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Vaughn Barry Date

The effect of perfluorooctanoic acid (PFOA) on health outcomes in an exposed community

By

Vaughn Barry, MPH

Doctor of Philosophy

Epidemiology

_____

Kyle Steenland, Ph.D.
Advisor

_____

Lyndsey Darrow, Ph.D.
Committee Member

_____

Mitchel Klein, Ph.D.
Committee Member

_____

Andrea Winquist, M.D., Ph.D.
Committee Member

Accepted:

_____

Lisa A. Tedesco, Ph.D.
Dean of the James T. Laney School of Graduate Studies

_____

Date

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By

Vaughn Barry

M.P.H., University of Minnesota, 2006

B.A., Mount Holyoke College 2002

Advisor: Kyle Steenland, Ph.D.

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Abstract

The effect of perfluorooctanoic acid (PFOA) on health outcomes in an exposed community

By Vaughn Barry

Perfluorooctanoic acid (PFOA) is a synthetic chemical used in manufacturing processes. It is found at low levels in the serum of most people in the U.S. with higher levels often observed in occupationally exposed workers. Animal studies suggest PFOA can cause adverse health events - including liver, testicular, and pancreatic tumors - but human health effects are unclear.

DuPont chemical plant in Washington West Virginia released PFOA into the Ohio River and air over a fifty year period. Residents living near the plant filed a class action lawsuit in 2001 alleging health damage due to PFOA contaminated drinking water. A pretrial settlement required DuPont to provide funding for several health surveys conducted among community residents and DuPont workers during 2005-2011.

This dissertation assessed whether PFOA was associated with certain health outcomes in this mid-Ohio valley population and explored how results could be impacted by diseases with different survival patterns.

Three manuscripts were developed from the work conducted in this dissertation. The first manuscript describes the association between estimated lifetime PFOA exposure and cancer incidence in this population. The second manuscript describes conditions for bias in exposure-disease estimates in this survivor cohort. The third manuscript describes whether PFOA exposure in early life was associated with overweight and obesity risk in adulthood.

Results suggest that PFOA may cause kidney and testicular cancer. The hazard ratios and 95% confidence intervals for incident kidney and testicular cancers were 1.10 (0.98 - 1.25) and 1.34 (1.00 - 1.79) for each 1-unit increase in ln-transformed estimated serum PFOA. Simulated data indicated that survivor bias existed when time to death after disease differed between those with low and high exposure. In this situation, bias was greatest when the disease of interest was highly fatal. High levels of PFOA exposure experienced during the first three years of life were not associated with overweight and obesity risk in adulthood and results did not vary by sex. Findings suggest that PFOA may be associated with particular adverse health events.

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CHAPTER 1: OVERVIEW AND MOTIVATION

Overview

Perfluorooctanoic acid (PFOA, or C8) is a synthetic chemical used since the late 1940's in manufacturing. It is ubiquitous in serum of most people living in the U.S. It has a relatively long half-life in humans (estimated 2-4 years), does not breakdown in the environment, and even though it has been phased out of manufacturing over the past few years, serum levels have not significantly decreased. It is unclear whether PFOA poses health risks to humans or not. It has been linked to kidney cancer mortality in workers and testicular tumors in mice. The Science Advisory Board to the U.S. Environmental Protection Agency has concluded that PFOA is "likely carcinogenic". There is growing concern that exposure to PFOA during important developmental periods in early life may be associated with overweight and increased adiposity in adulthood. Previous studies have typically been limited to animal experiments and studies of male occupationally exposed workers. The differing study designs, outcomes, and participants make it challenging to firmly conclude what the relationship is between PFOA and health.

DuPont chemical plant in Washington, West Virginia released PFOA into the Ohio River and air over a fifty year period. PFOA in the river contaminated the drinking water of several nearby communities. In 2001, community residents living near the chemical plant filed a class action lawsuit alleging health damage due to PFOA contaminated drinking water. A pre-trial settlement in 2005 resulted in a community-wide health study called the "C8 Health Project". The C8 Health Project surveyed 69,030 residents and collected information about residents' drinking water consumption, residential history, and health. Lifetime PFOA exposure was estimated for each participant in the C8 Health Project. Data from the C8 Health Project were used to examine the relationship between PFOA and cancer and obesity risk in this mid-Ohio

valley population. Simulations were run to explore and quantify the magnitude of possible bias in the study results.

Study Motivation

Due to its wide-spread environmental buildup, expected bioaccumulation, potential toxicity, and possible relationship with adult adiposity, there is rising concern about whether PFOA is associated with cancer and obesity in humans (Steenland et al. 2010; Environmental Protection Agency 2005; Environmental Protection Agency 2006; Halldorsson et al. 2012). The study design and methodology used to investigate these concerns may influence the amount of bias present in the results for different health outcomes.

Previous research on PFOA and cancer has been primarily restricted to animal experiments, mortality studies of male workers with occupational exposure, and community studies of populations with low exposure levels. Human studies have been limited by small numbers of cancer cases. Dissertation study 1 will examine PFOA and cancer incidence in a large community (n=32,254) with a range of exposure levels. Over 2,500 validated cancers covering 21 different cancer types were included in the analysis, making it one of the largest cohorts ever used to examine PFOA and cancer.

This is largely a survivor cohort: the cohort is mostly comprised of participants who had to be alive in 2005-2006 to enroll in the C8 Health Project. If participants who were more highly exposed to PFOA were less likely to enroll in the study, results could be impacted. Dissertation study 2 will examine the magnitude of bias associated with diseases with varying fatality patterns in the context of survivor cohorts.

Prior research examining the relationship between PFOA exposure in early life and overweight and obesity risk in adulthood are sparse because of the long follow-up time needed.

The few studies investigating the relationship have been limited to general populations with low PFOA exposure levels. Dissertation study 3 will examine whether PFOA exposure experienced during the first three years of life is associated with adult overweight and obesity risk in participants with a range of exposure levels.

Specific Aims of Dissertation

The overall purpose of this dissertation is to examine the possible effects of PFOA on cancer and obesity risk in this exposed mid-Ohio valley population and explore the amount of bias possible in the results. Specifically,

1. Determine whether PFOA is associated with incident cancer among this mid-Ohio valley population living near DuPont's Washington Works chemical plant.
2. Use Monte Carlo simulations to examine the presence and magnitude of bias associated with diseases with varying fatality patterns in the context of this study.
3. Determine whether PFOA exposure in early life is associated with adult overweight or obesity risk.

CHAPTER 2: BACKGROUND AND LITERATURE REVIEW

Perfluorooctanoic Acid

Perfluorooctanoic acid (PFOA, or C8) is a synthetic chemical used since the late 1940's in manufacturing. Its chemical properties make it stable in air at high temperatures, nonflammable, and not easily degraded. It is widely used in the production of water and stain-resistant clothing, upholstery, and carpet including Gore-Tex and Teflon as well as in the production process of industrial products and fluoropolymers. It is persistent in the environment and has been detected in people, animals, oceans, groundwater, surface water, and soils around the world (Lau et al. 2007; Olsen et al. 2007a; Seals et al. 2011; Yamashita et al. 2005).

PFOA is found in the serum of most people living in the US with higher levels observed in occupationally exposed workers (Calafat et al. 2007b; Lau et al. 2007). The geometric mean PFOA serum concentration in the U.S. population 12 years of age and older in 2007-2008 was 4.1 ng/mL (Kato et al. 2011). Serum levels in occupationally exposed workers have been reported to range from 350 ng/mL to 1100 ng/mL (Steenland et al. 2012; Olsen et al. 2007b). PFOA exposure varies to some extent by age, sex, and race. PFOA levels are often higher among those who are young or old (compared to middle aged), males (compared to females), those who are of white race (compared to other races), and those with higher education (Steenland et al. 2010; Calafat et al. 2007a). PFOA has a long serum half-life in humans estimated to range from 2.3 to 3.8 years (Olsen et al. 2007a).

Although PFOA in US manufacturing has been phased out over the past five years, PFOA serum concentrations have not considerably decreased. The geometric mean of PFOA concentration in serum (ng/mL) for the U.S. population 12 years of age and older for the years 1999-2000, 2003-2004, 2005-2006, and 2007-2008 were 5.21, 3.95, 3.92, and 4.13 (Kato et al. 2011).

Exposure sources in the general population are unclear but are assumed to include drinking water and also possibly food packaging, household products, and air (Lau et al. 2007). Exposures in highly exposed populations are thought to be primarily from drinking water (Emmett et al. 2006).

Because PFOA is ubiquitous in the serum of most people in the U.S. and because it is detected globally, efforts have increased over the past few years to better understand any possible hazards it may pose to human health.

PFOA and Health Outcomes

The influence of PFOA on specific health effects is not well established. PFOA causes testicular, liver, and pancreatic tumors in exposed rats but not in monkeys (Biegel et al. 2001; Butenhoff et al. 2002). It causes neonatal death and low birth weight in mice (Lau et al. 2007). PFOA exposure in rats causes spleen and thymus atrophy, hepatomegaly, and decreases cholesterol levels. Results from animal studies may not be generalizable to humans, particularly because the estimated PFOA half-life in rodents is much shorter than in humans (days in rodents compared to years in humans). Studies using rats suggest that PFOA is present primarily in the liver, kidney, and blood (Kennedy et al. 2004; Lau et al. 2007).

Epidemiologic studies examining PFOA exposure and health effects in humans are sparse and results are often modest and conflicting. PFOA was associated with kidney cancer mortality among a group of occupationally exposed male workers, and possibly pancreatic and prostate cancer mortality, too (Lundin et al. 2009; Leonard et al. 2008; Steenland et al. 2012). Some studies have found an association between PFOA exposure and low birth weight (Maisonet et al. 2012; Apelberg et al. 2007; Fei et al. 2007) but other studies show no association with low birth weight (Darrow et al. 2013; Stein et al. 2009; Savitz et al. 2012a; Savitz et al. 2012b). PFOA

may be associated with higher cholesterol (Sakr et al. 2007a; Sakr et al. 2007b; Steenland et al. 2009b), higher uric acid levels, and impaired liver function (Olsen et al. 2007b).

The differences in study designs, exposure measurements, and study populations are important to consider when reviewing the literature about PFOA and health outcomes. Many studies are cross-sectional which prevent causal conclusions. Others measure PFOA in serum only once or have small numbers of cases. Most studies are of occupationally exposed workers. These studies are usually limited to male participants and are not powered to study rare diseases. It is challenging to consider measuring the lifetime PFOA exposure any person may have, especially if PFOA exposure can vary over time.

As part of a class action lawsuit in the mid-Ohio valley, a panel of scientists was tasked with determining whether PFOA was more "probably linked" than not to several health outcomes among residents of the mid-Ohio valley. On the basis of their own studies in the region as well as the prior literature, the panel made "probable link" judgments on 55 diseases between December 2011 and October 2012. They reported probable links between PFOA and preeclampsia, ulcerative colitis, thyroid disease, testicular cancer, kidney cancer, and high cholesterol. They did not report any probable links between PFOA and any of the 49 other examined health outcomes including birth defects, miscarriage, kidney disease, liver disease, diabetes, coronary heart disease, hypertension, and the other 19 analyzed cancers.

PFOA and Cancer

PFOA induced liver, testis, and pancreatic tumors in male rats over a 2 year period (Biegel et al. 2001). A similar study that fed both male and female rats varying amounts of PFOA over a 2 year period found that PFOA caused an increased incidence of testicular adenomas in the male rats (Sibinski 1987). However, there was no evidence of hepatocellular, testicular, or pancreatic tumors in male cynomolgus monkeys exposed to PFOA for 26 weeks and observed for 90 days after exposure (Butenhoff et al. 2002). The PFOA-exposed monkeys were

more likely to have hepatomegaly suggesting that the liver is the primary target organ in male monkeys (Butenhoff et al. 2002).

