

CANCER MORTALITY IN 3M CHEMICAL WORKERS

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KATHERINE KOEHLER RALEIGH

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BRUCE H. ALEXANDER, PH.D., RESEARCH ADVISOR

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Dedication

To my husband and two sons—Ryan, Jasper and Olyn, your unwavering support will forever be unmatched. And to my father who passed away from kidney cancer when my youngest, Olyn, was a baby, before I began this endeavor.

Organization

The organization of this thesis provides initial chapters (chapters one-three) that include the study introduction, literature review, research design and methodology. The first three chapters are followed by chapters four and five, which are stand-alone papers to be submitted for peer-reviewed publication. There is some redundancy in literature review due to this. The final chapter (chapter six) provides a synthesis of the research including a discussion of results and a sensitivity analysis.

Abstract

At the 3M facility in Cottage Grove, Minnesota, Ammonium Perfluorooctanoate (APFO) was formulated as a polymerization aid used in the production of commercial and consumer products from 1947-2002. APFO is in a class of perfluorocarbons (PFCs)—inert chemicals—that for many years were thought to be nontoxic. More recently, however, a growing interest has surfaced and attention has turned to the health effects from exposure to APFO, and its dissociated anion perfluorooctanoic acid (PFOA). Researchers and public health officials have noted the persistence of APFO and PFOA in the environment along with data that show it remains for a long period in the human body (Houde et al., 2006; and Olsen et al., 2007). Adverse health outcomes reported from several animal studies in conjunction with the noted positive association between APFO exposure and an increased risk of dying from prostate cancer found in workers at the 3M Cottage Grove, Minnesota location, have been the impetus for this research (Lundin et al., 2009; Butenhoff et al., 2002; and Kennedy et al., 2004). The current study evaluated the risk of death in workers based on a newly developed APFO/PFOA quantitative exposure data matrix. We evaluated workers' health outcomes using annual exposure estimates. These estimates were based on two exposure models; a cumulative model (potential cumulative dose, PCD) and a cumulative clearance weighted model (CCWD). Compared to previous epidemiological studies with this cohort, this study estimated a quantitative level of exposure that potentially contributes to negative health

effects in humans. This was a retrospective occupational mortality study of 9,027 3M employees who worked a minimum of one year at one of two 3M locations (Cottage Grove and St. Paul plants) in Minnesota. The workers were followed from their first date of employment (beginning in 1947) until their date of death or until the study ended in 2008. Standardized mortality ratios (SMRs) were calculated using the Minnesota population as the referent population. The risk of a cancer death was evaluated using a time-dependent Cox proportional hazards (PH) model, which compared the workers' exposure over time to the non-occupationally exposed workers at the St. Paul location. There were a total of 2,979 identifiable deaths in the cohort, and of these there were 72 prostate cancer deaths, 48 pancreatic cancer deaths, 16 bladder cancer deaths, 24 kidney cancer deaths, 25 female breast cancer deaths, and 15 liver cancer deaths. The SMR for prostate cancer deaths in the Chemical Division workers was 1.18 based on 16 observed versus 13 expected deaths. All cancer-specific hazard ratios (HRs) using annual continuous exposure estimates were at or below no effect level with increasing exposure to APFO/PFOA. The population was additionally divided into six exposure groups. The HRs for workers showed an increase in the risk of a prostate cancer death with increasing exposure groupings compared to the St. Paul group [HR=1.22, (95% CI: 0.57-2.61), and HR=1.27 (95% CI: 0.30-5.28)], for the top two exposure groups. These results were imprecise, but showed a dose-response relationship.

This study explored the risk of cancer mortality associated with age and intensity of exposure. The findings show a non-significant elevated risk of cancer deaths in the highest exposure groups for cumulative exposure during age 40-49 [HR=1.96, (95% CI: 0.67-5.68), and HR=1.51 (95% CI: 0.47-4.90)], for bladder and liver cancers respectively. These results were imprecise and based on six bladder and seven liver cancer deaths.

Previous studies of the Cottage Grove population have shown an increased risk of death from prostate cancer with increasing exposure to APFO/PFOA using qualitative exposure groupings (Gilliland and Mandel, 1993; and Lundin et al., 2009). Risk of death was determined based on workers' job title and did not explore quantitative dose estimates from air measurement data, as was done in the current study. In this study we evaluated risk based on worker-specific quantitative annual exposure estimates from a reconstruction of the inhalation exposure at Cottage Grove. Prostate cancer results for the Chemical Division and top two exposure groups from the current study support the previous results. The overall results for all cancers are inconsistent when evaluating the continuous exposure compared to exposure group rankings, with the exception of kidney cancer. Kidney cancer was consistently below unity across all analyses, while the remaining cancers only show modest increases in risk based on few cases.

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CHAPTER 1: INTRODUCTION

Ammonium Perfluorooctanoate (APFO) is in a class of synthetic polyfluorinated chemicals—perfluorocarbons (PFCs)—used primarily as a processing aid in the production of fluoropolymers and fluoroelastomers. APFO is an eight-chain fully fluorinated organic compound that is thermally inert and chemically stable. In the presence of water and other liquids, APFO dissociates to perfluorooctanoate (PFOA) and the ammonium ion. In the late 1930s the controlled synthesis of PFCs became available with electrochemical fluorination, which made commercial production of APFO/PFOA possible (Simons and Bryce, 1954). It lacks affinity for fat, water and oil, (i.e., it is lipophobic, hydrophobic, and oleophobic, respectively) making it an ideal chemical surfactant. The carbon-fluorine bonds are extremely strong and resistant to decomposition. APFO is a chemical intermediate used in many industrial and commercial products that require surface protection such as: carpets, textiles, electronics, cookware, paper and wood board surfaces because of its unique properties to repel water and oil (US EPA, 2005). At the 3M facility in Cottage Grove, Minnesota, APFO was formulated as a polymerization aid used in the production of high-performance materials and other consumer products from 1947-2000—when the company began its phase out. Production ended and was completely eliminated in 2002.

Exposure to APFO/PFOA can occur from various environmental sources including: airborne dust, contaminated water and food, and contact with various consumer products including the degradation of other fluoropolymer-containing

products (Paustenbach, 2007). When ingested, inhaled or absorbed through dermal contact, APFO dissociates to the anion form $[\text{CF}_3(\text{CF}_2)_6\text{COOH-NH}_3 = \text{CF}_3(\text{CF}_2)_6\text{COO}^- + \text{H}^+ + \text{NH}_3]$ of perfluorooctanoic acid (PFOA) inside the body. PFOA is the unconjugated metabolite measured in blood-serum samples of humans and other animals following exposure to APFO (Kuslikis et al., 1992).

For many years PFCs were considered non-toxic because of their inert chemical and physical properties. However, over the past decade there has been an increased focus directed towards evaluating the potential adverse human health impact, in part, due to biological monitoring results of APFO production workers showing PFOA-serum levels two to three orders of magnitude greater than the general public (Olsen and Zobel, 2007). With these markedly high blood-serum concentration findings in production workers, compared to the public, in conjunction with the observation that PFOA produces a large range of toxic effects in animals including: carcinogenesis, hepatotoxicity, immunotoxicity, developmental toxicity and endocrine toxicity, it is possible that exposure may not be innocuous in humans (Biegel et al. 2001; Butenhoff et al., 2002; Griffith and Long, 1980; Kennedy et al., 2004; and Yang et al., 2001). What is unknown is the level of exposure in humans that may put them risk of adverse health outcomes. Considering the long chemical half-life of serum-PFOA in humans compared to animals, and the potential that some workers had high and/or long-term chronic exposure, if there are health repercussions from exposure then it may affect APFO/PFOA chemical workers—putting them at a greater risk of adverse outcomes when compared to workers who were not directly involved in chemical

production or when compared to the general population. Consequently, there is a need to establish what the health impact of APFO/PFOA on humans is and what level of exposure would contribute to an increase in the risk of an adverse health outcome.

Investigators of two previous occupational mortality studies reported a positive association between working in the highest APFO/PFOA exposure group, of three groups, and dying from prostate cancer for chemical workers at the 3M Cottage Grove facility (Gilliland and Mandel, 1993 and Lundin et al., 2008). This outcome was based on the internal comparison of exposed to non-exposed workers using qualitative exposure categories determined by job title. However, when the standardized mortality ratios were estimated from the general Minnesota population compared to the entire working population, there was no observed risk. Therefore, depending on the choice of referent population, results varied.

To elucidate potential health effects associated with APFO/PFOA exposure and the impact of exposure on the risk of death—specifically cancer mortality—we completed a quantitative exposure assessment. The goal was to determine the amount of APFO/PFOA over time each worker was exposed to using occupational air monitoring data, professional input, and the workers' sequence of job titles and locations throughout their career. This was conducted with the workers at the Cottage Grove plant and their cancer mortality rates were compared to workers in St. Paul, who had a similar demographic background to Chemical Division workers, however this working population did not have direct APFO/PFOA occupational exposure. Our study population included workers

from the two 3M plants who worked for a minimum of one year over the course of more than half a century. All Cottage Grove workers in our cohort have an exposure history profile that was created based on the exposure reconstruction—which captured daily exposure levels categorized by job title, location and year—from each employee’s work history records. Our primary goal in the context of this research is to evaluate the risk of a cancer death that is associated with APFO/PFOA exposure in the workers by utilizing their updated exposure profiles with extended follow-up time. We accomplished this by utilizing the individual-level time-dependent exposure profiles and compared the rates of six *a-priori* cancer deaths and other health outcomes based on exposure levels. In summary, we calculated the mortality rates using the following analyses: the standardized mortality ratios (SMRs) with Minnesota population-level data, hazard rate ratios based on exposure groupings and continuous annual exposure data, exposure during two decades, and finally the intensity of exposure with the referent working population as a sensitivity analysis.

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CHAPTER 2: BACKGROUND AND SIGNIFICANCE

Ammonium perfluorooctanoate (APFO) is the primary salt of perfluorooctanoic acid (PFOA). PFOA is globally distributed and has been detected in a variety of environmental media including surface water, air and soil samples (Yamashita et al., 2005 and Lau et al., 2007). The presence of PFOA in blood sera found in the general public may arise from a combination of sources such as environmental contamination of water, air and food. In addition, exposures can occur from the metabolic degradation of precursor compounds found in commercial and industrial products (Andersen et al., 2008). PFOA is resistant to decomposition and bio-accumulates in humans and other animals—with a residence time of several years in humans compared to weeks or less in animals. In toxicological studies, animal models have shown an increase in the incidence of tumors and other adverse health outcomes at large doses (Biegel et al., 2001 and Kennedy et al., 2004). Due to its bio-persistence, widespread environmental distribution, long human serum half-life and uncertain toxicological effects in humans, PFOA has become a public health concern among the scientific community and the public.

PFOA is one of many perfluorinated compounds (PFCs) released into the air, soil and water from production activities and from the breakdown of precursor chemicals. It has been detected in both the Pacific and Atlantic oceans with a mean concentration of 439 picograms per liter (pg/L). PFOA has also been measured in the atmosphere in close proximity to production facilities with concentrations ranging from 0.1–3.84 micrograms per cubic meter of air ($\mu\text{g}/\text{m}^3$)

(Yamashita et al., 2005 and Barton et al., 2006). The ionic forms of PFCs are transported with ocean currents and the volatile, precursor PFCs (e.g., fluorotelomer alcohols, FTOHs) are transported in the atmosphere and later breakdown to form PFOA and other PFCs (Dreyer et al., 2009; and Stock et al., 2007). PFOA is mobile in soil and can leech into drinking water. It bioaccumulates in fish and other animal species (Houde et al., 2006). The major non-occupational exposure pathway of PFOA comes from oral exposure including the consumption of contaminated water and food due to chemical migration of packaging, and indirectly from dust ingestion (Emmett et al., 2006; and Begley et al., 2005).

At this time there are no promulgated standards for PFOA in drinking water, consumer products, or for occupational exposures in the U.S. However, the American Conference of Governmental Industrial Hygienists (ACGIH) has set guidelines for the threshold limit value (TLV) at 0.01 mg/m^3 for workers who are exposed to APFO 40 hours a week, annually as the time weighted average (Miller et al., 2007). PFOA is on the priority list to be evaluated for carcinogenicity by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO). A formal review of the literature conducted by the U.S. EPA's Science Advisory Board (SAB) suggested that PFOA is "likely to be carcinogenic to humans" (US EPA, 2006). In addition to the occupational guidelines, in 2006 the U.S. EPA set an action level in drinking water at 0.5 parts per billion (ppb)—which was a sharp decrease from the 150 ppb level set in 2002 (US EPA, 2006). Furthermore, at the local level, in Minnesota the state health

department lowered its health-based value in drinking water from 1.0 to 0.5 ppb (MDH, 2007).

In the early 1990s, scientists in 3M's Medical Division began a voluntary program to collect and test the blood of workers for PFOA. In 2000, approximately 70% of employees involved in APFO production participated in 3M's medical surveillance program. The blood-serum PFOA concentrations were log normally distributed, and ranged from 7 ppb to 92 ppm (3M Final Report, 2003b). The same year 3M established a biological limit value (BLV) of 5 ppm ($\mu\text{g}/\text{mL}$) in the workers engaged in APFO production. This BLV was based on a 10-fold safety factor applied to sera concentrations that were associated with liver weight increases in a sub-chronic feeding study of cynomolgus monkeys (Butenhoff et al., 2002).

In the 1999-2000 National Health and Nutrition Examination Survey (NHANES) investigators collected US population based blood serum PFOA samples for the first time. The serum-PFOA median value for the US population was approximately 5 ppb. A second NHANES survey conducted in 2003-2004 showed a downward trend in blood PFOA concentrations with a median value of 3.9 ppb (Calafat et al., 2007). More recently, however, the Fourth National Exposure Report from March 2013 showed the serum-PFOA geometric mean concentration for the U.S. population was 5.21 ppb—an increase from the former survey (CDC, 2013).

Since the 1950s perfluoroalkyl chemistry—including ammonium perfluorooctanoate—has shown utility in surface applications by repelling oil-

and water-based materials (Kennedy et al., 2004). There are two main chemical processes used to manufacture APFO: electrochemical fluorination (ECF) and telomerization. The ECF method was developed by Simons in 1941 (Simons and Bryce, 1954). ECF reactions are fueled by electrical currents causing all the hydrogen atoms on the carbon backbone to be replaced with fluorine atoms using a low voltage, high current nickel anode (Lau et al., 2007). The telomer process results in a linear APFO carbon chain, while the ECF process yields a partially branched chain (Kennedy et al., 2004). Loveless et al. (2006) conducted a comparative study on mice and rats to evaluate the differences in toxicological properties for linear, linear/branched and branched APFO. The overall response for both mice and rats was that the toxicity of the linear and linear/branched isomers was similar while the branched isomer was less toxic.

Employees at the 3M Cottage Grove, Minnesota plant's Chemical Division manufactured and processed APFO for more than fifty years. The production included five stages: electrochemical fluorination, followed by the formation of a salt slurry mixture, the conversion to a salt cake, drying the cake, and finally packaging and shipping the ammonium salt (3M Final Report, 2003a). There was ample opportunity for exposure to the final product (APFO) in its particulate (ammonium salt) form and also to the vapor intermediate, PFOA, throughout the various phases of production. Exposure occurred at low-dose levels from various daily work responsibilities/tasks and at high-dose levels—but short term—from less frequent spills and releases. Routes of exposure primarily included inhalation exposure with some dermal exposure and possibly some

incidental oral dust exposure. The fabrication of APFO at 3M was phased out starting in May of 2000, and the final manufacturing and handling ceased in late 2002.

In January of 2006, the U.S. Environmental Protection Agency and eight companies that produced APFO created a “2010/2015 PFOA Stewardship Program”. These eight companies utilized APFO at lower levels compared to their high production years in the 1980s and 1990s. Some have eliminated production completely within the U.S. They all pledged to reduce their production and application by ninety-five percent in 2010 and work towards eliminating all uses by 2015 (US EPA, 2006). Although these companies within the U.S. have pledged to phase out production of this bio-persistent chemical, currently other U.S. based and some international companies are involved in APFO production—and/or its application in the making of several industrial and commercial products. It is unknown exactly what quantities of APFO/PFOA are released world-wide, however the estimate of global production from 2000-2002 was between 5 and 6.5 million kilograms per year (Telomer Research Update, 2002).

Chemical, Physical and Toxic Properties

Ammonium perfluorooctanoate (CAS Registry No. 3825-26-1) is a fully fluorinated eight-chain carbon compound with an extremely low surface tension coefficient. The carbon-fluorine bond is one of the strongest organic bonds in nature (Asakawa et al., 2008). It structurally resembles fatty acids with its hydrophilic carboxylate head and its hydrophobic and lipophobic fluorocarbon

tail (Lemal, 2004). It is a nonvolatile white powder at standard temperature and pressure, which can become readily airborne (Kennedy et al., 1986). Its molecular weight is 431.10 g/mol, it has a vapor pressure of 7×10^{-5} mmHg at 20°C and it sublimates (passes directly from the solid to gas phase) at 130°C. It is nonflammable and is not readily degraded by oxidizing agents, or by strong acids or bases. In the presence of aqueous media, APFO dissolves to form PFOA [$\text{CF}_3(\text{CF}_2)_6\text{CO}_2\text{NH}_4 = \text{CF}_3(\text{CF}_2)_6\text{COOH} + \text{NH}_3$] (Griffith and Long, 1980).

Perfluorooctanoic acid (CAS registry number 335-67-1) has a molecular weight of 414.16 g/mol with a melting point of 59-60°C, and boiling point of 189°C, at standard conditions. PFOA is among the strongest acids known with a $\text{pK}_a = 2.5$ (Olson C. and Andersen M., 1983). PFOA is an intermediate in the production of the ammonium salt (APFO) and is produced as a mixture of branched chain isomers.

For clarification, when discussing human exposure and uptake of the chemical, APFO will be used to refer to all pre-absorption discussion and PFOA will be referenced after exposure has occurred and thus the chemical is no longer the ammonium salt, rather it is the dissociated anion of APFO, i.e. the metabolite found in biological media that is from APFO exposure. In addition, in proceeding chapters APFO/PFOA will be utilized to show potential for exposure to both particulate and the vapor, respectively.

Several acute, sub-chronic and chronic studies have been conducted with various animal species and by different pathways of exposure (e.g., inhalation, dermal and oral exposures). There are many recorded observed toxic effects in

multiple species. In acute toxicity studies the lethal dose for half of the animals (LD₅₀) tested in a study by Glaza (1997) was greater than 500 mg/kg of body weight for male rats and 250-500 mg/kg for female rats. Acute inhalation and dermal toxicity of APFO was tested in Sprague-Dawley rats and Hra(NZW)SPF rabbits, respectively (Glaza, 1995). The inhalation dose applied to the rats was 18.6 mg/m³ for one hour with no observed deaths. The LD₅₀ for dermal exposure to the rabbits was determined to be greater than 2000 mg/kg of body weight. APFO is a mild skin irritant and a moderate eye irritant in rabbits. It exhibits a moderate acute oral and inhalation toxicity and mild dermal toxicity (Griffith and Long, 1980; Kennedy et al., 1986; and Kennedy et al., 2004). The liver is the primary target for both acute and chronic effects of PFOA in rats (Griffith and Long, 1980) and in cynomolgus monkeys (Butenhoff et al., 2002).

PFOA is in a sub-class of peroxisome proliferators (PPs) that induce oxidative stress, which results in increased tumor formation associated with β -oxidation (Reddy et al., 1980). In rats, PFOA acts as an agonist for the peroxisome proliferator activated alpha-receptor (PPAR α) signaling pathway. The interaction of PFOA with PPAR α up-regulates several genes, which lead to a significant increase in hepatocellular tumors in rats. A triad of tumors was observed including hepatocellular adenomas, testicular Leydig cell tumors, and pancreatic acinar cell tumors, after a lifetime of oral exposure in male Sprague-Dawley rats (Biegel et al., 2001). In a study by Yang et al. (2002), reductions in spleen and thymus weight were observed only in wild-type mice, not in PPAR α -null mice, suggesting that PPAR α activation may be a factor in the observed toxic

effects. Likewise, PPAR α -null mice do not exhibit the typical PP-mediated response of carcinomas. However, PFOA may act as a cancer promoter in PPAR α -null mice and cynomolgus monkeys and has been shown to cause hepatomegaly—an enlarged liver (Butenoff et al., 2002).

