expected; CI, confidence interval; CHD, coronary and atherosclerotic heart disease; CD, Chemical Division.

TABLE 4.2.25 AGE ADJUSTED STANDARDIZED RATE RATIOS (SRRs) FOR ALL CAUSE, CANCER, AND CARDIOVASCULAR MORTALITY BY DURATION OF EMPLOYMENT, AMONG MALE EMPLOYEES, 1947-1989.

Cause of death	SRR*	95%CI
all causes	.81	.63-1.03
all cancers	1.04	.67-1.61
all cardiovascular	.91	.62-1.34

Abbreviations used are: SRR, standardized rate ratio ; CI, confidence interval.
* less than 10 years of employment as referent category

TABLE 4.2.26 AGE ADJUSTED STANDARDIZED RATE RATIOS (SRRs) FOR ALL CAUSE, CANCER, LUNG CANCER, GI CANCER, AND CARDIOVASCULAR MORTALITY BY EVER/NEVER EMPLOYED IN THE CHEMICAL DIVISION, AMONG MALE EMPLOYEES, 1947-1989.

Cause of death	SRR*	95%CI
all causes	1.18	(.95,1.47)
all cancers	1.10	(.74,1.65)
lung cancer	1.09	(.67,2.31)
GI cancer	1.16	(.50,2.69)
all cardiovascular	1.05	(.76,1.48)

Abbreviations used are: SRR, standardized rate ratio ; CI, confidence interval; GI, gastrointestinal.
* Never employed in the Chemical Division as referent category

TABLE 4.2.27 AGE STRATIFIED, YEARS OF FOLLOW-UP ADJUSTED RATE RATIOS (RR_{MH}) FOR ALL CAUSE, CANCER, AND CARDIOVASCULAR MORTALITY BY EVER/NEVER EMPLOYED IN THE CHEMICAL DIVISION, AMONG MALE EMPLOYEES, 1947-1989.

Age at employment	RR_{MH}*	95%CI
All causes		
15-19 years	1.22	(.62, 2.40)
20-29 years	.95	(.68, 1.32)
30-39 years	.95	(.61 1.50)
40-65 years	1.02	(.72-1.44)
All cancers		
15-19 years	.95	(.21, 4.34)
20-29 years	.72	(.38, 1.35)
30-39 years	1.10	(.62, 1.90)
40-65 years	.66	(.27, 1.60)
All cardiovascular		
15-19 years	1.40	(.39, 5.03)
20-29 years	.86	(.44, 1.67)
30-39 years	.78	(.44, 1.29)
40-65 years	1.11	(.73, 1.82)

Abbreviations used are: RR_{MH}, Mantel-Haenszel age adjusted rate ratio ; CI, confidence interval.
* Adjusted for years of follow-up and stratified by four age categories. Never employed in the Chemical Division as referent category

TABLE 4.2.28 AGE STRATIFIED, YEARS OF FOLLOW-UP ADJUSTED RATE RATIOS (RR_{MH}) FOR ALL CAUSE, CANCER, AND CARDIOVASCULAR MORTALITY BY DURATION OF EMPLOYMENT IN THE CHEMICAL DIVISION, AMONG MALE EMPLOYEES, 1947-1989.

Age at employment	RR_{MH}*	95%CI
All causes		
15-19 years	1.30	(.58, 3.28)
20-29 years	1.16	(.81, 1.65)
30-39 years	2.16	(1.52, 2.70)
40-65 years	1.69	(1.07, 2.60)
All cancers		
15-19 years	2.17	(.40, 11.61)
20-29 years	.84	(.44, 1.51)
30-39 years	1.75	(.95, 3.21)
40-65 years	2.67	(.995,7.14)
All cardiovascular		
15-19 years	.88	(.25, 3.33)
20-29 years	1.38	(.73, 2.60)
30-39 years	3.53	(1.68, 6.21)
40-65 years	1.50	(.81, 2.79)

Abbreviations used are: RR_{MH}, Mantel-Haenszel age adjusted rate ratio ; CI, confidence interval.

* Adjusted for years of follow-up and stratified by four age categories. less than 10 years employment as referent category

TABLE 4.2.29 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE ALL CAUSE MORTALITY AMONG 2788 MALE WORKERS.

Variable	ß	SE(ß)	p-value	RR#
Year of first employment	-.55	.009	.0001	.946
Age at first employment*	.079	.006	.0001	1.082
Duration of employment*	-.34	.001	.0001	.967
Months in chemical division	.001	.001	.24	1.001

Abbreviations used are: ß, regression parameter; SE(ß), standard error of the slope parameter; RR, relative risk.
relative risk for one unit change in independent variable
* years

TABLE 4.2.30 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE CARDIOVASCULAR MORTALITY AMONG 2788 MALE WORKERS.

Variable	ß	SE(ß)	p-value	RR#
Year of first employment	-.075	.016	.001	.928
Age at first employment*	.119	.009	.0001	1.126
Duration of employment*	.230	.294	.45	.852
Months in chemical division	.0002	.001	.85	1.00

Abbreviations used are: ß, regression parameter; SE(ß), standard error of the slope parameter; RR, relative risk.
relative risk for one unit change in independent variable
* years

TABLE 4.2.31 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE CANCER MORTALITY AMONG 2788 MALE WORKERS.

Variable	ß	SE(ß)	p-value	RR#
Year of first employment	-.031	.019	.11	.969
Age at first employment*	.078	.011	.0001	1.081
Duration of employment*	-.028	.009	.002	.972
Months in chemical division	.002	.001	.20	1.002

Abbreviations used are: ß, regression parameter; SE(ß), standard error of the slope parameter; RR, relative risk.
relative risk for one unit change in independent variable
* years

TABLE 4.2.32 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE LUNG CANCER MORTALITY AMONG 2788 MALE WORKERS.

Variable	ß	SE(ß)	p-value	RR#
Year of first employment	-.019	.042	.65	.981
Age at first employment*	.070	.021	.001	1.072
Duration of employment*	-.062	.133	.64	.940
Months in chemical division	-.026	.016	.11	.975

Abbreviations used are: ß, regression parameter; SE(ß), standard error of the slope parameter; RR, relative risk.
relative risk for one unit change in independent variable
* years

TABLE 4.2.33 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE GI CANCER MORTALITY AMONG 2788 MALE WORKERS.

Variable	ß	SE(ß)	p-value	RR#
Year of first employment	.015	.038	.71	1.015
Age at first employment*	.130	.021	.001	1.139
Duration of employment*	.005	.020	.82	1.005
Months in chemical division	.001	.002	.56	1.001

Abbreviations used are: GI, Gastrointestinal; ß, regression parameter; SE(ß), standard error of the slope parameter; RR, relative risk.
relative risk for one unit change in independent variable
* years

TABLE 4.2.34 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE PROSTATE CANCER MORTALITY AMONG 2788 MALE WORKERS.

Variable	ß	SE(ß)	p-value	RR#
Year of first employment	.010	.081	.90	1.011
Age at first employment*	.082	.045	.06	1.085
Duration of employment*	-.070	.052	.18	.932
Months in chemical division	.010	.005	.03	1.010

Abbreviations used are: ß, regression parameter; SE(ß), standard error of the slope parameter; RR, relative risk.
relative risk for one unit change in independent variable
* years

TABLE 4.2.35 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE PANCREATIC CANCER MORTALITY AMONG 2788 MALE WORKERS.

Variable	ß	SE(ß)	p-value	RR#
Year of first employment	.046	.066	.48	1.047
Age at first employment*	.136	.034	.0001	1.146
Duration of employment*	-.012	.035	.73	.988
Months in chemical division	-.002	.006	.73	.998

Abbreviations used are: ß, regression parameter; SE(ß), standard error of the slope parameter; RR, relative risk.
relative risk for one unit change in independent variable
* *years

TABLE 4.2.36 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE DIABETES MELLITUS MORTALITY AMONG 2788 MALE WORKERS.

Variable	ß	SE(ß)	p-value	RR#
Year of first employment	-.405	.221	.06	.667
Age at first employment*	.092	.044	.04	1.096
Duration of employment*	.009	.030	.75	1.009
Months in chemical division	-.001	.004	.76	.999

Abbreviations used are: ß, regression parameter; SE(ß), standard error of the slope parameter; RR, relative risk.
relative risk for one unit change in independent variable
* *years

TABLE 4.2.37 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE ALL CAUSE MORTALITY AMONG 749 FEMALE WORKERS.

Variable	ß	SE(ß)	p-value	RR#
Year of first employment	-.02	.03	.41	.977
Age at first employment*	.08	.02	.0001	1.08
Duration of employment*				
2-10 years	1.31	.54	.01	3.72
>10 years	.85	.57	.14	2.33
Months in chemical division	-.003	.004	.48	.997

Abbreviations used are: ß, regression parameter; SE(ß), standard error of the slope parameter; RR, relative risk.
relative risk for one unit change in independent variable
* years

TABLE 4.2.38 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE CARDIOVASCULAR MORTALITY AMONG 749 FEMALE WORKERS.

Variable	ß	SE(ß)	p-value	RR#
Year of first employment	-.034	.048	.48	.966
Age at first employment*	.119	.024	.0001	1.126
Duration of employment*	-.011	.025	.67	.986
Months in chemical division	-.015	.017	.37	.985

Abbreviations used are: ß, regression parameter; SE(ß), standard error of the slope parameter; RR, relative risk.
relative risk for one unit change in independent variable
* years

TABLE 4.2.39 PROPORTIONAL HAZARD REGRESSION MODEL OF FACTORS PREDICTING THE CANCER MORTALITY AMONG 749 FEMALE WORKERS.

Variable	ß	SE(ß)	p-value	RR#
Year of first employment	-.043	.053	.42	.958
Age at first employment*	.085	.025	.001	1.089
Duration of employment*	-.021	.025	.65	.980
Months in chemical division	.001	.005	.87	1.001

Abbreviations used are: ß, regression parameter; SE(ß), standard error of the slope parameter; RR, relative risk.
relative risk for one unit change in independent variable
* years

FIGURE 1. Free testosterone and total serum fluorine 1990 3M Chemolite study

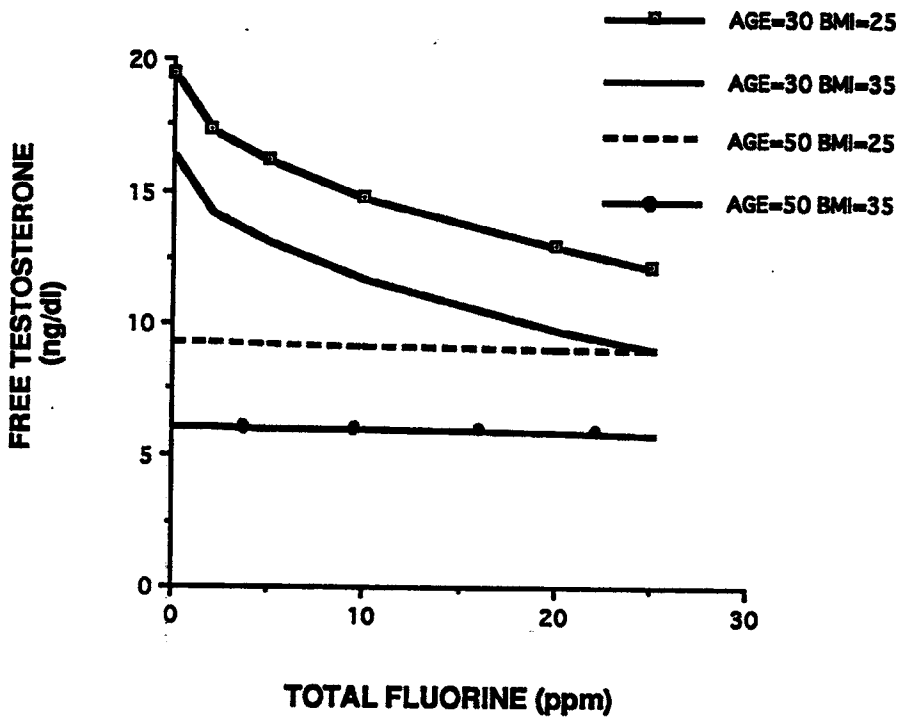


Figure 2. Bound testosterone and total serum fluorine
1990 3M Chemolite study

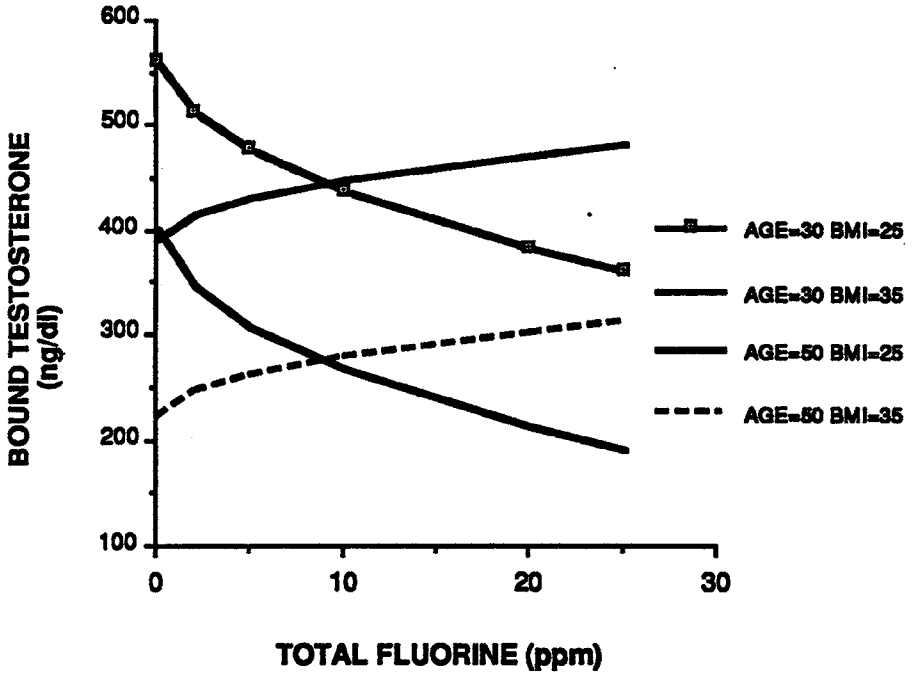


Figure 3. Estradiol and total serum fluorine
1990 3M Chemolite study

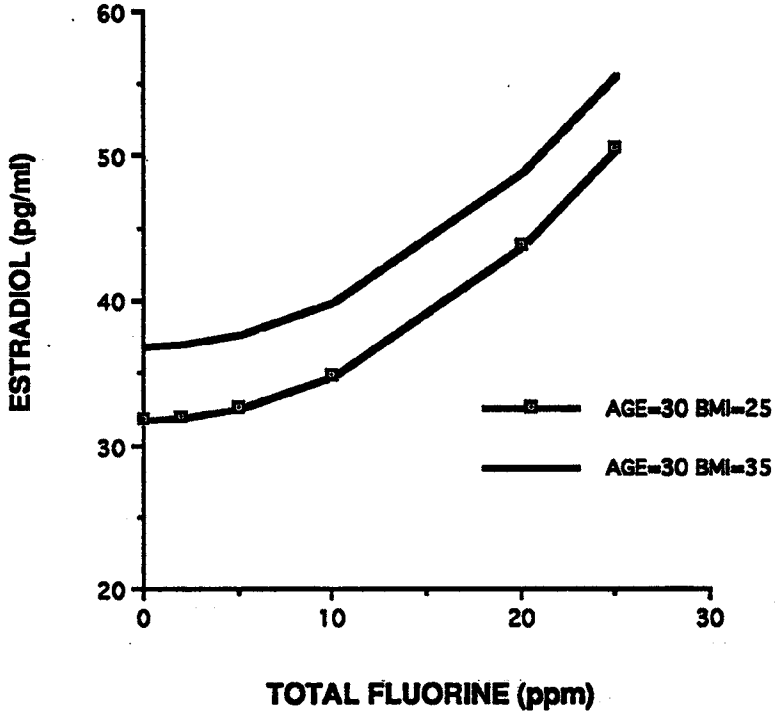


Figure 4. Lutenizing hormone and total serum fluorine
1990 3M Chemolite study

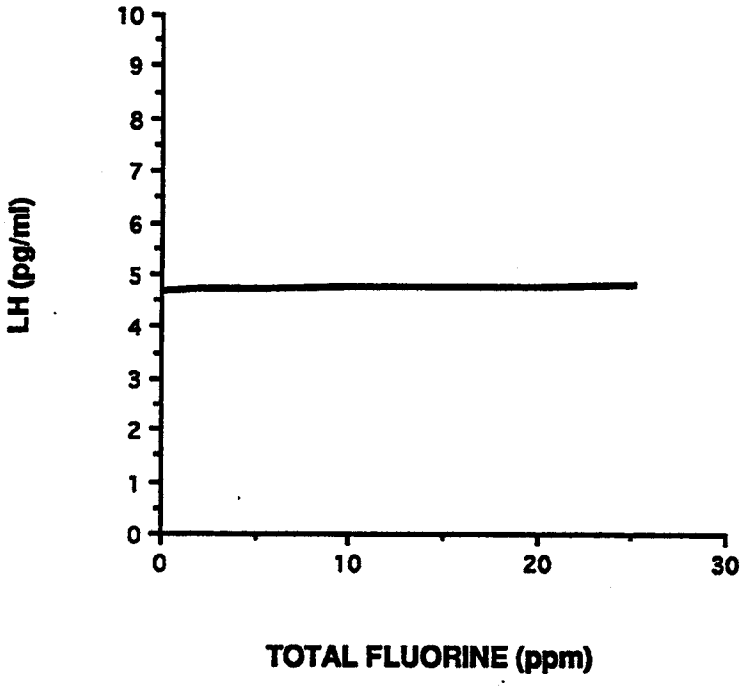


Figure 5. Follicle stimulating hormone and total serum fluorine 1990 3M Chemolite study