The biologic mechanisms by which PFOA caused rat tumors and the pertinence of the animal findings to humans are unclear. PFOA activation of peroxisome proliferator receptors may cause liver tumors in rats (Kennedy et al. 2004) and PFOA-induced increases in serum estradiol levels (Biegel et al. 2001) may have caused testicular tumor growth. The exact role these processes may play with regard to human cancer is unclear (DeWitt et al. 2009; Koeffler 2003; Suchanek et al. 2002). Pancreatic acinar tumors are uncommon in humans so it is uncertain whether the PFOA relationship with pancreatic tumors seen in rats is generalizable to humans.

Several animal studies have examined the relationship between PFOA and mammary tumors with inconclusive results. The study that fed male and female rats PFOA over a 2 year period found a greater incidence of mammary gland fibroadenomas in the female rats in the high PFOA-dose group compared to the low-dose group and female control rats (Sibinski 1987). However, there was controversy regarding the choice of the control groups used in this particular study. A pathology working group independently re-reviewed the study data and concluded that PFOA did not increase mammary gland neoplasms in the study (Hardisty et al. 2010). A different study of mice found that female mice with gestational exposure to PFOA had altered mammary gland development after birth (White et al. 2006, White et al. 2009). It is unclear whether this has anything to do with tumor production in mice later in life but it does suggest that the mammary gland is sensitive to PFOA exposure.

Human studies examining the association between PFOA and cancer are mostly limited to mortality studies of occupationally exposed workers with few cancer deaths. One study followed workers employed at a Minnesota PFOA production plant between 1947 and 1997 (Lundin et al. 2009, Gilliland et al. 1993). These investigators found some positive trends for

prostate and pancreatic cancer across job categories with increasing PFOA exposure, but these trends were based on only 16 and 13 deaths respectively.

A second mortality study followed workers who had ever been employed between 1948 and 2002 at the same DuPont Washington Works plant considered here (Leonard et al. 2008). The authors found that plant workers had a non-significant approximately two-fold elevated risk of kidney cancer mortality compared with other regional DuPont workers (Standardized Mortality Ratio (SMR)=181.0, 95% CI=93.5, 316.2). This study was recently updated by Steenland et al. (Steenland et al. 2012), who found a significant increase in kidney cancer mortality with increasing estimated cumulative PFOA serum concentrations among the workers at the DuPont Washington Works plant compared to other regional DuPont workers, based on small numbers. SMRs (and 95% confidence intervals) by increasing exposure quartile were 1.07 (0.02, 3.62), 1.37 (0.28, 3.99), 0 (0, 1.42) and 2.66 (1.15, 5.24), based on 12 kidney cancer deaths (p=0.02, trend test).

There are two PFOA-cancer incidence studies among general populations (Bonefeld-Jorgensen et al. 2011; Eriksen et al. 2009). PFOA levels are typically low and widespread in general populations. There were no strong associations found in these studies. One measured PFOA in the serum of 57,053 Danish citizens and then followed them for 10 years for cancer incidence (Eriksen et al. 2009). The authors reported a borderline significant positive trend between PFOA level and prostate cancer (p=0.06) but with none of the other cancers examined. A case-control study measured PFOA in serum of an Inuit population that traditionally has high PFOA levels and found no relationship between PFOA and breast cancer (Bonefeld-Jorgensen et al. 2011).

Potential for Survivor Bias when Examining PFOA and Cancer Risk

When study participation is dependent on survival to a certain time point after disease risk has already begun, measures of effect describing the relationship between exposure and disease onset may be subject to bias. This concern about bias stems from the fact that some exposed subjects may develop disease and subsequently die before study enrollment begins. Selection bias could occur if those that die before study enrollment are different than subjects that survive long enough to enroll. It is natural to assume that the presence and magnitude of this bias may be a function of the specific survival pattern associated with the disease outcome of interest. For example, a non-fatal disease outcome could mean that everyone survives to enroll which would presumably result in no survivor bias in the study's exposure-disease estimates. However, there may be times where study estimates are unbiased even when many subjects die before study enrollment and are thus excluded from the study. Disease fatality and the specific relationships between the exposure, disease, and death may all be factors that can determine whether bias is induced or averted in studies where participation is dependent on survival. Our focus is on examining the presence and magnitude of bias associated with diseases with different survival patterns in these survivor cohorts.

PFOA in Early Life and Adult Obesity

Research from the growing fetal origins of adult disease field suggests that the body's organs and systems are malleable while developing and are particularly susceptible to the environment experienced while in utero and during the first few years of life (Barker 2004; Lynch et al. 2005; Newbold et al. 2007; Oken et al. 2003). One hypothesis that has emerged over the last ten years suggests environmental causes could play a role in obesity development, especially exposure to environmental chemicals during early life (Baillie-Hamilton 2002; Grun 2010).

Infants and young children may be particularly vulnerable to PFOA's effects because per body weight, they consume more fluid than adults and thus have higher relative exposure for their size (Post et al. 2012). Infants and toddlers often receive exposure from breast milk from mothers

who drink contaminated water and/or from formula prepared with contaminated drinking water. In a large mother-child pair study, PFOA serum levels in children under age 5 years were 44% higher than maternal PFOA levels (Mondal et al. 2012). Additionally, PFOA serum levels are higher in children than adults, both in the general population and in highly exposed populations (Calafat et al. 2007a; Steenland et al. 2009a).

There are only two studies that examine possible effects of early life PFOA exposure on adult adiposity. One animal study found that mice exposed to low doses of PFOA while in utero were overweight in mid-life compared to both unexposed and highly exposed mice (Hines et al. 2009). The same study also reported no mid-life effect when the same low-dose PFOA regimen was instead administered to mice in young adulthood.

In an epidemiologic study, researchers measured PFOA in the serum of 665 pregnant women recruited from the general population in Denmark and then measured the BMI of the offspring 20 years later (Halldorsson et al. 2012). Twenty-year-old women who had been more highly exposed to PFOA in utero (PFOA serum concentrations of 4.8 - 19.8 ng/mL in the pregnant mother) were more likely to be overweight or obese compared to their less exposed counterparts (PFOA serum concentrations of 0.1 - 4.8 ng/mL in the pregnant mother). Adjusted relative risks and 95% confidence intervals by increasing PFOA quartile were 1.0, 1.3 (0.6 - 2.8), 1.7 (0.8 - 3.5), and 2.5 (1.3 - 5.0). There was no relationship between *in utero* PFOA exposure and adult overweight or obesity in the twenty-year-old men (all relative risks = 1.0). Participants in this study had low PFOA levels typical of general populations limiting the reported dose-response relationship to a narrow window of exposure levels.

It has been hypothesized that early life PFOA exposure may affect adult weight by permanently altering weight controlling hormones, perhaps by its known activation of the peroxisome proliferator-activated receptor alpha, which is a hormone receptor that plays a role in metabolism. A relatively newer hypothesis is that PFOA may alter hormone levels through

ovarian effects and consequently women may be more susceptible to the effects than men (White et al. 2011; Zhao et al. 2010).

CHAPTER 3: STUDY POPULATION

DuPont Chemical Plant

The DuPont chemical plant in Washington, West Virginia began using PFOA in its manufacturing process in 1951. The plant released PFOA into the Ohio River and air beginning in the 1950s, peaking in the 1990s, and decreasing after 2001. PFOA emitted from the plant entered the groundwater which was the public drinking water source. In 2001, residents living near the chemical plant filed a class action lawsuit alleging health damage due to PFOA contaminated drinking water. A pretrial settlement required DuPont to provide funding for an independent community health study called the "C8 Health Project" (C8 Health Project 2012; Frisbee et al. 2009), and also resulted in the creation of the C8 Science Panel (C8 Science Panel 2012) tasked with determining whether there was a probable link between PFOA and disease in the community living near the plant. The members of the Science Panel were chosen jointly by the lawyers for the community residents and DuPont and their work and conclusions are independent of either party to the lawsuit. The Science Panel members include Drs Kyle Steenland (Emory University), Tony Fletcher (London School of Hygiene and Tropical Medicine), and David Savitz (Brown University).

C8 Health Project

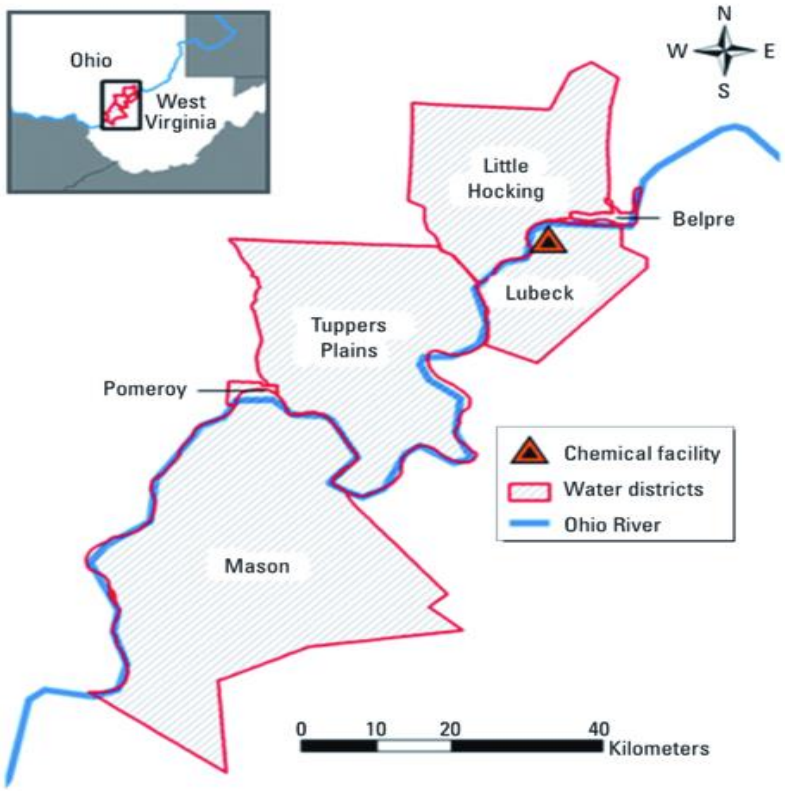
The C8 Health Project was designed to investigate the effect of PFOA in this community. The C8 Health Project enrolled 69,030 people between August 2005 and August 2006. Participants were eligible if they lived, worked, or attended school for at least one year in one of six contaminated water districts near the DuPont chemical plant between 1950 and December 3rd, 2004 (Frisbee et al. 2009). The estimated participation rate was high (81% among current

residents age 20 years and older) likely due to the significant publicity surrounding the circumstances (Steenland et al. 2009a).

Figure 3.1 shows the six water districts near the plant that were found to have PFOA drinking water contamination attributed to industrial releases from the DuPont plant (Frisbee et al. 2009; Steenland et al. 2009a). The water districts are all situated near the DuPont plant and located on the Ohio River (either on the Ohio or West Virginia sides).

Figure 3.1 Six contaminated water districts of the C8 Health Project

(Steenland K, Jin C, MacNeil J, Lally C, Ducatman A, Vieira V, et al. 2009. Predictors of PFOA levels in a community surrounding a chemical plant. Environ Health Perspect 117(7):1083-1088)



C8 Health Project participants took a survey where they reported demographic and health characteristics as well as an extensive residential history. They also gave blood samples for PFOA serum concentration measurements. PFOA levels measured in the C8 Health Project participants during 2005-2006 were eight times the level found in the general population. PFOA levels varied by water district and decreased going downstream: Little Hocking water district had the highest PFOA levels, followed by Lubeck, then Tuppers Plains and Belpre, then Pomeroy and Mason County.

Subsequent Surveys

To ascertain more recent and potentially incident health outcomes in this community, subsequent surveys were conducted between August 2008 and May 2011 on adult C8 Health Project participants and on a group of DuPont workers. Most C8 Health Project participants age 20 years and older consented to and participated in at least one of the follow-up surveys. Follow-up survey participants reported demographic and health characteristics and most consented to have their medical charts reviewed by the study investigators. A cohort of 32,254 adult worker and resident participants who completed at least one subsequent survey was assembled (Winquist et al. 2012). Figure 3.2 shows the enrollment of study participants.

Figure 3.2 Enrollment of study participants (Winquist A, Lally C, Shin H-M, Steenland K. 2013. Design, methods, and population for a study of PFOA health effects among highly exposed mid-Ohio valley community residents and workers. Environ Health Perspect 121(8):893-899.)