Non-carcinogenic toxic effects reported in animals include: liver and kidney-weight increases, decreased overall weight gain, alterations in lipid metabolism, uncoupling of oxidative phosphorylation, decrease in testosterone levels, increase in estrogen circulating hormones and a modest increase in total cholesterol (Griffith and Long, 1980; Haughom and Spydevold 1992; and Sakr et al., 2007). PFOA has been shown to induce mitochondrial proliferation in rats—which may account for an increase in liver mass (Butenhoff et al., 2002). Inhibitory feeding behavior and delayed gastric emptying were seen in mice examined after they were given intraperitoneal injections of PFOA (Asakawa et al., 2008). There are no known teratogenic effects and there is no evidence of deleterious reproductive effects from a two-generation reproductive study (Butenoff et al., 2004). Data suggest that APFO is not genotoxic since it was not found to be mutagenic in the presence or absence of metabolic activation in the Ames test using five strains of *Salmonella typhimurium* (Griffith and Long, 1980). Yang et al (2001) reported on the immunotoxic effects of oral APFO exposure showing decreases in thymus and spleen weights noted in male mice. These changes have only been established in animal studies and it is unclear if these toxic effects are significant for humans.

Pharmacokinetics

Based on the results of several animal studies, there is no evidence that PFOA is metabolized in mammals (Kuslikis et al., 1992; and Vanden Heuvel et al., 1991). APFO is rapidly and completely absorbed following inhalation and oral exposure—and to a lesser extent after dermal exposure. After absorption, PFOA is distributed primarily to the liver, kidneys and plasma. To a lesser extent, it is distributed to various tissues including the lungs, heart, skin, testes, muscle, fat and brain (Kennedy et al., 2004). PFOA binds strongly to plasma and other proteins in the body with saturation of binding sites occurring at 30 mg/kg (Hanhijavri et al., 1988). Covalent binding of PFOA was reported for proteins in liver, plasma, and testes of rats (Vanden Heuvel et al., 1992). More recent data show that over 90% of PFOA is bound to blood albumin in the rat, monkey, and in humans (Han et al., 2003). Biochemical changes reflect changes in hepatic cell morphology. In rats, PFOA is a potent inducer of hepatic cytochrome P450, cytochrome P450 reductase and epoxide hydrolase (Pastoor et al., 1987).

PFOA does not undergo de-fluorination and there is no evidence of phase two metabolism following a single dose (Ylinen et al., 1989). There is evidence of enterohepatic recirculation—the circulation of bile from the liver to the small intestine and back to the liver—with the primary route of excretion occurring in the urine (Kennedy et al., 2004). There are marked differences in the rate of inter and intra-species PFOA elimination. In rats, differences in clearance rates between females and males were evaluated using ¹⁴C-PFOA intravenously administered. Females excreted almost 100% of the administered dose within 24 hours, whereas males excreted less than 25% in the same time frame (Johnson et

al., 1979). Male rats appear to be more sensitive to PFOA presumably due to the long half-life compared to the half-life in female rats; with half-lives of hours for females compared to days for males (Kennedy et al., 1986). The rapid excretion found in female rats is due to an estrogen-dependent active renal tubular secretion mechanism of the organic ion transport system (Andersen et al., 2006). This secretion system may be hormonally controlled since castrated male rats treated with estradiol have similar excretion rates as female rats (Ylinen et al., 1989).

The biological half-life of serum-PFOA has been established for several species including rats, rabbits, mice, dogs and monkeys. Rats have been studied extensively and as previously stated, the half-lives range from 5-10 days and 5-7 hours for males and females, respectively (Ohmori et al, 2003; and Vanden Heuval et al., 1991). The half-life in male and female rabbits was 5-7 hours after a single dose of 20 mg/kg; and the serum half-life of PFOA in male and female mice was 12 and 20 days, respectively. The half-life in male and female dogs was three weeks and one week, respectively after an intravenous dose of 30 mg/kg (Hanhijarvi et al., 1988). In a six-month oral dose study of cynomolgous monkeys, the half-life of PFOA in sera was reported to be approximately 20 days for males and 30 days for females (Butenhoff et al., 2002).

Human half-life estimates are on the order of three to five years based on retired occupationally exposed workers (Olsen et al., 2007a). Humans have a decreased ability to excrete PFOA compared to animals, which suggests the potential for ongoing low-level toxicity. Traces of PFOA from production workers were found to remain at detectable levels long after cessation from work

place exposures (Ubel et al., 1980). The difference in half-lives between animals and humans is dramatic, and there appear not be significant sex differences in excretion rates for humans—unlike many other animals. The closest animal model to humans (non-human primates) shows a half-life range of several weeks for monkeys compared to several years for humans. When assessing risk to humans, scientists may be underestimating the long-term health-related impacts of APFO exposure.

Toxic Effects

There is a large database of information related to PFOA toxicity. It includes carcinogenicity, reproductive toxicity, developmental toxicity, immunotoxicity, hepatotoxicity, and mode of action studies. The following information highlights data specific to these toxicities and provides study details that elaborate on the previous overview of APFO/PFOA toxicity:

Carcinogenicity

PFOA has been found to act as a cancer promoter of liver tumors from a rat initiation-selection-promotion bioassay (Nilsson et al., 1991). Investigators from two separate two-year feeding studies of rats evaluated APFO and its carcinogenic potential. Biegel et al. (2001) administered a maximum dose of 300 ppm to the test animals. APFO was found to increase the incidence of benign hepatocellular adenomas, testicular Leydig cells, and pancreatic acinar-cell tumors at 300 ppm. In a two year feeding study of female rats, an increased number of mammary fibroadenomas was observed in the PFOA-fed rats compared to the control group (Sibinski, 1987).

Tilton et al. (2008) examined gene expression in an *in-vivo* trout cancer model to evaluate the potential of PFOA as a carcinogen in the absence of peroxisome proliferation—which may be more relevant to human health compared to other animal models due to the mode of action. This chronic feeding study demonstrated that at a dose of 50 mg/kg/day, PFOA was a cancer promoter of the liver. The tumor promotion was correlated with estrogenic signaling in trout, which was independent of the peroxisome proliferation mechanism of toxicity.

Reproductive Toxicity

Butenhoff et al. (2004) studied male and female Sprague-Dawley rats given 0, 1, 10, and 30 mg/kg/day of APFO orally. This was a two-generation study with the first generation dosed prior to mating, and their offspring dosed after weaning. No adverse effects were observed in mating, fertility or natural delivery in either generation. However, sexual maturation was delayed for the highest dosed group in the second generation.

Developmental Effects

Midasch et al. (2007) showed that PFOA crosses the placental barrier after analyzing human maternal and cord blood samples taken from eleven mother-infant pairs. Similar effects of PFOA readily crossing the placenta in addition to being detected in breast milk have been found in Sprague-Dawley rats (Fei, 2008).

The toxic effects of fetal development have been studied in rats and rabbits for both inhalation and oral exposures to APFO. Pregnant rats were

exposed to five dose-levels of APFO ranging from 0.14 -21 ppm. Mean fetal body weight decreased for the fetuses of the surviving dams exposed to 9.9 ppm and higher concentrations of APFO (Staples et al., 1984). In another developmental study, rabbits were given oral doses of 1.5, 5 or 50 mg/kg from gestation day six through day 18. No evidence of fetus abnormalities was seen—even at the highest dose group where the dams showed a reduction in maternal weight gain (Gortner, 1982).

Immunotoxicity

In a feeding study of monkeys, both lymphoid tissue and bone marrow were found to be sites of histopathology. The monkeys in the highest and second highest dosed groups had moderate hypocellularity of the bone marrow and atrophy of lymphoid follicles in the spleen and lymph nodes (Griffith and Long, 1980). Yang et al. (2001) conducted a dietary study of male mice for ten days. As previously stated, there were observed decreases in the thymus and spleen weights, and an increase in liver weight. These effects were dose dependent. In addition to these findings, PFOA was found to be immunosuppressive in the mice. Another study confirmed these findings and indicated that the toxic effects were related to the suppression of the immune mediated inflammatory response (Guruge et al., 2006). Fairley et al. (2007) examined the effects of dermal exposure in mice and found an increased Immunoglobulin E (IgE) response. These results suggest that PFOA may be immunotoxic and that it may augment the IgE response to allergens.

Hepatotoxicity

Griffith and Long (1980) studied male and female rats. In this dietary study, there were statistically significant differences seen between the control group and the APFO fed groups. The mean female and male liver weights, in the 300 ppm and 30 ppm groups, were greater than the controls. In a 90-day dietary study of rats, doses as low as 100 ppm (5mg/kg/day) for males and 1000 ppm (76.5 mg/kg/day) for females, showed a significant increase in liver weight and hepatocellular hypertrophy (Goldenthal, 1978). Palazzolo (1993) studied male rats fed *ad libitum* a diet containing 1, 10, 30, and 100 ppm of APFO. Mean body weight gains were significantly lower for the 100 ppm group compared to the control group. Hepatic palmitoyl-CoA oxidase activity was significantly higher at the 30 and 100 ppm groups when compared to the control group. A lowest observed adverse effect level (LOAEL) was observed for the 10 ppm group.

Mode of Action: PPAR α activation and lipid metabolism

PFOA has been observed to activate the PPAR α receptor, which acts as peroxisome proliferator (PP) in rodents. PPARs are a structurally diverse group of nongenotoxic carcinogens (e.g., Trichloroethylene, is a solvent and a PP). They belong to a family of nuclear hormone receptors. These are ligand-activated transcription factors that play a large role in lipid metabolism (Michalik et al., 2006). Prolonged exposure to PPs has been shown to increase the incidence of liver tumors in rats (Abdellatif et al., 1991). The increase in liver tumors is thought to be from sustained oxidative stress and the regulation of cell proliferation and differentiation. The effects of PFOA on PPAR α -null mice were

studied showing hepatomegaly—which suggested that PFOA might cause liver toxicity independently of PP (Yang et al, 2002).

Cook J. et al. (1991) conducted a set of experiments in rats. The results showed that decreased testosterone levels were associated with PFOA treated rats, which resulted from altered steroidogenesis in the Leydig cell.

In rats and monkeys, PFOA has been shown to induce mitochondrial proliferation. These effects may be associated with oxygen stress and apoptosis, as PFOA disrupts the inner membrane which affects respiratory functions (Keller et al., 1992 and Strakov and Wallace, 2002).

The mechanism of action of PFCs has been reported as mediated by the cell membranes. PFOA has been suggested to alter cell membrane function through changes in fatty acid composition and oxidation status (Olson and Andersen, 1983). PFOA has the ability to interfere with fatty acid metabolism and cholesterol synthesis in the liver (Haughom and Spydevold, 1992). The serum cholesterol levels in the rats tested decreased by 50-70% after the initial 24 hours of dosing. Conclusions from this study indicate that the hypo-lipidemic effect was from the reduced synthesis of esterification of cholesterol in conjunction with the enhanced oxidation of the liver fatty acids. In a study of workers, hormone levels were examined along with concentrations of PFOA (Olsen et al., 1998). Serum cholesterol and triglycerides were positively correlated with blood serum-PFOA levels. This is inconsistent with the animal data showing that increased levels of PFOA are related to decreased lipid concentration.

Many of the results observed in the toxicological studies may not relate directly to humans, in particular the mode of action in humans is unknown. Animal toxicity is observed at relatively high doses of APFO/PFOA compared to what the general population is exposed to from the environment. Therefore the body burden is much lower outside the laboratory. However, the length of time for the clearance of PFOA in humans is almost three-fold greater when compared to other animals. The absolute toxic properties and subsequent health effects on humans remain uncertain. What may help clarify this uncertainty and make for a more justified extrapolation for the general population is to evaluate the varying levels of occupational exposure and the health outcomes seen in the current 3M study population using a quantitative exposure assessment.

Preliminary Epidemiologic Studies

U of MN: Cancer Mortality Among 3M, PFOS Workers in Decatur, Alabama

PFOA is related to a synthetic perfluorinated chemical, perfluorooctanesulphony fluoride (PFOS), which was manufactured at another 3M location in previous decades (Alexander et al., 2003). Study investigators conducted a retrospective cancer mortality study based on three levels of exposure to PFOS. The findings showed a potential association between exposure and adverse health outcomes—specific to bladder cancer. This was based on only three bladder cancer cases and the authors stated the possibility of a chance finding could not be ruled out.

U of MN: Initial Mortality Study of Workers Employed at the 3M Cottage Grove Facility

A retrospective mortality study was conducted with employees at the 3M Cottage Grove plant by investigators at the University of Minnesota (Gilliland and Mandel, 1993). This was the first study to look at workers' exposures to APFO and evaluate any potential health outcomes associated with exposure. This cohort of workers included 2,788 males and 749 females who were employed for a minimum of six months between the years 1947-1984. The employees were placed into two groups; the first group consisted of workers in the Chemical Division, and the second group consisted of employees who never worked in the Chemical Division. Vital status was ascertained and death certificates were obtained from the National Death Index (NDI). The records were searched for the contributing cause of death. Estimates of APFO exposure were based on job history and worker status of ever versus never employed in the Chemical Division. The total time spent working in the Chemical Division provided the cumulative exposure metric for analysis. Standardized mortality ratios were adjusted for covariates and compared to the state of MN and US general populations. Age at first employment was positively, but not statistically, associated with prostate cancer death. The statistically significant observed health outcome was for employees who worked in the Chemical Division. They had an increased risk of dying from prostate cancer when compared to the MN population. Men had a three-fold increase of prostate cancer with ten years of employment in the Chemical Division. The authors were cautious in any over interpretation of the outcome based on the small number of cancers, but suggested

that if the excess in mortality from prostate cancer was related to APFO that it may be through endocrine alterations.

Gilliland and Mandel (1996) studied 115 occupationally exposed workers in a cross-sectional study design to evaluate cholesterol, hepatic enzymes and lipoproteins in association to APFO exposure. This was an ancillary study from their primary mortality study conducted to evaluate a sample of the entire worker population previously described. The researchers observed a positive association for increased serum-PFOA levels with an increase in liver enzymes and hepatocyte necrosis, suggesting irreversible cell damage. Serum-PFOA was positively associated with estradiol and thyroid stimulating hormone, while it was negatively associated with free testosterone. The authors stated that these results suggest that PFOA affects male reproductive hormones.

U of MN: Follow-up Mortality Study of Workers Employed at the 3M Cottage Grove Facility

A study was conducted as a follow-up to the initial mortality study to assess the death experience of employees at an ammonium perfluorooctanoate production facility (Alexander, 2001; and Lundin et al., 2008). The purpose of this study was to determine if occupational exposures to APFO were related to the workers' mortality experience. The cohort was enumerated starting in 1947 through the end of 1997. There were 3,993 eligible workers who met the minimum criteria, which was one year of employment. Data records were abstracted from 3M and exposure to APFO was calculated based on job titles that were independently reviewed by a panel of experts from 3M (including veteran workers and industrial hygienists). The job titles recorded for each worker were

static—meaning the analysis was based on only one job title per employee. Three categories of exposure were evaluated in this cohort (definite, probable, and none). The death experience of this occupational group was determined through 2002 and standardized mortality ratios (SMRs) were estimated for all cause and cause-specific deaths. The referent group for the SMRs was the state of Minnesota population. There were 807 deaths reported and the SMRs for all causes of death and for cancer mortality were 0.8 (95% CI 0.7-0.9) and 0.9 (95% CI 0.8-1.0), respectively. Also, analyses included a Cox proportional hazard regression model to estimate cancer risk with an internal referent population. Workers who were categorized as part of the high or moderate exposure group, compared to the low exposure group, had an elevated risk of death from prostate cancer (HR=7.0, 95% CI 1.4-18.6 and HR=3.0, 9.5% CI 0.8 -11.0, respectively). The results indicated no association between APFO exposure and cancer when using the general population as the reference group; however, there were findings associated with an elevated risk for prostate cancer death for both definite and probable exposure groups when compared to an internal non-exposed group.

U of MN: Cancer Mortality Among 3M, Oil, Chemical and Atomic Workers Union, Local 6-75

A retrospective mortality study was conducted by the University of Minnesota's School of Public Health. It included workers from the 3M oil, chemical, and atomic workers union at the St. Paul location from 1947 through the end of 1992. The employees were divided into six job groups based on department numbers and there were five activity classifications included in each individual's record. Vital status was determined through the National Death

Index (NDI) and also from drivers' licenses records, MN and WI death certificates and active tracing to determine residential status of the workers. Cause and date of death were recorded for all individuals. The analysis of this mortality study included 3,995 men and 564 women and SMRs were calculated based on the state of MN and the seven county metropolitan area populations. The main findings included an elevated risk of stomach cancer in female employees and large intestinal cancer in male production tape employees.

Summary

Overall, many studies—both animal and occupational studies—have been conducted in order to improve the level of understanding regarding the health effects of APFO/PFOA related to human health. The ACGIH rates APFO as a confirmed animal carcinogen, with unknown relevance to human health. Carcinogenic endpoints observed in animal studies that may impact human health include: cancers of the liver, pancreas, testicles, breast, and thyroid gland (Butenoff et al., 2004, and Biegel et al. 2001). In the aforementioned occupational mortality studies of APFO exposure have shown an increase in risk of death from prostate cancer and bladder cancer, albeit with few workers (Lundin et al., 2008; and Alexander et al., 2003).

Globally, it remains that production is ongoing, exposure is wide-spread and the human health endpoints related to various levels of exposure are uncertain. In this research, our aim was to assess the levels of exposure in our working cohort over time in relation to their mortality experience in order to better understand the long-term potential health impact of the chemical on human health.

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CHAPTER 3: RESEARCH DESIGN AND METHODS

Specific Aims

The over-arching goal for this area of research was to evaluate health outcomes from exposure to ammonium perfluorooctanoate (APFO) and perfluorooctanoic acid (PFOA). Specifically, the purpose of this study was to examine the relationship between occupational exposure to APFO/PFOA and the risk of death from six *a priori* selected cancers; bladder, breast, kidney, liver, pancreatic and prostate. To achieve this goal, our plan was to evaluate cancer mortality from an occupational cohort with employees who had a broad range of APFO/PFOA exposure estimates spanning more than fifty years. The exposure estimates were completed by conducting a historical exposure reconstruction, using air measurements and calculating daily inhalation values for workers at an APFO/PFOA manufacturing plant. The exposure values assigned to each worker were based on their job title, location and date of employment. To ascertain the risk of cancer mortality we explored the relationship between the time-dependent cumulative estimates of APFO/PFOA exposure by linking individuals to death records and their mortality experience. This was completed using a retrospective occupational cohort consisting of two 3M locations. The workers completed a minimum of one year of work from their start date through the end of 2008. The following *specific aims* were addressed in pursuit of our long-term study goals:

1. We established a complete occupational cohort of all eligible employees from two 3M locations from 1947-2008; the Cottage Grove facility (APFO/PFOA exposed workers) and the St. Paul plant (non-

occupationally exposed workers). To meet the eligibility criteria the employees must have worked for a minimum of one calendar year as of December 31st, 2008.

2. We developed a work history profile for each eligible employee to include specific job titles and department combinations in addition to all job start and end dates for each unique combination. This was carried out by updating and standardizing job titles, building on a previously enumerated work history data set of Cottage Grove workers, which ended in 1997.
3. We ascertained updated mortality records from the National Death Index (NDI) for all eligible workers to record cause and date of death through the end of 2008.
4. We constructed an APFO/PFOA-exposure data matrix (EDM) for the period of 1947-2008 (starting when APFO was first synthesized) based on air sampling data, and professional input for all job titles by year of exposure at the 3M Cottage Grove facility. An example of an EDM for a few workers is provided (Figure 1).
5. We developed a time-dependent exposure profile for each Cottage Grove worker in the study population based on exposure estimates from the EDM linked to job title, dates worked per job title and department location. The EDM was used to create each individual's unique exposure profile based on the inhalation in mg/m³ of APFO/PFOA. Examples of workers are provided (Figure 2).

6. We calculated two annual estimates of exposure to APFO/PFOA based on the sum of exposure and the biologically effective dose for each worker in milligrams (mg) of exposure and placed workers into categories of exposure. Two exposure models were used. The first model was a standard cumulative exposure model and the second was a clearance weighted cumulative model—used to account for the elimination of serum-PFOA in addition to exposure over time (Figure 3).
7. We evaluated cancer mortality risk based on cumulative exposure, during two distinct decades, and exposure intensity. Both time-dependent dose estimates were evaluated (i.e., the cumulative and the clearance weighted estimates in mg).
8. We determined the association between the estimated APFO/PFOA exposures and risk of death from cancer for bladder, breast, kidney, liver, pancreatic, and prostate *a priori* specific cancers. We evaluated uncertainty by the re-parameterization of exposure groupings.