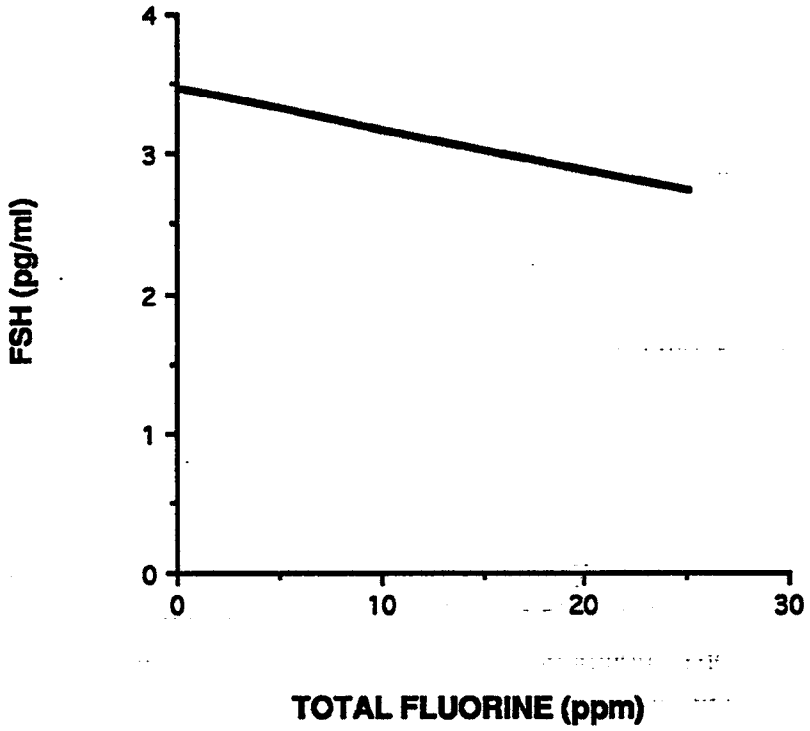


Figure 6. Prolactin and total serum fluorine
1990 3M Chemolite study

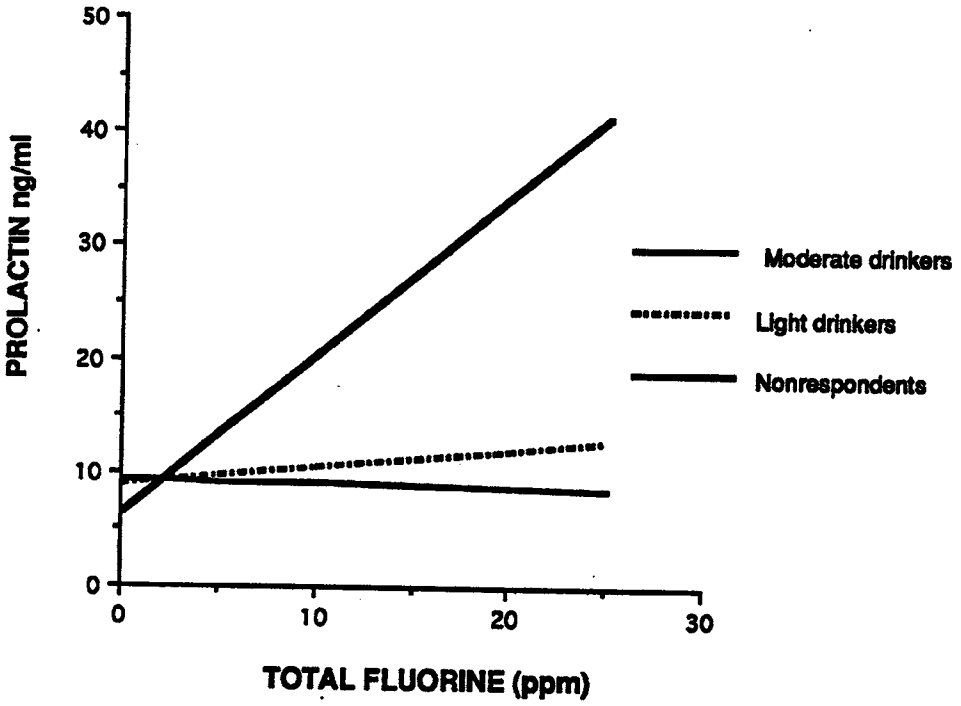


Figure 7. Thyroid stimulating hormone and total serum fluorine 1990 3M Chemolite study

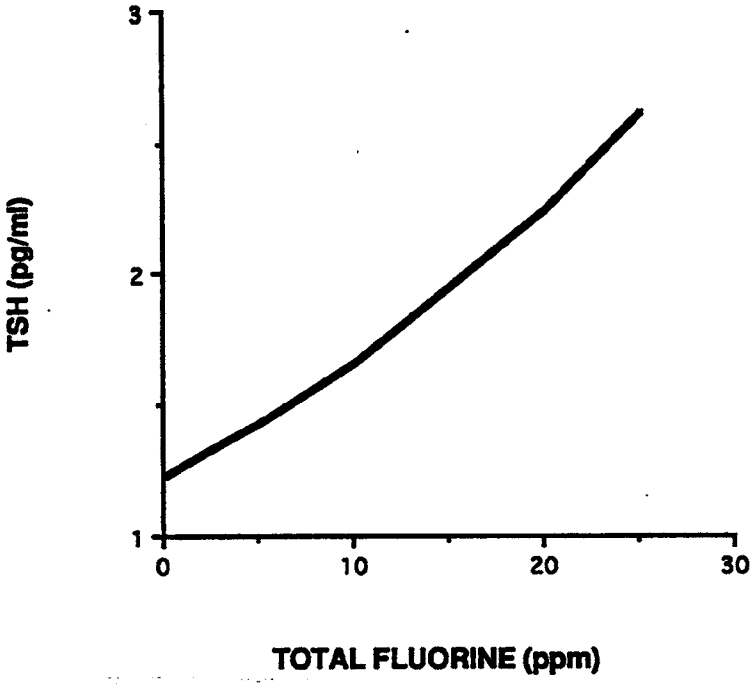
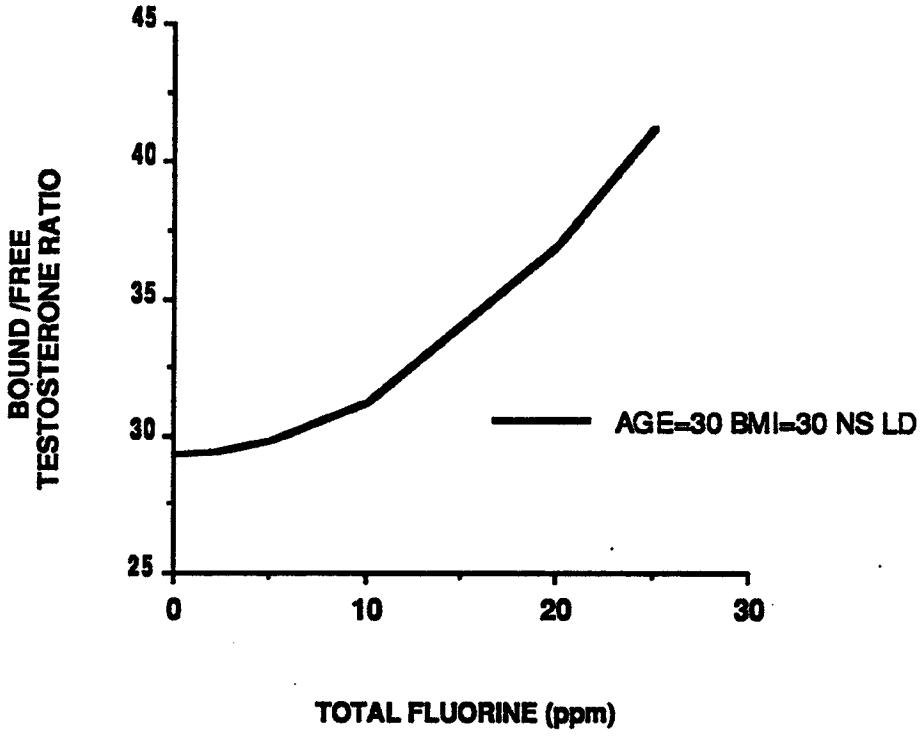


Figure 8. Bound to free testosterone ratio and total serum fluorine 1990 3M Chemolite study



5. DISCUSSION

5.1 Physiologic Effects Study

5.1.1 Introduction

This was a cross-sectional study of the relationship between selected physiologic parameters and PFOA exposure which was assessed using total serum fluorine. Participants were recruited from workers employed during November, 1990 in the Chemical Division of the 3M Chemolite Plant in Cottage Grove, Minnesota. All current workers who had worked in high exposure jobs at any time in the five previous years were invited to participate. A sample of workers employed in low exposure jobs was frequency matched to the age distribution of workers in high exposrue jobs.

Participants completed a corporate medical history questionnaire and had vital parameters measured by an occupational health nurse. Blood was drawn for assays of total serum fluorine, seven hormones involved in the hypothalamic-pituitary-gonadal axis, serum lipids, lipoproteins, hepatic function parameters, and hematology indices. Blood was drawn in the morning after workers were assigned to the day shift for at least three days.

In 93% of participants serum fluorine levels were at least 10 times the background levels in the general population and in 3M workers not employed at Chemolite. Many workers who lacked PFOA exposure by job history had elevated PFOA levels. The sources of the unexpected PFOA exposure are unknown.

5.1.2 Hormones

The findings from this study are consistent with the hypothesis that perfluorooctanoic acid (PFOA) affects the human hypothalamic-pituitary-gonadal axis. This study showed that relatively low levels of serum PFOA (20µM) depressed free testosterone and elevated estradiol but did no affect LH or FSH levels. The association between free testosterone and PFOA was different in

older men than in younger men. In older men, free testosterone (FT) was depressed below 10 ng/ml at serum fluoride levels below one part per million (estimated PFOA levels below 1 µM). In younger men, FT decreased toward 10 ng/ml at serum fluoride levels above 15 ppm (estimated PFOA levels below 15 µM). Increasing age may increase men's susceptibility to the testosterone lowering effects of PFOA. The associations between PFOA and the hormone levels may reflect a true causal relationship, or may be a result of chance, bias, or uncontrolled confounding. There are no human studies of PFOA associated reproductive toxicity available for comparison. Studies of the effects of PFOA in rodents have demonstrated a similar decrease in testosterone, increase in estradiol, and little change in LH [19].

The association between PFOA and free testosterone may have been mediated by elevated estradiol and prolactin. Elevated estradiol decreases testosterone and other steroid hormone synthesis in Leydig cells. LH response to low testosterone is attenuated by estradiol through negative feedback mechanisms at the pituitary and hypothalamic levels [112, 113]. Elevated prolactin sensitizes the hypothalamus and pituitary to estrogens feedback. The combined effect of elevated estradiol and prolactin could have reduced the secretion of LH and the subsequent Leydig cell response. Estradiol has direct effects on Leydig cell testosterone synthesis. In rats, PFOA decreased androstenedione and testosterone, but not 17 alpha-hydroxyprogesterone [19]. The metabolism of 17 alpha-hydroxyprogesterone to androstenedione was inhibited at the step of the C-17,20 lyase. The activity of the rate limiting C-17,20 lyase has been reported to be under estradiol regulation in rat Leydig cells [114, 115]. Thus, in PFOA treated rats, elevated levels of estradiol may inhibit the C-17,20 lyase and thereby reduce testosterone synthesis. The increase in the estradiol-testosterone ratio observed in workers is compatible with this mechanism for decreased free testosterone.

The primary source of estradiol in males is the P450 (P450 19) mediated aromatization of testosterone [116, 117]. Additional estradiol is secreted directly from Leydig cells. The observed increase in estradiol may be the result of increased production from one of these two sources or may be the result of inhibition of P450 mediated estradiol metabolism [118]. Perfluorooctanoic acid, a

prototype peroxisome proliferator, may regulate steroidogenesis by binding to a member of a new family of cytosolic receptors (PPAR) belonging to the nuclear hormone receptor superfamily and transactivating the transcription of genes involved in steroid synthesis [119-121].

PFOA was positively associated with the TB/TF and E/TF ratios. PFOA binding to sex hormone bindinf globulin (SHBG) may have produced changes in the bound to free testosterone ratio. However, this would result in a change in the TB/TF that is in the opposite direction to the observed association between PFOA and TB/TF. The associations of PFOA with these ratios are consistent with a mechanism that involves decreased production of testosterone and increased production of estradiol.

The HPG axis of older men appeared to be more susceptible to PFOA compared to that of younger men. No animal data has been reported concerning age related sensitivity to the effects of PFOA. However, the onset of Leydig cell tumors has been reported to occur late in two year rat feeding studies [122]. This finding may represent increased susceptibility for hormonal alterations in aged rats. Further animal research is needed to define any age related susceptibility factors.

Prolactin levels were positively associated with total serum fluoride in participants who reported moderate drinking (1-3 drinks/day). Since the function of prolactin in men is uncertain, the clinical significance of such an association is unclear. Alcohol ingestion is a stimulus for prolactin secretion. The mechanism of this effect appears to be mediated by alterations in calcium mediated signal transduction pathways [123]. This suggests that the elevation of prolactin associated with PFOA and alcohol may be mediated by alterations in calcium mediated events such as transmembrane signal transduction pathways.