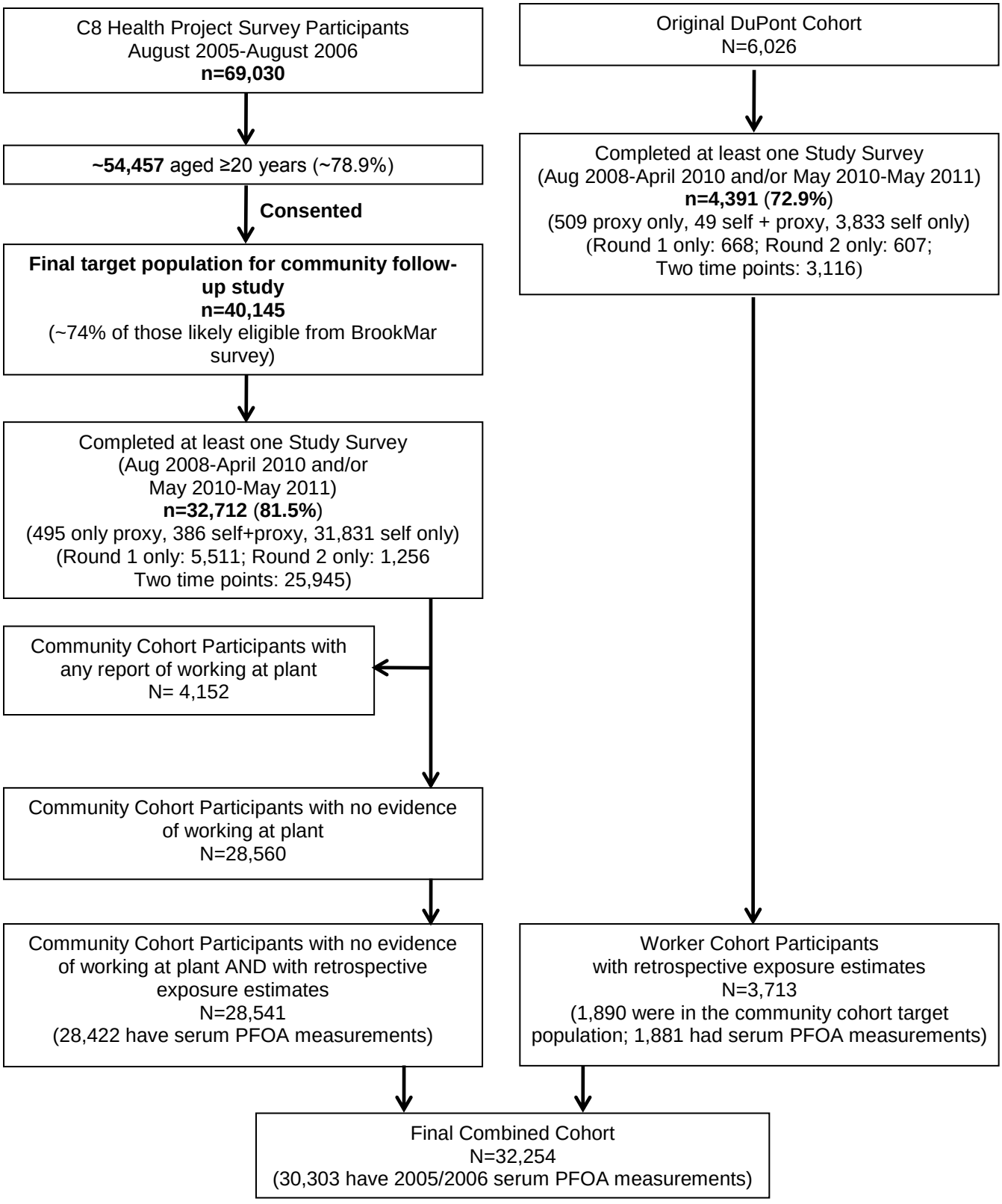
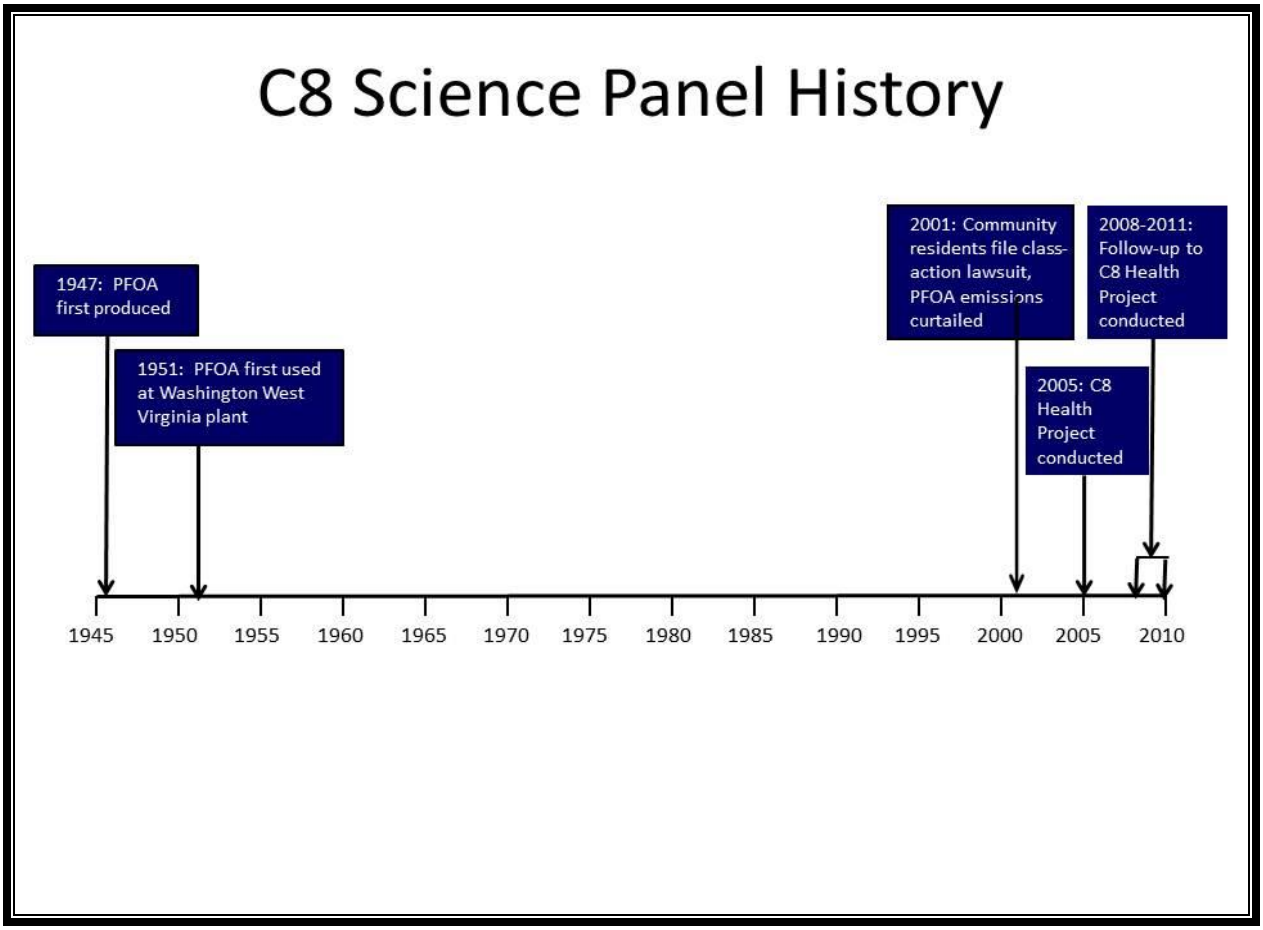


Figure 3.3 shows the timeline of the emissions and studies over time.

Figure 3.3 Timeline of PFOA emissions and studies



CHAPTER 4: METHODS

PFOA Estimation Procedure

Annual retrospective PFOA serum concentrations were estimated for each participant for each year of their life beginning in 1952 or the participant's birth year, whichever was most recent, through 2011 (Shin et al. 2011b). Estimates accounted for exposure received from nearby chemical plant emissions and also for background exposure not originating from the facility. Estimates were based on historical regional data including the PFOA amounts emitted by the DuPont facility, wind patterns, river flow, and groundwater flow (Shin et al. 2011a). Exposure estimates took into account the participant's reported residential history, drinking water source, tap water consumption, work place water consumption, and a PFOA absorption, distribution, metabolism, and excretion model. The exposure estimates for participants who had ever worked at the DuPont plant took into account occupational exposure they may have received at their specific job (Woskie et al. 2012). Exposure estimates in early life took into account approximated maternal PFOA serum level estimates because PFOA serum concentration in young children is strongly correlated with maternal serum concentration (Mondal et al. 2012).

Health Outcomes

Participants were asked in the follow-up studies "Have you ever been told by a doctor or other health professional that you had cancer or a malignancy of any kind?" Participants could report all cancer types and also reported the age of diagnosis for each cancer type. Self-reported cancer diagnoses were confirmed though medical records review and state cancer registry matching. The confirmation process also verified that the diagnosed cancer was a primary cancer and not a metastasis of another cancer.

Participants also reported height and weight. Participants were asked "how tall are you, to the nearest inch, without shoes?" and "how much do you weigh now, to the nearest pound, without shoes?" Pregnant women were asked to report pre-pregnancy weight. Body mass index (BMI) was calculated by dividing weight in pounds by height in inches squared multiplied by 703.

Analysis Methods

Dissertation study 1 will use proportional hazards regression models with a time-varying exposure to determine whether cumulative PFOA exposure is associated with incident cancer. A model will be run for each cancer type with time to cancer diagnosis as the outcome and cumulative PFOA as the exposure. Time for each person will begin at age 20 years if the person's 20^{th} birthday was in 1952 or later. Otherwise, time will start at the age the person was in 1952. Time will end at age of cancer diagnosis, age at the time of the last follow-up survey, or age at death (if died), whichever came first. PFOA exposure is time-dependent since each participant has an estimated PFOA exposure measurement for each year of life.

Table 4.1 shows the data layout for the regression models. The number of observations per participant varies depending on the participant's birth year and survey year or diagnosis year. The maximum number of exposure measurements a person can have is 60 which would represent annual exposure measurements for somebody born before 1952 who participated in a follow-up survey in 2011.

Table 4.1: Data layout for proportional hazards regression modeling

ID	AGE	YEAR	PFOA EXPOSURE
1	2	1952	0.0001
1	3	1953	0.0004
1	4	1954	0.0008
…	…	…	…
1	61	2011	56.86
2	0	1962	0.0012
2	1	1963	0.0024
…	…	…	…
2	49	2011	1.60
3	0	1979	0.003
3	1	1980	0.006
…	…	…	…
3	32	2011	0.59
4	5	1952	0.01
4	6	1953	0.03
…	…	…	…
4	64	2011	0.64

Dissertation study 2 will use simulated data to explore how assumptions regarding the exposure-disease relationship and disease fatality may influence the presence and magnitude of bias in survivor cohorts. The study methods will involve simulating a group of participants with different exposure levels and then simulating following them through time to see who develops disease and who dies (i.e. the inception cohort). We will calculate the "true" effect of exposure on disease in this inception cohort. Next, we will create a subset of the inception cohort (i.e. the survivor cohort). We then will calculate the estimated effect of exposure on disease in the survivor cohort and compare it to the inception cohort estimate. We will examine how different assumptions about fatality impact bias in the survivor cohort estimates.

Dissertation study 3 will use logistic regression models to examine whether PFOA experienced during the first three years of life is associated with reporting a BMI indicating

overweight or obesity in adulthood. Models will include overweight or obesity as a dichotomous outcome (yes/no) and the total estimated PFOA received during the first three years of life as the exposure. Separate models will be run for men and women since previous hypotheses indicated that early life PFOA exposure could influence men and women differently with respect to weight in adulthood.