The three central *hypotheses* for this study to address the specific research goals included:

1. Occupational exposure to APFO/PFOA is associated with an increased risk of cancer; specifically bladder, breast, kidney, liver, pancreatic, prostate, and all-cause cancers.
2. Risk of cancer is associated with age at exposure to APFO/PFOA.

3. Risk of cancer is associated with the intensity of exposure to APFO/PFOA.

Collaboration

This study builds on previous work conducted by the Division Of Environmental Health within the School of Public Health at the University of Minnesota in collaboration with the 3M Company of Minnesota. 3M personnel provided historical employment records, information on the manufacture of Ammonium Perfluorooctanoate and the jobs that were involved in its production and handling. They also provided historical air monitoring data. This collaboration served the primary goal of this research, which was to evaluate the health of APFO/PFOA exposed workers by utilizing quantitative dose estimates with mortality data.

Research Overview

For more than half a century 3M workers manufactured, packaged and shipped APFO to various locations. These workers had the opportunity for a wide range of exposures—depending on job location and job tasks—which varied over time. The effects of APFO/PFOA exposure on human health were studied in the employees of the Cottage Grove, 3M facility using a retrospective cohort design. Previously, two populations of 3M employees from the Cottage Grove and St. Paul Minnesota plants were enumerated by University of Minnesota researchers in the Division of Environmental Health Sciences, School of Public Health. The work history of these two groups was combined and updated with eleven

additional years (1998-2008) of employment information to create one large cohort. This cohort includes workers with and without occupational exposure to APFO/PFOA. Workers at the St. Paul plant had no direct exposure to APFO/PFOA and the Cottage Grove workers had a wide range of annual exposure estimates. More than half of the workers at the Cottage Grove facility were exposed to APFO/PFOA at some point during their work history by means of direct or indirect exposure. The remaining workers had estimates of only background exposure. All workers were placed into exposure groups after completing our comprehensive exposure reconstruction and based on the individual's exposure profile created from linking their job history to exposure estimates from a newly created exposure data matrix.

All cause and cause-specific cancer mortality rates were compared among the referent and the exposure groups within the working population. Additionally, the workers' continuous exposure estimates were evaluated to assess the risk of a cancer death. Standardized mortality ratios (SMRs) were calculated for the cohort using the Minnesota state population reference rates. The hazard rates for the APFO/PFOA exposure groups were compared to the referent group—St. Paul workers. The hazard ratios (HR) and odds ratios (OR) along with the 95% confidence intervals (CI) were calculated using time-dependent Cox proportional hazard ratios and logistic regression models (Breslow and Day, 1987). The Cox regression analyses were completed using the PHREG procedure in SAS 9.2 (SAS, 2011). The HR and OR were stratified by gender and adjusted for first year of employment.

The final cohort included 9,027 current and former 3M workers whom had up to 59 years of follow-up time. Cancer rates for all-cause cancers in addition to bladder, breast, kidney, liver, pancreatic and prostate cancer were ascertained and the mortality rates of the exposed groups were compared to the St. Paul workers.

Study Population

We included all workers with a minimum of one year of employment at the 3M Cottage Grove and St. Paul locations beginning in 1947 through the end of follow-up, either date of death or December 31st, 2008 when the study ended. There were a total 4,668 workers from Cottage Grove and 4,359 workers from the St. Paul plant who met our minimum eligibility requirement. Worker's mean age at first and last employment was 30 and 44 years, and 29 and 49 years for St. Paul and Cottage Grove plants, respectively. Females represented only 12% of the St. Paul group compared to 20% of Cottage Grove workers. St. Paul workers on average were employed on average six additional years, 20 years, compared to the average number of years, 14 years, for all Cottage Grove employees. There were a total of 323,456 person-years for both locations.

The Cottage Grove campus was divided into the Chemical and Non-Chemical Divisions, with APFO/PFOA production limited to the Chemical Division. The workers were initially categorized in either the Chemical or Non-Chemical Division based on the department they worked in—some worked in both Divisions. There were a total of 121 departments at the Cottage Grove facility and 23 were part of the Chemical Division. More than half of all Cottage Grove workers held a minimum of one job in the Chemical Division at some point

during their work history. More males were represented in the Chemical Division (87%) compared to the workers in the Non-Chemical Division (72%). Workers who only worked in the Chemical Division, on average, worked fewer years compared to all Cottage Grove workers, 11 and 15 years respectively.

We did not have data on race or ethnicity; however, from personal correspondence/ retired employees (and as reported in the most recent mortality analyses by Lundin et. al) the population at both locations was predominately Cuacasion—which is consistent with state and county level data reported by the US Census Bureau (US Census Bureau; Quick Fact Sheet, MN).

Data Collection

The human resource records were evaluated for work history information. Data from all workers with more than 365 days of cumulative employment at the Cottage Grove and St. Paul locations were abstracted. For each employee, all unique combinations of job title, job department and dates of employment were recorded. Job titles were standardized based on professional input after a thorough assessment of job responsibilities per job title.

The exposure data were obtained from more than 800 air measurements. Expert judgment was provided regarding the amount of time spent working with APFO/PFOA on a daily basis by specific job title, location and year of employment. The final exposure values for the exposure data matrix (EDM) were calculated using the amount of time spent working with APFO/PFOA in conjunction with the air measurements to provide daily time-weighted averages

(TWAs). These TWAs were summed for each employee to provide a unique exposure history profile.

Date and cause of death were recorded from national data for all deceased cohort members using the National Death Index (NDI). The cause of death for each person was coded by a certified nosologist using the International Classification of Disease (ICD) revision codes in effect at the time of death—the most current being the 10th revision.

Data Analysis

The goal of this research was to evaluate the risk of death from cancer as a result of occupational exposure to APFO/PFOA. The outcomes of interest included cancers of the bladder, breast, kidney, liver, pancreas and prostate within the 3M worker population at the Cottage Grove and St. Paul, Minnesota locations. We attempted to control for confounding with the aid of a causal model (Figure 4). The following three hypotheses were addressed:

Hypothesis 1: Occupational exposure to APFO/PFOA is associated with an increased risk of cancer mortality; specifically bladder, breast, kidney, liver, pancreatic, prostate cancers.

To evaluate the risk of death based on the worker's exposure level we calculated the amount of person-time for each worker and estimated the hazard ratio using the time-dependent exposure estimates from two exposure models. Specifically, we modeled the relationship between the level of exposure of APFO/PFOA and the occurrence of a cancer death event using a time-dependent Cox proportional hazard regression model. The hazard ratios (HRs) and 95%

confidence intervals (CIs) were estimated by comparing the rates between the exposure groups (using both estimates of exposure from the two models independently) for the Cottage Grove workers and the St. Paul workers as the non-exposed referent group. To account for cancer latency, we lagged the models by ten years. In addition, we calculated the standard mortality ratios (SMRs) using the state of Minnesota death rates comparing both the Cottage Grove workers and the St. Paul works to the state population. We controlled for year of entry into the cohort.

Hypothesis 2: Risk of a cancer death is associated with age at exposure to APFO/PFOA.

We calculated the risk of a cancer death based on the cumulative and clearance weighted exposure estimates during two decades. This included estimates for the fourth and fifth decades of life. We modeled the relationship between the level of exposure and the cancer events using a Cox PH regression model and the hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated while controlling for year of entry into the cohort.

Hypothesis 3: Risk of a cancer death is associated with the intensity of exposure to APFO/PFOA.

To evaluate workers' risk of death based on differences in their intensity of exposure (i.e., how quickly they reached a relatively low, medium, or high exposure estimate) we used *dose-rate* parameters as a measure of intensity. We evaluated cancer death for each *dose-rate* group compared to the referent group, stratified by “total years worked” categories. We calculated the odds ratios (ORs)

and 95% confidence intervals (CIs) to estimate the probability of death from cancer based on the dose-rate level using a logistic regression model, controlling for year of entry into the cohort and gender.

Sensitivity Analysis

To account for the effects on the direction and magnitude of our risk estimates from bias and potential confounding, we evaluated risk after re-parameterizing the exposures for the workers using various exposure classifications. We explored workers risk of death by the redistribution of exposure groups. We estimated the hazard ratios (HRs) using the time-dependent Cox proportional hazard models. Finally, we explored workers who had similar final cumulative estimates of exposure and evaluated their risk of death after placing these workers in dose-rate groupings. This was explained in the aforementioned intensity-exposure and risk of death hypothesis.

Bias Evaluation

Information Bias

To ensure the validity of any epidemiological study—including occupational studies—it is important to account for potential systematic errors in the assignment of exposure and health outcomes. The data for the EDM that we reconstructed was a rigorous process of utilizing available air measurements of APFO and PFOA in addition to the professional input of former 3M employees who knew the production process and exposure scenarios. A structured evaluation of the amount of time spent working directly and indirectly with

APFO/PFOA was a key determinant to calculate the exposure estimates for all employees in order to decrease or prevent bias. The assignment of exposure for each worker was based on their unique work history and the sum of the daily TWAs for their profile. This was all conducted in order to reduce the potential for exposure misclassification and the potential for information bias.

The chance of information bias from the health outcomes—specifically, cancer deaths—is minimal due to our data collection of outcomes recorded using the NDI data. Vital status is recorded through the national database and records provide the cause and date of death verified by certified nosologists with the ICD codes from the revision in effect at the time of death.

Selection Bias

In the current study selection bias was not of primary concern. This is due to the fact that we compared the exposed population (Cottage Grove workers) to a cohort of 3M workers with a similar socioeconomic status (SES). To the best of our knowledge, the St. Paul workers were similar to the Cottage Grove workers in every way except with regards to the exposure of interest. This may be most relevant when comparing the Chemical Division workers to St. Paul workers—as both populations were production workers (albeit working with distinctly different chemicals). This is a key component to reducing selection bias.

Human Subjects' Protection

All work performed was approved by the University of Minnesota's Institutional Review Board (IRB). This was a follow-up cancer mortality study

with minimal or no risk to the subjects. The IRB 3M Cancer Study was #0809S47221.

As stated, potential risks were negligible and the work history records with personal identifying information were kept in encrypted files on a secure password protected computer server. Each participant was assigned a unique study identification number and the personal identifying information was kept separate from other study records. Access to this server was limited to specific internet provider addresses.

Summary

Results from two previous mortality studies of 3M Cottage Grove workers showed a higher than expected rate of prostate cancer deaths in employees with exposure to APFO compared to the employees with no known exposure. In addition to this observed outcome, several animal studies have documented adverse health outcomes of exposure to APFO/PFOA including an increase in cancer of the liver, Leydig cells, and pancreatic acinar cells. Other toxic endpoints from animal studies have shown an increase in liver and thymus weights, altered lipid metabolism, decreases in body weight, and endocrine effects. This research focused on understanding the potential health effects of occupational exposure to APFO/PFOA by using the workers' updated exposure profiles, derived from an in-depth exposure assessment, and evaluating the risk of a cancer death from six *a-priori* and other health outcomes based on the time-dependent exposure estimates of the workers.

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Figures and Tables

Figure 1. Exposure Data Matrix: Daily TWAs in mg/m³ of APFO/PFOA Exposure by Job Title, Department, and Year

DEPT	YEAR	Administrative					Process		
		Assistant	Analyst	Chemist	Custodian	Engineer	Manager	Engineer	Supervisor
MMM	1954	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
MMM	1955	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
MMM	1956	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
MMM	1957	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001	0.00001
MMM	1958	0.000015	0.000015	0.000015	0.000015	0.000015	0.000015	0.000015	0.000015
MMM	1959	0.000015	0.000015	0.000015	0.000015	0.000015	0.000015	0.000015	0.000015
MMM	1960	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002
MMM	1961	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002	0.00002
MMM	1962	0.000025	0.000025	0.000025	0.000025	0.000025	0.000025	0.000025	0.000025
MMM	1963	0.000025	0.000025	0.000025	0.000025	0.000025	0.000025	0.000025	0.000025
MMM	1964	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003
MMM	1965	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003
MMM	1966	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003
MMM	1967	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003	0.00003
MMM	1968	0.00004	0.00004	0.00004	0.00004	0.00004	0.00004	0.00004	0.00004
MMM	1969	0.00004	0.00004	0.00004	0.00004	0.00004	0.00004	0.00004	0.00004
MMM	1970	0.00004	0.00004	0.00004	0.00004	0.00004	0.00004	0.00004	0.00004
MMM	1971	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005
MMM	1972	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005
MMM	1973	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005
MMM	1974	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005
MMM	1975	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005
MMM	1976	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005	0.00005

**Figure 2. Chemical Division Workers with Low-Level Exposure
Groups: Two Worker's Exposure Profiles**

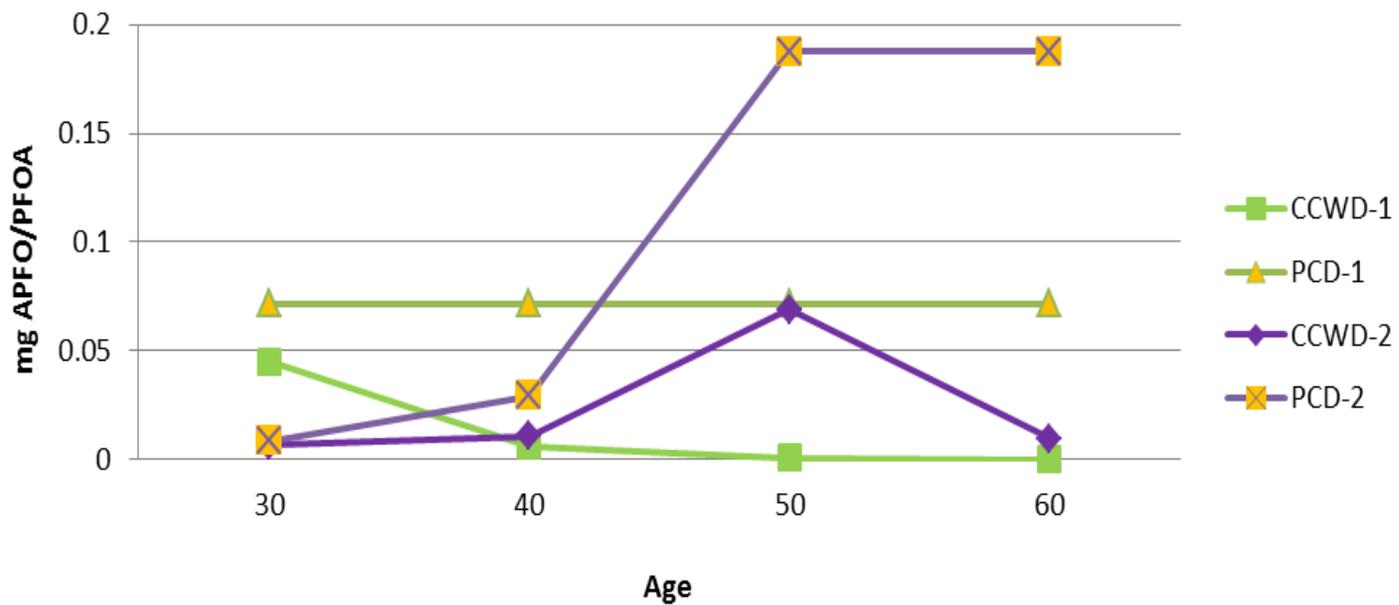


Figure 3. APFO Exposure Equations: Cumulative and Clearance-Weighted

In the equations below the estimated dose is in milligrams (mg), j refers to the specific year of exposure, n is number of years worked, i is the i^{th} worker, and k is the elimination constant, and is equal to $\ln 2/T_{1/2}$.

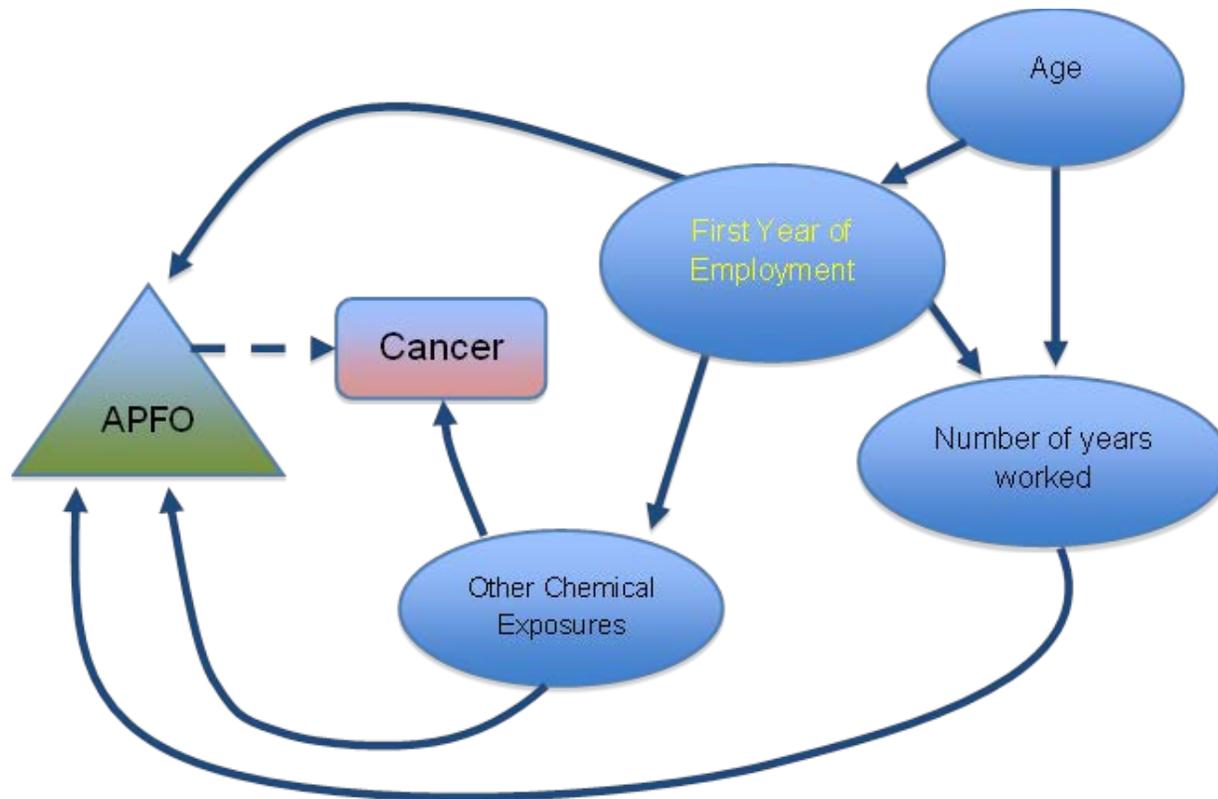
Potential Cumulative Dose (PCD) estimate of body burden in mg:

$$PCD_i = \sum_{j=1}^n E_{ij}$$

Cumulative Clearance Weighted Dose (CCWD) estimate of body burden in mg:

$$CCWD_i = \sum_{j=1}^n \{ >(CCWD_{j-1}) \text{ or } (E_{ij}) * (e^{-k}) \}$$

Figure 4. Directed Acyclic Graph: Prostate Cancer



CHAPTER 4: OCCUPATIONAL EXPOSURE TO AMMONIUM PERFLUOROCTANOATE: AN INHALATION EXPOSURE RECONSTRUCTION EVALUATING TWO TIME DEPENDENT MODELS

Workers at a chemical production facility in Cottage Grove, Minnesota manufactured ammonium perfluorooctanoate (APFO) for over fifty years. Employees who were directly involved in the development of APFO had potential for high-level exposure compared to non-production employees. Other Chemical Division and Non-Chemical workers had potential for indirect, lower exposures to APFO. The overarching goal for this paper was to reconstruct the APFO exposure history for all eligible employees and estimate each worker's exposure over time. Specifically, the purpose was three-fold: To demonstrate the methods used to build a facility-wide exposure data matrix; to display the range of exposures over time; and to explore exposure misclassification. A primary limitation of some occupational studies is the choice of exposure model to reflect a time-dependent biological dose that represents true body burden. To address this limitation we calculated a daily time weighted average (TWA) of APFO in milligrams per cubic meter (mg/m^3) using historical air measurements for each worker catalogued by job title, location and year of employment. Next, we used two exposure models to estimate time-dependent dose in milligrams (mg)—a standard cumulative model that describes the potential cumulative dose (PCD), and a cumulative clearance weighted dose (CCWD) model to account for retention and elimination of serum-PFOA. We placed workers into one of six exposure groups using orders of magnitude to define the groups. The daily TWA

of inhalation exposure for all workers in the Non-Chemical Division ranged from 1×10^{-8} to 1×10^{-5} mg/m³. In the Chemical Division the range was up to five orders of magnitude greater. For production workers within the Chemical Division, the daily exposure ranged from 0.001 mg/m³ to 0.379 mg/m³. Overall, when comparing the two annual estimates we found workers who were most susceptible to potential misclassification were individuals assigned to the middle to high-dose groups and in their later years of life. The exposure models and annual estimates provide options to examine specific assumptions about time related exposures, chemical retention and elimination, and disease onset.