Thyroid stimulating hormone was positively associated with total serum fluoride. Animal studies have shown that perfluorodecanoic acid depressed peripheral thyroid hormone levels without producing a hypothyroid response [75, 79, 101]. In the present study, peripheral thyroid hormone levels were not assayed. Therefore, it is not possible to assess whether the observed association between

PFOA and TSH could be a direct hypothalamic effect, a pituitary regulatory effect, or an effect mediated by changes in peripheral thyroid hormone levels.

In summary, this is the first report of hormonal changes associated with PFOA in humans. The present findings in humans are consistent with those previously reported in animal studies [19].. The consistent findings include low free testosterone, increased estradiol, and unchanged LH. Rodent and human reproductive endocrine systems differ greatly, yet the suggested effects of PFOA are similar. In light of the observed similarities in effect, it is tempting to speculate that PFOA may effect the humans and rodents reproductive endocrine system through the same mechanism. A hypothesis that PFOA alters a calcium mediated cellular signal transduction pathway, such as the cAMP or inositol triphosphate mediated second messenger response, may provide a unified mechanism for the multiple loci of putative effects.

No adverse health effects have been observed in exposed [8]. The present study did not examine adverse health effects, although several adverse outcomes associated with hormonal alterations are possible. The etiology of a number of cancers including adenocarcinomas of the prostate, endometrium, colon, rectum, pancreas and breast, have been linked to changes in endogenous hormones [124]. Cancers in this etiologic category include.

Perfluorooctanoic acid is not a genotoxic carcinogen in standard assays [9]. However, PFOA is a nongenotoxic rodent carcinogen. In rats exposured to PFOA over a two year period, there was associated increase in Leydig cell tumors [125]. Leydig cell tumors have been observed in association with other peroxisome proliferators in rats [122]. It has been hypothesized that chronically elevated LH produced testicular neoplasms [19, 122]. However, in PFOA treated rats, LH was not elevated. This may be due to estrogens feedback inhibition as discussed previously, or due to insufficient experimental induction time [19]. Alternatively, another mechanism may have been operative in producing Leydig cell tumors. Exogenous estradiol produces Leydig cell tumors in mice [126]. High estradiol levels are associated with Leydig cell tumors in both rats and humans [127, 128]. The tissue surrounding the Leydig cell adenomas also produces increased estrogens [127]. High estradiol may be a stimulus for Leydig cell

proliferation and tumor formation. This hypothesis is supported by the observation that estradiol stimulates TGF-a secretion in Leydig cells TGF-a binds to EGF receptors expressed on Leydig cells [129] and stimulates cell proliferation. The hormonal changes associated with PFOA may be a mechanism for nongenotoxic carcinogenesis. The role of PFOA in human nongenotoxic carcinogenesis needs to be clarified.

Adequate androgen levels are necessary for maintenance of potency, spermatogenesis, libido and male reproductive organs. Low testosterone and high estrogens may decrease libido, and fertility in males [130]. Decreased male fertility may be one potential adverse outcome of PFOA. The reproductive toxicity of PFOA has not been extensively studied. No studies have been conducted in humans. PFOA was not teratogenic in rats [9, 131, 132]. No adverse effects on fertility were noted for female rats in a teratogenesis study [9]. Male rats were not studied. No other reproductive studies in animals have been reported. Studies of human reproductive function are needed since human reproductive processes are thought to be more sensitive to xenobiotic insults compared to other animal species [133].

5.1.3 Cholesterol, Triglycerides, and Lipoproteins

Cholesterol, triglycerides, and LDL were not significantly associated with PFOA. The lack of association of PFOA with cholesterol or triglycerides is consistent with observations in experimental animal models. No animal studies of PFOA's effect on LDL are available for comparison. The are no studies in humans concerning the relationship of PFOA with LDL, cholesterol, or triglycerides.

In light drinkers, PFOA had little effect on HDL levels. In moderate drinkers, increasing PFOA reduced HDL. The putative effects of PFOA and alcohol may be mediated by alteration of a common HDL regulatory process. The findings are limited by the small number of exposed workers, the limited range of total fluoride values, and the limitations of the study design. The conclusion and suggested mechanism must be considered preliminary.

The mechanism by which PFOA modifies the alcohol-HDL relationship could be mediated by alterations in fatty acid metabolism or fatty acid binding. Alcohol intake induces specific P450 metabolic enzymes including 2E1 and alters lipid metabolism [134]. PFOA induces a specific P450 A1 family of metabolic enzymes and alters lipid metabolism in rodents. The joint effect of alcohol and PFOA on P450 mediated lipid metabolism could alter HDL dynamics. The primary structure of PFOA suggests that PFOA could affect the ligand binding of fatty acid in hepatocytes and HDLs. The competition for NEFA binding sites could reduce the effect of alcohol on HDL levels. Studies of the joint effect of PFOA and alcohol on HDL may clarify the regulatory mechanisms for HDL.

The decrease in HDL associated with increasing PFOA levels may be clinically significant. In a meta-analysis of 12 prospective studies of the relationship between HDL levels and coronary heart disease (CHD), Gordon estimated that the change in CHD risk associated with a one mg/dl change in HDL level is approximately the same as the change in risk associated with a 2-4 mg/dl change in LDL level [135]. The predicted drop in HDL for a moderate drinking participant with a total fluoride of 20 ppm is 30 mg/dl. A change of this order of magnitude may have a measurable impact on the occurrence of cardiovascular disease. In the retrospective mortality study, there was no increase in mortality from cardiovascular disease. However, there are a limited number of workers with total serum fluorine levels of 20 ppm of more. Any increase in risk for cardiovascular diseases among a small group of highly exposed workers may not be readily apparent in a study of all Chemolite or CD employees. Further research is needed to confirm and clarify the association between PFOA and HDL level. Future studies could test the hypothesis that PFOA and alcohol jointly alter NEFA metabolism resulting in a decrease in HDL and an increase in cardiovascular morbidity and mortality risks for exposed workers who drink alcohol.

5.1.4 Hepatic Parameters

Changes in SGOT (AST) and SGPT (ALT) appear to be associated with total serum fluoride through an interaction with adiposity. In obese participants, both SGOT and SGPT increased with increasing PFOA. However, there did not

appear to be an independent effect of PFOA on SGOT after adjusting for SGPT. The findings are limited by the small number of exposed workers, the limited range of total fluoride values, and the previously discussed limitations of the study design. The conclusion and suggested mechanisms must be considered preliminary.

Compared to SGOT, SGPT is a relatively specific marker for hepatocyte disruption [136]. The lack of association of SGOT with PFOA after adjusting for SGPT suggests that the liver is the primary source for the small PFOA associated changes in transaminases. Since SGPT is a enzyme associated with the ER membrane, the increase in SGPT may have been the result of PFOA associated ER proliferation. It may indicate a disruption in the integrity of hepatocyte membranes which allows increased release of cytosolic hepatic enzymes. The tissue specific effect suggested for hepatocyte membranes could be due to a higher hepatic concentration of PFOA.

Liver injury is generally considered to be a multifactorial process. There is evidence that interactions between endogenous and exogenous factors play a role in hepatotoxicity observed in workers [137]. The modification of the adiposity-SGPT association by PFOA suggests that the mechanisms of transaminase elevation may be linked. Obesity has been associated with elevation of transaminases as well as clinically important hepatitis [138, 139]. The observation that some obese individuals evidence little adiposity effect while other obese individuals develop hepatic fibrosis has not been explained. It has been hypothesized that metabolic polymorphisms or other hepatotoxin exposure may play a role [140]. Animal studies and limited human data suggest that xenobiotics, such as certain solvents and alcohol, may potentiate the effects of other hepatotoxins [141, 142]. Following this model, PFOA may directly or indirectly potentiate the hepatotoxic effect of obesity.

A mitochondrial site of PFOA action may occur. The mitochondria plays an essential role in fat metabolism. Disruption of mitochondrial function can produce impairment of mitochondrial oxidation of long chain and medium chain fatty acids. Studies of fatty acid metabolism in PFOA exposed humans have not been carried out. Valproic acid, an eight carbon branched chain fatty acid (2 propyl-pentanoic

acid) that impairs mitochondrial function and fatty acid metabolism, is an example of a hepatotoxic xenobiotic of similar carbon structure to PFOA [143]. Commercial grade PFOA contains isomers with carbon backbones identical to valproic acids structure [39]. The valproate-like isomers of PFOA could produce toxicity similar to that of valproate. The modification of the association between PFOA and the transaminases by adiposity could be mediated by disturbances of mitochondrial fatty acid metabolism in humans.

GGT increased as alcohol use increased. The increase in GGT was smaller as PFOA increased. This association was independent of changes in SGOT, SGPT, and AKPH. Perfluorooctanoic acid may inhibit the hepatotoxic effects of alcohol. The GGT-alcohol dose response relationship is thought to be secondary to the induction and increased release of GGT. Increased serum GGT levels indicate proliferation of the endoplasmic reticulum and induction of cytochrome P450 system, leakage from hepatocytes, or injury to other tissues [144-147]. Perfluorooctanoic acid may decrease serum GGT by altering cell membrane permeability, by reducing the alcohol mediated induction of GGT, or by changing alcohol oxidation pathways and reducing the production of toxic intermediates such as acetaldehyde.

Perfluorooctanoic acid was negatively associated with AKPH in non-smokers. In workers who smoke greater than five cigarettes per day, PFOA was positively associated with AKPH. The association of AKPH with PFOA was independent of GGT, transaminases, and hormones. Smoking has been reported to elevate AKPH [148]. The mechanism of this effect is thought to be the result of AKPH induction by compounds in cigarette smoke. The joint effect of smoking and PFOA could increase the induction of AKPH.

In summary, the associations between PFOA and hepatic enzymes are weak and are not clinically significant. In the retrospective mortality study, there was no increased in mortalityassocaited with liver disease. Future studies of the effects of PFOA may elucidate possible mechanisms of action of nongenotoxic hepatic carcinogens. The hepatic enzyme results are illustrative of the problem of extrapolating findings observed in rodent animal models to other species, including humans [149]. In humans, PFOA does not cause the dramatic hepatic

effects observed in rodents. Instead, the observed associations may result from PFOA modification of the hepatic effects of obesity, alcohol consumption, and smoking. Each of these factors are independently associated with hepatotoxicity. Further studies of the joint effects of PFOA and BMI, alcohol, and smoking on hepatic enzymes are needed.

5.1.5 Hematology Counts and Parameters

PFOA was weakly, but significantly associated with hemoglobin levels, MCV, and MCH. The associations between PFOA and erythrocyte indices appeared to be mediated through interactions with smoking, and perhaps alcohol consumption. The findings in animal studies [9, 150] are consistent with a decrease in red cell volume and a larger decrease in red cell number. Together, these changes produce an increase in cellular hemoglobin concentration. The estimated changes in erythrocytes indices are not of clinical significance over the range of total serum fluoride. However, these findings suggest that further studies of the effect of PFOA on red cell regulation and function are needed. The findings are limited by the small number of exposed workers, the limited range of total fluoride values, and the previously discussed limitations of the study design.

Pharmacological doses of androgens increase erythrocyte number and mass but produce little change in MCV or MCH [151, 152]. The mechanisms by which androgens increase hemoglobin appear to mediated by modulating the erythropoietin responsiveness of multi-potential stem cells and by stimulating erythropoietin production [151, 153-155]. In physiologic doses, the effect of testosterone on erythrocyte indices is controversial. Palacios et al. and Cunningham et al. reported that testosterone is associated with a small increase in hemoglobin, but no change in MCV or MCH [156, 157]. Mauss et al. reported no change in red cell indices for physiologic levels of testosterone [158]. In the present study, the testosterone level was not strongly or significantly related to the red cell indices. Estradiol was weakly association with HGB but not MCV or MCH. The effect of physiologic estradiol levels on the male hematological system is poorly understood. Tell et al. reported that the effect of smoking on red cell indices was different in male than in female adolescents [159]. This suggests that estrogen levels may play a role in the effect of xenobiotics on red cell

indices. Taken together, the evidence suggests that the association between PFOA and erythrocyte indices was not mediated by the PFOA associated changes in testosterone, but may have been mediated in part by changes in estradiol.

Thyroid hormone was associated with changes in HGB and MCV. A decreased availability of thyroxin (T_4) to myxedma levels produces a mild macrocytic anemia in humans. The increased cell volume is due to alterations in lipid deposition in erythrocyte membranes that occurs during ineffective erythropoiesis [160]. TSH confounded the association between PFOA and MCV. Decrease in T_4 could explain some of the increase in MCV and TSH. However, PFOA appeared to have an independent and opposite effect on MCV. Therefore, the association between PFOA and changes in red cell indices was probably not related to changes in thyroid function.

The immune system effects associated with PFOA present a complex picture. As expected, smoking had a strong effect on leukocyte counts. Smoking modified the association between cell count and PFOA for total lymphocytes, eosinophils, platelets and basophils. However, smoking did not modify the estimated PFOA effect on WBC, PMN, band count, or monocyte count. Alcohol modified the association between PFOA and cell count for WBC, PMN, and lymphocyte count. Adiposity modified the association between PFOA and lymphocyte count, monocyte count, and platelet count. Taken together, this preliminary data suggests that PFOA is associated with changes in peripheraly leukocyte counts. The negative association with lymphocyte count is consistent with the lymphocytes effects observed in primate studies. PFOA could modulate cell counts by altering the effects of smoking, alcohol consumption, and adiposity on peripheral leukocyte counts.

The magnitude of the WBC and PMN associations were not clinically significant from an infectious disease perspective. Increased WBC is positively associated with mortality from all causes, cardiovascular diseases, cancer and myocardial infarction [161-168]. It is unclear if the alteration in WBC is a consequence of, or the cause of, ongoing pathological processes. Judgment as to the clinical relevance of the PFOA associated changes in WBC must await further study.

Adiposity modified the association between cell count and PFOA for monocytes. Alcohol and cigarette consumption were independent determinants in the present study. Monocyte counts have been reported to be low in massively obese individuals [169]. The biological basis for these effects are not clear. The univariate and joint effects of adiposity and PFOA on monocyte count may a fruitful area for future research.

In the present study, the complex relationships between lymphocyte count, PFOA, alcohol use, cigarette use, and body mass may have been the result of the differential effect on T cell subsets. In order to clarify these associations, specific subsets need to be measured. The association of lymphocyte subsets with disease endpoints have yet to be clarified. The interpretation of the observed association requires further research.

Smoking was negatively associated with basophil count. As PFOA level increased, the smoking effect was diminished. Taylor et al. reported an increase in blood basophils in smokers compared to nonsmokers [170]. Walter et al. studied smokers and nonsmokers and found that acute smoking causes degranulation and loss of basophils. However, chronic smoking is associated with an elevated basophil count. [171-174]. No attempt was made to prohibit subjects from smoking prior to the time of blood sampling. The negative association observed in this study may reflect recent smoking by participants prior to blood drawing. The apparent reduction in the degranulating effect of smoking suggests that PFOA may interact with the basophil degranulaltion process.

Exposure to PFOA may be associated with changes in immune function beyond simple changes in cell number. The avid oxygen binding by PFCs may alter the effectiveness of peroxidatic killing by PMNs. Cytokine signaling is important in immune function and could be altered by PFOA exposure [175]. The response to antigen binding depends upon rearrangement of membrane proteins. Changes in the membrane physical characteristics produced by the potent surfactant action of PFOA could alter immune responses. More research is needed in the area of PFOA immunotoxicity. The findings of the present study need to be confirmed. Lymphocyte could be immunophenotyped using well established flow

cytometry methods [176, 177]. The standard immunotoxicologic assessment defined by the National Toxicology Program [178] should be carried out for PFOA.