CHAPTER 5: PERFLUOROOCTANOIC ACID (PFOA) AND INCIDENT CANCERS AMONG ADULTS LIVING NEAR A CHEMICAL PLANT

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Vaughn Barry, Andrea Winquist, and Kyle Steenland

Rollins School of Public Health, Emory University, Atlanta, Georgia, USA

Corresponding Author:

Vaughn Barry

Departments of Epidemiology and Environmental Health

Rollins School of Public Health, Emory University

1518 Clifton Road, Atlanta, GA 30322

E-mail: vbarry@emory.edu

Phone: 612-669-9098

Fax: 404-727-8744

Running Title: PFOA and cancer risk among adults exposed to PFOA through drinking water

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are administered by an agency that reports to the court. Our work and conclusions are independent of either party to the lawsuit.

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ABSTRACT

Background: Perfluorooctanoic acid (PFOA) is a synthetic chemical ubiquitous in serum of US residents. It causes liver, testicular, and pancreatic tumors in rats. Human studies are sparse.

Objectives: Examine cancer incidence in mid-Ohio valley residents exposed to PFOA in drinking water due to chemical plant emissions.

Methods: The cohort consisted of adult community residents who resided in contaminated water districts or worked at a local chemical plant. Most participated in a 2005/2006 baseline survey in which serum PFOA was measured. We interviewed the cohort in 2008-2011 to obtain further medical history. Retrospective yearly PFOA serum concentrations were estimated for each participant from 1952-2011. Self-reported cancers were validated through medical records and cancer registry review. We estimated the association between cancer and cumulative PFOA serum concentration using proportional hazards models.

Results: Participants (n=32,254) reported 2,507 validated cancers (21 different cancer types). Estimated cumulative serum PFOA concentrations were positively associated with kidney and testicular cancer (HR=1.10; 95% CI: 0.98, 1.24 and HR=1.34; 95% CI: 1.00, 1.79, respectively, for 1-unit increases in ln-transformed serum PFOA). Categorical analyses also indicated positive trends with increasing exposures for both cancers (kidney cancer HRs for increasing exposure quartiles= 1.0, 1.23, 1.48, and 1.58, linear trend test p=0.18; testicular cancer HRs = 1.0, 1.04, 1.91, 3.17, linear trend test p=0.04).

Conclusions: PFOA exposure was associated with kidney and testicular cancer in this population. Because this is largely a survivor cohort, findings must be interpreted with caution, especially for highly fatal cancers such as pancreatic and lung cancer.

INTRODUCTION

Perfluorooctanoic acid (PFOA) is a synthetic chemical used since the late 1940's in manufacturing to create industrial and household products (Steenland et al. 2010). It is persistent in the environment and has a long human half-life (Lau et al. 2007; Olsen et al. 2007; Seals et al. 2011). PFOA is found at low levels in the serum of most people living in the U.S., with higher levels observed in occupationally exposed workers (Calafat et al. 2007; Lau et al. 2007). Exposure sources in the general population are not well established, but likely include diet, drinking water, food packaging, and household products (Lau et al. 2007). PFOA induced liver, testes, and pancreatic tumors in male rats over a 2-year period (Biegel et al. 2001). However, there was no evidence of hepatocellular, testicular, or pancreatic tumors in male monkeys exposed to PFOA for 26 weeks and observed for 90 days after exposure (Butenhoff et al. 2002). Exposure levels used in the animal studies were higher than human levels typically seen from drinking water or occupational exposure. Due to its potential for environmental persistence, long human half-life, and possible toxicity, there is rising concern about whether PFOA might be associated with human cancers (Environmental Protection Agency 2005; Environmental Protection Agency 2006).

The biologic mechanisms by which PFOA caused rat tumors and the pertinence of the animal findings to humans are unclear. PFOA activation of peroxisome proliferator receptors may cause liver tumors in rats (Kennedy et al. 2004) and PFOA-induced increases in serum estradiol levels (Biegel et al. 2001) may have caused testicular tumor growth. It is not known if these processes are relevant to human cancer (DeWitt et al. 2009; Koeffler 2003; Suchanek et al. 2002).

Most previous human studies of the association between PFOA and cancer have been mortality studies of occupationally exposed workers with few cancer deaths. One study followed

workers employed at a Minnesota PFOA production plant between 1947 and 1997 (Lundin et al. 2009). These investigators reported some evidence of positive trends for prostate and pancreatic cancer across job categories with increasing PFOA exposure, but estimates were based on only 16 and 13 deaths respectively.

A second mortality study followed workers who had ever been employed between 1948 and 2002 at the same DuPont Washington Works plant considered here (Leonard et al. 2008). The authors reported that kidney cancer mortality was almost doubled among plant workers compared with other regional DuPont workers (Standardized Mortality Ratio (SMR)=181.0, 95% CI=93.5, 316.2). This study was recently updated by Steenland and Woskie (2012), who reported a significant increase in kidney cancer mortality with increasing estimated cumulative PFOA serum concentrations, based on 12 kidney cancer deaths. SMRs (and 95% confidence intervals) by increasing exposure quartile were 1.07 (95% CI: 0.02, 3.62), 1.37 (95% CI: 0.28, 3.99), 0 (95% CI: 0, 1.42) and 2.66 (95% CI: 1.15, 5.24) (trend test p=0.02).

There are two PFOA-cancer incidence studies among general populations (Bonefeld-Jorgensen et al. 2011; Eriksen et al. 2009). One enrolled 57,053 cancer-free Danish adults age 50-65 years (Ericksen et al. 2009). PFOA plasma concentrations were measured during enrollment and participants were followed for approximately ten years for incident prostate, pancreas, liver and bladder cancers. Positive associations between PFOA and prostate and pancreatic cancers were reported but were not significant and no significant linear trends were seen for any of the four cancers. A case-control study of 31 breast cancer cases from the Inuit population reported no relationship between PFOA and breast cancer (Bonefeld-Jorgensen et al. 2011). The unadjusted odds ratio (and 95% confidence interval) was 1.07 (95% CI: 0.88, 1.31). PFOA levels are typically low and widespread in general populations.

The DuPont chemical plant in Washington, West Virginia began using PFOA in its manufacturing process in 1951. The plant released PFOA into the Ohio River and air beginning in the 1950s, peaking in the 1990s, and decreasing emissions after 2001. PFOA emitted from the plant entered the groundwater which was the public drinking water source.

In 2001, residents living near the plant filed a class action lawsuit alleging health damage due to PFOA contaminated drinking water. A pretrial settlement required DuPont to provide funding for an independent community health study called the "C8 Health Project" (C8 Health Project 2012; Frisbee et al. 2009), and also resulted in the creation of the C8 Science Panel (C8 Science Panel 2012) tasked with determining whether there was a probable link between PFOA and disease in the community living near the plant.

The C8 Health Project surveyed mid-Ohio valley residents in 2005-2006. The survey collected medical history and also measured serum PFOA concentrations. The median serum PFOA concentration in this population was 28 ng/ml in 2005-2006, compared with 4 ng/ml in the US (Calafat et al. 2007; Steenland et al. 2009a).

Using the C8 Health Project cohort in combination with a DuPont worker cohort, the C8 Science Panel conducted subsequent interviews in 2008-2011 to gather disease incidence data. Cancer incidence results from that investigation are reported here.

METHODS

Data Sources/Study Participants

The C8 Health Project surveyed 69,030 people between August 2005 and August 2006. Participants were eligible if they lived, worked, or attended school for at least one year in one of six contaminated water districts near the plant between 1950 and December 3rd, 2004. Participants reported demographic and health characteristics and an extensive residential history. Serum was collected for PFOA measurements. The estimated C8 Health Project participation

rate was high (81% among current residents 20 years and older) (Frisbee et al. 2009). A detailed study description has been published (Frisbee et al. 2009).

The C8 Science Panel sought to enroll adult C8 Health Project participants in subsequent surveys to study disease incidence; 74% of the participants 20 years and older consented to further contact by the C8 Science Panel. Of these, 82% participated in one or two surveys during 2008-2011. The C8 Health Project participants who completed at least one subsequent survey did not differ significantly from the original adult C8 Health Project participants with respect to age, sex, education, water district, or 2005-2006 measured PFOA serum concentrations. They reported demographic information, health-related behaviors, and medical history. Additionally, we obtained a list of DuPont workers who formed a cohort that was originally constructed for a mortality study (Leonard et al. 2008; Steenland and Woskie 2012). This DuPont cohort was formed by DuPont and included 6,026 workers who were employed at the Washington, West Virginia plant for at least one day between January 1st 1948 and December 31st 2002. Of these, we interviewed 4,391, including 1,890 who were also enrolled in the C8 Health Project.

Figure 3.2 describes how the analysis cohort was compiled. The analysis included 32,254 people 20 years or older, who participated in at least one subsequent survey, and had exposure estimates.

All participants gave informed consent to participate, to match personal information to state cancer registries, and to release medical records to study personnel. Medical records were protected in accordance with Health Insurance Portability and Accountability Act (HIPAA) regulation. The study was approved by the Emory University Institutional Review Board.

PFOA Estimates

Cumulative PFOA serum concentration estimates were calculated retrospectively for each community participant for each year of their life beginning in 1952 or the participant's birth

year, whichever was most recent, through 2011. Estimation procedure details have been published (Shin et al. 2011a; Shin et al. 2011b). Estimates were based on historical regional data including the PFOA amounts emitted by the DuPont facility, wind patterns, river flow, and groundwater flow. Exposure estimates took into account the participant's reported residential history, drinking water source, tap water consumption, work place water consumption, and a PFOA absorption, distribution, metabolism, and excretion model.

The exposure estimates for participants who had ever worked at the DuPont plant took into account occupational exposure they may have received at their specific job. Estimated serum levels over time for workers in different plant jobs were based on over 2,000 PFOA serum measurements taken over time from workers (Woskie et al. 2012). These estimates were used to create a job-exposure matrix to estimate serum levels for workers across time in different jobs and departments. After employment ended, exposure estimates decayed at a rate of 18% per year based on a presumed half-life of 3.5 years (Olsen et al. 2007). These estimates were then combined with estimated serum levels from residential exposure to contaminated drinking water. We estimated combined residential and occupational exposure for 3,713 (84%) of the interviewed workers.

Cancer Data/Confirmation Process

Participants were asked, "Have you ever been told by a doctor or other health professional that you had cancer or a malignancy of any kind?" Participants reported the cancer type and diagnosis age. Those reporting cancer were asked to allow us to review their medical records. For all self-reported cancers, we sought diagnosis validation though medical chart review or Ohio/West Virginia state cancer registry matching.

The Ohio state cancer registry began in 1992 and the West Virginia registry in 1993. If a participant who self-reported a cancer type was found in either of the state cancer registries to

have that cancer, we confirmed their cancer using the registry. We also sought medical records for participants who reported cancer and who consented for us to do so. Some participants who reported cancer were not identified in the registries (possibly due to living out of state or receiving a cancer diagnosis prior to 1992) and in these cases, we used their medical records to confirm self-reported cancer. Medical records were received from doctors the participant reported were relevant to the specific condition and ranged from primary care physician records to oncologist records. We confirmed cancers if there was sufficient information in the record to confirm it. This information could include mention of cancer diagnosis, treatments received, ICD 9/10 codes, or specific cancer or tumor descriptive characteristics.

Statistical Analysis

Our main analyses were restricted to validated primary cancers. Participants who reported a cancer that was not validated were excluded from the specific cancer model and thus did not contribute any person-time to the model.

A proportional hazards regression model was run for each cancer type with the cancer as the outcome, time-varying cumulative PFOA serum concentration as the independent variable, and age as the time scale. Participants were followed from age 20 or age in 1952 (year after first PFOA emissions), whichever was later, to cancer diagnosis age, last survey age, or death age (if deceased), whichever came first. Each model was adjusted for time-varying smoking, time-varying alcohol consumption, gender, education, and 5-year birth year period. We checked the proportional-hazards assumption for each model by including an exposure-age interaction, and found no violation of the proportional-hazards assumption (all interaction p-values > 0.05).