INTRODUCTION

Perfluorooctanoic acid (PFOA), an eight chain fully fluorinated carboxylic acid, is a synthetic chemical surfactant with broad commercial and industrial application. Its primary salt, ammonium perfluorooctanoate (APFO), was manufactured via electrochemical fluorination for more than fifty years at a 3M Cottage Grove facility in Minnesota. Employees who worked in chemical production had potential for significant exposure to both PFOA and APFO primarily through inhalation. Two mortality studies of this manufacturing cohort have been published in peer-reviewed literature. The original retrospective cohort included 3,537 workers who were employed for a minimum of six months between 1947 and 1983. Exposure categories were based on work history records of ever or never working in the Chemical Division of the plant (Gilliland and Mandel, 1993). Lundin et al. updated the employment and mortality experience through 1997 to include a total of 3,993 workers. The investigators classified

exposure by three categories based on the worker's job title and department combination: 1) definite exposure, 2) probable exposure, and 3) non-exposed (Lundin et al., 2009). These classifications were combined with duration of employment for a weighted cumulative exposure. Both studies reported an association between increasing exposure and death from prostate cancer, but the findings were dependent on the choice of referent population and statistically unstable. A noted weakness in these studies was the limited nature of the exposure model.

Historical exposure reconstruction, accounting for timing and intensity of exposure, is a critical step to understanding how exposure is related to long-term health outcomes, including cancer (Nieuwenhuijsen et al., 2006). Occupational studies frequently rely on work history information to evaluate cumulative exposure, but less frequently evaluate time varying exposures related to health outcomes. When analyzing the amount of chemical exposure and the likelihood of it being causal for a specific health outcome, it is essential to determine time-varying dose estimates. Workers may often have a similar cumulative exposure estimate at the end of their career, but may have a different risk of disease because of variable exposure scenarios over time. Their real body burden may be affected by variables such as intensity of the exposure, time since last exposure and chemical retention and elimination from the body—all factors that are not always part of a standard exposure assessment. In the most recent 3M mortality study, Lundin et al. (2009) stated that the use of duration of employment, instead of the range of exposure estimates that vary with calendar time, may have potentially

contributed to some exposure misclassification. It is possible that some workers who had job titles in the “probable” exposure category had short but intense exposures. These workers, therefore, may have had a body burden similar to workers in the “definite” exposure group who had sustained, low dose exposures. The exposure categories captured a single job title, whereas many workers switched job titles—and therefore different levels of exposure—over time. The current study captures exposure using quantitative estimates of exposure based on job title changes throughout each employee’s career.

As part of an ongoing cancer incidence study and mortality update, the goal of this research was to develop a quantitative exposure metric that closely reflects body burden. The exposure models will estimate cumulative and time-varying exposure to APFO in this cohort.

METHODS

This section describes our approach to: 1) estimate the daily exposure for each worker using air measurements, production records, and professional input; 2) develop exposure data matrices (EDMs) for each department in the Chemical Division based on job title, job location and year; 3) calculate quantitative exposure profiles for each worker in our cohort by linking work history records and the EDMs; and 4) display and evaluate two time dependent exposure profiles at the worker and cohort level.

Study Population

The cohort included all workers employed at the Cottage Grove 3M plant for a minimum of one year of employment between 1947 through 2008

(N=4,668). The Cottage Grove campus was divided into Chemical and Non-Chemical Divisions, with APFO production limited to the Chemical Division. Within the Chemical Division a few departments were directly involved with the production of APFO and these changed over time. Other departments may have had some involvement with APFO, but were not the main production sites. The APFO chemical group locations were verified by reviewing company production records and with input from former employees.

Production Process

The production of APFO was a multi-step process that included many tasks with various opportunities for workers to be exposed. Inhalation exposure occurred from both the acid vapor and ammonium salt particulate phase during regular production duties and other less frequent responsibilities such as cleaning equipment, changing filters, and quality control checks. Production workers had the potential for high-level exposure during rare events such as incidental spills, filter clogs and dust releases. Low-level continuous exposure to APFO occurred from working in the general production environment without direct involvement in chemical production.

The manufacture of APFO initially began in a small chemistry pilot plant in the late 1940s and expanded to an entire building with four main areas starting in 1951. The production process evolved over time including changes in equipment and volume output. It increased steadily by decade until the 1980s when production fell from approximately 60,000 to 2,000 pounds per year. In the 1990s there was sharp increase until the end of production in 2002 (Table 1).

The production process included the following steps: electrochemical fluorination, stabilization, fractionation, distillation, purification, the addition of ammonium, drying, and packaging the final product. Electrochemical fluorination (ECF) reactions took place in the Cell Room. The ECF reactions were conducted with the use of electrical currents that replaced all of the hydrogen atoms with fluorine atoms by adding hydrogen fluoride (HF). HF was added to the eight-chain carbon compound inside 1,000 gallon stainless steel cells with encapsulated metal plates. The material was piped through a closed system from the Cell Room to the reactors in the Kettle Room where the perfluorooctanoic acid (PFOA) was fractionated by separating out the eight-chain carbon compound. PFOA was purified after high and low vapor pressure constituents were boiled off from the mixture by charging, distilling, and draining the material. After the acid was purified it was drained into large drums and stored in a hot room at 150 °F. Next, ammonium (anhydrous ammonia) was added to the acid to make a slurry mixture in 50 gallon reactors through 1978, after 1978 this was done in a larger reactor (400 gallons). The slurry was stored in 55 gallon drums or 200 gallon totes. There was potential for exposure during the purifying process of production from occasional leaks and spills. Other opportunities for exposure occurred when the workers replaced the filters, when the metal plates from the cells were cleaned or replaced, and when quality control samples were collected.

Through the end of 1977 the material was dried by a tray dry method. From 1978 until 1981, a variety of drying methods were attempted including a

filter press and oven with a pulverizer method and a Bird Young™ filter/blender-dryer method. After 1981 the inert material was evaporated from the acid using a spray dryer. The ammonium salt was blended, packaged, and finally shipped to various locations. During the drying process there was potential for very high inhalation exposure while spray-drying and packaging the powder. In the 1990s, a curtain barrier was used in the Spray Dry Room to isolate the ammonium salt and reduce contamination. In 1999 a plexi-glass barrier was installed in the Spray Dry Room, and in 2000 the use of full-face respirators was mandated for the production workers.

Work History

Work history records indicating the job department, job title, and start and end dates were used to identify the duration and calendar period of employment. Several thousand job titles were standardized to represent the workers' duties for each position. A total of forty-five unique job titles were identified and used for the Chemical Division from 1951-2002. All job titles for pre and post-production Chemical Division workers, and all workers in the Non-Chemical Division, were standardized by division and year of employment.

Exposure Data

There were a total of 205 personal and 659 area APFO/PFOA (APFO C₈HF₁₅O₂NH₃; and PFOA C₈HF₁₅O₂) air measurements used in the quantitative exposure assessment. Air data collection for APFO/PFOA began in 1977 and ended in 2000 (Table 2). Both PFOA and APFO were sampled depending on the process step and exposure (i.e., vapor or particulate phase). The following

sampling media were used to collect PFOA vapors; Impinger (0.01N NaOH methanol), silica gel tubes, and ethylene glycol coated Tenax tubes through the 1980s. After the 1980s, PFOA was captured using Tenax tubes, silica gel acid tubes and finally OVS-XAD-4 resin tubes. From 1977-1999, APFO was collected with tared 0.8 micrometer pore size Nuclepore filters; with a switch to OVS-XAD-4 resin tubes in 2000. The PFOA anion (PFO⁻) was the measured analytic compound using gravimetric gas chromatography, flame ionization and electron capture analyses.

All the personal air samples were breathing zone samples taken during various exposure tasks including; charging, draining, fractionation, stabilization, changing filters, spray drying, grinding, manual crushing, dumping trays, packaging the material, and cleaning. The area samples were taken in the production room and represented the background exposure value during production and non-production activities. Both personal and area samples were short term, task-based samples—the duration varied from twenty minutes to over two hours, depending on the task. Using the data from the air measurements and professional judgment regarding the amount of time spent at the various exposure tasks performed during a typical shift, we estimated daily inhalation exposure values.

Exposure Values: Daily Time-Weighted Averages

We created an exposure data matrix with annual estimated time weighted average (TWA) exposure values for all jobs held from 1947 through 2008. For Chemical Division workers during production years (1951-2002), we estimated a

daily TWA in mg/m^3 for each year-job title-department combination using the task-based arithmetic mean and duration of task per shift. We calculated close to 3,000 TWAs from 1951 through 2002 to create the EDMs. Exposures for the concurrent year were used when available. Fewer than 20% of the TWAs were computed directly from concurrent year measurements. There were 2,462 imputed TWAs using air measurements from a specific job title and department combination, but different year(s). The imputed TWAs in years without sampling data were calculated by adjusting for production rates—reflected in the amount of time spent conducting an exposure task. The amount of time for an eight hour shift was divided into three parts; 1) time spent outside of the production room (“**Outside Production Room**”), 2) time spent in the production room without directly performing a APFO/PFOA-related exposure task (“**Inside Production Room: No Exposure Task**”), and 3) time spent in the production room conducting APFO/PFOA-related exposure tasks (“**Inside Production Room: Exposure Task**”). For the time spent “Outside Production Room”, we used a constant value of $0.001 \text{ mg}/\text{m}^3$.

The daily TWA exposure in mg/m^3 of air was calculated as:

$$C_j = \frac{\sum_{i=1}^n c_i t_i}{\sum_{i=1}^n t_i} \quad \text{Equation (1)}$$

C_j are the mean concentrations in mg/m^3 of PFO for a given job title for the i^{th} worker, t_i are the amounts of time in minutes and c_i are the air concentrations for each of n distinct work-time areas. A total of 480 minutes were used in the denominator for each calculation representing an eight-hour

work shift. The method for estimating the TWA that incorporates different task-based exposures for the same job are displayed in Tables 3 and 4.

All Non-Chemical Division workers' daily TWAs were estimated using an APFO/PFOA background exposure estimate—taken from facility area and public environmental sampling data. Likewise, Chemical Division workers' pre and post-production (1947-1951, and 2003-2008) daily TWAs were calculated with a similar method as all Non-Chemical workers. Specifically, prior to the start of production, we used a step-wise algorithm to estimate TWAs. Area samples from non-production measurements and from local and regional environmental air data provided by the Minnesota Pollution Control Agency (MPCA) and reported by Stock et al. (2004) were reviewed. Stock et al (2004) measured atmospheric fluorinated telomer alcohols (FTOHs), which degrade in the environment to PFOA, at several locations in North America with a range of concentrations of 1.65×10^{-7} to 1.1×10^{-8} mg/m³. We calculated a daily TWA for all Chemical Division workers by increasing exposure by 50% for each year from 1947-1951 starting with a baseline TWA established with expert input and the review of the aforementioned non-production area measurements and atmospheric data. We assumed the annual increase would be based on a gradual production rate increase, which would reflect background exposure levels. Workers in the Non-Chemical Division were assigned the same initial ambient measurements that were assigned to Chemical Division workers in 1947. These increased by 50% every three years through 1951. From 1952 through 1959 the daily TWA increased by one order of magnitude to account for transient exposures. For the

1960s we increased the TWA by one order of magnitude. The following decades through 2002, we increased exposure once more to reach $1.0 \times 10^{-5} \text{ mg/m}^3$ to account for the change in production rates.

After production ceased in 2002, we continued to assign exposure levels (daily TWAs) for all workers based on their division from the on-site chemical residuals. We decreased the Chemical Division workers' TWAs by 50% annually through 2008. The calculation for the Non-Chemical Division workers' TWAs followed the same method; however the TWAs were one order of magnitude lower than the Chemical Division workers (Table 6).

Exposure Models: Annual Exposure Estimates

Using the APFO/PFOA values from the EDMs linked to the workers' job history files we built two exposure models to estimate annual dose from the TWAs: 1) a standard cumulative exposure model: potential cumulative dose (PCD) and, 2) a clearance weighted exposure model: cumulative clearance weighted dose (CCWD) that accounts for the retention and elimination of PFOA from the body. The first model is a cumulative estimate of the inhaled exposure over time based on the sum of the daily TWAs from the individual's work history. The second model is a first order kinetics model based on the estimated human half-life of serum-PFOA used to predict clearance of PFOA from the body. While not a complete physiologically based pharmacokinetic model, the CCWD model provides an estimate of body burden in periods where exposure is decreased or eliminated. Methods similar to the exposure modeling for the CCWD model have

been previously described to capture an estimate of time-varying body burden compared to a standard cumulative exposure model (Checkoway, 1990).

To estimate total annual dose we converted the daily TWA in mg/m^3 (Equation 1) to milligrams (mg) of exposure by multiplying the TWA with a standardized breathing rate taken from the United States Environmental Protection Agency (US EPA) Exposure Factor Handbook (1997) for an average adult conducting light work—using 1.0 cubic meter (m^3) of air inhaled and exhaled per hour for an eight hour work shift. The sum of the total number of days an employee worked per year and the daily dose in mg gives a dose rate using the following equation:

$$E_j = C_j (\text{mg}/\text{m}^3)(1.0 \text{ m}^3/\text{1 hour})(8 \text{ hours}/\text{1 day})(\# \text{ work days}/\text{year}) \quad \text{Equation (2)}$$

The E_j represents the amount of APFO/PFOA (the exposure) entering the body in milligrams for each year worked, j , and C_j is the daily TWA in mg/m^3 taken directly from the EDM and linked to the worker's HR profile.

We calculated each worker's annual cumulative dose and clearance weighted dose profiles using the following equations:

Potential Cumulative Dose (PCD) estimate of body burden in mg:

$$PCD_i = \sum_{j=1}^{n_i} E_{ij} \quad \text{Equation (3)}$$

Cumulative Clearance Weighted Dose (CCWD) estimate of body burden in mg:

$$CCWD_i = \sum_{j=1}^{n_i} \{ > (E_j) \text{ or } (CCWD_{j-1}) * (e^{-k}) \} \quad \text{Equation (4)}$$

In Equations 3 and 4, the E_{ij} represents the estimated dose in mg, j refers to the specific year of exposure, n is number of years worked, and i is the i^{th} worker. In the Equation 4, k is the elimination constant, and is equal to $\ln 2/T_{1/2}$. $T_{1/2}$ is the half-life of serum-PFOA in humans, estimated to be between 2.3 and 3.8 years (Bartell et al., 2009, Brede et al., 2010, Olsen G.W. et al., 2007). We have chosen to use 3.5 years in the half-life model based on data from retired workers at the 3M production workers reported by Olsen G.W. et al. (2007). This model accounts for body burden following cessation of or reduction in exposure. After exposure ceases body burden will exponentially decline asymptotically.

Data Analysis

To analyze the workers' exposure profiles and compare the two annual dose estimates, we began by reviewing the distribution of cumulative dose estimates for all workers. We evaluated the range of data from the cumulative estimates to guide in the demarcation of the dose ranges and establish the exposure groups. We reviewed the workers' exposure profiles comparing estimates from the two models at specific ages for the total population. The four ages evaluated were each decade, age 30 through 60. This allowed us to explore differences in exposure levels between the PCD and CCWD model and determine which ages and exposure groups were most sensitive to exposure misclassification. We displayed three groups of chemical production workers representing a range of job titles in the main production APFO building.

RESULTS

Study Population

Workers averaged 15 years of employment with a maximum of 59 years. The average age at first and last employment was 30 and 43 years, respectively. Males represented more than three-quarters of the population with 3,716 males and 952 females. The data in Table 5 show workers' range of cumulative exposures for both models. The groups were split by workers who; ever worked in the Chemical Division, those who only worked in the Non-Chemical Division, and all workers. More males were represented in the Chemical Division, and on average they started younger and completed work later in life, compared to the Non-Chemical Division. Close to two-thirds (63%) of those who ever worked in the Chemical Division also held jobs in the Non-Chemical Division, often making the shift from a Chemical to Non-Chemical job with increasing age.

Exposure Analysis

The Chemical Division's range of daily TWAs was 1×10^{-8} to 3.79×10^{-1} mg/m^3 , and the Non-Chemical Division's range of TWAs was 1×10^{-8} to 1×10^{-5} mg/m^3 . Table 6 shows the range of TWAs for both divisions by year. It also includes job titles with specific APFO-exposure tasks within the Chemical Division. The data were log normally distributed. We used the range of the potential cumulative dose estimates to create six exposure groups representing orders of magnitude differences with a range starting from less than 0.001 mg up to greater than 10 mg (Table 7). The majority of all workers were located in the mid-range groups (groups 3-5; >0.01 - 10 mg of APFO/PFOA). The highest

exposure group (group 6; >10.0 mg of APFO/PFOA) contained few workers overall, and almost none were from the Non-Chemical Division.

The majority of workers' exposure group classifications changed with age. We evaluated the cumulative exposure for all workers at 30, 40, 50, and 60 years of age comparing estimates from the two models. The mean and median estimates of potential cumulative dose and cumulative clearance weighted dose in mg of APFO/PFOA for the workers by age are provided in Table 8. The age groups with the greatest potential for exposure misclassification were the final two decades evaluated. The trend for median values increases with age for both model estimates. However the highest mean exposure values occur at age 40 and age 50 for the PCD and CCWD models, respectively. When evaluating the whole population, the correlation between the estimates from the two models illustrates a greater gap between dose estimates with increasing age. Figure 1 displays the distribution of estimates on a log scale between the two models and the correlation co-efficient for all four of the selected ages.

Worker Exposure Profiles

We displayed the exposure profiles for three sets of workers with the same job title for part of their career. These data represent the comparison between the cumulative annual estimates using the PCD model and the annual exposure estimates from the CCWD model. Figures 2, 3, and 4 show a range of exposures for APFO production workers, starting with relatively low to very high-level exposed personnel. Figure 2 shows relatively low-dose estimates for production workers where worker 1 is in group 3 at age 30 for both model estimates, however

by age 40 s/he decreases to group 2 only for the CCWD model annual estimate. Worker 2 starts in the low group and by age 60 is part of exposure group 4 based on the PCD model estimate, compared to group 2 for the CCWD estimate.

In Figure 3, we display two workers with mid-level overall dose estimates for APFO production workers. At age 50, worker 1 is part of the highest exposure group (group 6) and a middle exposure group (group 3), for the PCD and CCWD model estimates respectively. Therefore, when using the values from the standard cumulative model this worker reaches a high exposure group early on and stays there—but by incorporating the serum elimination half-life, this same worker changes to lower rankings with time. Finally, figure 4 shows two workers with the same job titles, who held high-level exposure jobs compared to the aforementioned workers. Both workers show a distinct separation between the exposure group rankings by age 50, moving to a lower exposure group when using the CCWD estimates compared to the PCD estimates.

DISCUSSION

In this study we evaluated the APFO/PFOA exposure profiles of 4,668 workers at the Cottage Grove, MN 3M plant comparing dose estimates from two cumulative time-dependent exposure models. Each worker had a unique exposure profile based on the combination of their job title(s), work department(s), and year(s) of employment. Job assignments changed over time—as did each worker’s exposure. We expected a divergence in the two annual exposure estimates by worker due to the nature of the models; however, what was not apparent was the amount of potential misclassification depending on the age and

intensity of exposure. On average, the workers most vulnerable to potential misclassification were those individuals with estimates in the mid to high exposure groups and workers with increasing age. This may have ramifications for health-outcome analyses. Using the standard cumulative model negates any elimination that occurs and may inflate the exposure estimates with time. By contrast, the clearance-weighted model more closely reflects time varying body burden—especially after exposure has ceased.

Many workers who were part of the Chemical Division started at an early age, had high exposures early on, and switched to jobs with lower exposures later in their career. In this population, workers had their greatest exposure potential in the earliest two decades of their work-life, compared to the later years. When this is true, many workers with long tenure—and early age high-level exposure—often ended their career with a low body burden and a relatively low exposure group ranking when using the annual estimates from the clearance-weighted model instead of the traditional cumulative model. With increasing age, many workers show a greater difference between the CCWD and PCD estimates—making the choice of model more important with age.