Smoking has been observed to increase platelet number, survival, adhesiveness, activation, and aggregation when exposed to ADP [179-184]. Adhesiveness may change as a result of the effects of smoking on nonesterified fatty acids (NEFA). Smoking increases NEFA which may compete with PFOA for platelet membrane binding sites. Such competition could alter the smoking associated increase in platelet count. This hypothesized mechanism could be tested by in vitro modeling of platelet function in the presence of NEFA and PFOA. The relationship between obesity and platelet count has not been well studied. BMI has been reported to be negatively associated with platelet count [185]. The mechanism for this effect is not clear, but may be related to changes in NEFA associated with obesity. Thus, the effect modification of the PFOA effect by smoking and obesity may have resulted from a common effect on NEFA. Changes in platelet count have been associated with risk for cardiovascular disease [186, 187]. Direct and indirect mechanisms have been hypothesized for the observed increase in disease occurrence. Thus, PFOA associated changes in platelet count may be a marker for increased cardiovascular disease risk. Further study of potential effects of PFOA on platelet count and function are needed.

5.1.6 Total Fluorine

Smoking and total serum fluorine were weakly associated in participants. The adjusted estimate for the difference in mean fluorine between smokers and nonsmokers was small (0.1 ppm) and probably not of biological significance. Smoking intensity was not significantly correlated with total serum fluorine levels. It is unlikely that smoking affects the pharmacokinetics of serum fluorine or PFOA. It is unlikely that smoking was a primary route for absorption of PFOA.

Exposure reduction does not need to await the results of future studies. In rodents, removal from exposure results in the reversal of the marked hepatic responses to PFOA [188]. Intervention to reduce the PFOA body burdens of employees would prevent any potential adverse effects in the future. The reduction of exposure is especially important since PFOA has an unusually long

biological half-life. A significant reduction in body burden will require years of reduced exposure.

5.1.7 Methodological Considerations

5.1.7.1 Selection Bias

Given the occupational study setting, the voluntary participation, and the requirements for blood sample collection, the overall participation was unexpectedly high. Past medical screening programs at Chemolite had participation rates of 60% to 70%. The present study's participation rate exceeded 80%. Given the high participation, non-response bias is likely to be small.

Selection bias is an important validity issue for cross sectional studies [189]. Only active Chemical Division workers were included in this study. Workers not included may have had a different response pattern than those who were included. If continued employment depended on response to exposure and the exposure was associated with the endpoint of interest, then selection bias may have occurred. A finding of the present study was that PFOA was associated with decreased free testosterone and increased estradiol. If workers who had high susceptibility to the effect of PFOA changed jobs, then the overall slope of the dose response curve could be underestimated. Conversely, if workers with low testosterone associated with PFOA changed jobs less often, then the overall dose response curve may be overestimated. Migration out of the high exposure jobs is unlikely to be the result of subclinical changes in hormone levels. All current Chemical Division employees who worked in high exposure jobs over the last five years were included in the sample. Many workers who had been employed in the high exposure jobs, but who changed jobs were included as participants. The vast majority of workers who had significant exposure over the previous five years would be included in the study sample as the turn-over rate in Chemolite employees was low (three percent per year) and the study included all current employees with appropriate job histories. Selection bias is not a likely explanation for the findings in this study.

5.1.7.2 Information Bias

No worker was unexposed. The lowest potential exposure group had significantly elevated levels of total serum fluorine. In view of this, the observed effects may represent an underestimate of the true effect.

Total serum fluorine was used as a surrogate variable for PFOA exposure. The use of total serum fluorine has been validated in past biological monitoring in the Chemolite Plant and other plants using PFOA [8]. Direct measurement of PFOA using gas chromatographic techniques have been highly correlated with total serum fluorine in Chemolite workers. Approximately 90% of total serum fluorine in Chemolite workers was reported to be in the form of PFOA [8, 12]. The validity of using this surrogate measure was not directly assessed in the current study due to cost. Small amounts of PFCs other than PFOA may have been present in serum. The half-life of PFC compounds is directly related to molecular weight. Compounds with six or less carbon backbones are likely to be rapidly excreted by exhalation [190]. Short chain PFCs are unlikely to contribute appreciably to total serum fluorine. Longer chain PFC, such as perfluorodecanoic acid (PFDA), are not produced at Chemolite. The high toxicity of PFDA excludes it from commercial applications [63, 77, 79, 101, 102]. Longer chain PFC are unlikely to be a significant component of total serum fluorine. Other organic fluorine containing compounds exist in biological systems and the environment. However, the small amounts absorbed from the environment in the form of drugs or plant products are rapidly metabolized and excreted [191]. Inorganic fluorine was not a large constituent of the total fluorine levels. Serum ionic fluorine levels in the 1-5 ppm range are associated with death in unintentional occupational exposures [192]. Total serum fluorine is a good surrogate measure for PFOA in this cohort.

The coefficient of variation for total serum fluorine was 66%. The repeatability of the assay was better at total serum fluorine levels above five ppm. At the low end of the spectrum (< 1 ppm), where the assay is limited by sensitivity, the total serum fluorine values may overestimate the true value. These measurement errors are likely to lead to an underestimate of the effect of PFOA on the physiologic endpoints.

Commercial PFOA is a complex mixture of isomers and related compounds [39]. It is clear that structurally related compounds, such as valproic acid, exhibit toxicity for certain isomeric forms, but not others [143, 193]. It is widely recognized that different drug enantomers have different pharmacokinetic and pharmacodynamic properties [191]. Thus, different isomers of PFOA may have different toxicities. If one isomer of PFOA is associated with toxicity, then the use of total serum fluorine or total PFOA levels could have produced an under-estimate of the true strength of association. However, in animal studies using straight chain PFOA, the spectrum of toxicities is similar to those observed in studies using mixed isomer of PFOA [37, 38, 88, 194]. Further research is needed to clarify the role of PFOA isomers.

The toxicokinetics of PFOA in humans are different from those observed in rodents. Extrapolating the tissue distribution of PFOA from animals to humans may not be valid. No data exist on the relationship between serum and tissue PFOA distribution or body burden in humans. The use of serum levels to extrapolate to the concentrations at the site of PFOA action may have been inappropriate. Obtaining pharmacokinetic data in humans or appropriate animal models is an important area for future research efforts.

The temporal variability of physiologic parameters is recognized. The ultraridian, circadian, and circannual variability of the study endpoints was not assessed directly. Instead, blood samples were drawn at the same time of day, on the same shift for all participants. One sample was drawn to estimate mean parameter values. Considerable measurement error is inherent in this procedure for hormones with short pulsatile intervals such as LH, FSH, and testosterone. However, studies have shown that one sample is as good as three samples in estimating mean values [195]. The use of a single sample to estimate mean hormone level produced random measurement error and would be expected to attenuate the observed relationships. Mean serum values of the assayed hormones may not represent the biologically important quantities at the site of action.

Validation studies of self-reported smoking status, using biochemical markers such as exhaled carbon monoxide, serum and urine thiocyanate, and serum

thiocyanate, have shown that smokers underreport their smoking [196]. Smoking is associated with changes in physiologic parameters such as hematological counts [197-199], cholesterol [200], lipoproteins [201, 202], and hepatic enzymes [148]. The strength and direction of the association between self-reported smoking information and these parameters can be used to indirectly assess the validity of the smoking information [203].

In this study, smoking status and intensity was strongly and significantly associated with leukocyte count, band count, eosinophil count, platelet count, and monocyte count. As expected, smoking intensity was negatively associated with basophil count.

No participant reported drinking more than 3 ounces of alcohol per day. This may reflect the company's success in discouraging heavy alcohol consumption in employees, a reporting bias, or the fact that heavy drinkers may not be able to continue employment due to the demands of Chemical Division jobs.

Alcohol consumption is associated with changes in physiologic parameters such as hepatic enzymes [204], erythrocyte mean corpuscular volume, triglycerides, and high density lipoprotein [144]. As with smoking, the strength and direction of the association between self-reported alcohol consumption information and these parameters can be used to indirectly assess the validity of the alcohol information. Increased serum HDL is associated with moderate alcohol intake [144, 205, 206]. The expected relationship between alcohol intake and HDL was observed in this study in individuals with low PFOA levels. As expected, there was a positive association between alcohol intake and triglycerides. Alcohol has a direct toxic effect upon erythrocyte size, maturation, and number [207, 208]. The specificity of MCV is 90% in identifying alcoholics from social drinkers with a positive predictive value of 96% [209]. In the present study alcohol consumption of 1 to 3 drinks per day was associated with an increase in MCV of the same order as reported previously [208]. Alcohol induces GGT. The sensitivity of GGT in detecting alcohol use varies from 52% to 94%. GGT is highly non-specific for alcohol consumption or for hepatic abnormalities [146, 210]. Heavy drinking of two to five drinks per day over one week or more are necessary to induce GGT [145, 208]. GGT may be the only commonly assayed hepatic enzyme to increase with

heavy drinking. A significant positive association between GGT and self-reported alcohol use was observed. The presence of these associations indicates that the misclassification of alcohol use was unlikely to produce a bias large enough to explain the observed associations.

Alcohol use was weakly associated with hepatic transaminases. SGOT and SGPT are less sensitive indicators of alcohol use than GGT. In alcohol induced liver disease, SGOT may be slightly elevated and SGPT little changed. SGPT has been shown to decrease in some cases of alcohol induced liver disease [211]. Considering the relationship known to exist between alcohol use, SGOT and SGPT, little alcohol associated change in transaminases would be expected. The observed weak association is not an unexpected finding and therefore probably does not reflect misclassification of alcohol use.

Nonrespondents to the alcohol item were different than respondents. They were treated as a separate group in the analysis since the difference could not be explained by measured covariates. Although the power of the study is diminished by treating alcohol information as a nominal categorical variable, the potential for bias was reduced.

5.1.7.3 Confounding Bias

Information on the duration of employment in exposed jobs was not collected. Plant records did not contain sufficient information to reconstruct exposures more than five years in the past. The duration of exposure may be an important determinant of PFOA effect. Duration of employment may be related to PFOA level since PFOA has the potential for bioaccumulation. The duration of exposure may have been a confounder for peptide hormonal endpoints in this study. In rodents, steroid hormonal and hepatic enzyme effects of PFOA exposure occur after two weeks of exposure, whereas peptide hormonal effects may require longer exposures [19]. Leydig cell tumors may require a considerable length of exposure or latency to develop [122].

There are many compounds in complex androgen-estrogen system. The present study measured only a few of them. Other biologically important steroid

hormones include cortisol, androstenedione, dihydroepiandrostenedione sulfate (DHEAS), estrone, estriol, estrogenic catechols, and dihydrotestosterone (DHT). A total estrogen index or estrogen to testosterone ratio (E/T) may be more important than assays of individual compounds [212]. Sex hormone binding globulin (SHBG), a major determinant of the estrogen to testosterone balance at the tissue level [213], was not assayed. More research is needed to clarify the potential role of these hormones as confounders of the observed associations.

The relationship for bound testosterone may have been confounded by steroid hormone binding globulin (SHBG). Sex hormone binding globulin is an important determinant of testosterone and estradiol levels in different tissues as well as their metabolism [213]. Plasma SHGB levels are positively associated with estrogens and negatively associated with androgens [214]. Thyroid hormone levels affect SHBG [215]. The ratios of estradiol to testosterone and testosterone to DHT may be regulated by SHBG levels [213]. The association between PFOA and bound testosterone may have been, in part, related to estradiol and thyroid hormone changes in SHBG levels. Adult rats do not express SHBG [216]. The decline in total testosterone observed in rats is not the result of changes in the amount or binding characteristic of SHBG. The observed depression of free testosterone in men is analogous to changes in total testosterone in rats and is probably not significantly related to changes in SHGB binding.

Major stresses, such as surgical procedures, have been shown to markedly affect hormones in men [217]. It is unlikely that major physical stresses were associated with PFOA. Therefore, stress was not a significant confounder in the present study.

Shiftwork has been shown to affect a variety of physiologic endpoints including biochemical parameters, hematologic indices, and hormones [218]. Study participants rotated weekly through three shifts. All samples were collected on the day shift at least three days post shift change. Given the rotating shifts and standard day shift sampling, it is unlikely that shiftwork and PFOA were associated. Shiftwork did not appear to be a significant confounder of the estimated dose response relationships.

Several dietary factors are determinants of the endpoints considered in this study. The effects of dietary fat and cholesterol on serum lipoproteins and lipids is widely appreciated [200]. Dietary calories, fat, and carbohydrates affect steroid hormones [219, 220]. Diet can also affect the metabolism of steroid hormones [221, 222]. Since it is unlikely that diet is associated with PFOA, it is probably not a confounding covariate in this study.

Physical activity affects many physiologic parameters including hormones [223], enzymes [224], lipoproteins [200], and hematologic indices. For hormones, only maximal exercise produced an effect. No effect was noted for submaximal physical activity. It is unlikely that many participants engaged in maximal physical activity. Therefore, in this group, it is unlikely that physical activity is a determinant of the hormonal endpoints under study. Physical activity may effect HDL levels, but it is unlikely that physical activity was associated with PFOA. Therefore, physical activity was unlikely to be a significant confounder in this study.

Medication usage and diseases such as diabetes mellitus are important determinants for some of the physiologic parameters measured in this study [193, 195]. Questionnaire items concerning medication use and medical history were incomplete and were not validated. PFOA exposure has not been associated with any medical conditions [8]. If the use of medication or the diagnosis of a medical condition that affects one of the physiologic endpoints is associated with PFOA exposure, then confounding may occur. However, no such relationships have been described.

Inflammatory processes, which are major determinants of WBC, were not assessed in this study. There is no evidence that inflammatory processes are related to total serum fluorine or serum PFOA. Therefore, these determinants of leukocyte count are unlikely to confound the estimated relationships.

5.1.7.4 Analytic Model Specification Bias

The analytic multivariate approach used in this study assumed that a linear model with additive effects was an adequate model with which to summarize the

data. A normal error term was used. Similar models of physiologic variables have been extensively used in the past and their assumptions tested [225]. The model form was partially defined a priori based on a biological hypothesis. The choice of a final model was based on biological knowledge plus best predictive power. The variable transformations used were not based on a specific biological mechanism, but instead reflect the basic form of dose response relationships observed in nature.

5.2 1990 Chemolite Mortality Study

5.2.1 Introduction

This was a retrospective cohort study of mortality in workers employed in a PFOA production plant for greater than six months during the period from January 1, 1947 to December 31, 1989. Completeness of the cohort was assessed from independent sources. Demographic and work history data were collected from plant records and verified from independent sources where possible. Cohort members were not individually contacted for additional information on confounding variables such as smoking. Vital status was confirmed for 100% of the cohort. Cause of death was obtained from death certificates for 99.6% of deaths and other sources for 0.4% of deaths. Cause of death was coded by ICD-8 categories by a nosologist. Reliability of death certificate coding was assessed by random resubmission of death certificates for recoding. The concordance was 100% for three digit ICD-8 codes.

5.2.2 Participant Characteristics

The 749 women were observed for 19,309 person-years, had a mean age at first employment of 27 years and mean follow-up of 26 years. The number of expected events given the age and size of the cohort was small. The study had limited power to detect moderate increases in cause-specific mortaltiy.

The 2788 men were observed for over 70,000 person years. The mean age at first employment was 27 years,the mean length of follow-up was 25 years and a the mean age of death was 56 years. Non-CD men were older on average than

CD men and had more person-years in the older age groups where mortality was the highest. Internal comparisons were confounded by age as well as other time correlated factors such as length of follow-up.