Our primary exposure metric was cumulative PFOA serum concentration (ng/ml-years), which was calculated as the sum of all yearly serum concentration estimates up to a given age. We considered models that included the natural log of cumulative PFOA serum concentration as

a continuous variable (a test for trend), and models that included categorical variables for cumulative serum concentration quartiles. The log of cumulative serum concentration consistently fit better than the linear untransformed cumulative serum concentration (based on the Akaike Information Criterion (AIC)), presumably because log transformation diminished the influence of relatively sparse data with very high cumulative exposure. The interpretation of the log cumulative exposure coefficient is that an increase of one unit of log cumulative exposure results in an RR of e^{β} compared to those with one unit less. We also tested for a linear trend in log RRs in categorical analyses by assigning the mid-point to each quartile and conducting a weighted linear regression of the log RRs on these mid-points.

Quartile cutpoints were calculated among the cumulative PFOA serum concentration estimates for the cancer-specific cases at diagnosis time. We also considered models that lagged cumulative PFOA serum concentration by ten and twenty years to consider scenarios in which cancer could have been caused by exposure further in the past. We report the models that lagged cumulative PFOA serum concentration by ten years. We also ran models limited to community residents who did not work at the plant to explore whether results were driven by the high PFOA exposure experienced by workers. Quartile cutpoints were re-calculated for every cancer and population subgroup model.

RESULTS

Demographic Characteristics

Table 5.1 displays descriptive data for the 32,254 participants. Participants were, on average, 53 years old at the time of their final survey, with male participants slightly older than female (54 years vs. 52 years). Most participants were of white race and community residents. Eleven percent had ever worked at the DuPont plant. Female participants were more likely to have some college education compared with male participants (36% of women, 29% of men).

Participants who had ever worked at the DuPont plant were more likely to be male and older at the time of interview compared to participants without DuPont work experience (80% vs. 42% and 59 years vs. 52 years).

Participants who had worked at the plant had higher PFOA serum levels in 2005-2006 and also had higher estimated annual PFOA serum levels compared with participants who never worked at the plant (Table 5.2). On average, each participant contributed 33 follow-up years after age 20 years but estimated serum levels were low prior to 1980.

Participants reported 3,589 different cancer diagnoses covering 21 cancer types; 2,507 cancer diagnoses were validated (70%). Table 5.3 shows the number of cancer diagnoses reported, the number with a received medical record or state cancer registry entry, and the number validated. We obtained a record to review for 88% of self-reported cancers. Reasons for non-validation included living in a different state, having a cancer prior to the existence of the two cancer registries, or failing to consent for medical record review. The accuracy of self-reported cancer varied by cancer site. Breast, bladder, kidney, prostate, thyroid, colorectal, lung, leukemia, and lymphoma cancers were more likely to be confirmed compared with other cancer types. Cervical cancer had a low validation rate, possibly due to participants misinterpreting abnormal pap smear results. Cancer was more often validated in DuPont worker participants compared to community residents who never worked at DuPont (75% vs. 69%) (see Supplemental Material, Table A1).

Exposure-outcome Associations

Table 5.4 shows adjusted proportional hazards model results for each cancer type based on validated cases only. Thyroid, kidney, and testicular cancer risk increased with an increase in the log of estimated cumulative PFOA serum concentration (Table 5.4), this association was statistically significant only for testicular cancer at the p=0.05 level. The hazard ratios and 95%

confidence intervals were similar between models where exposure was unlagged, models where exposure was lagged 10 years, and models where exposure was lagged 20 years (results not shown). The models generally fit slightly better for unlagged exposure compared to 10 and 20-year lagged exposures, as measured by the Akaike Information Criterion (AIC). Results based on all self-reported cancer cases were similar to estimates based on validated cases only (data not shown). The increase in testicular and kidney cancer risk by increasing log of estimated cumulative PFOA serum concentration was stronger in community residents compared to DuPont workers (see Supplemental Material, Table A2). However, the association between thyroid cancer risk and PFOA was positive and significant in DuPont workers but not community residents (see Supplemental Material, Table A2).

Table 5.5 reports proportional hazards model results for selected cancers using estimated cumulative PFOA serum concentration quartiles. Relative risks estimated for kidney cancer and testicular cancer generally increased monotonically across quartiles, while the pattern across thyroid cancer quartiles was less consistent. P-values for linear trend tests of log rate ratios across quartiles of unlagged exposures (using exposure category mid-points, and inverse variance weighting in a no-intercept linear regression model) were 0.25, 0.18, and 0.04 for thyroid, kidney, and testicular cancers, respectively. The p-values for thyroid, kidney, and testicular cancer trend tests with a 10-year lag were 0.57, 0.34, and 0.02. When stratified by occupational status, estimated relative risks for thyroid cancer increased monotonically across quartiles among DuPont workers but did not increase monotonically for kidney cancer among DuPont workers (see Supplemental Material, Table A3). Results for the worker cohort are limited by low sample size for cancers of interest.

As thyroid cancer is more common in women, perhaps reflecting different mechanisms from men, we ran separate analyses for men and women (24 and 74 cases, respectively). Results were similar in each group (data not shown).

Sensitivity Analyses

We conducted several sensitivity analyses. We looked back at each participant's residential history and estimated the time when they were first known to have begun living or working in one of the 6 contaminated water districts, excluding prior time. We then considered survival models that started each person's time on this "qualifying date", excluding years before that date. These analyses resulted in slightly less person time and slightly fewer cancer cases than original analyses; again, results were similar to reported results. Hazard ratios for a 1-unit increase in ln-transformed cumulative exposure in relation to thyroid, kidney, and testicular cancers were 1.06 (95% CI: 0.92, 1.23), 1.12 (95% CI: 0.99, 1.26), and 1.37 (95% CI: 0.99, 1.90) for unlagged exposures, and 1.02 (95% CI: 0.87, 1.19), 1.10 (95% CI: 0.98, 1.24), and 1.31 (95% CI: 0.95, 1.81) for exposures lagged by 10 years.

DISCUSSION

We estimated associations between estimated cumulative PFOA exposures and incident cancers among a group of individuals exposed through drinking water or work at the local DuPont chemical plant. Positive associations between PFOA and cancer were found for kidney, testicular, and thyroid cancer.

The positive exposure-response trend for kidney cancer is consistent with a previous DuPont worker mortality analysis, which indicated a positive exposure-response trend for kidney cancer deaths (Steenland and Woskie 2012). Our findings are also in agreement with an

ecological study of incident cancer rates in relation to PFOA exposure levels between 1996-2005 in five Ohio and eight West Virginia counties (Vieira et al. 2013), which included some cancers diagnosed among participants in the present study population. They reported a significant positive association between kidney cancer and the two highest estimated PFOA serum exposure categories. Finally, the kidney is of a priori interest because studies using rats, mice, hamsters, rabbits, and chickens have shown that PFOA is distributed mainly in the kidneys, liver, and serum (Han et al. 2005; Kennedy et al. 2004; Lau et al. 2007).

Testicular cancer was of *a priori* interest, because PFOA has been shown to induce testicular tumors in male rats (Biegel et al. 2001) and increase estradiol production in male rats, which may increase testicular tumor risk (Biegel et al. 2001). In the ecological study performed by Vieira et al. (2013), estimated PFOA exposures were positively associated with testicular cancer. As noted above, cases included in the ecological study would have partly overlapped with cases diagnosed in our study population.

To our knowledge, there are no reports of an association between PFOA and thyroid cancer from experimental studies of animals or observational studies of human populations. However, there is evidence that PFOA is associated with incident non-malignant thyroid disease in this population (Winquist et al. 2012).

We confirmed self-reported cancers through state cancer registry matching and medical record review. Our cancer validation rates for breast, prostate, lung, and melanoma cancers are similar to previous studies suggesting that breast, prostate, and lung cancers are typically reported accurately, while rectal cancer and melanoma of the skin may be reported less accurately (Bergmann et al. 1998; Stavrou et al. 2011). We tried to avoid these problems by grouping self-reported cases of "colon" and "rectal" cancer as "colorectal" cancer cases. Similarly, we did not

evaluate non-melanoma skin cancer as an outcome and limited melanoma cases to participants confirmed for melanoma.

Community cohort participants (n=30,431) had to be alive in 2004-2005 to participate in the C8 Health Project, and thus to be eligible for inclusion in our community cohort. Worker cohort participants who were not in the C8 Health Project (1,823) did not have to be alive in 2004-2005 to be included in the study. Nevertheless, because of difficulties in obtaining proxy respondents for deceased target cohort members at time of interview in 2008-2011, most of the participants from both cohorts were alive at the time of their interview in 2008-2011. It is possible that some potentially eligible kidney cancer cases would not have been enrolled or interviewed because they died prior to 2005, given that the five-year survival rate for kidney cancer based on 2002-2008 SEER data was only 70% (National Cancer Institute 2012). In contrast, cancers with low fatality rates, such as thyroid and testicular cancer, would not be expected to be missing from the study cohort. If cancer cases with higher exposure were more likely to die before they could be enrolled in our cohort, associations with PFOA may be biased toward the null, particularly for highly fatal cancers like pancreatic cancer and lung cancer; consequently our results must be interpreted with caution. On the other hand, associations could be biased away from the null if a disproportionate number of highly exposed cancer cases participated in the study.

This study has several other limitations. PFOA was estimated individually for each year of each participant's life based on their self-reported residential history, DuPont PFOA emission patterns, and a PFOA absorption, distribution, metabolism, and excretion model. There is likely misclassification in exposure estimates, although we did find good agreement between model-predicted and measured serum levels in 2005-2006 among the C8 Health Project participants who had never worked at the DuPont plant (r=0.67) (Shin et al. 2011b). Misclassification could cause

bias if it was differential according to the outcomes evaluated. Non-differential misclassification is more likely to result in bias toward the null than away from the null, but not always (Armstrong 1998; Steenland et al. 2000). Also, the cancer validation process was implemented only for those who self-reported a cancer. There could have been participants who had a history of cancer but did not report it. However, potential misclassification of cases as non-cases would have a smaller impact on the analysis than misclassification of non-cases as cases because the number of cases misclassified as non-cases is likely small relative to the total number of non-cases.

CONCLUSION

In summary, previous research on PFOA and cancer has been primarily restricted to animal experiments, mortality studies of male workers with occupational exposure, and community studies of populations with low exposure levels, and human studies have been limited by small numbers of cancer cases. The present study estimated relative risks of incident cancers in association with cumulative PFOA exposure in a large community with a range of exposure levels. Over 2,500 validated cancers covering 21 different cancer types were included in the analysis, making it one of the largest cohorts ever used to examine PFOA and cancer. Our findings indicated that PFOA exposure was positively associated with kidney and testicular cancer in this mid-Ohio valley population. Results for highly fatal cancers must be interpreted with caution since this is largely a survivor cohort.