When interpreting the results of our exposure group rankings and the models chosen, some study limitations are clear. We made several assumptions related to our daily TWA calculations. The majority of our air monitoring samples came from the final years of production. We assumed that these represented earlier years' exposures with the adjustment of production rates. We used a percentage of the mean concentration based on the relative production rates

by year. Additionally, sample collection methods and analysis methods of APFO/PFOA changed over time with increasing sensitivity to detect lower level airborne concentrations in the later years of production. With the data from earlier years, we may not have captured very low airborne concentrations of APFO/PFOA, which may lead to an underestimate of exposure.

Another study limitation of note is the use of the CCWD model as a surrogate measure of body burden. We did not have a complete pharmacokinetic model that considers all routes of exposure (inhalation, dermal, oral) and several other biological parameters. However, we calculated the internal dose based on the main exposure route, i.e. inhalation, a novel approach. We did not evaluate other routes of exposure such as dermal absorption and/or oral exposure; however, our assumption is that the majority of workers' exposure is through inhalation compared to other routes of exposure and that the other routes of exposure would be equally negligible for all workers.

PFOA is on the priority list to be evaluated for carcinogenicity by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO). A formal review of the literature conducted by the US EPA's Science Advisory Board (SAB) suggested that PFOA is "likely to be carcinogenic to humans" (US EPA, 2006). The validity of any health outcome study that presupposes an association between a specific level of exposure and occurrence of disease is contingent on utilizing exposure models that best represent an individual's true body burden.

Various occupational studies have attempted to evaluate real time exposures. In a study on lung cancer, researchers found that workers with two unique time varying exposure profiles, yet the same cumulative exposure, resulted in different lung cancer rates when compared to controls (Hauptmann et al., 2002). Other researchers have attempted to eliminate exposure misclassification and more accurately represent exposure by using half-life and other time-dependent exposure models. An exposure reconstruction conducted by Gallagher et al. (2011) from a case-control study on perchloroethylene (PCE)-contaminated drinking water and the risk of breast cancer found that misclassification might have occurred in a prior analysis mainly among subjects with low exposure levels. The initial exposure assessment used a proxy measure of PCE while in the updated assessment the investigators utilized more precise estimates of time-dependent exposure. The results of this study indicated that with a more accurate estimate of exposure, women in the highest exposure group had a greater risk of breast cancer. In another occupational exposure study investigators showed that exposure to pesticides in apple thinners when using a time-integrated exposure model, compared to a traditional model, was more predictive of dose when compared to biological samples. The model incorporated exposure over time instead of assuming a fixed exposure independent of time (Doran et al., 2003).

Occupational studies that have estimated APFO/PFOA exposures have relied on work history profiles and serum-PFOA data. Kreckman et al. (2009) completed an exposure reconstruction with workers using the distribution of serum concentrations to determine exposure intensity with concurrent job titles

that were recorded at the time of blood draw. They found high variability in the blood levels of workers with the same job titles. This may be, in part, due to the long half-life of serum-PFOA and the possibility of frequent job changes or the occasional high-exposure event. Ubel et al. (1980), in his paper of the health status of fluorochemical workers at 3M, reported blood levels of total organic fluorine to be elevated related to intensity of exposure and duration of employment. This shows that the precision of individual-level dose estimates can be enhanced when using the workers' full employee history, including the time spent working in each job title and the related exposure estimates. We attempted to include these variables in our exposure assessment.

Our aim was to demonstrate methods for reconstructing the daily exposure of this cohort by building an exposure data matrix with daily TWAs for each job title, department and year of exposure combination. Additionally, the purpose of this paper was to provide individual-level annual dose estimates of APFO/PFOA that closely represent time-dependent body burden. These data capture a wide range of cumulative estimates, which will help inform the scientific community regarding what level of APFO/PFOA exposure affects disease outcomes. The exposure estimates from both models provide alternatives for study investigators to examine specific assumptions about time related exposures, chemical retention and elimination, and disease onset. In our cohort, the choice of exposure model becomes critical with increasing exposure estimates and age, and in particular when evaluating the risk of long-latency disease outcomes. We have hypothesized that the annual dose estimates from the CCWD model capture

variability of body burden over time accounting for retention and elimination of APFO. These annual estimates, along with cumulative estimates of the CCWD model, provide an alternative way to characterize exposure. However, confirmation of this assumption can only be made through the examination of biological data correlated to dose estimates. Overall, these data allow for a future exploration of risk of disease related to dose during potential etiologic ages.

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Figures and Tables

Table 1. Cottage Grove: APFO/PFOA Production by Year

Year	Pounds	Year	Pounds
1951-1959	200-500	1976	31,200
1960-1969	900-1,200	1977	56,400
1970-1971	1,200-2,000	1978	60,000
1972	18,000	1979	36,000
1973-1974	36,000*	1980-1989	2,000*
1975	20,400	1990-1999	360,000*

**Values are an average annual value*

Table 2. Air Sampling Data: Analytic Method Measuring the Carboxylate Anion (PFO-)

Years	Personal Samples	Area Samples	Total Samples
1977-1980*	67	95	162
1981-1990*	45	101	146
1991-1999*	19	42	61
2000 APFO	23	214	237
2000 PFOA	27	143	170
2000 Both*	24	64	88
2000 Total	205	659	864

**Approximately half PFOA (vapor) and half APFO (particulate) samples*

Table 3. Cottage Grove: Time Weighted Average (TWA) Exposure Calculation: 1990

1990: 8-Hour Tasks: Chemical Department Operator- Kettle Room APFO	Number of Samples	Average mg/m ³	480 min/day
Outside Production Room	Professional Judgment*	0.001	434**
Inside Production Room: No Exposure Task	101	0.012	32**
Inside Production Room: Exposure Task	12	0.051	14**
			0.003 mg/m ³

*Professional Judgment is a best estimate of exposure without PFO- measurements from experienced industrial hygiene staff.
 **These minutes were estimated based on exposure task sampling time, expert input, and production records.

Table 4. Cottage Grove: Time Weighted Average (TWA) Exposure Calculation: 2000

2000: 8-Hour Tasks: Chemical Department Operator- Kettle Room APFO	Number of Samples	Average mg/m ³	480 min/day
Outside Production Room	Professional Judgment*	0.001	434**
Inside Production Room: No Exposure Task	101	0.012	2**
Inside Production Room: Exposure Task	12	0.051	44**
			0.006mg/m ³

*Professional Judgment is a best estimate of exposure without PFO- measurements from experienced industrial hygiene staff.
 **These minutes were estimated based on exposure task sampling time, expert input, and production records.

Table 5.**Cottage Grove Workers: 1947-2008 (N=4,668)**

	Chemical Division (ever)	Non-Chemical Division (only)	All Workers
Number of Workers	2,366	2,302	4,668
Number of Males (%), Females (%)	2,059 (87%), 307 (13%)	1,657 (72%), 645 (28%)	3,716 (80%), 952 (20%)
Average age at first employment	29, (16-64)	30, (17-73)	30, (16-73)
Average age at last employment	46, (19-92)	41, (19-85)	43, (19-92)
Average number of years worked	17, (1-59)	10, (1-47)	15, (1-59)
Range mg APFO/PFOA (PCD model)	1.36×10^{-5} - $2.12 \times 10^{+3}$	3.66×10^{-5} - $2.37 \times 10^{+1}$	3.66×10^{-5} - $2.12 \times 10^{+3}$
Range mg APFO/PFOA (CCWD model)	1.02×10^{-5} - $5.12 \times 10^{+3}$	1.36×10^{-5} - $8.29 \times 10^{+1}$	1.02×10^{-5} - $5.12 \times 10^{+3}$

Table 6. Daily Time Weighted Average (TWA) Range: APFO Exposure

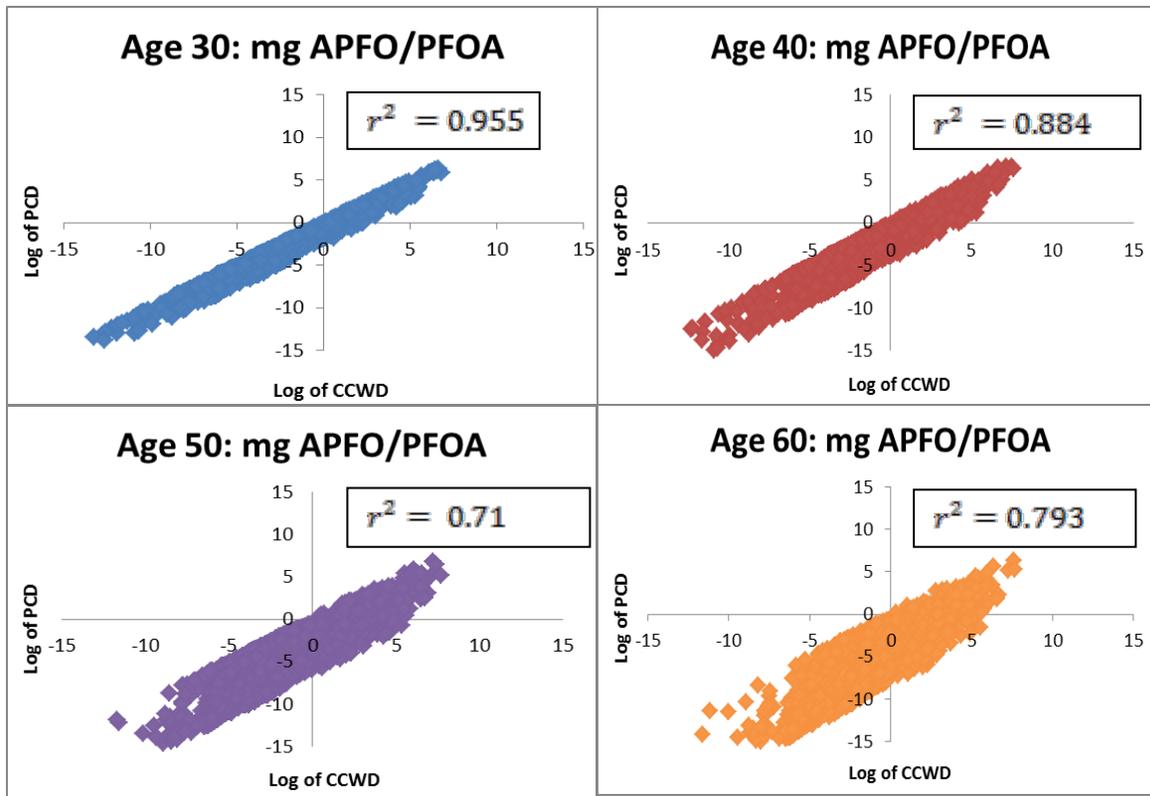
Job Title	Years	Range of daily TWAs (mg/m³)
All Jobs Non-Chemical Division	1947-1952	1×10^{-8} - 1×10^{-7}
All Jobs Non-Chemical Division	1953-2002	1×10^{-7} - 1×10^{-5}
All Jobs Non-Chemical Division	2003-2008	1.56×10^{-7} - 5×10^{-6}
Non-APFO Jobs Chemical Division	1947-1952	1×10^{-8} - 1×10^{-4}
Non-APFO Jobs Chemical Division	2003-2008	1.56×10^{-6} - 5×10^{-6}
Clerk	1956-1961	0.001
Custodian	1959-2002	0.0011-0.002
Finished Goods-Floor Checker	1997-2002	0.002
Foreman	1954-1976	0.0026-0.007
Head Cell Operator	1956-1999	0.0015-0.003
Helper	1956-1978	0.006-0.379
Lab Technician	1956-1966	0.017-0.0023
Manager	2000-2002	0.0003
Operator-Spray Dryer	1978-2002	0.011-0.124
Operator- Kettle Room APFO	1956-2002	0.0017-0.04
Operator-Kettle Room non-APFO	1956-2002	0.001-0.008
Pilot Plant Technician	1954-1962	0.0017-0.0023
Process Engineer	1956-1961	0.0006-0.0011
QC Foreman	1956-1960	0.0001
Senior Pilot Plant Assistant	1956	0.0017
Supervisor	1954-2002	0.0001-0.0003

Table 7.		Number of Workers by Category: Cumulative Dose Estimate: APFO/PFOA (mg)		
		Chemical Division (ever)	Non-Chemical Division (only)	All Workers
1	(0-.001)	36	83	119
2	(>.001-.01)	20	447	467
3	(>.01- 0.1)	217	793	1010
4	(>0.1- 1.0)	816	849	1665
5	(>1.0-10)	904	127	1031
6	(>10)	373	3	376
Total Number of Workers		2366	2302	4688

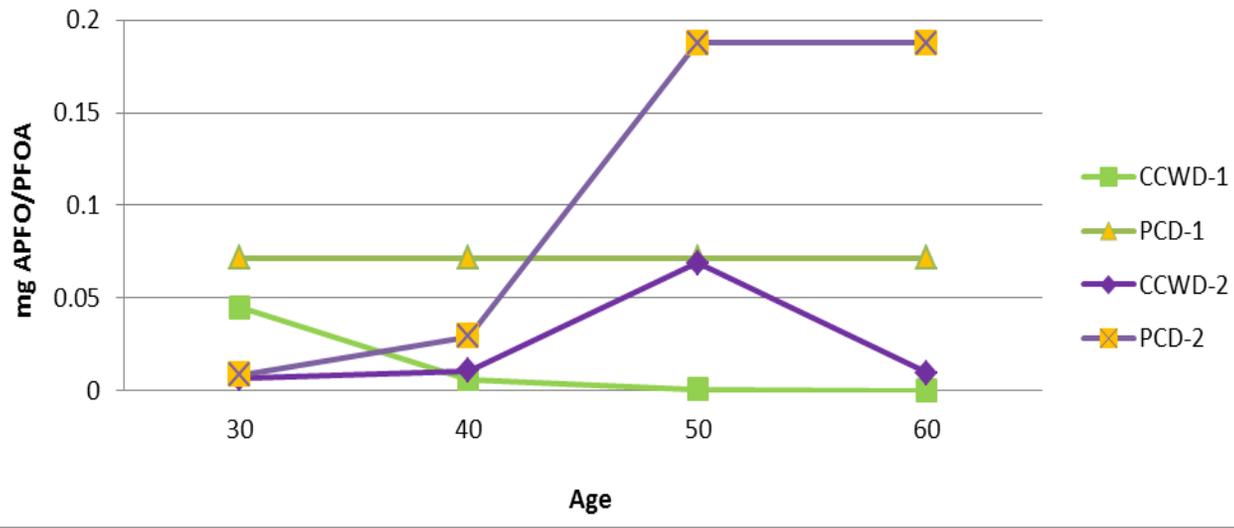
Workers' APFO/PFOA Dose Estimates: Mean and Median Value in mg by Age:
Table 8. Potential Cumulative Dose (PCD) and Cumulative Clearance Weighted Dose (CCWD) Models

Age	PCD Model		CCWD Model	
	Mean	Median	Mean	Median
30	4.8	0.07	23.1	0.15
40	8.9	0.13	35.3	0.30
50	8.2	0.18	37.6	0.41
60	8.3	0.24	31.6	0.49

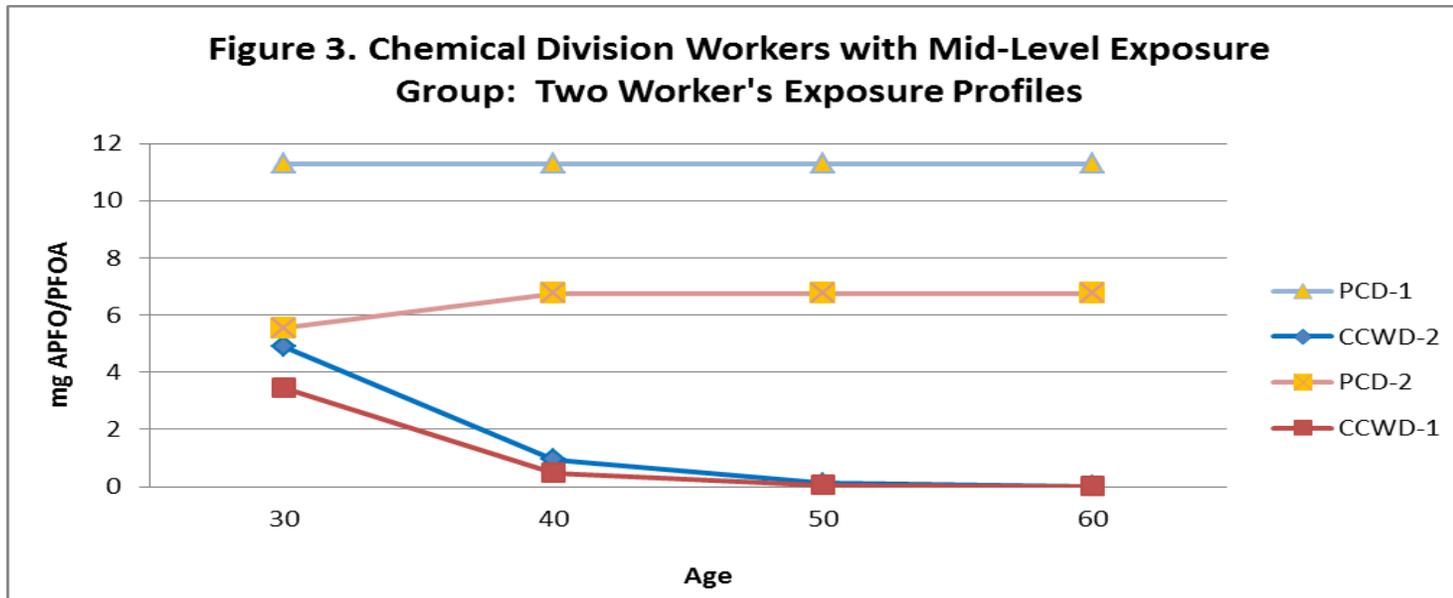
Figure 1. Distribution by Age: Comparing the Potential Cumulative Dose (PCD) and the Cumulative Clearance Weighted Dose (CCWD) Model Estimates in mg APFO/PFOA



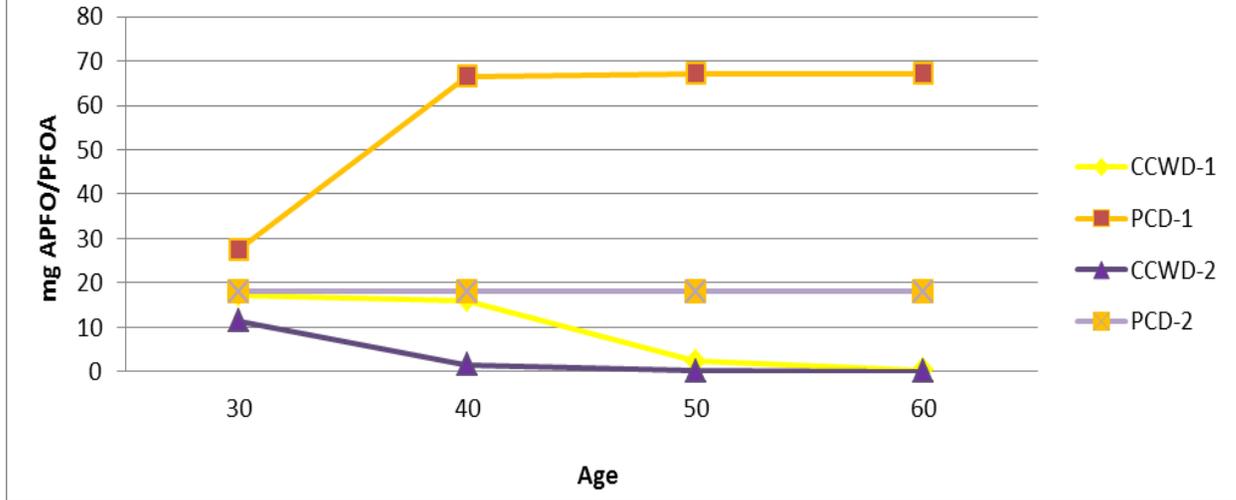
**Figure 2. Chemical Division Workers with Low-Level Exposure
Groups: Two Worker's Exposure Profiles**



**Figure 3. Chemical Division Workers with Mid-Level Exposure
Group: Two Worker's Exposure Profiles**



**Figure 4. Chemical Division Workers with High-Level Exposure
Group: Two Worker's Exposure Profiles**



CHAPTER 5: OCCUPATIONAL EXPOSURE TO PERFLUOROOCCTANOIC ACID AND ITS PRIMARY SALT, AMMONIUM PERFLUOROOCCTANOATE AND RISK OF CANCER MORTALITY IN WORKERS

The extent of human health effects from exposure to perfluorooctanoic acid (PFOA) and its primary salt, ammonium perfluorooctanoate (APFO) are unknown. In toxicological and occupational studies, exposure to APFO/PFOA has been associated with an increased risk of non-malignant tumors and cancer mortality, respectively. These effects were observed in the context of laboratories where animals were given high doses of APFO/PFOA, and in occupational settings with relatively high-level exposure for workers who produced APFO. The purpose of this paper was to evaluate the risk of cancer mortality in a cohort of 3M workers with various levels of APFO/PFOA exposure. To accomplish this, we utilized a newly created exposure data matrix (EDM). The EDM was constructed using air measurements for specific jobs—in place of previous analyses that evaluated a worker’s job title or location as the exposure measure. This was a retrospective occupational mortality study with 9,027 workers from two 3M plants in Minnesota, evaluating the risk of a cancer death associated with an individual’s exposure over time. We examined workers’ risk of cancer mortality for six *a priori* cancers—bladder, breast, kidney, liver, pancreatic, and prostate cancers using extended Cox proportional hazard models and standardized mortality ratios (SMRs) were calculated from Minnesota population death rates. Hazard Ratios (HRs) for the internal evaluation were calculated using the

workers' continuous exposure estimates, exposure group rankings, and exposure during time-windows—all as potential risk factors for cancer mortality. To account for the effect of cancer latency, we lagged the models by ten years. Models were stratified by gender, and adjusted for the workers' first year of employment.