5.2.3 Mortality Results

In females, 6.7% were deceased compared to 12.5% in the males. Given that the mean age at first employment and mean length of follow-up was similar for males and females, this reflects the expected survival advantage of women. For both males and females the proportion of deaths was smaller in the CD cohort. Employment in the Chemical Division did not produce a large survival disadvantage.

The all causes, all cancer, and all cardiovascular mortality among women was less than expected in the overall cohort. The SMRs were remarkably stable when stratified on ten year exposure groups, and ten, fifteen, and twenty year latency periods. The all causes SMR was .75 in the total cohort, .75 in those employed for at least ten years or for those employed longer than ten years, and .75 in all three latency periods. Cardiovascular diseases and cancer mortality followed a similar pattern.

In males, the all causes, cardiovascular diseases, all gastrointestinal, and all respiratory diseases SMRs were significantly less than one. The all causes SMR was .77 using Minnesota mortality rates and .73 using national rates. The low SMRs are most likely a result of the healthy worker effect (HWE). As expected, the cancer SMR is less affected by the HWE. The all causes SMRs were .75 for all three latency groups. Latency did not have a strong relationship with the HWE. The all causes SMR was .80 in the greater than five year employment duration group and .68 in the greater than 20 year employment group. The low all causes SMR in the greater than 20 year duration group suggests that working for 20 or more years was associated with continued selection based on good health. The all causes SMR decreased with duration of employment in one meta-analysis of retrospective cohort studies [226] .but increased in the meta-analysis by Fox and Collier [227].

The SRRs for all causes, all cancer, and all cardiovascular diseases for less that ten years employment to more than ten years employment were not significantly different from one. Because the rates were based on small numbers of events, the 95% CI were wide. Due to the small number of events in the females, SRRs were not calculated. The SRRs are similar to the SMRs for the less than ten year employment and greater than ten year employment groups.

The SRRs for CD versus non-CD male workers for all causes, all cancer, and all cardiovascular diseases were not significant and were similar to the SMRs. Working in the CD did not substantially alter the rates of death. The small number of events observed for rare causes of death or specific causes of death make it unlikely that moderate increases in rates could be detected in this cohort for the follow-up period through 1989. More follow-up time will be needed to allow sufficient power to detect moderate increases in rates for specific causes of death.

The results from the adjusted RR_{MH} contrasting the mortality rates for all causes, all cancer, and all cardiovascular diseases between CD and non-CD male workers were similar to those for the SRRs and SMRs. None of RR_{MH} point estimates were statistically different from one. The contrast of rates between less than ten years of employment and greater than ten years of employment presented a different picture. All cause RR_{MH} were significantly elevated in the oldest two age groups, while the RR_{MH} for cardiovascular diseases was significantly elevated in the 30 to less than 40 year age group. The all cancer RR_{MH} displayed a trend toward a statistically significant elevation in the oldest two groups. The RR_{MH} were not adjusted for year of first employment. They may have been substantially confounded by changes of exposure over time since year of first employment. As seen in several PH regression models, year of first employment was significantly associated with the mortality. After age and length of follow-up, calendar time is the strongest time factor associated with mortality [189]. Hence, it is likely that the elevated RRs for composite categories of cause of death in the oldest groups were a result of uncontrolled confounding by calendar period. Given the small number of events in strata, it was not feasible to further stratify the data on year of first employment.

In the PH regression analysis, prostate cancer mortality was positively and significantly related to time in the Chemical Division. Ten years of employment in the CD was associated with a 3 fold increase in prostate cancer mortality compared to men never employed in the CD. This trend was evident in the SMR analysis stratified by CD and non-CD employment. This association was independent of duration of employment and year of first employment. As expected, age at first employment was positively related to prostate cancer mortality rate. The interpretation of this estimated relative rate is tempered by a number of factors. The estimates were based on six prostate cancer deaths, four in the CD cohort and two in the non-CD cohort. A change of one case could significantly alter the estimates. Ascertainment of all prostate cancer deaths may have been incomplete. Diagnosis may have been more complete in the CD cohort. Given that death certificate cause of death information is known to be imperfect, misclassification of one or more deaths could occur. The use of mortality as the event of interest for etiologic studies of prostate cancer is not the best study endpoint because of the long natural history and low mortality of prostate cancer. The majority of incident prostate cancers do not progress and cause death [228, 229]. For localized disease, an 80% ten year survival in untreated patients have been reported [230]. Studies of prostate cancer incidence in this workforce are needed to clarify the suggested increase in prostate cancer risk. The findings of hormonal alterations in PFOA exposed men suggests a possible biologic mechanism for the increase in prostate cancer mortality [231]. Incidence studies of other diseases that are hormonally mediated may be indicated if the PFOA associated hormonal changes are confirmed.

5.2.4 Methodological Considerations

5.2.4.1 Information Bias

The use of death certificates to categorize cause of death imperfect [232-235]. The size of the potential bias depends on the cause of death. In one study cancer as a cause of death was under-reported by 13% [232]. Leukemias and lymphomas were underreported in 19% of autopsy confirmed cases. Colorectal cancers were underreported in 12% of cases. Therefore, it appears that cancer deaths were not severely misclassified. All cardiovascular diseases as a group may

have been inaccurate. Individual disease with the whole may be severely misclassificated and may produce large biases. For example, specific causes of death in the cardiovascular group, such as cerebrovascular disease, are inaccurately designated on death certificates.

Three measures of PFOA exposure based on job history were used in this study. First, the cohort was dichotomized into those who ever worked in the CD and those who never worked in the CD. Second, the number of months worked in the CD until 1985 was used as a continuous parameter for PFOA exposure. Third, the total duration of Chemolite employment was used as a continuous parameter for the effect of work in a plant producing PFOA among a large number of products. Each of these surrogate variables may produce a different spectrum of misclassification. Categorization of workers into ever versus never employed in the CD may not reflect the biological effective dose of PFOA. Many CD jobs do not entail PFOA exposure. A number of workers were employed in the CD for short periods in the distant past. Their exposure may not have been significant. This categorization may misclassify unexposed workers as exposed. Conversely, PFOA exposure was widespread among Chemical Division (CD) employees working in jobs with no exposure to PFOA. No exposure measurements have been done in non-CD employees. It is possible that non-CD employees had significant body burdens of PFOA. If this was the case, exposed workers would have been classified as unexposed. Such misclassification would be expected to bias the effect estimates toward the null. The months of employment in the CD was the best available estimate of PFOA dose. Not all CD jobs have PFOA exposure. The misclassification produced by classifying unexposed workers as exposed could have biased the estimate toward the null. The use of duration of employment at Chemolite as a continuous exposure parameter is less specific for PFOA than time in the CD. If another xenobiotic exposure in the plant has modulated disease occurrence rates, the use of duration may produce less misclassification than use of duration in the CD.

5.2.4.2 Confounding and Selection Bias

The healthy worker effect strongly affects the validity of many occupational studies [189, 236]. It is a complex bias that results, in part, from the selection of

individuals for employment who are healthier than those in the comparison population. The HWE is usually stronger for cardiovascular diseases and respiratory diseases. Because cardiovascular diseases mortality accounts for a significant portion of all causes mortality, the HWE usually reduces the all causes SMR. The age at first employment, age at risk, length of follow-up, and duration of employment are four time factors that are associated with changes in the HWE [189]. Generally, the HWE diminishes with age and time.

Collection of confounder information for individuals is difficult in retrospective cohort mortality studies. The present study included workers followed for more than 40 years. It was not feasible to collect individual information on such covariates as smoking, health status, medical history, or dietary habits. The proportion of workers at Chemolite who smoke has been lower than in other facilities owned by the same corporation. In recent health maintenance studies, the self-reported smoking prevalence (25%) is lower than the statewide smoking prevalence. The observation that all respiratory diseases and lung cancer rates are lower than expected may be the result of historically low smoking prevalence. The low smoking prevalence may depress the all causes SMR, all cancer SMR, and all respiratory disease SMR. The use of internal comparison groups may reduce this smoking related bias [237].

Time factors such as age at risk, age at first employment, year of first employment, and duration of employment are associated with the occurrence of many diseases [189]. The use of an internal comparison group may reduce certain selection effects, but may not control confounding if the exposure defined internal comparison groups have different distributions of these time factors. Although the mean age at first employment and mean year of first employment are similar in the CD and non-CD cohorts of men and women, the comparisons of the rates of disease are confounded by differences in the distribution of age at risk. These time factors are strongly correlated, with some being exact linear combinations of others. The relationship between measures of exposure and disease occurrence may be complex functions of these inter-related time factors. Adjustment for time factors may reduce the effects of confounding, but may not control confounding [238]. If the disease occurrence relationship is defined in terms of cumulative

exposure, the true effect of exposure may be biased toward the null by uncontrolled confounding due to the complex time factors [189].

Some workers were exposed to many other potentially disease causing xenobiotics, such as benzene and asbestos, during their employment at Chemolite. Adjustment for their effects was not possible in this study. Even if information was available, exposures are often highly correlated making the separation of individual effects impossible.

5.2.4.4 Analytic Model Specification Bias

Comparison of SMRs and RR_{MH} between exposure groups may not be strictly valid. However, if the distribution of the person time in the comparison groups is not strongly discordant, then such a comparison may be useful. In the current study, the person-time distributions are different in the exposed groups. However, the differences appear to be of a magnitude that makes useful comparisons of SMRs possible.

Although the proportional hazard (PH) model has been used frequently for cohort studies and clinical trials, it has not been widely used in occupational studies. In the past, it has been suggested that Poisson regression was the analytic strategy of choice because computational costs were less and the conceptualization of the model straight forward [189]. However, PH models are now easily run with standard computer packages [109]. Their wide application in clinical trials and cohort studies has fostered the understanding of the PH models. Poisson models appear less frequently in the literature and may not be as well understood. Poison regression and PH models have theoretical links and have been shown to give similar results when used to analyze the same data set [189]. Cox PH regression was chosen as the multivariate model to employ in this study. The validity of the proportional hazards assumptions was examined using the two standard techniques. The assumptions did not appear to be grossly violated. However, in analyses involving a small number of events, the assessment of the validity of assumptions may be limited. The use of the factors as continuous variables was based on lack of statistical evidence for a significant nonlinear

effect. Although this strategy has been widely used for control of confounding, it has not been extensively validated in simulation studies.

6. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Cross-Sectional Study of the Physiologic Effects of PFOA

This was a cross-sectional study of selected physiologic effects of PFOA, as quantified by total serum fluorine. Participants were recruited from workers employed during November 1990 in the Chemical Division of the 3M Chemolite Plant in Cottage Grove, Minnesota. All current workers who were employed in high exposure jobs at any time during the previous five years and an age matched sample of workers employed in low exposure jobs were invited to participate.

Participants completed a corporate medical history questionnaire and had vital parameters measured by an occupational health nurse. Blood was drawn for assays of total serum fluorine, seven hormones involved in the hypothalamic-pituitary-gonadal axis, serum lipids, lipoproteins, hepatic function parameters, and hematology indices. Blood was drawn in the morning after workers were assigned to the day shift for at least three days.

In past studies, the majority of total serum fluorine found in Chemolite workers was in the form of PFOA. Thus, total serum fluorine is a valid surrogate measure of PFOA in Chemolite employees. For 93% of workers, total serum fluorine levels were 20 times greater than community and corporate background levels. Findings in the current study are consistent with other data suggesting that PFOA has a long biological half-life in both men and women. The long half-life of PFOA may result in significant bioaccumulation from small frequent doses or large, infrequent doses.

The hormonal findings from this study are consistent with the hypothesis that PFOA depresses the human hypothalamic-pituitary-gonadal axis. The results show that low levels of serum PFOA (20μM) depressed free testosterone and elevated estradiol with little observed change in LH levels. In older men, free testosterone was depressed below 9 ng/dl at serum fluorine levels below one ppm (estimated PFOA levels below 1 μM).

Mean prolactin levels were positively associated with PFOA in moderate drinkers, but not in light drinkers. Since the function of prolactin in men is uncertain, the clinical significance of this finding is unclear.

Mean thyroid stimulating hormone was positively associated with PFOA. Since peripheral thyroid hormone levels were not assayed, it was not possible to assess whether the observed association between PFOA and TSH was the result of a direct effect on the hypothalamus, pituitary, thyroid gland, or peripheral thyroid hormone metabolism.

Cholesterol, triglycerides, and LDL were not significantly associated with PFOA. PFOA was negatively associated with HDL in moderate drinkers.

PFOA was not associated with the marked hepatic changes in humans that have been observed in rodents. PFOA appeared to alter the hepatic response to endogenous factors and xenobiotics.

PFOA was significantly associated with hemoglobin levels, MCV, and MCH. The estimated changes in erythrocytes are not of clinical significance over the range of observed total serum fluorine.

The changes in leukocyte counts associated with PFOA exposure presented a complex picture. For example, the negative association between PFOA and lymphocytes was increased by smoking more than 10 cigarettes per day and decreased by alcohol use and adiposity. The magnitude of these associations are not clinically significant from an infectious disease perspective. However, elevated WBC has been associated with increased all causes, cardiovascular diseases, and cancer mortality as well as increased incidence of myocardial infarction.

6.2 Retrospective Cohort Mortality Study Of The Chemolite Workforce, 1947-1990

This was a retrospective cohort study of mortality in workers employed in a PFOA production plant. All causes mortality in both male and female Chemolite employees were significantly less than expected based on comparisons to the

mortality experience of the Minnesota and United States population. The SMRs for several other causes of death including all respiratory diseases were less than expected. Since the healthy worker effect was apparently strong in the Chemolite cohort, internal comparisons of SMRs were made between Chemical Division (CD) and non-Chemical Division (non-CD) employees. These comparisons did not suggest any significant excesses in mortality in CD or non-CD employees.

Generally, the findings from this study provide no evidence that employment at Chemolite results in elevated mortality rates from any cause. However, prostate cancer mortality may be associated with length of employment in the Chemical Division. Ten years of employment in the CD was associated with a significant three fold increase in prostate cancer mortality. There was no association between prostate cancer mortality and employment (ever/never) in the Chemical Division. Given the small number of deaths from prostate cancer in this study and the natural history of the disease, the association between employment in the CD and prostate cancer must be viewed as hypothesis generating and should not be over interpreted. However, the biological plausibility for any association between CD employment and prostate cancer is increased by animal and human toxicological data suggesting an association between PFOA and steroid sex hormone changes.

6.3 Conclusion

Perfluorooctanoic acid was associated with reproductive hormonal changes in exposed workers. The clinical significance of these findings are unknown. The associations of PFOA with hormones, HDL, hematology parameters, prostate cancer mortality in men indicates the need for further research.

6.4 Recommendations

Research is needed in five areas.

1. An assessment of the hormonal effect of PFOA in women is needed. A cross-sectional study should be conducted using specific assays for PFOA and accounting for temporal hormonal variations.

2. The clinical significance of the associations of PFOA with the physiologic parameters need clarification. Since morbidity from diseases such as prostate cancer is reflected in mortality, an update of the retrospective mortality study is needed in five years. Morbidity studies should be conducted of endpoints that may be produced by hormonal changes . Since exposed workers are relatively young and are limited in number, the feasible endpoints for a short term morbidity study are limited. Pooling of workers from a number of plants could increase the number of exposed workers and allow endpoints with lower incidence to be studied. The morbidity study should be a long term which would allow the study of endpoints that occur at higher frequency in older age groups. In men, endpoints should include the incidence of benign prostatic hypertrophy and prostate cancer. The feasibility of including inflammatory bowel disease and colorectal cancer as endpoints should also be evaluated. In women, endpoints should include the age of menopause, the incidence of osteoporosis and related fractures, uterine fibroids, and cholelithiasis. If there are a sufficient number of events, endometrial cancer and inflammatory bowel disease should be evaluated. If the cross-sectional hormonal study in women finds no association between PFOA and hormones, then the morbidity study can be limited to men.