Table 5.1 Demographic characteristics of cohort (n=32,254) by community and occupational groups

Group	Entire cohort	Community group	Occupational group
N	32,254	28,541	3,713
Characteristic	N (%) or mean ± SD		
Sex			
Male	14,894 (46.2)	11,939 (41.8)	2,955 (79.6)
Female	17,360 (53.8)	16,602 (58.2)	758 (20.4)
Race/Ethnicity[a]			
White, non-Hispanic	31,144 (97.4)	27,860 (97.6)	3,284 (96.1)
Other	815 (2.6)	681 (2.4)	134 (3.9)
Education[b]			
Less than highschool	3,063 (9.5)	3,026 (10.6)	37 (1.0)
Highschool or General Equivalency Degree (GED)	12,971 (40.2)	11,706 (41.0)	1,265 (34.1)
Some college	10,522 (32.6)	9,441 (33.1)	1,081 (29.1)
Bachelor or higher	5,694 (17.7)	4,366 (15.3)	1,328 (35.8)
Mean age in years at final interview	53.0 ± 15.6	52.2 ± 15.6	59.3 ± 14.1
Mean year of birth	1957 ± 15.6	1958 ± 15.6	1951 ± 14.1
Type of participant			
Community only	28,541 (88.5)	28,541 (100.0)	---
Worker only	1,823 (5.7)	---	1,823 (49.1)
Community & worker	1,890 (5.9)	---	1,890 (50.9)

[a] 295 missing race/ethnicity info (all from occupational group)
[b] 4 missing education info (2 from community group and 2 from occupational group)

Table 5.2. Measured and estimated PFOA exposure concentrations in the cohort (n=32,254)

Cohort	Median (range) PFOA exposure in ng/mL
Measured PFOA serum level in 2005-2006	
Community (n=28,541)	24.2 (0.25 – 4,752)
Worker (n=1,881) [a]	112.7 (0.25 – 22,412)
Estimated annual PFOA serum level [b]	
Community (n=28,541)	19.4 (2.8 – 9,217)
Worker (n=3,713)	174.4 (5.2 – 3,683)

[a] workers who did not participate in the C8 Health Project did not have serum levels measured (n=1,823) and other workers were missing measurements (n=9)

[b] community residents were followed for an average of 32 years, workers were followed for an average of 38 years

Table 5.3 Number of reported and validated[a] primary cancer cases among the cohort (n=32,254)

Cancer	# reported	# reported that had a medical record reviewed or a cancer registry entry	# validated (% validated) among those reported
Bladder	115	115	111 (96.5)
Brain	33	31	23 (69.7)
Breast	608	600	581 (95.6)
Cervical	383	245	22 (5.7)
Colorectal	311	297	276 (88.7)
Esophagus	21	19	15 (71.4)
Kidney	124	117	113 (91.1)
Leukemia	79	71	69 (87.3)
Liver	18	15	10 (55.6)
Lung	133	124	113 (85.0)
Lymphoma	164	158	142 (86.6)
Melanoma	519	414	245 (47.2)
Oral	35	34	20 (57.1)
Ovarian	87	65	43 (49.4)
Pancreatic	35	31	26 (74.3)
Prostate	515	476	458 (88.9)
Soft Tissue	25	19	17 (68.0)
Stomach	29	24	12 (41.4)
Testicular	32	21	19 (59.4)
Thyroid	98	97	87 (88.8)
Uterine	225	173	105 (46.7)
TOTAL	3589[b]	3146	2507 [c] (69.9)

[a] Validated cases were limited to participants who reported the cancer and were subsequently confirmed either by Ohio/West Virginia cancer registry or medical record review; participants reported whether a doctor had ever told them they had a cancer or malignancy of any kind

[b] These 3,589 cancers were self-reported by 3,292 participants; some participants reported more than 1 cancer type

[c] These 2,507 cancers are among 2,361 participants

Table 5.4 Hazard ratios and 95% confidence intervals assessing the effect of logged estimated cumulative PFOA serum concentration on cancer risk in the cohort (n=32,254)

		NO LAG		10 YEAR LAG	
Cancer[a]	# cases [b]	HR (95% CI)[c]	p-value	HR (95% CI) [c]	p-value
Bladder	105	1.00 (0.89, 1.12)	0.98	0.98 (0.88, 1.10)	0.77
Brain	17	1.13 (0.84, 1.51)	0.43	1.06 (0.79, 1.41)	0.70
Breast	559	0.94 (0.89, 1.00)	0.05	0.93 (0.88, 0.99)	0.03
Cervical	22	0.89 (0.63, 1.24)	0.48	0.98 (0.69, 1.38)	0.90
Colorectal	264	0.99 (0.92, 1.07)	0.84	0.99 (0.92, 1.07)	0.77
Esophagus	15	0.96 (0.70, 1.32)	0.82	0.97 (0.72, 1.31)	0.84
Kidney	105	1.10 (0.98, 1.24)	0.10	1.09 (0.97, 1.21)	0.15
Leukemia	66	1.01 (0.87, 1.18)	0.88	1.02 (0.88, 1.18)	0.80
Liver	9	0.73 (0.43, 1.23)	0.23	0.74 (0.43, 1.26)	0.26
Lung	108	0.88 (0.78, 1.00)	0.05	0.92 (0.81, 1.04)	0.17
Lymphoma	136	1.01 (0.91, 1.12)	0.88	0.98 (0.88, 1.10)	0.78
Melanoma	241	1.00 (0.92, 1.09)	0.97	1.04 (0.96, 1.13)	0.30
Oral	18	0.89 (0.65, 1.22)	0.46	0.66 (0.43, 1.02)	0.06
Ovarian	43	0.95 (0.76, 1.19)	0.64	0.90 (0.69, 1.16)	0.42
Pancreatic	24	1.00 (0.78, 1.29)	0.99	0.96 (0.75, 1.22)	0.72
Prostate	446	0.99 (0.93, 1.04)	0.63	0.99 (0.94, 1.05)	0.80
Soft Tissue	15	0.75 (0.51, 1.10)	0.14	0.72 (0.48, 1.09)	0.12
Stomach	12	0.72 (0.45, 1.14)	0.16	0.77 (0.49, 1.22)	0.27
Testicular	17	1.34 (1.00, 1.79)	0.05	1.28 (0.95, 1.73)	0.10
Thyroid	86	1.10 (0.95, 1.26)	0.20	1.04 (0.89, 1.20)	0.65
Uterine	103	1.05 (0.91, 1.20)	0.53	0.99 (0.86, 1.15)	0.94

[a] A proportional hazards regression model was run for each cancer. Each model was adjusted for time-varying smoking, time-varying alcohol consumption, gender, education, and stratified by 5 year period of birth year. Time began at age 20 if the person's 20th birthday was in 1952 or later. Otherwise time began at the age the person was in 1952. Time ended at the age of cancer diagnosis, age at the last follow-up survey, or age on December 31st 2011, whichever came first.

[b] refers to the number of cancer cases used in the regression model (i.e. no missing data for any of the model's covariates).

[c] per unit of log estimated cumulative PFOA serum concentration (ng/mL)

Table 5.5 Hazard ratios and 95% confidence intervals by PFOA quartile[a] for thyroid, kidney, and testicular cancer cases among the cohort (n=32,254)

		Hazard Ratio (95% CI)[b]					
Cancer	#cases	Quartile 1 (Reference)	Quartile 2	Quartile 3	Quartile 4	p-value[c]	p-value[d]
Kidney –no lag	105	1.00	1.23 (0.70, 2.17)	1.48 (0.84, 2.60)	1.58 (0.88, 2.84)	0.18	0.10
Kidney –10 yr lag	105	1.00	0.99 (0.53, 1.85)	1.69 (0.93, 3.07)	1.43 (0.76, 2.69)	0.34	0.15
Testes –no lag	17	1.00	1.04 (0.26, 4.22)	1.91 (0.47, 7.75)	3.17 (0.75, 13.45)	0.04	0.05
Testes –10 yr lag	17	1.00	0.87 (0.15, 4.88)	1.08 (0.20, 5.90)	2.36 (0.41, 13.65)	0.02	0.10
Thyroid–no lag	86	1.00	1.54 (0.77, 3.12)	1.48 (0.74, 2.93)	1.73 (0.85, 3.54)	0.25	0.20
Thyroid–10 yr lag	86	1.00	2.06 (0.93, 4.56)	2.02 (0.90, 4.52)	1.51 (0.67, 3.39)	0.57	0.65

[a]Quartiles were defined by the estimated cumulative PFOA serum concentration among the thyroid, kidney, or testicular cancer cases at the time of cancer diagnosis

[b] A proportional hazards regression model was run for each cancer. Each model was adjusted for time-varying smoking, time-varying alcohol consumption, gender, education, and stratified by 5 year period of birth year. Time began at age 20 if the person's 20th birthday was in 1952 or later. Otherwise time began at the age the person was in 1952. Time ended at the age of cancer diagnosis, age at the last follow-up survey, or age on December 31st 2011, whichever came first.

[c] P-value is for linear trend test in the log rate ratios across quartiles. P-values were calculated using exposure category mid-points and inverse variance weighting in a no-intercept linear regression model.

[d] P-value is from the continuous log estimated cumulative PFOA serum concentration models

CHAPTER 6: HOW DISEASES WITH DIFFERENT SURVIVAL PATTERNS IMPACT THE MAGNITUDE OF SURVIVOR BIAS IN THE CONTEXT OF THIS STUDY

Authors: Vaughn Barry, Mitchel Klein, Andrea Winquist, Lyndsey Darrow, Kyle Steenland

Affiliations: Departments of Environmental Health and Epidemiology, Rollins School of Public Health, Emory University, 1518 Clifton Road, Atlanta, Georgia, 30322 USA

Corresponding author:

Vaughn Barry

Departments of Epidemiology and Environmental Health

Rollins School of Public Health, Emory University

1518 Clifton Road, Atlanta, GA 30322

E-mail: vbarry@emory.edu

Phone: 404-727-8058

Fax: 404-727-8744

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ABSTRACT

Background: When study participation is dependent on survival to a certain time point after disease risk has already begun, measures of effect describing the relationship between exposure and disease onset may be subject to bias.

Objectives: Explore how assumptions regarding disease fatality and the effect of exposure on disease fatality influence the presence and magnitude of bias in survivor cohorts.

Methods: We simulated an inception cohort of 50,000 subjects who were exposed to a disease-causing exposure over time and followed for up to forty years. We simulated this cohort under ten different assumptions about disease fatality and two different assumptions about the effect of exposure on disease fatality. We then excluded all subjects that died from each inception cohort to create corresponding survivor cohorts. We compared the exposure-disease associations in each inception cohort to those in the corresponding survivor cohorts to determine how the different assumptions impacted bias in the survivor cohorts. We used cox proportional hazards regression to calculate measures of effect.

Results: There was no bias in the survivor cohort estimates when case fatality among diseased subjects was independent of exposure. This was true even when the disease was fatal and highly exposed subjects were more likely overall to develop disease and die compared to less exposed subjects. There was bias in the survivor cohort estimates when case fatality differed by exposure status. This bias increased as the difference in disease severity between the less and most exposed diseased subjects increased.

Conclusions: Survival cohort estimates associated with fatal outcomes of interest are not always biased although precision associated with the estimates can decrease.

INTRODUCTION

When study participation is dependent on survival to a certain time point after disease risk has already begun, measures of effect describing the relationship between exposure and disease onset may be subject to bias (Delgado-Rodríguez et al. 2003, Rothman et al. 1998). This concern about bias stems from the fact that some exposed subjects may develop disease and subsequently die before study enrollment begins. Selection bias could occur if those that die before study enrollment are different than subjects that survive long enough to enroll. It is natural to assume that the presence and magnitude of this bias may be a function of the specific survival pattern associated with the disease outcome of interest. For example, a non-fatal disease outcome could mean that everyone survives to enroll which would presumably result in no survivor bias in the study's exposure-disease estimates. However, there may be times where study estimates are unbiased even when many subjects die before study enrollment and are thus excluded from the study. It is commonly assumed that bias will automatically be present in a survivor cohort just based on disease fatality, even if unrelated to exposure. This may not always be the case. Disease fatality and the specific relationships between the exposure, disease, and death may all be factors that can determine whether bias is induced or averted in studies where participation is dependent on survival. Our focus is on examining the presence and magnitude of bias associated with diseases with different survival patterns in survivor cohorts.

This work was motivated by a specific situation that involved emissions of a chemical from a chemical plant over a fifty year period (C8 Health Project 2012). The chemical contaminated the local drinking water supply of several nearby communities beginning in the 1950's and continuing through the year 2000. In the year 2005, a series of community health studies was initiated to determine whether exposure to the chemical had caused adverse health and disease in the community residents (Frisbee et al. 2009; Winquist et al. 2013). Most of the

resident participants had to survive until 2005 to enroll in these studies. One common assumption about this survivor cohort is that any measure of effect assessing the relationship between exposure and a highly fatal disease will be biased because those who developed highly fatal diseases were presumably more likely to die before 2005 and thus less likely to be included as participants in the studies.

We wondered specifically how to interpret some of our own results from the studies. For example, we examined whether exposure to the chemical was related to cancer incidence in the residents living near the chemical plant (Barry et al. 2013). One of the cancer types we were interested in was pancreatic cancer since the chemical had previously caused pancreatic tumors in male rats (Biegel et al. 2001). We found no relationship between the chemical and pancreatic cancer incidence in the cohort of 32,254 residents living near the chemical plant who each, on average, contributed 33 adult follow-up years. However, there were not a large number of pancreatic cancer cases in our study (n=24) likely because pancreatic cancer tends to have higher mortality risk compared to other cancers. For example, there were more than three times as many thyroid cancer cases (n=86) in the same cohort even though thyroid cancer incidence in the U.S. is reported to be the same as pancreatic cancer incidence (Howlader et al. 2013). We wondered if our pancreatic cancer estimates were biased because pancreatic cancer is often a fatal disease. We also wondered which types of assumptions influenced whether estimates were biased or not.