There were a total of 2,979 identifiable deaths in our cohort, and of these there were 72 prostate cancer deaths, 48 pancreatic cancer deaths, 16 bladder cancer deaths, 25 (female) breast cancer deaths, 24 kidney cancer deaths, and 15 liver cancer deaths. The SMRs for Cottage Grove showed no excess of cancer deaths. Within Cottage Grove, workers who were ever involved in chemical production of APFO observed 16 prostate cancer deaths compared to 13 expected. The internal exposure group evaluations showed two of the six cancers examined have a potential dose-response relationship. There was an elevated, but imprecise risk of dying from prostate cancer for the highest two exposure groups compared to the workers in St. Paul [HR=1.22, (95% CI: 0.57, 2.61) and HR=1.27, (95% CI: 0.30, 5.28)]. The risk of a pancreatic cancer death was elevated for the second highest exposure group—there were no pancreatic cancer deaths in the top exposure group. All other cancers evaluated were at or near no effect level when evaluating time-dependent exposure data and specific time-windows of exposure. All internal estimates were unstable. There was limited evidence of consistent outcomes to confirm previous study results. Future mortality and morbidity studies based on the EDM may be warranted.

INTRODUCTION

Perfluorooctanoic acid (PFOA) is an eight chain fully fluorinated carboxylic acid. The salts of PFOA have been used as surfactants and processing aids for several decades. As a synthetic chemical surfactant it has a broad range of commercial and industrial applications including the manufacture of electronics, flame-retardants, non-stick cookware and waterproof textiles (Kennedy et al., 2004). It bio-accumulates and does not break down easily in the environment due to the carbon-fluorine bonds. Although PFOA is not naturally occurring, it has been detected in many animal species and environmental matrices around the world (Houde et al, 2006). In the body, it binds to blood proteins and is found primarily in blood-serum, liver and kidneys. PFOA has a long serum half-life in humans and there is evidence that its excretion is biphasic, where early excretion is more rapid than later excretion (Steenland et al., 2011). The production and use of APFO has been phased out over the last decade due to concerns about effects on human and environmental health.

PFOA's primary salt, ammonium perfluorooctanoate (APFO), was manufactured for more than fifty years at the 3M Cottage Grove facility in Minnesota. Workers were predominately exposed to APFO particulate and PFOA vapors through inhalation, followed by dermal contact and cross-contaminated oral routes of exposure. Production workers at the Cottage Grove plant had potential for significant exposure to APFO and PFOA compared to other non-production workers and the general public. The median blood serum-PFOA concentration for Cottage Grove APFO production workers ranged from 100 to

5,000 parts per billion (ppb) (Olsen et al., 2003). In 1999-2000 the National Health and Nutrition Examination Survey (NHANES) data on the U.S. population reported a geometric mean serum-PFOA concentration of 5 ppb that declined by 25% to 3.9 ppb in the years 2003-2004 (Calafat et al., 2007). The most recent Fourth National Exposure Report from March 2013 showed the serum-PFOA geometric mean concentration for the U.S. population is 5.21 ppb—an increase from the former survey (CDC, 2013). The three-fold difference in the concentrations of measured serum-PFOA levels between production workers and the general U.S. population emphasizes the magnitude of occupational exposure and raises concern regarding its long-term health impact.

Several animal studies have evaluated the toxicological impact of APFO and PFOA. The health outcomes are broad and include an array of health effects. These include an altered immune response, changes in lipid metabolism, low birth weight, developmental toxicity and an increase in tumor incidence in some animal species. Data from rodent studies show an increase in the incidence of a triad of tumors—pancreatic, liver and testicular cancers (Lau et al., 2007; Loveless et al., 2008; and Kennedy et al., 2004). APFO and PFOA are rapidly and completely absorbed following inhalation and oral exposure—and to a lesser extent after dermal exposure. There is no evidence that PFOA is metabolized in mammals (Kuslikis et al., 1992 and Vanden Heuvel et al., 1991). PFOA is a known hepatocarcinogen in rodents; however, it is not established whether it is carcinogenic in humans. In rats, PFOA acts as an agonist for the peroxisome proliferator activated alpha-receptor (PPAR α) signaling pathway, which initiates

a sequence of biochemical events known to promote carcinogenesis (Biegel et al., 2001). PFOA's PPAR α mode of action and toxicity in rodents has been established; however, its toxicological applicability to humans is still uncertain. Yao and Zhong (2005) evaluated human hepatoma cells and concluded that PFOA exerts a genotoxic effect on the HepG2 cells most likely by way of oxidative DNA damage. In a more recent study, Tilton et al. (2008) examined gene expression of an *in-vivo* trout cancer model to evaluate the potential of PFOA's role as a carcinogen in the absence of peroxisome proliferation. Tilton found that PFOA was a cancer promoter of the liver in the fish. Like humans, fish lack the PPAR α mode of action—thus emphasizing the potential health impact of PFOA exposure and relevance in humans.

PFOA is on the priority list to be evaluated for human carcinogenicity by the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO). A formal review of the literature conducted by the US Environmental Protection Agency's (EPA) Science Advisory Board (SAB) suggested that PFOA is "likely to be carcinogenic to humans" (US EPA, 2006). There are limited data on human exposure to PFOA and the possible link to cancer. A recent study of Inuit women in Greenland evaluated PFOA exposure from cases and controls with findings of elevated breast cancer cases associated with serum-PFOA levels (Bonfeld-Jorgeson et al., 2011). To date, two occupational cohorts have been studied for risk of cancer mortality based on human exposure to APFO and PFOA. A polymer production plant in West Virginia was evaluated for various health outcomes. Leonard et al. (2008) found

some evidence of an excess of kidney, liver, and bladder cancers among workers at the DuPont polymer production facility, however there were too few cases to draw clear conclusions. An updated analysis of that cohort reported an excess risk of malignant and non-malignant renal disease in higher exposed workers (Steenland and Woskie, 2012). It is important to note that these workers are also exposed to tetrafluoroethylene, a known kidney carcinogen. The second occupational cohort evaluated is the 3M Cottage Grove APFO production plant. Two mortality studies with this cohort showed a positive association between increasing exposure to APFO and the risk of a prostate cancer death (Gilliland and Mandel, 1993; Lundin et al., 2009). A recent study that examined proportional morbidity in a non-occupationally exposed cohort of a community residing next to the DuPont plant in West Virginia found a positive association between serum-PFOA and kidney cancer for high and very high exposure groups (Vieira et al., 2013).

The goal of this research was to use a newly created inhalation exposure data matrix and evaluate 3M workers' cumulative exposure and the workers' mortality experience. The exposure values for this study include the duration and intensity of exposure over time and were taken from air measurements collected at the Cottage Grove plant. This work explores risk by using estimates from two exposure models that provide options regarding body burden measurements. Specifically, we present an analysis of the risk of death for six *a priori* cancer classifications including bladder, breast, kidney, liver, pancreatic and prostate cancers. These cancers and other health outcomes—such as diabetes and

ischemic heart disease (IHD)—have been highlighted as health outcomes associated with exposure to APFO and PFOA and were selected based on the aforementioned positive findings in toxicological data and epidemiological health studies.

METHODS

Study Population

3M Workers from both Cottage Grove and St. Paul Minnesota plant locations were part of this study. The Cottage Grove campus has operated in more than 30 buildings including production and pilot plants. The facility has developed numerous products and various goods ranging from Scotchgard to Post-it-Notes and has produced specialty chemicals, including APFO, as well as products such as films. The plant was constructed in 1947 and APFO production began soon thereafter. APFO production increased substantially over time but was phased out completely by the end of 2002. The Cottage Grove population consisted of 4,668 members, including 3,993 workers enumerated for the previous study and another 675 who were hired between 1997 and 2002 when APFO production stopped. The St. Paul worker cohort had been previously enumerated by the University of Minnesota and included 4,359 workers. The total cohort population of both locations included 9,027 workers. At the St. Paul location workers manufactured tape, abrasives and adhesive products but never were involved with APFO production. Production at the St. Paul facility was gradually phased out between 1998 and 2005. There was a small group of 200 workers who

had employment records in both locations. We included all workers who accrued a one-year minimum cumulative employment through 2008 for the Cottage Grove population and 1998 for St. Paul. The cohort members were followed starting January 1st, 1947 or their first date of employment, until either their date of death or the end of follow-up, December 31st, 2008. Human resource records were abstracted for work history information; job title(s), job department(s), start and stop dates for each position a worker held, and personal identifying data. A job exposure data matrix (EDM) was created to estimate exposure across the life of the Cottage Grove plant for all chemical workers. The non-production Chemical Division workers, Non-Chemical Division and St. Paul workers were assigned estimated exposures from ambient air measurements and professional input.

Determination of Vital Status

We conducted a vital status assessment on all cohort members not determined to be deceased in the previous follow-up. Vital status was first determined using the Social Security Administration's (SSA) data records. A commercial skip-tracing vendor was used to confirm the vital status of workers who were not clearly identified as alive or deceased through the SSA. We used the National Death Index (NDI) to obtain the date of death, state of death, and underlying and contributing cause of death for all workers through 2008. The causes of death were coded to the International Classification of Disease (ICD) version in effect at the time of death.

Exposure Assessment and Exposure Models

Using work history records we characterized APFO/PFOA exposure as an estimate of internal dose based on the inhalation route of entry for all 3M workers. We utilized two cumulative exposure models—one is a standard model and the other incorporates the serum-half-life of PFOA to ascertain extended burden of this persistent chemical.

A complete description of the methods to create the worker's time-dependent exposure profiles can be found elsewhere (Chapter Four: *Occupational Exposure to Perfluorooctanoic Acid and its Primary Salt, Ammonium Perfluorooctanoate: An Inhalation Exposure Reconstruction Evaluating Two Time Dependent Models*). However, a brief summary of the exposure reconstruction methods is presented below.

We calculated a daily time-weighted average (TWA) in milligrams per cubic meter of air (mg/m^3) using occupational air measurements of both APFO and PFOA. For jobs in the APFO production area of the Chemical Division we incorporated the amount of time spent during an eight hour shift in up to three pre-defined work-task areas; 1) time conducting exposure tasks in the production room, 2) time conducting non-exposure tasks in the production room, and 3) time outside of the production room. A TWA was calculated for each combination of job title, location and year to create an EDM that contained 23 departments and 45 job titles within the Chemical Division for all production years starting in 1947 through the end of 2008. Job and department combinations in the non-APFO production areas of the Chemical Division and the Non-Chemical Division were assigned an estimated TWA based on expert judgment. Cohort members from the

St. Paul Plant were assigned a background level based on estimated exposures in the general population. The estimated exposures for each job varied by the amount of APFO produced over time. We linked the department, job and year-specific TWAs to the employees' work history records. We calculated cumulative estimates of exposure based on the summation of daily TWAs by year. Annual dose estimates were calculated in milligrams (mg) of APFO/PFOA after converting mg/m^3 into mg by using the standardized breathing rate of $1.0 \text{ m}^3/\text{hour}$ for an eight hour work shift, taken from the US EPA Exposure Factor Handbook (1997) to represent an average adult conducting light work.

We used two cumulative exposure models to estimate an annual dose estimate for each model. The first is a standard cumulative model: **“Potential Cumulative Dose”** or PCD. It is the sum of the workers' daily exposure estimates in milligrams calculated by linking their work history profile to the EDM: The sum of exposures for the *i*th worker for all job titles held (*j*), for each year (*n*).

(Equation 1)

$$PCD_i = \sum_{j=1}^{n_i} E_{ij}$$

The second model: **“Cumulative Clearance Weighted Dose”** or CCWD, accounts for retention and elimination of the chemical from the body by incorporating the estimated serum-half life into the cumulative sum of annual exposures: The sum of exposures for the *i*th worker for all job titles held (*j*), for

each year (n). This model accounts for the residual impact from the body burden of this persistent chemical.

(Equation 2)

$$CCWD_i = \sum_{j=1}^{n_i} \{ > (E_j) \text{ or } (CCWD_{j-1}) * (e^{-k}) \}$$

We divided the workers into six exposure groups, using the range of exposure estimates.

Cancer Mortality: Outcome of Interest

The following causes of death were selected based on observations from animal and occupational studies. These specific selections were also considered identifiable based on the size of our study cohort and the national data from cancer-specific death rates provided by the National Cancer Institute (NCI, SEER statistics 2003-2007). Three of the *a priori* causes of death include: cancers of the liver, pancreas, and breast, which were selected based on results from animal studies (Butenoff et al., 2004; Biegel et al. 2001). In addition, we chose to evaluate the risk of death from prostate, kidney and bladder cancers as a follow-up to the epidemiological studies that have shown a positive association with increasing exposure to PFOA and higher rates of cancer mortality (Vieira et al., 2013; Leonard et al., 2008; Lundin et al, 2009; Alexander et al, 2003; Steenland and Woskie, 2012).

The International Classification of Diseases (ICD) 10th revision codes were used to classify the underlying cause of death and include: liver cancer, c22-c24 (formally 155-156 in the ICD 9th revision), pancreatic cancer, c25 (ICD 9th

revision: 157); breast cancer c50 (ICD 9th revision: 174-175); prostate cancer, c61 (ICD 9th revision: 185); and bladder cancer c67-c68 (ICD 9th revision: 188, 189.3-189.9); and kidney cancer c64-c66 (ICD 9th revision: 189.0-189.2).

Data Analyses

We initially compared the cohort mortality rates to the state of Minnesota's population mortality rates from the National Center for Health Statistics data. Age, sex, race, and calendar period, SMRs and 95% confidence intervals (CIs) were computed using the National Institute of Occupational Safety and Health's (NIOSH) software for Life Table Analysis System (NIOSH, 1998). Complete referent data were available from 1960 onwards, thus our analysis was limited to events occurring after 1960. We estimated SMRs for each location and by Division within the Cottage Grove location.

We further evaluated associations with APFO exposure and risk of dying from specific cancers with an internal analysis. The risk of a cancer death was estimated as a function of APFO/PFOA time-dependent dose estimates by calculating hazard ratios (HR) with 95% CIs using extended Cox proportional hazards regression models (Breslow and Day, 1987 and Kleinbaum, 1996). Exposure was classified in six categories based on order of magnitude differences on a log scale ($\leq 10^{-3}$ through 10 mg APFO). We modeled the risk of death using the cumulative exposure estimates for all workers with both models. The analyses were conducted using the PHREG procedures in SAS 9.2. The time covariate was age—starting with age at date of entry into the cohort until censored at time of death or the end of the study. We adjusted for year of entry

into the cohort and stratified by gender. To evaluate the potential effects of latency the exposure was lagged by 10 years.

To explore the possibility of biologically important ages of exposure related to the development of disease, we evaluated cancer risk based on cumulative exposure estimates calculated during specific ages. We utilized the workers' exposure estimates calculated during two time-windows—the 4th and 5th decades of life. The estimates during these periods capture the annual exposure from each employee's time-window. We proposed that the selected age-windows may be etiologically relevant periods regarding the sensitivity to disease based on biological functions and age-related physiological changes. Thus, we wanted to explore the possibility that dose during distinct ages may have the potential to affect the risk of cancer.

RESULTS

The cohort of 9,027 experienced 2,979 deaths. The cohort members from the two plants were similar in age at entry into the cohort, but the St. Paul Plant was on average six years older at the end of follow-up (Table 1). Of the 2,979 deaths for the entire cohort 72 prostate cancer deaths, 48 pancreatic cancer deaths, 16 bladder cancer deaths, 25 female breast cancer deaths, 24 kidney cancer deaths, and 15 liver cancer deaths were identified. Almost 33% of the entire population was deceased by the end of 2008, and the percentage of those who had died was higher in the St. Paul group (42%) compared to the Cottage Grove group (25%). St. Paul workers on average were employed more years, 20, compared to the average number of years, 14, for all Cottage Grove employees. Females

represented only 12% of the St. Paul group compared to 21% of Cottage Grove workers. We did not have data on race or ethnicity; however, from personal correspondence with current and retired workers the population at both locations consisted of workers who were predominately of Caucasian background—which is consistent with state and county level data reported by the US Census Bureau (US Census Bureau; Quick Fact Sheet, MN).

All workers were assigned to one of six exposure groups (Table 2). The range of cumulative dose estimates for the entire working cohort was log-normally distributed—many workers had low-level relative exposure values and very few had high-level exposure values. The groupings were formed using orders of magnitude from the APFO/PFOA mg estimates.

The characteristics of the workers from each exposure group vary substantially. The workers classified in the top half of the exposure groups (groups 4-6) worked for more years, approximately three times more on average, and were predominately male compared to the workers in lower exposure groups (groups 2-3). Additionally, the lowest exposure group had the highest percentage of employees who were deceased and the oldest mean age of exit from the cohort. Table 3 presents the number of cancers per group. Of these, the highest two exposures groups (>1.0 mg APFO/PFOA) contain few cancer deaths compared to the lowest exposure group (<0.001 mg APFO/PFOA), which includes the referent group of workers in St. Paul.

External Analysis: Standard Mortality Ratios

The overall and all-cause cancer SMRs for the entire cohort were 0.92 (95% CI: 0.89, 0.96), and 0.96 (95% CI: 0.90, 1.03) respectively, demonstrating that the 3M workers as a whole have a lower risk of all deaths compared to the Minnesota general population. In Tables 4 and 5, the SMRs are provided for the *a-priori* cancers and ischemic heart disease (IHD) and diabetes stratified by location and division. As a whole, Cottage Grove workers compared to the Minnesota population showed lower rates of all cancers selected. However, workers who ever were employed in the Chemical Division at Cottage Grove had an increased risk of prostate cancer deaths [SMR=1.18 and 95% CI (0.67, 1.91)]. The rate ratio was not statistically significant and based on few cases (16 prostate cancer deaths). In the St. Paul, referent group, kidney, breast, pancreatic and prostate cancer deaths were elevated.

Internal Analysis: Hazard Rate Ratios

The overall risk estimates for all cancer deaths evaluated were at or below no effect level based on the time-dependent cumulative and weighted estimates of dose (Table 6). Tables 7 and 8 show the risk estimates of the selected cancers, comparing all exposure groups to the St. Paul workers. The risk of death from prostate cancer increased by 22% and 27% for exposure groups 5 and 6, respectively, but the estimates were imprecise and not beyond chance. Both prostate and pancreatic cancer deaths showed a dose-response relationship, however the results are imprecise. Additionally, breast, bladder, and liver cancers were elevated comparing groups 3, 4 and 5, relative to the St. Paul workers. However, there was no apparent dose-response relationship and only four or

fewer cancer deaths resulted by group (Table 7). Table 8 data show an increase only for bladder and liver cancer deaths combining the top three exposure groups compared to the St. Paul workers (six and four cancers respectively). When lagging the two dose estimates by 10 years there were no significant changes in the results (Table 9).

We evaluated the risk of a cancer death based on the exposure groupings for two time-windows (Tables 10 and 11). We found negative associations with exposure to APFO/PFOA for many of the selected cancers, during the fourth decade of life (age 30-39 years) except there was an increase in pancreatic and prostate cancer deaths observed in the low exposure group (using the clearance weighted model). Both bladder and liver had elevated risks for the high exposure groups—these results were all imprecise. During the fifth decade (age 40-49), again bladder and liver cancer deaths were elevated for the high exposure groups, but based on small numbers (five and six deaths respectively). The risk of a prostate cancer death decreased for both low and high exposure groups in the fifth decade of life.