3. Studies of reproductive outcomes in both men and women are needed. Libido, potency, and fertility are directly associated with steroid hormones levels. The feasibility of a retrospective study of reproductive endpoints or a prospective study of time-to-pregnancy needs to be explored.

4. The mechanisms of action of PFOA need to be studied concurrently with morbidity. Mechanistic studies are needed to define the relevance of animal studies for humans and provide a firm biological basis for the findings of the mortality, morbidity, and reproduction studies.

In vitro and cell line studies could clarify the mechanisms of action of PFOA on the pituitary secretion of LH, FSH, TSH, and prolactin. Pituicyte cultures may be

helpful in evaluating the direct effect of PFOA pituitary function. The effect of PFOA on other autocrine or paracrine factors such as TGF-a, TGF-ß, FGF, and TNF could also be evaluated. Human adiptocyte cultures could be used to study the effect of PFOA on aromatase activity. Additionally, studies are needed to clarify the relationship between PFOA and the temporal variability of reproductive hormones.

5. Studies are needed to better define the PFOA exposure profile of all workers employed at Chemolite, to ascertain the source of their PFOA exposure and route of continued absorption and to clarify the toxicokinetics and toxicodynamics of PFOA in humans.

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APPENDIX 1

PHYSIOLOGIC EFFECTS STUDY QUESTIONNAIRE

Medical History Questionnaire

Medical History Questionnaire

Form 2500e - PHO

Name	Employee Number	Date
Location	Birthdate	Social Security Number
Sex ☐ Male ☐ Female	Height	Weight	Blood Pressure

Medical Symptoms

(The presence of these symptoms may be medically significant. Consultation with your personal physician is recommended if any have occurred.)

Check all that you have had in the last two years.	Yes	No
1. Persistent, bothersome cough	☐	☐
2. Recurrent wheezing	☐	☐
3. Becoming easily short of breath with exertion	☐	☐
4. Recurring chest pain	☐	☐
5. Ongoing difficulty with swallowing	☐	☐
6. Persisting abdominal pain	☐	☐
7. Blood in stool or black stools	☐	☐
8. Change in bowel habits	☐	☐
9. Change in mole or other skin lesion	☐	☐
10. Persistent swollen lymph nodes	☐	☐
11. Blood in urine	☐	☐
12. Burning with urination	☐	☐
13. Difficulty with balance or coordination	☐	☐
14. Temporary loss of vision or other visual disturbance	☐	☐
15. Persisting numbness in hands or feet	☐	☐
16. Fainting	☐	☐
17. Trouble speaking	☐	☐
18. Weakness in arm or leg	☐	☐

(The presence of these symptoms may be medically significant. Consultation with your personal physician is recommended if any have occurred.)

19. Do you take medications?	☐	☐

20. If, so please list (include non prescription medications.)

Medical Diagnoses

(Check yes for all medical problems you have that have been diagnosed by a physician.

	Yes	No
21. Allergies that cause nose or eye symptoms	☐	☐
23. Chronic bronchitis	☐	☐
23. Emphysema	☐	☐
24. Asthma	☐	☐
25. Other lung disease	☐	☐
26. Please specify		
27. Coronary artery disease	☐	☐
28. Heart attack	☐	☐
29. Angina	☐	☐
30. Arrhythmia (heart rhythm disturbance)	☐	☐
31. Heart failure	☐	☐
32. Hepatitis	☐	☐
33. Cirrhosis of the liver	☐	☐
34. Gall bladder disease	☐	☐
35. Other liver disease	☐	☐
36. Stomach ulcer	☐	☐
37. Duodenal ulcer	☐	☐
38. Colon polyps	☐	☐
39. Kidney failure or insufficiency	☐	☐
40. Bladder polyps or tumors	☐	☐
41. Diabetes	☐	☐
42. Anemia (low blood)	☐	☐
43. Low white blood cell count	☐	☐
44. Blood clotting disorder	☐	☐
45. Other blood disorder	☐	☐
46. Please specify		

	Yes	No
47. Neuropathy (nerve abnormality in arms or legs.	☐	☐
48. Seizure disorder	☐	☐
49. Multiple sclerosis	☐	☐
50. Other nervous system disease	☐	☐
51. (Please specify)		
52. Carpal Tunnel sydrome	☐	☐
53. Chronic low back pain	☐	☐
54. Herniated or ruptured disc in the low back	☐	☐
55. Herniated or ruptured disc in the neck	☐	☐
56. Any cancer	☐	☐
57. (Please specify)		

Tobacco Smoking

	Yes	No
Have you ever smoked cigarettes? (No means less than 20 packs of cigarettes or 12 oz. of tobacco in a lifetime or less than 1 cigarette a day for 1 year.) 58.	☐	☐
If yes: (Complete the following questions)		
Do you now smoke cigarettes (as of one month ago?) 59.	☐	☐
How old were you when you first started regular cigarette smoking? 60.		
If you have stopped smoking cigarettes completely, how old were you when you stopped? Age stopped ➤ 61.		
How many cigarettes do you smoke per day now? Per day ➤ 62.		
On the average of the entire time you smoked, how many cigarettes did you smoke per day? Per day ➤ 63		

Do or did you inhale the cigarette smoke?

64. ☐ Does not apply ☐ Slightly ☐ Deeply ☐ Not at all ☐ Moderately

	Yes	No
Have you ever smoked a pipe regularly? (Yes means more than 12 oz. of tobacco in a lifetime.) 65.	☐	☐

If yes:

How old were you when you started to smoke a pipe regularly? Age ➤ 66.

If you have stopped smoking a pipe completely, how old were you when you stopped? Age stopped ➤ 67. Still smoking ☐

On the average over the entire time you smoked a pipe, how much pipe tobacco did you smoke per week? ➤ 68. (Standard pouch of tobacco contains 1 1/2 oz. per week.)

How much pipe tobacco are you smoking now? Oz. per week ➤ 69. Not smoking a pipe ☐

Do you or did you inhale the pipe smoke?

70. ☐ Never smoked ☐ Slightly ☐ Deeply ☐ Not at all ☐ Moderately

	Yes	No
Have you ever smoked cigars regularly? (Yes means more than 1 cigar a week for a year) 71.	☐	☐

If yes:

How old were you when you first started smoking cigars regularly? Age in year ➤ 72.

If you have stopped smoking cigars completely, how old were you when you stopped? Age stopped ➤ 73. Still smoking ☐

On the average over the entire time you smoked cigars, how many cigars did you smoke a week? Cigars per week ➤ 74.

How many cigars are you smoking per week now? Cigars per week ➤ 75. Not smoking cigars ☐

Do or did you inhale the cigar smoke?

76. ☐ Never smoked ☐ Slightly ☐ Deeply ☐ Not at all ☐ Moderately

Check if you use snuff or chewing tobacco. 77 ☐

If yes, how many years have you used it? Years used ➤ 78.

What is the best description of the number of alcoholic beverages you consume (1 drink = 1 12oz. beer, 1 glass of wine or 1 oz. of hard liquor)

79. ☐ None ☐ Less than 1 drink per day ☐ 1 to 3 drinks a day ☐ 4 or more drinks per day

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APPENDIX 2

TABLES OF HORMONE RATIOS BY BODY MASS INDEX, AGE, SMOKING STATUS, AND ALCOHOL CONSUMPTION

TABLE A4.1.1 BOUND TESTOSTERONE TO FREE TESTOSTERONE RATIO (TB/TF) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 1990 PERFLUOROCHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		TB/TF				
	N	MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	40	37.2	9.06	37.1	22.3-62.5	F=1.47
25-30	56	37.6	9.31	37.1	19.3-62.4	p=.23
>30	17	33.3	9.18	31.2	19.7-52.4	
AGE						
<31	20	32.4	6.92	31.2	20.0-43.6	F=.2.39
31-40	48	37.3	9.37	37.1	19.3-62.6	p=.07
41-50	26	37.1	9.30	38.8	19.7-58.8	
51-60	19	39.9	9.90	39.9	22.3-62.4	
Alcohol						
<1oz/d	86	37.4	9.90	37.2	19.3-62.6	F=2.06
1-3oz/d	19	33.9	6.70	32.2	22.7-44.1	p=.15
missing	8	38.0	5.70	38.6	26.4-43.5	
Tobacco						
smoker	27	37.9	7.96	37.2	25.0-58.8	F=.32
nonsmoker	84	36.7	9.64	36.3	19.3-62.6	p=.57
missing	2	27.5	1.57	27.5	26.4-28.6	
TOTAL	113					

#univariate Anova

TABLE A4.1.2 ESTRADIOL TO FREE TESTOSTERONE RATIO (E/TF)
BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS
1990 PERFLUOROCHEMICAL EFFECTS STUDY,
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	E/TF MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	40	2.07	0.88	1.94	0.73-5.0	*
25-30	56	2.28	0.92	2.17	0.76-5.39	>30vs<=30
>30	17	2.56	0.98	2.42	1.44-5.31	T=2.35
						p=.02
AGE						
<31	20	1.94	0.56	1.81	1.44-3.27	F=1.19
31-40	48	2.25	0.81	2.17	0.77-4.18	p=.32
41-50	26	2.31	1.20	2.04	0.77-5.39	
51-60	19	2.48	1.05	2.42	1.07-5.31	
Alcohol						
<1oz/d	86	2.23	0.92	2.10	0.74-5.31	F=.01
1-3oz/d	19	2.21	0.76	2.21	0.73-4.18	p=.92
missing	8	2.46	1.96	2.09	1.41-5.39	
Tobacco						
smoker	27	2.19	0.98	2.06	0.74-5.39	F=.15
nonsmoker	84	2.27	0.92	2.19	0.73-5.31	p=.70
missing	2	1.88	0.32	1.88	1.65-2.11	
TOTAL	113					

#univariate Anova
*Student t test, Prob>T

TABLE A4.1.3 ESTRADIOL TO BOUND TESTOSTERONE RATIO (E/TB) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 1990 PERFLUOROCHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		E/TB x100				
	N	MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	40	5.8	2.65	5.7	11.8-13.5	F=3.70
25-30	56	6.3	2.73	5.5	2.2-13.4	p=.03
>30	17	8.0	2.91	7.6	3.7-14.4	
AGE						
<31	20	6.1	1.79	5.9	3.0-9.5	F=.07
31-40	48	6.4	2.78	5.6	11.8-13.5	p=.98
41-50	26	6.5	3.53	5.0	1.7-14.4	
51-60	19	6.4	2.76	6.0	2.3-11.6	
Alcohol						
<1oz/d	86	6.3	2.90	5.7	1.2-14.4	F=.08
1-3oz/d	19	6.5	1.98	6.8	3.0-10.5	p=.98
missing	8	6.6	3.45	5.4	3.9-13.5	
Tobacco						
smoker	27	5.9	2.73	5.1	11.8-13.5	F=1.01
nonsmoker	84	6.5	2.84	6.0	2.2-13.4	p=.32
missing	2	6.9	1.55	6.9	3.7-14.4	
TOTAL	113					

#univariate Anova

TABLE A4.1.4 ESTRADIOL TO LUTENIZING HORMONE RATIO (E/LH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 1990 PERFLUOROCHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	E/LH MEAN	E/LH SD	E/LH MEDIAN	E/LH RANGE	TEST#
BMI						
<25	40	7.0	3.11	7.3	2.0-16.4	F=2.59
25-30	56	7.0	4.29	6.5	1.0-20.6	p=.08
>30	17	9.3	3.92	8.8	3.3-18.4	
AGE						
<31	20	8.7	4.58	7.6	2.3-20.6	F=2.51
31-40	48	7.8	3.35	7.6	1.5-16.4	p=.06
41-50	26	6.4	4.58	5.1	1.6-18.8	
51-60	19	5.8	2.89	6.2	1.0-11.5	
Alcohol						
<1oz/d	86	7.2	3.95	7.0	1.0-20.6	F=.04
1-3oz/d	19	7.4	4.16	6.7	1.6-16.4	p=.86
missing	8	8.5	3.19	8.7	4.3-15.4	
Tobacco						
smoker	27	7.0	4.42	6.2	1.5-16.4	F=.34
nonsmoker	84	7.5	3.77	7.1	1.0-20.6	p=.56
missing	2	4.7	3.35	4.7	2.3-7.0	
TOTAL	113					

#univariate Anova

TABLE A4.1.5 FREE TESTOSTERONE TO LUTENIZING HORMONE RATIO (TF/LH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 1990 PERFLUOROCHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		TF/LH				
	N	MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	40	3.6	1.74	3.3	1.1-11.3	F=1.47
25-30	56	3.2	1.74	2.9	0.7-9.1	p=.24
>30	17	3.9	1.75	3.4	1.4-7.1	
AGE						
<31	20	4.8	2.55	3.9	3.9	F=7.21
31-40	48	3.6	1.43	3.3	3.3	p=.0002
41-50	26	2.9	1.30	2.7	2.7	
51-60	19	2.5	1.14	2.5	2.5	
Alcohol						
<1oz/d	86	3.4	1.85	3.2	0.7-11.3	F=.06
1-3oz/d	19	3.3	1.44	3.2	0.7-6.4	p=.81
missing	8	3.8	1.43	3.6	2.1-6.2	
Tobacco						
smoker	27	3.2	1.34	3.2	1.1-6.9	F=1.20
nonsmoker	84	3.6	1.87	3.2	0.7-11.3	p=.28
missing	2	2.4	1.38	2.3	1.4-3.3	
TOTAL	113					

#univariate Anova

TABLE A4.1.6 BOUND TESTOSTERONE TO LUTENIZING HORMONE RATIO (TB/LH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 1990 PERFLUOROCHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		TB/LH				
	N	MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	40	131	57.9	125	39-298	F=.79
25-30	56	116	60.6	107	24-288	p=.46
>30	17	122	43.4	121	55-199	
AGE						
<31	20	147	69.7	136	39-298	F=4.72
31-40	48	133	58.0	131	36-288	p=.004
41-50	26	101	36.7	93	29-163	
51-60	19	96	47.6	95	24-208	
Alcohol						
<1oz/d	86	122	57.1	121	24-298	F=.32
1-3oz/d	19	114	56.1	92	29-202	p=.57
missing	8	147	63.2	142	77-234	
Tobacco						
smoker	27	116	52.4	114	41-288	F=.54
nonsmoker	84	125	58.8	121	24-298	p=.46
missing	2	64	34.3	64	39-88	
TOTAL	113					

#univariate Anova

TABLE A4.1.7 THYROID STIMULATING HORMONE TO LUTENIZING HORMONE RATIO (TSH/LH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 1990 PERFLUOROCHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		TSH/LH x10				
	N	MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	40	3.2	1.78	3.2	0.6-8.3	F=3.40
25-30	56	3.5	2.80	3.0	0.4-17.0	p=.02
>30	17	5.3	3.34	4.4	1.7-13.5	
AGE						
<31	20	3.7	2.45	3.1	1.0-9.9	F=.14
31-40	48	3.8	3.16	3.2	0.4-17.0	p=.93
41-50	26	3.4	1.89	2.9	0.8-8.3	
51-60	19	3.8	2.48	3.5	0.4-11.0	
Alcohol						
<1oz/d	86	3.5	2.29	3.1	0.4-11.0	F=2.92
1-3oz/d	19	4.7	4.05	3.2	1.0-17.0	p=.09
missing	8	3.3	1.74	3.6	0.8-5.8	
Tobacco						
smoker	27	3.0	1.68	2.8	0.4-7.6	F=2.89
nonsmoker	84	4.0	2.89	3.3	0.4-17.0	p=.09
missing	2	1.7	0.01	1.7	1.7-1.7	
TOTAL	113					