In this paper, we used simulated data to explore how assumptions regarding the disease fatality and the effect of exposure on disease fatality influence the presence and magnitude of bias in survivor cohorts.

Our overall strategy was to simulate a group of subjects with different exposure levels and then simulate following them through time to see who develops disease and who die (i.e. the inception cohort). We calculated the "true" effect of exposure on disease in this inception cohort.

Next, we created a subset of the inception cohort (i.e. the survivor cohort). We then calculated the estimated effect of exposure on disease in the survivor cohort and compared it to the inception cohort estimate. We examined how different assumptions about disease fatality impacted bias in the survivor cohort estimates.

METHODS

Inception Cohort: Exposure

We simulated a cohort of 50,000 subjects. We assumed that each subject was followed from age 20 to age 60 years (40 years per subject) or death with no loss to follow up. Each subject was assigned an age at which he or she began experiencing exposure during the 40-year period. Additionally, each subject was exposed for a specific number of years (i.e. cumulative exposure). We assumed that exposure intensity was constant over time and at a rate of 1 unit exposure per year. This design was roughly based on the idea of an exposure being present in a city over a 40 year period. We imagined that subjects could move in and out of the city at different ages and for different numbers of years and thus be exposed for different amounts of time.

We generated age at first exposure for each subject by drawing ages randomly from a uniform distribution between 20 and 40. Thus, age at first exposure was equally likely to occur anytime between age 20 and 40 years with an average of 30 years (range= 20-40 years). We assumed that the cumulative amount of exposure each subject had would follow a normal distribution with a mean of 20 and standard deviation of 5. Consequently, on average, each subject was exposed to 20 units of exposure over their lifetime but any given subject could have been exposed from 1 to 40 units of exposure during their lifetime. We chose these numbers

because they gave us a cohort with a wide-range of exposure levels, similar to the original cohort of residents living near the chemical plant.

Note that using these assumptions, some subjects (~8%) will have an assigned cumulative exposure that is too large given the age at which they entered the study. For example, a subject with an assigned cumulative exposure of 25 units who begins the exposure at age 39 years will have hit only 21 units at the age of 60 years (which is the end of follow-up). This means that the right tail of the normal distribution curve (that generated cumulative exposure) is partially truncated but not meaningfully so. After taking this into account, on average each subject was exposed to 20 units of exposure during their lifetime and any given subject could have been exposed to anywhere from 1 to 37 units of exposure.

Inception Cohort: Disease

Each subject had an assigned probability of developing disease at any given age during their 40-year follow-up period. The specific probability was determined by the exponential equation: p=exp[-7 + (.07*exposure)]. Disease (yes or no) was generated for each subject at each age by random draws from a binomial distribution with probability p. This equation was loosely based on a similar equation used in a different simulation (Richardson et al. 2004). The probability of disease for each subject took into account each subject's cumulative exposure at any given year, assumed that the risk of disease increased with increasing exposure, and did not depend on age. The ".07" suggests that we should see a hazard ratio near 1.07 for every increase in 1 unit of exposure. The "-7" generates a relatively common disease. There were some instances where a subject developed the disease twice (i.e. at age 40 and then again at age 50) or even three times during their life. In these cases, we counted only the disease that occurred first.

Inception Cohort: Case Fatality

We assumed that each subject that developed disease would die at some point after disease. Death could occur either before the age of 61 years (i.e. during follow-up) or sometime after age 60 years (i.e. after the end of follow-up). We generated this time to death for diseased subjects by drawing randomly from an exponential distribution with mean X. X was varied to simulate different disease survival patterns. For example, when X=8, subjects with disease died, on average, about eight years after they developed disease (Figure 6.1). We chose ten different exponential means that corresponded to varying disease fatality rates: 1, 3, 5, 8, 12, 16, 25, 41, 65, and 160. The highest mean of 160 generated a non-fatal disease since it caused subjects with disease to die on average, well after death from natural causes would occur. The lowest mean of 1 generated a highly fatal disease that caused subjects with disease to die on average, only one year after they developed disease.

We simulated case fatality in different inception cohorts using two different assumptions: (1) case fatality was independent of exposure and (2) case fatality was not independent of exposure. In the inception cohort using the first assumption, all diseased subjects had the same probability of dying regardless of exposure level.

In the inception cohort using the second assumption, subjects with disease who were less exposed were assumed to have a non-fatal disease while diseased subjects who were most exposed had more fatal disease. We considered the "most exposed" subjects to be either the 10% or 50% of diseased subjects with highest cumulative exposure at the time of disease. Thus, the "least exposed" subjects were the remaining 90% or 50% diseased subjects. We assumed that the least exposed diseased subjects always had the lowest case fatality (exponential mean = 160). However, the most exposed diseased subjects were assigned a different exponential mean for the fatality parameter to see how results compared when case fatality was not independent of exposure. For example, in one simulation, we assumed that the least exposed diseased subjects

had the lowest case fatality (exponential mean = 160) while the most exposed diseased subjects had a higher case fatality (exponential mean = 65). In a different simulation we assumed the same low case fatality in the least exposed (exponential mean = 160) but assumed a highly fatal disease among the most exposed (exponential mean = 1).

Survivor Cohort

For each simulation, the survivor cohort included everyone in the inception cohort (n=50,000) who lived until age 60 years (i.e. everyone who survived until the end of follow-up). If a subject in the inception cohort died before age 60 years, they were not included in the survivor cohort. If a subject in the inception cohort died after age 60 years, they were included in the survivor cohort. Thus, the survivor cohort is a subset of the inception cohort.

Statistical Analysis

We ran separate proportional hazards (time-to-event) regression models for the inception and survivor cohorts with disease (yes/no) as the event of interest, time-varying cumulative exposure as the independent variable, and age as the time scale. Subjects were followed from age 20 to age of disease onset or age 60, whichever came first. We did not control for any variables as we did not introduce confounders into the cohort design. We verified the proportional-hazards assumption by testing an exposure-age interaction. We expected that the assumption would be met because disease was assigned using an exponential distribution that depended on exposure but not age or any form of interaction between exposure and age. We report hazard ratios per 10-unit increase in exposure. All analyses were done with SAS version 9.3 (SAS Institute, Cary, North Carolina).

To summarize: we assumed that exposure was of a constant intensity, that exposure caused disease, and that subjects could die only from disease (i.e. no competing risks). Disease

occurred during the 40 year follow-up period while death from disease could occur during or after the follow-up period.

RESULTS

Inception Cohort Characteristics

The inception cohort included 50,000 subjects that were followed from age 20 to age 60 years. As expected, the average age at first exposure was 30 years and the average exposure duration was 20 years. Of the 50,000 subjects in the cohort, 4,234 developed disease during the 40 years of follow-up. Total person-years used in the inception cohort regression model (after censoring subjects at the time of disease) were 1,940,390 years. The average age at the time of disease onset was 45.9 years (median=48 years, range=22-60 years). The average cumulative exposure at the time of disease onset was 15.1 units (median=16 units, range=0-34 units). The average cumulative exposure at age 60 for the 45,766 subjects that did not develop disease was 19.7 units (median=20 units, range=1-37 units).

Ten different disease survival patterns were simulated by using an exponential distribution with ten different means (Table 6.1). When the exponential mean was highest (mean=160), the disease was less fatal and only 9% of the diseased subjects died during the observed follow-up time (between the ages of 20 and 60 years old). When the exponential mean was lowest (mean=1), the disease was highly fatal and 96% of the diseased subjects died during follow-up. Further, when the exponential mean was 8, then the average time to death after disease among the 4,234 subjects who developed disease was 8.2 years (median=6 years, range=0-80 years). Using this exponential mean, 2,940 of the 4,234 diseased subjects (69%) died during follow-up while 1,294 (31%) died after follow-up time ended.

Survivor Cohort Characteristics

The number of subjects in the survivor cohort varied depending on the specific disease survival pattern simulated in the inception cohort and whether case fatality was independent of exposure or not. When case fatality was independent of exposure and the disease in the inception cohort was not especially fatal (exponential mean = 160), the survivor cohort was made up of 49,637 subjects (compared to the 50,000 in the inception cohort) (Table 6.2). Similarly, when the disease in the inception cohort was highly fatal (exponential mean = 1), the survivor cohort included 45,930 subjects. Average age at disease onset in the survivor cohorts was older compared to the inception cohort with the difference increasing with increasing disease fatality. Diseased subjects in the highly fatal disease survivor cohort were more likely to have recent disease onset because subjects who developed disease at earlier ages died during follow-up and were not included in the survivor cohort.

Estimates: Non-differential Fatality by Exposure

When case fatality did not vary by exposure, there was no meaningful bias in the exposure-disease estimates in the survivor cohorts (Table 6.3). Even when the disease was highly fatal in the inception cohort and the survivor cohort was thus missing most of the diseased subjects, bias was not present in the estimates. However, as expected, precision decreased in the survivor cohort estimates because fewer diseased individuals were present in the survivor cohort.

Estimates: Differential Fatality by Exposure

When case fatality was differential across exposure levels, there was bias in the survivor cohort estimates and the bias varied by disease survival pattern. When the 10% of diseased subjects who were most exposed were assumed to have higher disease fatality compared to the 90% less exposed with lower disease fatality, the survivor cohort estimates became biased (Table 6.4). Here, the survivor cohort hazard ratios became more biased downward toward the null as

the disease in the subjects who were most exposed became more fatal compared to the less severe disease in the less exposed. When diseased subjects were divided equally into two categories of least and most exposed (i.e., top 50% of exposures considered high), estimates were more biased than when the subjects with disease were split into 10% and 90% groups (Table 6.5). When the most extreme situation occurred - where half the diseased subjects had a low-fatality disease but the other half had a highly fatal disease - the hazard ratio was so biased it crossed the null and incorrectly suggested that the exposure could prevent disease.

DISCUSSION

We found that there was bias in the exposure-disease estimate in this type of survivor cohort when case fatality differed by exposure status. There was no bias when case fatality among diseased subjects was independent of exposure. This was true even when the disease was fatal and highly exposed subjects were more likely overall to develop disease and die compared to less exposed subjects. When the disease was highly fatal and case fatality was independent of exposure, there were fewer diseased survivors in the survivor cohort which reduced precision of the exposure-disease estimates but still did not bias the estimates.

We assumed that everyone in our cohorts was uniformly susceptible to the effect of exposure. This assumption may hold true in some real world settings. However, there may be times where only a certain proportion of the exposed population is susceptible to disease caused by exposure. For example, some in an exposed population may have a gene that protects them from the exposure's effects while others in the exposed group do not (Christiani et al. 2008; Khoury et al. 2004). In this situation, the susceptible individuals could develop disease early on, leaving a more resistant population later on (Aalen 1994; Hernán 2010). This frailty phenomenon may bias hazard ratios over time. However, when susceptibility to the effect of exposure is 100%, there is no bias in the hazard ratios over time, assuming no other biases or confounding

(Applebaum et al. 2011; Hernán 2010). We considered only the scenario where all were susceptible to the effect of exposure to focus on how different disease fatality assumptions influenced the amount of bias present in the survivor cohort estimates.

Our conclusions are dependent on other assumptions we made, too. In our simulations, we generated a disease that was fairly common. If we considered a more rare disease, the patterns of bias should still exist under the assumptions we've used but the magnitude of bias may be less since there would be fewer subjects lost to death. Similarly, we assumed that exposure was constant over time and the hazard of disease in the population was constant across the study period. Conclusions may differ if one or both of these varied over time.