DISCUSSION

In this cohort we did not observe a clear association between APFO exposure and the causes of death of interest. There was no excess in mortality for all six specific cancer-related deaths when evaluating the workers' cumulative exposure over time. When examining the time-dependent APFO/PFOA exposure groupings we observed a small, but imprecise increase in risk of prostate and pancreatic deaths for the highest exposure groups compared to the St. Paul

workers. This and the SMR of 1.18 (95% CI 0.7, 1.9) for prostate cancer death in workers who had ever worked in the Chemical Division is similar to that observed in the previous mortality study of the Cottage Grove population. Bladder cancer deaths were elevated for three exposure groups compared to the referent group, but the estimates were imprecise and did not exhibit an exposure-response relationship. Death from liver cancer was inconsistent—and higher in the mid-range exposure group—group three, than in higher exposure groups, four and five. These results were based on fewer than five cancers by exposure group and therefore are limited by number. All other cancers were not associated with APFO/PFOA exposure and the results lacked statistical significance. Diabetes mortality was observed as increased in the St. Paul workers overall, and in the Chemical Division workers within Cottage Grove. When lagging the models by ten years, we observed a similar, but weaker association for all outcomes in the highest exposure groups with the exception of bladder and liver cancers.

This was the third mortality study using the Cottage Grove cohort to evaluate cancer mortality related to APFO/PFOA exposures at work; however the defined populations vary by study. The first study by Gilliland and Mandel (1993) required six months of employment for study inclusion—while the follow-up (Lundin et al, 2009) and the current studies have used a minimum of one year of employment. The current study population has more than a decade of additional working years and includes the non-occupationally exposed workers from a 3M St. Paul plant. The purpose of this inclusion was to have a referent population that represented the overall Cottage Grove workers in order to

minimize potential confounding. The St. Paul Plant employees are unionized employees working for the same company. This allowed for a more in-depth internal evaluation of cancer mortality using a group of 3M workers with a similar socio-economic status as the referent population.

When interpreting the results of this cancer mortality analysis some limitations are apparent and should be considered. Specifically, there was potential for exposure misclassification from the inhalation exposure data matrix. Exposure misclassification can depend on many factors (e.g., transient exposures) that could affect a worker's annual exposure estimates. The CCWD exposure model included the half-life value of serum-PFOA to account for retention of the chemical in an attempt to represent body burden. We did not, however, utilize blood-serum data to validate the exposure estimates. The half-life value used came from a sub-set of 3M Chemical Division workers—based on a range of half-life values (2.3-3.8 years), and we chose to use 3.5 years in our model. If a smaller half-life value had been used, the estimates from our CCWD model would have decreased more rapidly after exposure stopped, potentially altering the composition of the exposure groups.

Other limitations include the lack of information on smoking status and other potential confounders including family history of cancer, access to health care services, and other chemical exposures.

Prostate cancer was inconsistently associated with increasing exposure to APFO/PFOA from previous cancer mortality studies with this cohort. Based on past results, prostate cancer was the cause of death of most interest. In the current

study we evaluated each worker's individual APFO/PFOA exposure to determine the risk outcomes. We observed a positive dose-response relationship with increasing exposure to APFO/PFOA for prostate cancer deaths, but the risk was not beyond chance. It is established that age-related changes in the central region and the peripheral zones of the prostate show an increase in size with age (Allen et al., 1989). Since the prostate takes on physiological changes with age, exposures during certain ages may increase sensitivity and susceptibility to negative health outcomes. Our examination of specific windows of exposure did not find high exposure during these times to be associated with prostate mortality. Past studies have alluded to the causes of prostate cancer but have shown mixed results, with the exception of a few specific factors. These factors include age, family history, and race, which are all related to prostate cancer (Leitzmann and Rohrmann, 2012). In a recent meta-analysis, prostate cancer mortality was positively associated with smoking status. Smokers had a 14% increase in their risk of death compared to non-smokers (Huncharek M. et al., 2010). Other factors, such as prostate cancer screening, have an effect on early diagnosis and prognosis. Potentially, inconsistent findings in prostate mortality studies could be related to many uncontrolled variables.

Another cancer of concern that has been positively associated with APFO/PFOA exposure includes kidney cancer. Vieira et al. (2013) who studied residents living near the DuPont Teflon-manufacturing plant in West Virginia (WV) noted evidence of increasing risk for kidney cancer based on high and very high exposure categories. Additionally, an analysis of the DuPont chemical

workers evaluated the SMRs of the employees based on estimated serum-PFOA categories from a job-exposure matrix. Results showed a significant exposure-response trend for malignant and non-malignant renal disease (Steenland and Woskie, 2012). The findings from the current 3M occupational cohort do not support the community or occupational health findings in WV of a positive association with exposure to APFO/PFOA and kidney cancer mortality.

The primary strengths of this study are the size of the cohort, the almost complete case ascertainment and the use of an internal referent-working group, which helped reduce potential confounding from the healthy worker effect or dissimilar SES. Additionally, this study was the first to analyze the mortality experience of this cohort using time-varying worker exposure profiles from a recently created 3M-exposure data matrix. The validity of future cancer mortality and morbidity analyses with this population will be enhanced due to the available exposure data.

3M completely phased out the production of APFO by the end of 2002. Exposure in this setting is no longer of concern and there is limited evidence that a definitive risk of a negative health outcome exists. The results of the current study show that while a modest association is observed for prostate mortality, it is not observed across all analyses. There is not a conclusive link between APFO/PFOA and a cancer death due to the inconsistent findings depending on the chosen referent group and exposure group classifications. This ongoing work is essential to ensure the safety of workers in other locations who may continue to have relatively high occupational exposure to the chemical.

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Tables

Table 1.	Characteristics of Cottage Grove and St. Paul 3M Workers		
	St. Paul	Cottage Grove	Total Population
No. Workers	4,359	4,668	9,027
No. Male (%), Females (%)	3,833 (88%), 526 (12%)	3716 (79%), 952 (21%)	7,549 (84%), 1478 (16%)
	(range)		
Mean Age at First Employment	29 (16-63)	30 (16-73)	29 (16-73)
Mean Age at Last Employment	49 (19-77)	44 (19-92)	46 (19-92)
Mean Years Worked	20 (1-45)	14 (1-59)	17 (1-59)
Mean Person-Years	39	33	36
Mean Exit Cohort Age	68	62	65
No. of Deaths	1,834 (42%)	1,145 (25%)	2,979 (33%)

Table 2. Characteristics of APFO/PFOA Workers by Exposure Group: All Workers (N=9,027)

Exposure Group	Range APFO/PFOA mg	Age at First Work Year*	Age at Exit*	No. Years Worked *	No. Males (%)	No. Females (%)	Person Years *	No. Deaths**
1	0 to 1.0×10^{-3}	29	68	19	3,972 (88%)	546 (12%)	39	1,889 (42%)
2	$>1.0 \times 10^{-3}$ to 1.0×10^{-2}	28	67	3	257 (58%)	184 (42%)	39	118 (27%)
3	$>1.0 \times 10^{-2}$ to 1.0×10^{-1}	30	61	6	720 (74%)	248 (26%)	30	228 (24%)
4	>0.1 to 1.0	31	63	14	1,372 (80%)	338 (20%)	32	458 (27%)
5	>1.0 to 10	29	61	23	878 (87%)	127 (13%)	32	226 (22%)
6	>10	27	59	24	351 (91%)	35 (9%)	33	61 (16%)

**Mean, **Row percent*

Table 3. Number of Cancer Deaths by APFO/PFOA Exposure Group: All Workers (N=9,027)

Exposure Group	Range APFO/PFOA mg	No. Kidney Deaths	No. Bladder Deaths	No. Breast Deaths	No. Liver Deaths	No. Pancreatic Deaths	No. Prostate Deaths	Total No. Workers
1	0 to 1.0×10^{-3}	18	8	14	7	31	49	4518
2	$>1.0 \times 10^{-3}$ to 1.0×10^{-2}	0	0	1	0	1	1	467
3	$>1.0 \times 10^{-2}$ to 1.0×10^{-1}	1	2	4	4	2	3	1010
4	>0.1 to 1.0	4	4	4	3	6	9	1665
5	>1.0 to 10	1	2	2	1	8	7	1031
6	>10	0	0	0	0	0	3	376

Standardized Mortality Ratios (SMR): Selected Mortalities for Cottage Grove and St. Paul 3M Workers (N=9,027)

Table 4.

Cause	Cottage Grove			St. Paul		
	Observed	SMR	95% CI	Observed	SMR	95% CI
Cancers						
<i>All Cancers</i>	332	0.87	0.78, 0.97	514	1.04	0.95, 1.13
Kidney	6	0.53	0.20, 1.16	18	1.23	0.73, 1.95
Bladder	8	0.89	0.38, 1.75	8	0.62	0.27, 1.22
Breast	11	0.82	0.41, 1.47	15	1.39	0.78, 2.29
Liver	8	0.81	0.35, 1.59	7	0.55	0.24, 1.14
Pancreas	18	0.85	0.50, 1.34	30	1.09	0.74, 1.56
Prostate	24	0.83	0.53, 1.23	48	1.03	0.76, 1.37
Other Health						
<i>All Cause</i>	1125	0.85	0.80, 0.90	1828	0.98	0.94, 1.03
Diabetes	27	0.76	0.50, 1.11	64	1.42	1.09, 1.81
IHD	248	0.84	0.74, 0.95	443	0.95	0.86, 1.04

***Referent rates from MN. Population (1960-2007, IHD=Ischemic Heart Disease*

**Standardized Mortality Ratios (SMR): Selected Mortalities for Cottage Grove
3M Workers (N=4,668)**

Table 5.

Cause	Chemical Division-ever			Non-Chemical Division-only		
	Observed	SMR	95% CI	Observed	SMR	95% CI
Cancers						
<i>All Cancers</i>	161	0.94	0.80, 1.10	171	0.81	0.69, 0.94
Kidney	4	0.75	0.20, 1.92	2	0.34	0.04, 1.21
Bladder	3	0.73	0.15, 2.13	5	1.02	0.33, 2.40
Breast	1	0.34	0.01, 1.87	10	0.96	0.46, 1.76
Liver	4	0.9	0.24, 2.29	4	0.74	0.20, 1.89
Pancreas	7	0.73	0.29, 1.51	11	0.94	0.47, 1.69
Prostate	16	1.18	0.67, 1.91	8	0.53	0.23, 1.04
Other Health						
<i>All Cause</i>	523	0.88	0.81, 0.96	602	0.82	0.75, 0.89
Diabetes	20	1.27	0.78, 1.96	7	0.36	0.14, 0.74
IHD	110	0.83	0.68, 1.00	138	0.85	0.72, 1.01

***Referent rates from MN. Population (1960-2007), IHD=Ischemic Heart Disease*

Hazard Rate Ratios (HR) for Selected Cancer Mortalities
Table 6. All 3M Workers: Time-Dependent Estimates from PCD and CCWD Models (N=9,027)

Cause	N	HR: PCD Model	95% CI	HR: CCWD Model	95% CI
Cancers					
Kidney	24	0.66	0.28, 1.56	0.81	0.44, 1.46
Bladder	16	0.95	0.71, 1.28	0.96	0.82, 1.14
Breast	25	0.86	0.54, 1.37	0.99	0.96, 1.02
Liver	15	0.62	0.18, 2.07	0.89	0.56, 1.44
Pancreas	48	0.98	0.91, 1.05	0.99	0.99, 1.004
Prostate	72	1.00	0.99, 1.005	1.00	0.99, 1.002

**Models adjusted for first work year and gender, PCD=Potential Cumulative Dose and CCWD=Cumulative Clearance Weighted Dose*

**Table 7. Hazard Rate Ratios (HR) for Selected Cancer Mortalities
All 3M Workers: Time-Dependent Exposure Groups from PCD Model (N=9,027)**

HR: Exposure Groups vs. St. Paul Workers												
Cause	N	Group 1 (95%CI)	N	Group 2 (95%CI)	N	Group 3 (95%CI)	N	Group 4 (95%CI)	N	Group 5 (95%CI)	N	Group 6 (95%CI)
Cancers												
Kidney	0	-	0	-	1	0.33 (0.04, 2.51)	4	0.75 (0.25, 2.24)	1	0.34 (0.05, 2.56)	0	-
Bladder	0	-	0	-	2	1.89 (0.39, 9.13)	4	1.73 (0.51, 5.84)	2	1.81 (0.38, 8.69)	0	-
Breast	0	-	1	0.22 (0.03, 1.68)	4	0.94 (0.30, 2.94)	4	0.65 (0.21, 2.07)	2	1.12 (0.24, 5.31)	0	-
Liver	0	-	0	-	4	3.62 (1.04, 12.6)	3	1.32 (0.34, 5.19)	1	0.86 (0.11, 7.08)	0	-
Pancreas	1	1.10 (0.15, 8.05)	1	0.37 (0.05, 2.80)	2	0.44 (0.11, 1.85)	6	0.69 (0.29, 1.67)	8	1.80 (0.82, 3.97)	0	-
Prostate	1	0.57 (0.08, 4.15)	1	0.38 (0.05, 2.77)	3	0.53 (0.17, 1.72)	9	0.71 (0.34, 1.44)	7	1.22 (0.57, 2.61)	3	1.27 (0.30, 5.28)

**Models adjusted for first work year and gender, PCD=Potential Cumulative Dose*

**Table 8. Hazard Rate Ratios (HR) for Selected Cancer Mortalities
All 3M Workers: Time-Dependent Exposure Groups from PCD and CCWD Models (N=9,027)**

HR: High and Low Exposure Groups in Cottage Grove vs. St. Paul Workers

Cause	N	Low: PCD (95%CI)	N	Low: CCWD (95%CI)	N	High: PCD (95%CI)	N	High: CCWD (95%CI)	(St. Paul)
Cancers									
Kidney	1	0.19 (0.03, 1.43)	0	-	5	0.54 (0.19, 1.48)	6	0.54 (0.21, 1.37)	18
Bladder	2	1.03 (0.21, 4.98)	1	0.82 (0.10, 6.66)	6	1.62 (0.55, 4.79)	7	1.59 (0.56, 4.53)	8
Breast	5	0.53 (0.19, 1.50)	5	0.88 (0.31, 2.47)	6	0.71 (0.26, 1.79)	6	0.47 (0.17, 1.29)	14
Liver	4	2.13 (0.69, 7.41)	1	0.89 (0.11, 7.28)	4	1.05 (0.30, 3.67)	7	1.54 (0.53, 4.51)	7
Pancreas	4	0.50 (0.17, 1.45)	2	0.40 (0.10, 1.69)	14	0.97 (0.51, 1.84)	16	0.91 (0.49, 1.69)	30
Prostate	5	0.50 (0.19, 1.26)	2	0.30 (0.07, 1.22)	19	0.91 (0.53, 1.56)	22	0.91 (0.97, 1.03)	48

**Models adjusted for first work year and gender, **Groups 1, 2, and 3 (low) and Groups 4, 5, and 6 (high)*

Hazard Rate Ratios (HR) for Selected Cancer Mortalities with a 10-Year Lag Period
Table 9. All 3M Workers: Time-Dependent Exposure Groups from PCD and CCWD Models (N=9,027)

HR: High and Low Exposure Groups in Cottage Grove vs. St. Paul Workers

Cause	N	Low: PCD (95%CI)	N	Low: CCWD (95%CI)	N	High: PCD (95%CI)	N	High: CCWD (95%CI)	(St. Paul)
Cancers									
Kidney	1	0.34 (0.08, 1.61)	0	-	5	0.44 (0.15, 1.31)	6	0.45 (0.17, 1.23)	18
Bladder	2	1.02 (0.21, 4.92)	1	0.80 (0.10, 6.51)	6	1.63 (0.55, 4.81)	7	1.60 (0.57, 4.55)	8
Breast	5	0.52 (0.19, 1.48)	5	0.85 (0.30, 2.40)	6	0.73 (0.26, 2.01)	6	0.49 (0.18, 1.32)	14
Liver	4	2.02 (0.58, 7.13)	1	0.79 (0.10, 6.57)	4	1.09 (0.32, 3.78)	7	1.58 (0.54, 4.59)	7
Pancreas	4	0.73 (0.30, 1.78)	2	0.97 (0.37, 2.52)	14	0.84 (0.42, 1.65)	16	0.75 (0.39, 1.45)	30
Prostate	5	0.59 (0.25, 1.39)	2	0.44 (0.14, 1.40)	19	0.86 (0.50, 1.49)	22	0.87 (0.52, 1.48)	48

**Models adjusted for first work year and gender, **Groups 1, 2, and 3 (low) and Groups 4, 5, and 6 (high)*

**Table 10. Time-Windows of Exposure: Age 30-39 Hazard Rate Ratios (HR)
All 3M Workers: Exposure Groups using PCD and CCWD Models (N=9,027)**

HR: High and Very Low Exposure Groups in Cottage Grove vs. St. Paul Workers

Cause	N	Very Low: PCD (95%CI)	N	Very Low: CCWD (95%CI)	N	High: PCD (95%CI)	N	High: CCWD (95%CI)	(St. Paul)
Cancers									
Kidney	1	0.15 (0.02, 1.11)	0	-	5	0.54 (0.19, 1.47)	6	0.47 (0.19, 1.21)	18
Bladder	4	1.50 (0.44, 5.15)	3	2.17 (0.56, 8.41)	4	1.15 (0.34, 3.86)	5	1.04 (0.34, 3.24)	8
Breast	6	0.43 (0.16, 1.15)	5	0.59 (0.21, 1.65)	5	0.82 (0.28, 2.38)	6	0.51 (0.19, 1.39)	14
Liver	4	1.40 (0.38, 5.12)	2	1.26 (0.23, 6.82)	4	1.06 (0.30, 3.68)	6	1.18 (0.39, 3.57)	7
Pancreas	9	0.89 (0.41, 1.93)	8	1.56 (0.70, 3.50)	9	0.68 (0.32, 1.45)	10	0.55 (0.27, 1.15)	30
Prostate	10	0.81 (0.40, 1.62)	9	1.42 (0.66, 2.91)	14	0.66 (0.36, 1.20)	15	0.55 (0.30, 0.99)	48

**Models adjusted for first work year and gender, **Groups 1 and 2 (very low) and Groups 3, 4, 5, and 6 (high)*

**Table 11. Time-Windows of Exposure: Age 40-49 Hazard Rate Ratios
All 3M Workers: Exposure Groups using PCD and CCWD Models (N=9,027)**

HR: High and Very Low Exposure Groups in Cottage Grove vs. St. Paul Workers

Cause	N	Very Low: PCD (95%CI)	Very Low: CCWD (95%CI)	N	High: PCD (95%CI)	N	High: CCWD (95%CI)	(St. Paul)
Cancers								
Kidney	2	0.25 (0.06, 1.10)	-	4	0.50 (0.17, 1.49)	6	0.50 (0.20, 1.29)	18
Bladder	2	0.62 (0.13, 3.00)	1.13 (0.23, 5.52)	6	1.96 (0.67, 5.68)	6	1.36 (0.46, 3.98)	8
Breast	6	0.43 (0.16, 1.14)	0.39 (0.13, 1.22)	5	0.82 (0.29, 2.34)	7	0.71 (0.27, 1.83)	14
Liver	3	0.87 (0.22, 3.52)	0.51 (0.06, 4.42)	5	1.51 (0.47, 4.90)	7	1.43 (0.49, 4.22)	7
Pancreas	6	0.50 (0.20, 1.23)	0.48 (0.14, 1.60)	12	1.04 (0.53, 2.04)	15	0.87 (0.46, 1.64)	30
Prostate	6	0.39 (0.16, 0.91)	0.63 (0.25, 1.58)	18	0.98 (0.57, 1.69)	19	0.74 (0.43, 1.27)	48

**Models adjusted for first work year and gender, **Groups 1 and 2 (very low) and Groups 3, 4, 5, and 6 (high)*

CHAPTER 6: DISCUSSION

The relationship between exposure to perfluorooctanoic acid (PFOA) and its ammonium salt, ammonium perfluorooctanoate (APFO), and the risk of disease in humans has been in question for decades. It is persistent in the environment and there are many routes of low-level environmental exposure, with 98% of the US population having detectable serum-PFOA levels. Ammonium Perfluorooctanoate is a synthetic polyfluorinated chemical and in the presence of aqueous media, APFO dissolves to form PFOA [$\text{CF}_3(\text{CF}_2)_6\text{CO}_2\text{NH}_4 = \text{CF}_3(\text{CF}_2)_6\text{COOH} + \text{NH}_3$] (Griffith and Long, 1980). It is an eight-chain fully fluorinated organic compound that is thermally inert, chemically stable and used as a processing aid in the production of fluoropolymers and fluoroelastomers. Several animal studies of high level dosing have shown adverse health outcomes—including cancer of the liver, testes and pancreas—for cases compared to controls (Butenoff et al., 2004, and Biegel et al. 2001). APFO and PFOA produce a range of toxic effects in animals including: carcinogenesis, hepatotoxicity, immunotoxicity, developmental toxicity and endocrine toxicity; and it is possible that exposure may not be innocuous to humans (Biegel et al. 2001; Butenhoff et al., 2002; Griffith and Long, 1980; Kennedy et al., 2004; and Yang et al., 2001).