#univariate Anova

TABLE A4.1.8 FOLLICLE STIMULATING HORMONE TO LUTENIZING HORMONE RATIO (FSH/LH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 1990 PERFLUOROCHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	FSH/LH					
	N	MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	40	1.0	0.42	0.8	0.4-2.3	F=2.54
25-30	56	1.0	0.37	0.9	0.4-1.9	p=.08
>30	17	1.2	0.46	1.1	0.4-2.0	
AGE						
<31	20	0.9	0.4	0.8	0.5-1.9	F=3.06
31-40	48	0.9	0.46	0.9	0.4-1.9	p=.03
41-50	26	1.1	0.45	1.0	0.4-1.8	
51-60	19	1.2	0.49	1.1	0.5-2.3	
Alcohol						
<1oz/d	86	1.0	0.42	0.9	0.4-2.3	
1-3oz/d	19	0.9	0.41	0.9	0.4-1.7	F=.48
missing	8	0.9	0.24	0.9	0.5-1.3	p=.49
Tobacco						
smoker	27	1.0	0.40	0.9	.04-1.8	
nonsmoker	84	1.0	0.42	0.9	0.4-2.3	F=0.0
missing	2	0.9	0.43	0.9	0.7-1.0	p=.98
TOTAL	113					

#univariate Anova

TABLE A4.1.9 PROLACTIN TO LUTENIZING HORMONE RATIO (P/LH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 1990 PERFLUOROCHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	P/LH MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	40	1.84	1.14	1.53	0.50-6.39	F=.73
25-30	56	1.78	1.40	1.46	0.39-9.11	p=.49
>30	17	2.21	1.20	1.83	1.18-4.91	
AGE						
<31	20	2.29	1.70	1.83	1.18-4.91	F=1.55
31-40	48	1.95	1.56	1.67	0.39-9.11	p=.21
41-50	26	1.61	0.93	1.18	0.56-3.70	
51-60	19	1.54	0.87	1.39	0.35-3.37	
Alcohol						
<1oz/d	86	1.78	1.04	1.61	0.35-6.39	F=3.19
1-3oz/d	19	2.37	2.14	1.70	0.56-9.11	p=.08
missing	8	1.57	0.71	1.47	0.88-3.00	
Tobacco						
smoker	27	1.27	0.73	1.12	0.39-2.74	F=8.25
nonsmoker	84	2.07	1.38	1.72	0.35-9.11	p=.005
missing	2	1.43	0.78	1.43	0.88-2.00	
TOTAL	113					

#univariate Anova

TABLE A4.1.10 BOUND TESTOSTERONE TO THYROID STIMULATING HORMONE RATIO (TB/TSH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 1990 PERFLUOROCHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		TB/TSH				
	N	MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	40	500	331	413	170-1682	F=2.42
25-30	56	461	384	329	51-2102	p=.09
>30	17	296	152	297	87-589	
AGE						
<31	20	522	367	416	122-1682	F=2.64
31-40	48	521	388	421	51-2102	p=.05
41-50	26	359	203	345	131-1035	
51-60	19	328	231	286	87-1122	
Alcohol						
<1oz/d	86	468	352	353	87-2102	F=2.74
1-3oz/d	19	329	210	278	51-900	p=.10
missing	8	563	326	456	184-1154	
Tobacco						
smoker	27	468	232	403	184-1185	F=.07
nonsmoker	84	448	364	321	51-2102	p=.80
missing	2	371	198	371	231-511	
TOTAL	113					

#univariate Anova

TABLE A4.1.11 FREE TESTOSTERONE TO THYROID STIMULATING HORMONE RATIO (TF/TSH)
BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS
1990 PERFLUOROCHEMICAL EFFECTS STUDY,
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		TF/TSH				
	N	MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	40	13.8	8.62	10.8	3.8-43.7	F=2.43
25-30	56	11.7	7.01	9.7	1.7-35.8	p=.09
>30	17	9.3	5.05	8.0	2.0-19.7	
AGE						
<31	20	15.8	9.87	10.8	6.1-43.7	F=5.36
31-40	48	13.4	7.62	11.9	1.7-35.8	p=.002
41-50	26	9.7	4.58	8.7	3.7-22.0	
51-60	19	8.1	4.46	7.8	2.0-21.3	
Alcohol						
<1oz/d	86	12.4	1.65	10.0	2.0-43.7	F=2.48
1-3oz/d	19	9.5	5.10	8.7	1.7-20.7	p=.12
missing	8	15.3	9.35	12.3	5.0-33.5	
Tobacco						
smoker	27	12.5	5.53	11.3	5.0-27.2	F=.12
nonsmoker	84	11.9	8.06	9.8	1.7-43.7	p=.73
missing	2	13.7	7.99	13.7	8.1-19.4	
TOTAL	113					

#univariate Anova

TABLE A4.1.12 ESTRADIOL TO THYROID STIMULATING HORMONE RATIO (E/TSH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 1990 PERFLUOROCHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	E/TSH MEAN	E/TSH SD	E/TSH MEDIAN	E/TSH RANGE	TEST#
BMI						
<25	40	26.7	17.73	23.1	3.3-108.1	F=.27
25-30	56	25.4	14.04	21.3	1.8-59.0	p=.76
>30	17	23.4	15.79	18.4	7.7-54.8	
AGE						
<31	20	27.8	12.00	24.6	9.7-50.0	F=3.21
31-40	48	29.5	18.40	22.3	1.8-108.1	p=.03
41-50	26	21.8	13.06	19.0	3.3-52.2	
51-60	19	18.2	10.80	16.8	7.8-54.8	
Alcohol						
<1oz/d	86	25.1	13.08	21.5	3.3-59.0	F=.57
1-3oz/d	19	22.4	15.30	18.0	1.8-54.2	p=.45
missing	8	37.8	31.81	28.5	10.4-108.1	
Tobacco						
smoker	27	26.7	15.13	23.4	10.3-59.0	F=.20
nonsmoker	84	25.1	15.85	20.7	1.8-108.1	p=.65
missing	2	27.1	19.40	27.1	13.4-40.8	
TOTAL	113					

#univariate Anova

TABLE A4.1.13 THYROID STIMULATING HORMONE TO FOLLICLE STIMULATING HORMONE RATIO (TSH/FSH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 1990 PERFLUOROCHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	TSH/FSH					
	N	MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	40	0.39	0.25	0.34	0.08-1.09	F=.39
25-30	56	0.41	0.39	0.31	0.06-2.34	p=.68
>30	17	0.48	1.29	0.43	0.15-1.26	
AGE						
<31	20	0.46	0.31	0.44	0.12-1.26	F=.93
31-40	48	0.46	0.40	0.35	0.06-2.34	p=.43
41-50	26	0.37	0.28	0.30	0.09-1.21	
51-60	19	0.33	0.18	0.31	0.06-0.75	
Alcohol						
<1oz/d	86	0.38	0.26	0.32	.06-1.26	F=5.36
1-3oz/d	19	0.58	0.54	0.39	0.15-2.34	p=.02
missing	8	0.40	0.24	0.39	0.08-0.76	
Tobacco						
smoker	27	0.34	1.04	0.26	0.06-0.92	F=2.39
nonsmoker	84	0.45	2.28	0.40	0.06-2.34	p=.12
missing	2	0.21	0.05	0.21	0.17-0.25	
TOTAL	113					

#univariate Anova

TABLE A4.1.14 FREE TESTOSTERONE TO FOLLICLE STIMULATING HORMONE RATIO (TF/FSH)
BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS
1990 PERFLUOROCHEMICAL EFFECTS STUDY,
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		TF/FSH				
	N	MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	40	4.3	2.66	3.6	1.3-15.6	F=1.03
25-30	56	3.6	2.18	3.0	0.7-11.1	p=.36
>30	17	4.0	2.86	3.1	0.8-11.3	
AGE						
<31	20	5.8	3.34	5.0	1.7-15.6	F=10.35
31-40	48	4.2	2.16	3.7	1.7-11.3	p=.0001
41-50	26	3.0	1.62	2.5	0.7-6.6	
51-60	19	2.2	1.37	2.0	0.7-6.3	
Alcohol						
<1oz/d	86	2.8	2.60	3.1	0.7-15.6	F=.01
1-3oz/d	19	3.9	2.18	3.7	1.3-10.1	p=.91
missing	8	4.4	1.67	4.7	2.2-7.3	
Tobacco						
smoker	27	3.5	1.76	3.0	0.7-7.5	F=1.14
nonsmoker	84	4.1	2.67	3.5	0.7-15.6	p=.28
missing	2	2.6	0.91	2.6	2.2-7.3	
TOTAL	113					

#univariate Anova

TABLE A4.1.15 BOUND TESTOSTERONE TO FOLLICLE STIMULATING HORMONE RATIO (TB/FSH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 1990 PERFLUOROCHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		TF/FSH				
	N	MEAN	SD	MEDIAN	RANGE	TEST
BMI						
<25	40	155	87.3	138	39-411	F=2.00
25-30	56	126	67.9	113	23-297	p=.14
>30	17	122	75.7	115	34-306	
AGE						
<31	20	182	90.3	184	39-411	F=8.75
31-40	48	154	74.5	141	45-348	p=.0001
41-50	26	101	52.9	91	39-227	
51-60	19	87	53.2	78	23-264	
Alcohol						
<1oz/d	86	133	76.5	120	23-411	F=0.0
1-3oz/d	19	133	78.6	112	39-325	p=.98
missing	8	173	80.6	190	82-303	
Tobacco						
smoker	27	130	74.4	106	39-325	F=.28
nonsmoker	84	139	78.5	131	23-411	p=.60
missing	2	72	70.9	72	57-86	
TOTAL	113					

#univariate Anova

TABLE A4.1.16 ESTRADIOL TO FOLLICLE STIMULATING HORMONE RATIO (E/FSH) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 1990 PERFLUOROCHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		E/FSH				
	N	MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	40	8.7	5.51	6.8	1.3-23.3	F=.50
25-30	56	7.8	5.76	6.6	1.4-29.6	p=.61
>30	17	9.3	7.04	7.6	2.65-33.1	
AGE						
<31	20	10.8	5.80	9.6	3.1-25.0	F=5.00
31-40	48	9.5	6.01	7.3	1.4-33.1	p=.003
41-50	26	6.9	6.13	4.8	1.3-29.6	
51-60	19	4.9	2.27	4.6	1.5-9.3	
Alcohol						
<1oz/d	86	8.1	5.83	6.6	1.3-33.1	F=.01
1-3oz/d	19	8.3	4.99	7.1	3.1-19.1	p=.91
missing	8	10.9	7.94	83	4.6-29.6	
Tobacco						
smoker	27	8.1	6.82	4.7	1.4-29.6	F=.13
nonsmoker	84	8.5	5.59	7.0	1.3-33.1	p=.72
missing	2	5.1	2.56	5.1	3.2-6.9	
TOTAL	113					

#univariate Anova

TABLE A4.1.17 THYROID STIMULATING HORMONE TO PROLACTIN RATIO (TSH/P) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 1990 PERFLUOROCHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

		TSH/P				
	N	MEAN	SD	MEDIAN	RANGE	TEST#
BMI						
<25	40	0.22	0.17	0.18	0.06-0.83	F=.71
25-30	56	0.25	0.22	0.19	0.02-1.21	p=.49
>30	17	0.28	0.19	0.26	0.07-0.81	
AGE						
<31	20	0.17	0.09	0.15	0.05-0.39	F=1.38
31-40	48	0.25	0.23	0.17	0.02-1.21	p=.25
41-50	26	0.26	0.18	0.22	0.06-0.83	
51-60	19	0.29	0.20	0.20	0.07-0.81	
Alcohol						
<1oz/d	86	0.24	0.19	0.19	0.04-1.21	F=2.15
1-3oz/d	19	0.29	0.24	0.17	0.02-1.00	p=.15
missing	8	0.21	0.08	0.20	0.09-0.30	
Tobacco						
smoker	27	0.29	0.25	0.21	0.09-1.21	F=1.00
nonsmoker	84	0.23	0.18	0.18	0.02-1.00	p=.32
missing	2	0.14	0.08	0.14	0.09-0.30	
TOTAL	113					

#univariate Anova

TABLE A4.1.18 FREE TESTOSTERONE TO PROLACTIN RATIO (TF/P) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 1990 PERFLUOROCHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	TF/P MEAN	TF/P SD	TF/P MEDIAN	TF/P RANGE	TEST#
BMI						
<25	40	2.5	1.47	2.2	0.6-7.8	F=.19
25-30	56	2.3	2.13	1.9	0.5-15.0	p=.83
>30	17	2.1	1.12	2.0	0.8-4.6	
AGE						
<31	20	2.4	1.67	2.0	0.7-7.8	F=.72
31-40	48	2.6	2.27	2.1	0.5-15.0	p=.54
41-50	26	2.1	1.05	2.0	0.7-4.2	
51-60	19	2.0	1.19	1.6	0.8-5.6	
Alcohol						
<1oz/d	86	2.4	1.94	2.0	0.6-15.0	F=.19
1-3oz/d	19	2.0	1.20	1.5	0.5-5.1	p=.37
missing	8	2.6	0.75	2.7	1.5-3.8	
Tobacco						
smoker	27	3.2	2.81	2.4	1.1-15.0	F=9.58
nonsmoker	84	2.1	1.18	1.9	0.5-7.8	p=.003
missing	2	2.2	2.20	2.2	0.7-3.8	
TOTAL	113					

#univariate Anova

TABLE A4.1.19 BOUND TESTOSTERONE TO PROLACTIN RATIO (TB/P)
BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS
1990 PERFLUOROCHEMICAL EFFECTS STUDY,
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	TB/P MEAN	TB/P SD	TB/P MEDIAN	TB/P RANGE	TEST#
BMI						
<25	40	87	46.4	82	20-221	F=.60
25-30	56	87	85.0	64	22-624	p=.55
>30	17	68	36.1	67	28-158	
AGE						
<31	20	75	47.1	68	20-206	F=.83
31-40	48	96	91.0	79	22-624	p=.48
41-50	26	78	41.6	72	23-163	
51-60	19	73	34.4	63	34-158	
Alcohol						
<1oz/d	86	87	73.0	72	20-624	F=1.23
1-3oz/d	19	68	49.9	49	22-221	p=.27
missing	8	95	20.8	100	55-117	
Tobacco						
smoker	27	121	122.8	94	27-624	F=11.31
nonsmoker	84	73	38.7	66	22-206	p=.001
missing	2	60	56.8	60	20-100	
TOTAL	113					

#univariate Anova

TABLE A4.1.20 ESTRADIOL TO PROLACTIN RATIO (E/P)
BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS
1990 PERFLUOROCHEMICAL EFFECTS STUDY,
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	E/P MEAN	E/P SD	E/P MEDIAN	E/P RANGE	TEST#
BMI						
<25	40	4.7	2.80	4.4	1.1-13.8	F=.19
25-30	56	5.1	4.88	3.9	1.1-32.5	p=.82
>30	17	5.1	2.68	4.3	1.9-9.7	
AGE						
<31	20	4.2	2.20	4.1	1.1-9.0	F=1.09
31-40	48	5.7	5.18	4.6	1.1-32.5	p=.36
41-50	26	4.7	3.22	4.0	1.1-15.1	
51-60	19	4.3	2.00	3.7	2.2-9.6	
Alcohol						
<1oz/d	86	5.0	4.11	4.1	1.1-32.5	F=.59
1-3oz/d	19	4.2	2.94	3.1	1.1-13.0	p=.45
missing	8	6.5	4.15	4.6	3.0-15.1	
Tobacco						
smoker	27	7.2	6.67	5.2	1.1-32.5	F=12.21
nonsmoker	84	4.3	2.16	4.0	1.1-10.2	p=.001
missing	2	4.6	4.84	4.6	1.1-8.0	
TOTAL	113					