At the 3M Cottage Grove plant in Minnesota, APFO was formulated as a polymerization aid used in the production of high-performance materials and other consumer products from 1947-2002. In addition to background exposure,

some workers had the opportunity for high-level exposures while producing the chemical. Two previously conducted occupational mortality studies with the Cottage Grove 3M cohort have shown an association between worker's exposure to APFO and risk of prostate cancer deaths; however, these studies have had mixed results depending on the choice of referent group and have imprecise estimates due to few cases (Gilliland and Mandel, 1993 and Lundin et al., 2008). These occupational studies employed qualitative measures of exposure—assigning exposure based on the workers' job title, department or length of employment from human resource records. In the current study we explored the relationship between a vast range of more specific quantitative exposure estimates and risk of a cancer death. These APFO/PFOA exposure values were derived from air measurements used to calculate daily time-weighted exposure scenarios unique for each combination of job title, department and year. Therefore, the current study population of workers was assigned a daily exposure value for each day the person was part of the cohort. From these, we calculated an annual dose for each year the person was part of the study. This occupational study was a follow-up study with more than a decade of additional person-time. There were 675 new Cottage Gove (to bring the total number of Cottage Grove workers to 4,668) workers and for the first time the cohort included 4,359 St. Paul workers as the referent-working group to bring the total to 9,027 eligible workers. The longer follow-up period and the addition of an internal comparison group of workers decreased the potential for systematic and random error. The aim was to reduce the potential effects of confounding with the inclusion of the workers from

St. Paul due to their similar demographic characteristics and socioeconomic status. We used Cox proportional hazard models to evaluate the risk of death. We also compared the entire cohort to the state of Minnesota's population and analyzed the two locations separately and two divisions (Chemical and Non-Chemical) within Cottage Grove using standardized mortality ratios (SMRs).

Standardized Mortality Ratios

Results of this study indicate that when comparing the Cottage Grove 3M workers to the Minnesota state population, 3M workers have a lower risk of death from all *a-priori* cancers. Evaluating the two locations separately, overall we see a greater risk of dying from cause-specific cancers including; kidney, breast, pancreas, and prostate cancers only in the St. Paul population. Whereas, there was no increase in risk observed in the Cottage Grove group. The SMRs for the workers who were ever employed in the Chemical Division at Cottage Grove observed an increase in the risk of a prostate cancer death. The results were imprecise, and based on few cases.

Hazard Ratios

The internal analyses showed that the 3M workers are not at a greater risk of death for all *a-priori* cancers when evaluating the increase in APFO/PFOA time-dependent exposure as the predictor variable. However, there was an increase in risk observed with increasing exposure for pancreas and prostate cancer deaths compared to the referent group, but these were not beyond chance. We observed a greater risk for bladder and liver cancer deaths when evaluating

cumulative exposure groups for exposure only during the 4th and 5th decades of life. However, these results were based on few cases and not significant.

Limitations

We were not able to utilize blood-serum measurements due to the limited number of samples and the changes in methodology. The employee exposure profiles were calculated summing the daily mg/m³ of exposure to APFO/PFOA from the exposure data matrix. We used the annual estimates of exposure and the breathing rate to calculate milligrams (mg) of inhaled APFO/PFOA—these were a proxy for body burden. The exposure estimates included an exhaustive review of historical air measurements in addition to expert knowledge of job locations and exposure tasks by job title. However, these estimates of exposure were not real-time personal air measurements assigned to individuals in the cohort. Therefore, we have estimates of dose, which are a proxy for body burden. The relationship between serum-PFOA values and inhalation exposure was not evaluated, but could prove useful as a verification tool.

Other limitations included the inability to explore other types of cancer-specific deaths (e.g., testicular and thyroid cancers) due to the lack of power from the sample size. The analyses with cancers that have low case-fatality rates, such as prostate cancer, would be best evaluated with cancer incidence studies. Whereas, pancreatic cancer deaths are a fairly good surrogate for incidence due to its high case-fatality rate.

Bias, Study Validity and Sensitivity Analyses

Confounding can occur when there are unknown or unmeasured variables that have not been controlled in the analysis (Greenland et al., 1999; Rothman et al., 2008). Specifically, when a variable that is not controlled for is a cause of the disease and also related to the exposure of interest, then the results will not represent the true risk. There may be variables that are causal to the cancers evaluated and related to APFO/PFOA exposure in this study but were not controlled for due to the lack of information. However, we created a directed acyclic graph (DAG) to aid in the selection of potential confounding covariates in this study (Figure 1). We attempted to control for these with the selection of first year of employment as a proxy for wage status, age, number of years worked, and other SES and behavioral factors related to both the exposure and outcome.

Our main cancer of interest was prostate cancer due to findings from the previous two occupational studies with this Cottage Grove 3M cohort. Prostate cancer is the sixth leading cause of death from cancer and second most commonly diagnosed cancer in men (IARC, 2008). Several potential factors that influence risk of prostate cancer remain and these may have affected the results of the current cohort. Specifically, prostate cancer mortality has been associated with smoking status and other factors such as family history and race, which were not controlled for in the current study (Huncharek M. et al., 2010, Leitzmann and Rohrmann, 2012).

The other *a-priori* cancers evaluated have shown various environmental and genetic factors associated with increasing risk. For example, smoking is also known to be a major risk factor in the development of bladder, pancreatic, and

breast cancers. Additionally, certain germ-line mutations and racial status are risk factors for both pancreatic and breast cancers (Lowenfels and Maisonneuve, 2005). Alcohol consumption has been shown to increase the risk of several cancers including pancreatic, breast, liver, and bladder cancers (Grewal P and Viswanathen VA, 2012). As the fifth and seventh most common cause of cancer in men and women, respectively—the risk of liver cancer increases with exposure to the hepatitis B and C virus, aflatoxins and from some occupational chemical exposures, such as vinyl chloride (Cuang Sc, La Vecchia C and Boffetta P, 2009).

Selection bias can affect the study outcome when the population is not representative of the entire cohort. In epidemiological studies this includes biases that come from methods by which participants are chosen from the source population and selection or follow-up are not complete (Pearce et al., 2007). In the case of this 3M study, the inclusion criteria were a minimum of one year of employment for both locations. Therefore, the population was similar for all exposure groups and the referent population.

Information bias is the differential classification of the exposure(s) and the outcome(s), which may occur from systematic differences in the accuracy of data collected (Vandenbroucke et al., 2007). In this cohort there was potential for exposure misclassification; however the probability of exposure misclassification would not have been related to disease status and therefore non-differential. According to Pearce et al. (2007), non-differential misclassification usually biases study results towards to null and can produce false-negative findings.

Sensitivity analyses were conducted to observe the effects from potential exposure misclassification and are reported here. The magnitude and direction of the potential impact were examined relative to the defined exposure groups, and in particular the middle and highest exposure groupings. In the most recent mortality study of 3M Cottage Grove workers, Lundin et al. (2009) discussed the potential for exposure misclassification based on the three designated groupings—and in particular the workers who may have had brief but high-level exposure episodes (e.g., custodial workers or others assigned to clean-up). In this study an exposure reconstruction with quantitative data were applied, however the possibility of misclassification still looms primarily for mid-to high exposure groups, where it is possible that some workers had short-term high level exposures—such as spills—that were not fully captured. In an attempt to evaluate the significance of potential exposure misclassification and the outcome of a cancer death, we have re-parameterized workers who were part of the mid-to upper-range exposure groups into one high exposure group. The hazard ratios were re-calculated with group rankings from the cumulative estimates of exposure with a time-dependent Cox PH model and the St. Paul group was used as the referent population (Table 1). The results remained unchanged regarding the direction of the exposure-response relationship for all cancers evaluated. There was a modest increase in magnitude for kidney, bladder, breast and liver cancer deaths when evaluating the workers in the newly formed high group compared to the formerly defined high exposure group. However, only bladder and liver cancer deaths were above unity. Prostate and pancreatic cancer deaths were the

main cancers of interest based on the results from previous studies and the current study with this cohort. The results reported in the current analysis show no increase in risk due to APFO/PFOA exposure.

Another sensitivity analysis for the current paper includes the exploration of the intensity of exposure, which was based on how quickly a worker achieved a high-level cumulative exposure. We evaluated three dose-rate groups in order to determine if high, yet relatively short-term doses of APFO/PFOA were closely associated with the risk of disease compared to low-dose chronic exposure. This could provide insight into effects of workers who may be grouped into a low or medium cumulative exposure group, yet may have had higher body burden due to one or many short-term intense exposure scenarios. We divided the total cumulative exposure estimates by the number of years worked to get the dose-rate groups. This evaluation was completed with a LOGISTIC procedure in SAS 9.2 and stratified on groups of “total years worked”. The three groups included workers who had; $1 < 10$ years, $10 < 25$ years, and ≥ 25 years of work. We were able to compare workers in similar work-length groups to one-another and focus on the intensity of exposure per group in an attempt not to introduce confounding. Table 2 shows the number of workers per dose-rate group, and table 3 provides the number of deaths for each cancer by dose-rate groups. Table 4 provides the Odds Ratios (OR) for each cancer by dose-rate group with group 1 as the referent group. Overall, we observed a non-significant greater risk of bladder cancer death for both the middle and high (groups 2 and 3) dose-rate groups compared to the referent group. Both breast and liver cancer deaths show a greater risk of death

only for the middle dose-rate group. Results are not statistically significant—and based on few cases.

FUTURE RESEARCH

Many new ideas surfaced throughout the development of the research hypothesis in this study. The primary need for future research is a cancer incidence study, specifically for prostate cancer. Although all three studies evaluating cancer mortality with this cohort indicate a positive trend for prostate mortality with increased exposure to APFO/PFOA, the results are contingent upon the referent group and are marked by sparse data. Prostate cancer has a low case-fatality rate (unlike pancreatic and other cancers), and it would be valuable to conduct an incidence study using the current exposure data. Another cancer associated with PFOA from toxicological data suggests testicular cancer increases with exposure. Due to small numbers, these analyses were not feasible to conduct in the current study and would be of use as well in a future cancer incidence evaluation. Outcomes on cancer incidence related to time-dependent exposure for diseases with low case-fatality rates are important in the determination of causality. In addition, a cancer incidence survey would be useful to verify the family history of disease, wage status, alcohol consumption, and smoking status—unknown variables in the current research and all important contributing factors to specific cancers.

A future analysis may include a different choice of the serum-PFOA half-life value. We chose to use the geometric mean of 3.5 years. This figure was taken from a study result based on data from a group of APFO/PFOA chemical

workers at the Cottage Grove location conducted by Olsen et al. (2007). To investigate the potential impact of a shorter half-life (the range was 2.3 to 3.8 years), it would be of value to re-assess the risk of death in the cohort after recalculating annual exposure estimates with an alternative half-life in place of 3.5 years (e.g., 2.8 years—the lower value within the range). A shorter half-life increases the difference between the primary cumulative model estimates and the annual estimate of the elimination model estimates. Changes in the exposure group distribution would be observed and the elimination of APFO at a quicker rate would decrease the annual values more rapidly with time. Another development is with the use of biological sampling data, specifically serum-PFOA measurements of the workers. There were limited data available; however this could be used in the future to validate findings with a sub-set of the cohort. Finally, the most pressing finding from the mortality analyses in the current study is the modest positive dose-response relationship for prostate and pancreatic cancer deaths found with increasing exposure to APFO/PFOA by groups. It would be useful to evaluate the significance of high exposure groups relative to the lowest exposure group and the impact of a negative health outcome—both with a prostate cancer incidence study and a follow-up mortality study.

CONCLUSION

The long-term goal for this research was to evaluate health outcomes from occupational exposure to APFO/PFOA in a group of 3M workers. Moreover, the purpose of this study was to examine the relationship between APFO/PFOA exposure and the risk of death from six *a priori* selected cancers; bladder, breast,

kidney, liver, pancreatic and prostate. To achieve this goal, we evaluated cancer mortality from an occupational cohort with employees who had a broad range of APFO/PFOA exposure estimates over the course of more than half a century, and compared these workers to other demographically similar workers who never directly worked with APFO/PFOA. The exposure estimates were calculated for each worker by using a historical exposure reconstruction matched to the workers' employment history records. This allowed for a robust, exposure profile specific to each worker for the entire length of each individual's follow-up. To ascertain the risk of cancer mortality we explored the relationship between the time-dependent cumulative annual estimates by linking individuals with their mortality experience from death records. The 3M occupational retrospective cohort included all workers with a minimum of one year of work beginning in 1947 and follow-up time ending in 2008. The findings indicating that an increase in occupational exposure to APFO/PFOA increases the risk of cancer mortality were not beyond chance. The overall SMR for the entire cohort was below unity and statistically significant 0.93 (95% CI: 0.90, 0.97) demonstrating that the 3M workers as a whole have a lower risk of all deaths compared to the Minnesota general population. The two sensitivity analyses showed no change in the direction of the outcome, only small changes in magnitude, in particular for bladder and liver cancer deaths. The overall synthesis of analyses and conclusions reported herein shows a modest non-significant trend with increasing exposure groups and prostate cancer deaths. This and the SMR of 1.18 (95% CI 0.7, 1.9) from the sub-group of workers who ever worked in the Chemical

Division compared to the Minnesota state population support the outcome of the previous mortality study with this cohort.

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Tables

Hazard Rate Ratios (HR) for Selected Cancer Mortalities
Table 1. All 3M Workers: Time-Dependent Exposure Groups from PCD Model (N=9,027)
HR: Med-High and Low Exposure Groups in Cottage Grove vs. St. Paul Workers

Cause	N	Very Low: (95%CI)	N	Low: (95%CI)	N	Med-High: (95%CI)	N	High:(95%CI)	(St. Paul)
Cancers									
Kidney	0	-	1	0.19 (0.03, 1.43)	6	0.49 (0.19, 1.26)	5	0.54 (0.19, 1.48)	18
Bladder	0	-	2	1.03 (0.21, 4.98)	8	1.69 (0.62, 4.60)	6	1.62 (0.55, 4.79)	8
Breast	1	0.20 (0.03, 1.49)	5	0.53 (0.19, 1.50)	10	0.81 (0.34, 1.93)	6	0.71 (0.26, 1.79)	14
Liver	0	-	4	2.13 (0.69, 7.41)	8	1.64 (0.58, 4.63)	4	1.05 (0.30, 3.67)	7
Pancreas	2	0.56 (0.13, 2.40)	4	0.50 (0.17, 1.45)	16	0.84 (0.45, 1.56)	14	0.97 (0.51, 1.84)	30
Prostate	2	0.45 (0.11, 1.87)	5	0.50 (0.19, 1.26)	22	0.83 (0.47, 1.38)	19	0.91 (0.53, 1.56)	48

**Models adjusted for first work year and gender,*

***Original Groups 1, 2 and 3 (low)*

Groups 1 and 2 (very low)

****Original Groups 4, 5, and 6 (high),*

Groups 3, 4, and 5 (med-high)

Table 2. 3M Workers: APFO/PFOA Dose-Rate Groups (N=9,027)

Work-Year Groups	Number of Workers by Groups		
	Group 1: (< 0.00001) mg/year	Group 2: (=> 0.00001 < 0.01) mg/year	Group 3: (=> 0.01) mg/year
Group 1: (1< 10 years)	258	1687	1310
Group 2: (10< 25 years)	1331	280	1005
Group 3: (=>25 years)	2016	110	1031

Table 3. 3M Workers: Number of Cancer deaths by Intensity of Exposure (N=9,027)

Dose-Rate Groups			
Cause	Group 1	Group 2	Group 3
Cancers			
Kidney	1	3	4
Bladder	7	4	5
Breast	12	8	5
Liver	6	6	3
Pancreas	27	9	12
Prostate	47	11	14

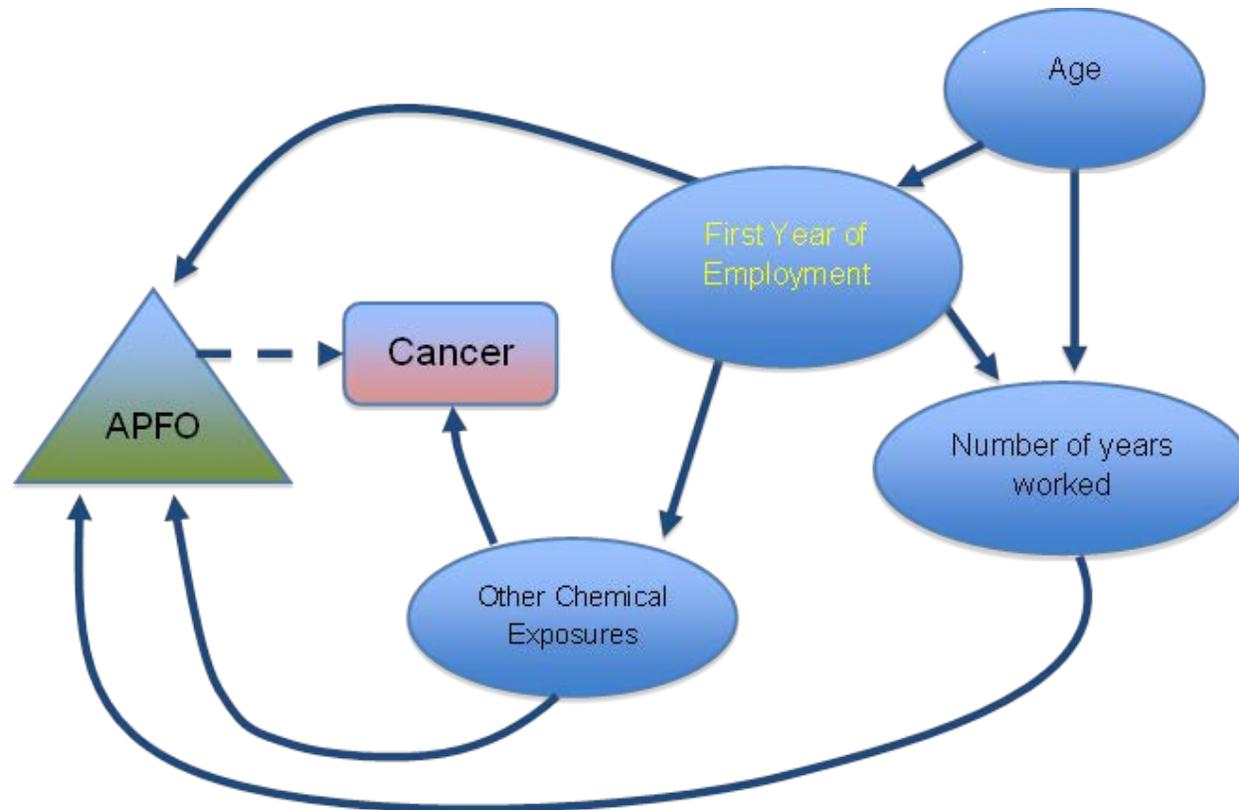
Odds Ratios for Selected Cancer Mortalities
3M Workers: Dose-Rate Exposure Groups from PCD Model

Table 4. **OR: Dose-Rate Groups vs. Lowest Dose-Rate Group**

Cause	N	Group 2 (95%CI)	N	Group 3 (95%CI)
Cancers				
Kidney	3	0.19 (0.04, 0.88)	4	0.27 (0.08, 0.88)
Bladder	4	1.95 (0.42, 9.04)	5	1.58 (0.48, 5.20)
Breast	8	1.11 (0.33, 3.81)	5	0.65 (0.19, 2.18)
Liver	6	2.35 (0.56, 9.82)	3	0.81 (0.19, 3.45)
Pancreas	9	1.0 (0.39, 2.58)	12	1.0 (0.51, 1.98)
Prostate	11	1.0 (0.41, 2.44)	14	1.0 (0.58, 1.73)

**Models adjusted for first work year and gender,*

Figure 1. Directed Acyclic Graph: Prostate Cancer



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