#univariate Anova

TABLE A4.1.21 FOLLICLE STIMULATING HORMONE TO PROLACTIN RATIO (FSH/P) BY BODY MASS INDEX, AGE, SMOKING AND DRINKING STATUS 1990 PERFLUOROCHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	FSH/P MEAN	FSH/P SD	FSH/P MEDIAN	FSH/P RANGE	TEST#
BMI						
<25	40	0.72	0.51	0.57	0.2-2.1	F=.22
25-30	56	0.79	0.52	0.65	0.1-2.6	.80
>30	17	0.74	0.58	0.57	0.2-2.6	
AGE						
<31	20	0.46	0.23	0.45	0.2-1.0	F=5.41
31-40	48	0.71	0.51	0.54	0.1-2.6	p=.002
41-50	26	0.88	0.50	0.73	0.2-2.1	
51-60	19	1.05	0.62	0.81	0.2-2.6	
Alcohol						
<1oz/d	86	0.81	0.57	0.66	0.2-2.1	F=3.18
1-3oz/d	19	0.57	0.32	0.50	0.1-2.6	p=.08
missing	8	0.66	0.31	0.60	0.2-2.6	
Tobacco						
smoker	27	1.00	0.57	0.79	0.3-2.6	F=7.90
nonsmoker	84	0.68	0.49	0.51	0.1-2.6	p=.006
missing	2	0.75	0.57	0.62	0.3-1.2	
TOTAL	113					

#univariate Anova

APPENDIX 3

TABLES OF HORMONE RATIOS BY TOTAL SERUM FLUORIDE

TABLE A4.2.1 HORMONE RATIOS BY TOTAL SERUM FLUORIDE:
ESTRADIOL/FREE TESTOSTERONE (E/TF)
ESTRADIOL/BOUND TESTOSTRONE (E/TB)
ESTRADIOL/THYROID STIMULATING HORMONE (E/TSH)
1990 PERFLUOROCHEMICAL EFFECTS STUDY,
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

TOTAL FLUORIDE ppm	N	MEAN	SD	MEDIAN	RANGE	TEST#
			E/TF			
<1	23	2.5	1.2	2.2	0.7-5.3	F=1.65
>=1-3	64	2.1	0.8	1.9	0.8-5.4	p=.16
>3-10	15	2.2	0.6	2.2	0.8-3.2	
>10-15	6	2.6	1.1	2.1	1.6-4.0	
>15-26	5	2.7	0.7	3	1.9-3.3	
TOTAL	113	2.25	.92	2.1	0.7-5.4	
			E/TB (X100)			
<1	23	7.3	3.5	6.3	1.1-14.4	F=1.17
>=1-3	64	5.9	2.5	5.5	1.7-13.5	p=.33
>3-10	15	6.7	2.8	5.9	2.9-12.3	
>10-15	6	6.9	2.8	5.6	4.3-11.7	
>15-26	5	6.3	1.8	5.3	4.8-8.4	
TOTAL	113	6.4	2.8	5.8	1.2-14.4	
			E/TSH			
<1	23	29.2	21.8	21.0	10.4-108.1	F=0.75
>=1-3	64	24.9	13.2	23.6	1.8-52.2	p=.56
>3-10	15	26.7	16.7	21.1	3.9-59.0	
>10-15	6	19.3	1.9	15.8	7.8-45.8	
>15-26	5	19.8	6.8	16.9	15.2-31.5	
TOTAL	113	25.5	15.6	21.3	1.76-108.1	

#univariate Anova

TABLE A4.2.2 HORMONE RATIOS BY TOTAL SERUM FLUORIDE:
BOUND TESTOSTERONE/FREE TESTOSTRONE (TB/TF)
BOUND TESTOSTRONE/FOLLICLE STIMULATING HORMONE (TB/FSH)
BOUND TESTOSTERONE/PROLACTIN (TB/P)
FREE TESTOSTERONE/PROLACTIN (TF/P)
1990 PERFLUOROCHEMICAL EFFECTS STUDY,
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	SD	MEDIAN	RANGE	TEST#
TOTAL FLUORIDE ppm			TB/TF			
<1	23	36.7	9.6	37.1	16.7-62.6	F=.94
>=1-3	64	36.8	9.6	35.6	19.2-58.8	P=.45
>3-10	15	34.5	7.0	33.3	25.0-43.6	
>10-15	6	38.3	7.6	38.9	29.3-47.6	
>15-26	5	43.6	10.6	39.9	36.9-62.4	<=10vs >10
TOTAL	113	36.8	9.2	37.0	16.7-62.6	T=2.10 P=.15
			TB/FSH			
<1	23	148.3	85.6	120.4	56.7-411.0	F=.40
>=1-3	64	130.9	76.3	114.2	23.1-347.7	P=.81
>3-10	15	135.3	81.4	86.9	49.0-303.3	
>10-15	6	122.7	48.3	135.8	34.4-169.8	
>15-26	5	162.9	76.4	143.5	67.1-253.5	
TOTAL	113	136.1	77.1	120.0	23.1-411.0	
			TB/P			
<1	23	82.1	43.2	67.5	19.8-205.5	F=.40
>=1-3	64	87.5	81.2	63.3	23.0-624.2	P=.81
>3-10	15	86.4	51.0	78.0	28.1-224.2	
>10-15	6	51.5	27.9	52.1	22.2-89.3	
>15-26	5	90.3	30.6	83.4	58.3-129.7	
TOTAL	113	84.5	67.3	70.7	19.8-624.2	
			TF/P			
<1	23	2.34	1.46	2.01	.7-7.8	F=.52
>=1-3	64	2.38	1.97	1.88	.6-15.0	P=.72
>3-10	15	2.64	1.84	2.40	.8-8.1	
>10-15	6	1.40	0.82	1.35	.5-2.3	
>15-26	5	2.20	0.91	2.16	.9-3.5	
TOTAL	113	2.35	1.78	1.95	.5-15.0	

#univariate Anova

TABLE A4.2.3 HORMONE RATIOS BY TOTAL SERUM FLUORIDE:
ESTRADIOL/PROLACTIN (E/P)
THYROID STIMULATING HORMONE/PROLACTIN (TSH/P)
FOLLICLLE STIMULATING HORMONE/PROLACTIN (FSH/P)
PROLACTIN/LUTENIZING HORMONE (P/LH)
1990 PERFLUOROCHEMICAL EFFECTS STUDY,
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	SD	MEDIAN	RANGE	TEST#
TOTAL FLUORIDE ppm			E/P			
<1	23	5.34	2.77	4.81	1.45-10.22	F=.64
>=1-3	64	4.75	4.38	3.77	1.09-32.50	p=.63
>3-10	15	5.87	4.65	4.76	1.87-17.89	
>10-15	6	3.13	1.08	3.45	1.13-4.07	
>15-26	5	5.55	1.69	6.17	3.11-7.09	
TOTAL	113	4.97	3.94	4.08	1.09-32.50	
			TSH/P			
<1	23	0.22	0.11	0.21	0.07-0.49	F=.40
>=1-3	64	0.24	0.21	0.17	0.04-1.20	p=.81
>3-10	15	0.29	0.76	0.20	0.07-0.85	
>10-15	6	0.24	0.14	0.25	0.02-0.41	
>15-26	5	0.29	0.09	0.27	0.20-0.44	
TOTAL	113	0.24	0.20	0.19	0.02-1.20	
			FSH/P			
<1	23	0.65	0.47	0.60	0.18-2.56	F=.89
>=1-3	64	0.80	0.52	0.66	0.15-2.17	p=.47
>3-10	15	0.67	0.68	0.57	0.15-2.58	
>10-15	6	0.52	0.36	0.47	0.13-1.04	
>15-26	5	0.66	0.36	0.47	0.33-1.13	
TOTAL	113	0.76	0.52	0.60	0.13-2.58	
			P/LH			
<1	23	1.71	0.65	1.64	0.35-3.02	F=1.72
>=1-3	63	1.85	1.75	1.57	0.41-6.39	p=.15
>3-10	15	1.79	1.20	1.45	0.39-4.00	
>10-15	6	3.14	3.00	1.88	1.10-9.11	
>15-26	5	1.5	0.44	1.33	1.03-2.10	
TOTAL.	112	1.87	1.29	1.62	0.35-9.11	

#univariate Anova

TABLE A4.2.4 HORMONE RATIOS BY TOTAL SERUM FLUORIDE: ESTRADIOL/LUTENIZING HORMONE (E/LH) ESTRADIOL/FOLLICLLE STIMULATING HORMONE(E/FSH) FOLLICLLE STIMULATING HORMONE/LUTENIZING HORMONE (FSH/LH) 1990 PERFLUOROCHEMICAL EFFECTS STUDY, 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	SD	MEDIAN	RANGE	TEST#
TOTAL FLUORIDE ppm			E/LH			
<1	23	8.64	4.73	8.28	1.51-18.81	F=.92
>=1-3	63	6.85	4.01	6.19	0.95-20.59	p=.45
>3-10	15	7.24	2.74	7.55	1.96-11.52	
>10-15	6	7.21	2.15	7.09	4.39-10.27	
>15-26	5	7.99	2.71	7.03	6.03-12.70	
TOTAL	112	7.34	3.91	7.01	0.95-20.59	
			E/FSH			
<1	23	10.27	6.85	8.69	1.36-33.12	F=1.00
>=1-3	64	7.71	5.98	6.10	1.30-29.60	p=041
>3-10	15	7.74	3.26	6.89	3.71-12.42	
>10-15	6	8.04	4.24	8.27	3.09-15.30	
>15-26	5	10.32	6.60	7.03	5.45-21.50	
TOTAL	113	8.37	5.81	6.85	1.30-33.12	
			FSH/LH			
<1	23	0.91	0.30	0.89	0.42-1.48	F=.41
>=1-3	63	1.04	0.44	0.94	0.37-2.25	p=.73
>3-10	15	0.99	0.43	0.91	0.41-1.95	
>10-15	6	1.08	0.50	1.03	0.53-1.78	
>15-26	5	0.91	0.35	0.99	0.55-1.40	
TOTAL	112	1.00	0.41	0.94	0.37-2.25	

#univariate Anova

TABLE A4.2.5 HORMONE RATIOS BY TOTAL SERUM FLUORIDE:
FREE TESTOSTERONE/THYROID STIMULATING HORMONE (TF/TSH)
BOUND TESTOSTRONE/THYROID STIMULATING HORMONE (TB/TSH)
FREE TESTOSTERONE/LUTENIZING HORMONE (TF/LH)
BOUND TESTOSTERONE/LUTENIZING HORMONE (TB/LH)
1990 PERFLUOROCHEMICAL EFFECTS STUDY,
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

TOTAL FLUORIDE ppm	N	MEAN	SD	MEDIAN	RANGE	TEST#
			TF/TSH			
<1	23	12.6	8.5	9.5	4.5-35.1	F=.93
>=1-3	64	12.7	7.5	11.1	1.7-43.7	p=.45
>3-10	15	11.9	6.8	10.4	3.2-27.2	
>10-15	6	8.5	1.7	7.5	12.0-20.7	
>15-26	5	7.5	1.9	7.9	4.6-9.6	
TOTAL	113	12.1	7.5	9.9	1.7-43.7	
			TB/TSH			
<1	23	456	330	320	170-1367	F=.54
>=1-3	64	479	363	370	51-2102	p=.70
>3-10	15	416	270	401	95-1185	
>10-15	6	334	296	226	87-900	
>15-26	5	314	49	317	247-370	
TOTAL	113	451	333	353	51-2102	
			TF/LH			
<1	23	3.7	2.3	3.3	1.2-11.3	F=.28
>=1-3	64	3.5	1.8	3.2	0.6-9.1	p=.89
>3-10	15	3.3	1.0	3.3	1.2-5.6	
>10-15	6	3.1	1.33	2.89	1.4-4.6	
>15-26	5	3.0	0.8	3.4	1.9-3.9	
TOTAL	113	3.4	1.7	3.2	0.6-11.3	
			TB/LH			
<1	23	127	66	125	39-298	F=.13
>=1-3	64	121	58	114	24-288	p=.93
>3-10	15	115	51	105	52-234	
>10-15	6	118	56	105	61-201	
>15-26	5	127	21	125	122-149	
TOTAL	113	122.1	57.3	118	24.3-298	

#univariate Anova

TABLE A4.2.6 HORMONE RATIOS BY TOTAL SERUM FLUORIDE:
THYROID STIMULATING HORMONE/FOLLICLE STIMULATING HORMONE (TSH/FSH)
THYROID STIMULATING HORMONE/LUTENIZING HORMONE (TSH/LH)
1990 PERFLUOROCHEMICAL EFFECTS STUDY,
3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

	N	MEAN	SD	MEDIAN	RANGE	TEST#
TOTAL FLUORIDE ppm			TSH/FSH			
<1	23	0.42	0.24	0.40	0.08-0.89	F=0.23
>=1-3	64	0.40	0.38	0.29	0.06-2.34	p=.92
>3-10	15	0.44	0.33	0.35	0.06-1.20	
>10-15	6	0.49	0.25	0.49	0.19-0.80	
>15-26	5	0.49	0.17	0.45	0.27-0.68	
TOTAL	113	0.42	0.33	0.35	0.06-2.34	
			TSH/LH			
<1	23	0.36	0.22	0.33	0.08-1.00	F=0.23
>=1-3	64	0.36	0.30	0.28	0.04-1.70	p=.92
>3-10	15	0.38	0.27	0.31	0.04-1.1	
>10-15	6	0.45	0.19	0.43	0.22-0.70	
>15-26	5	0.40	0.37	0.40	0.36-0.45	
TOTAL	113	0.37	0.26	0.31	0.04-1.70	
			TF/FSH			
<1	23	4.40	3.27	3.7	1.5-15.6	F=.34
>=1-3	64	3.77	2.38	3.1	.7-11.1	p=.85
>3-10	15	3.78	1.84	3.2	1.7-7.3	
>10-15	6	3.42	1.63	3.9	.8-5.3	
>15-26	5	3.93	2.15	3.6	1.8-6.6	
TOTAL	113					

#univariate Anova

TABLE 4.1.78 LINEAR MULTIVARIATE REGRESSION MODEL OF FACTORS PREDICTING THE HEMAGLOBIN AMONG 111 MALE WORKERS. 3M CHEMOLITE PLANT, COTTAGE GROVE, MINNESOTA

Variable	ß	SE(ß)	p-value
Intercept	14.51	.67	.0001
Total Fluorine (ppm)*	-.002	.0009	.02
Alcohol #			
low (<1oz/day)	.22	.20	.27
nonresponse (NR)	.56	.33	.09
Age (years)	.001	.009	.88
BMI (kg/m^2)	.01	.02	.65
Cigarettes/day	.01	.007	.20
Cigs/day X Fluorine^{2}**	.0003	.0001	.0005
Estradiol (pg/ml)	.01	.006	.07

R^2=.23
*square transformation of total fluoride
#Reference category is moderate drinkers who consume 1-3 oz ethanol/day.
** interaction term between cigarettes per day and square transformation of total fluoride