We simulated differential case fatality by exposure in several of our simulated cohorts. We assumed that this differential probability of death meant that exposure could cause a more fatal disease in highly exposed diseased subjects compared to less exposed diseased subjects. It is not known if or how much this occurs in the real world but is thought to be quite possible. For example, cancer tumors at the same site and of the same histology can have different genetic profiles and be characterized by different molecular pathways (Han et al. 2002, MacMahon et al. 1987, Stratton et al. 2009, Verhaak et al. 2013). Additionally, these tumors that seem similar with respect to site, histology, and morphology but are possibly genetically different may also be different with regards to survival and treatment (Gong et al. 2014, Stratford et al. 2010, Verhaak et al. 2013). In fact, in some cases like lung cancer, tumor markers may provide a better indication of prognosis and response to targeted therapy than the morphological characteristics of the tumor (Gerber et al. 2010, Soda et al. 2007). It is not a large next step to assume that an environmental exposure could cause a cancer to develop by way of a specific molecular pathway that could in turn, create a more (or less) severe cancer. Different molecular pathways have already been causally related to different environmental exposures but the tumor subtypes have

differed in their histologic appearance. For example, smoking is strongly related to mucinous ovarian cancer and colon cancers resulting from mismatch repair, but not to other histologically different subtypes of these tumors (Kurian et al. 2005, Limsui et al. 2010, Marchbanks et al. 2000, Modugno et al. 2002). Nevertheless, differential case fatality by exposure in the real world is a real possibility and can be studied. We hope to eventually sequence RNA in cancer tumor tissue of residents living near the chemical plant to determine whether high exposure resulted in a distinctive pattern of gene expression.

We found that there was no bias in the exposure-disease estimate in the survivor cohort when case fatality did not differ by exposure status. This was true even when the disease was highly fatal and highly exposed subjects were more likely overall to develop disease and die compared to less exposed subjects. In this situation, estimates were unbiased but precision in the estimate's 95% confidence intervals decreased.

Figure 6.1 Distribution of time to death in years after disease for the diseased subjects in the inception cohort (n=4,234) using an exponential distribution with mean=8 to generate case fatality.

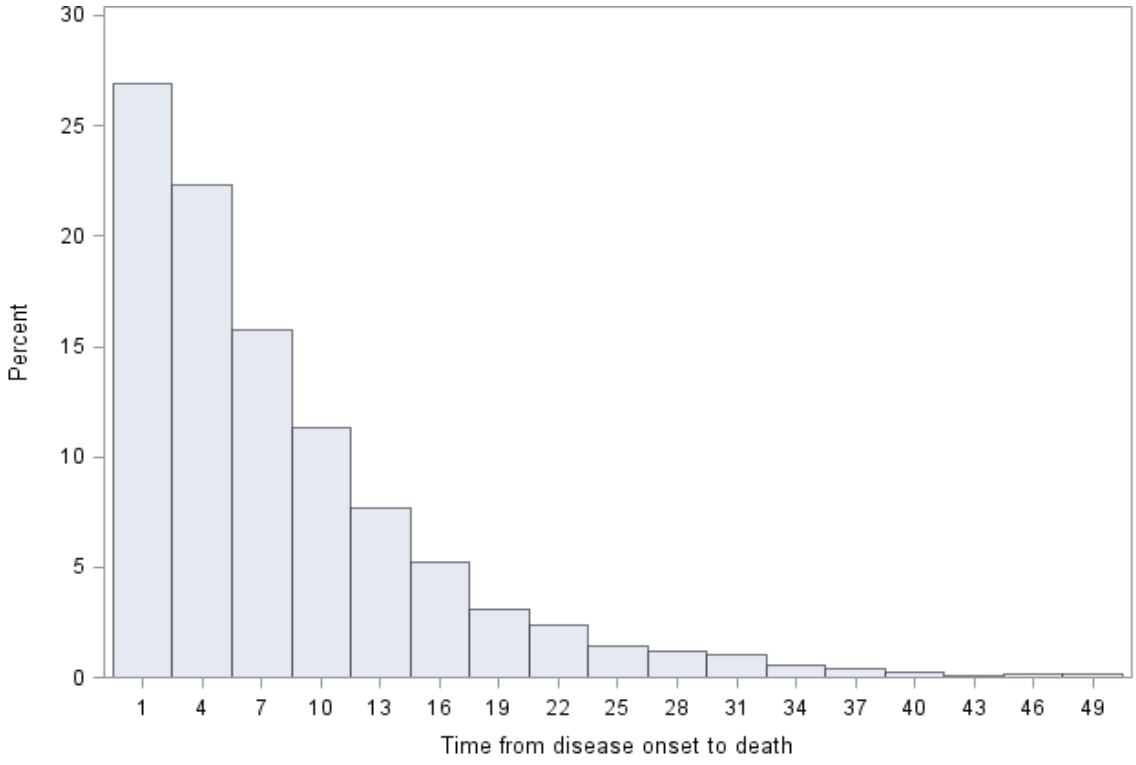


Table 6.1 Case fatality by different exponential means when case fatality is independent of exposure

Exponential Mean	% of diseased subjects that die during follow-up time period	Disease fatality
160	8.6%	Low fatality
65	18.9%	
41	27.5%	
25	39.4%	
16	51.3%	
12	59.1%	
8	69.4%	
5	79.9%	
3	87.8%	
1	96.1%	High fatality

Table 6.2 Characteristics of the 10 survivor cohorts generated by different case fatality patterns in the inception cohort when case fatality is independent of exposure*

Fatality	Exponential mean for disease survival pattern	# in survivor cohort	# diseased	Average age at disease onset in years	Average total exposure at disease onset in years
Low	160	49637	3871	47	16
	65	49200	3434	47	16
	41	48836	3070	48	17
	25	48331	2565	50	17
	16	47830	2064	51	18
	12	47498	1732	52	19
	8	47060	1294	54	19
	5	46619	853	56	20
	3	46283	517	58	21
High	1	45930	164	59	21

*Note that the inception cohort has 50,000 subjects and 4,234 subjects developed disease

Table 6.3 Hazard ratios* and 95% confidence intervals describing relationship between exposure and disease when case fatality is independent of exposure

Case fatality	Exponential mean for disease survival pattern	HR (95% confidence interval) inception cohort	HR (95% confidence interval) survivor cohort
Low	160	1.99 (1.86 - 2.12)	1.97 (1.84 - 2.11)
	65	1.99 (1.86 - 2.12)	2.01 (1.87 - 2.17)
	41	1.99 (1.86 - 2.12)	2.00 (1.85 - 2.16)
	25	1.99 (1.86 - 2.12)	2.00 (1.84 - 2.18)
	16	1.99 (1.86 - 2.12)	2.03 (1.84 - 2.23)
	12	1.99 (1.86 - 2.12)	1.98 (1.78 - 2.20)
	8	1.99 (1.86 - 2.12)	1.96 (1.73 - 2.21)
	5	1.99 (1.86 - 2.12)	1.95 (1.68 - 2.26)
	3	1.99 (1.86 - 2.12)	1.98 (1.63 - 2.40)
High	1	1.99 (1.86 - 2.12)	2.06 (1.47 - 2.88)

*Hazard ratios reported per 10-unit increase in exposure

Table 6.4 Hazard ratios* describing relationship between exposure and disease when case fatality differs by exposure: most exposed subjects are the 10% of diseased subjects with highest cumulative exposure at time of disease and less exposed subjects are the 90% of diseased subjects with least exposure

% less exposed	Exponential mean for less exposed	% most exposed	Exponential mean for most exposed	HR (95% confidence interval) inception cohort	HR (95% confidence interval) survivor cohort
90%	160	10%	160	1.99 (1.86 - 2.12)	1.97 (1.84 - 2.11)
90%	160	10%	65	1.99 (1.86 - 2.12)	1.93 (1.80 - 2.07)
90%	160	10%	41	1.99 (1.86 - 2.12)	1.89 (1.76 - 2.03)
90%	160	10%	25	1.99 (1.86 - 2.12)	1.84 (1.71 - 1.97)
90%	160	10%	16	1.99 (1.86 - 2.12)	1.78 (1.65 - 1.90)
90%	160	10%	12	1.99 (1.86 - 2.12)	1.72 (1.61 - 1.85)
90%	160	10%	8	1.99 (1.86 - 2.12)	1.64 (1.53 - 1.76)
90%	160	10%	5	1.99 (1.86 - 2.12)	1.54 (1.44 - 1.66)
90%	160	10%	3	1.99 (1.86 - 2.12)	1.46 (1.35 - 1.57)
90%	160	10%	1	1.99 (1.86 - 2.12)	1.36 (1.26 - 1.46)

*Hazard ratios reported per 10-unit increase in exposure

Table 6.5 Hazard ratios* describing relationship between exposure and disease when case fatality differs by exposure: most exposed subjects are the 50% of diseased subjects with highest cumulative exposure at time of disease and less exposed subjects are the 50% of diseased subjects with least exposure

% less exposed	Exponential mean for less exposed	% most exposed	Exponential mean for most exposed	HR (95% confidence interval) inception cohort	HR (95% confidence interval) survivor cohort
50%	160	50%	160	1.99 (1.86 - 2.12)	1.96 (1.83 - 2.10)
50%	160	50%	65	1.99 (1.86 - 2.12)	1.87 (1.74 - 2.00)
50%	160	50%	41	1.99 (1.86 - 2.12)	1.75 (1.63 - 1.88)
50%	160	50%	25	1.99 (1.86 - 2.12)	1.60 (1.48 - 1.72)
50%	160	50%	16	1.99 (1.86 - 2.12)	1.45 (1.34 - 1.57)
50%	160	50%	12	1.99 (1.86 - 2.12)	1.32 (1.22 - 1.43)
50%	160	50%	8	1.99 (1.86 - 2.12)	1.14 (1.05 - 1.24)
50%	160	50%	5	1.99 (1.86 - 2.12)	0.97 (0.89 - 1.06)
50%	160	50%	3	1.99 (1.86 - 2.12)	0.84 (0.77 - 0.92)
50%	160	50%	1	1.99 (1.86 - 2.12)	0.67 (0.61 - 0.74)

*Hazard ratios reported per 10-unit increase in exposure

CHAPTER 7: EARLY LIFE PERFLUOROOCTANOIC ACID (PFOA) EXPOSURE AND OVERWEIGHT AND OBESITY RISK IN ADULTHOOD IN A COMMUNITY WITH ELEVATED EXPOSURE

[At the time of dissertation submission, this chapter was under review for publication in the journal Environmental Research. Environmental Research allows authors to include their work in their dissertation.]

Vaughn Barry, Lyndsey Darrow, Mitch Klein, Andrea Winquist, Kyle Steenland

Rollins School of Public Health, Emory University, Atlanta, Georgia, USA

Corresponding Author:

Vaughn Barry

Departments of Epidemiology and Environmental Health

Rollins School of Public Health, Emory University

1518 Clifton Road, Atlanta, GA 30322

E-mail: vbarry@emory.edu

Phone: 612-669-9098

Fax: 404-727-8744

ABSTRACT

Background: Infants and young children may be susceptible to potential developmental effects of perfluorooctanoic acid (PFOA) exposure. Previous studies that examined general populations exposed to low PFOA levels found that early life exposure may cause high body mass index (BMI) in adulthood and effects may be stronger in women compared to men.

Objectives: Examine whether elevated early life PFOA exposure was associated with adult BMI among a group of mid-Ohio valley residents exposed to a wide range of early life PFOA levels due to emissions from a chemical plant.

Methods: The cohort consisted of 8,764 adults aged 20-40 years who reported height and weight on a 2008-2011 survey. Annual retrospective early life PFOA serum concentrations were estimated for each participant. Estimates accounted for exposure received from nearby chemical plant emissions and also for background exposure not originating from the facility. We defined early life exposure as the estimated average PFOA serum concentration over the first three years of life. We considered a participant to have elevated exposure if their early life estimate included exposure from the chemical plant. We examined the association between early life PFOA exposure and adult overweight (BMI $\geq$ 25 kg/m^2) and obesity (BMI $\geq$ 30 kg/m^2) risk using logistic regression models.

Results: Nearly half the participants (45%) had early life PFOA exposure serum concentration estimates above background levels. Using participants who were exposed only to background PFOA levels as the referent category with quintiles of exposure above background, adjusted odds ratios (and 95% confidence intervals) for overweight risk by increasing exposure category for women were 1.0 (ref), 1.0 (0.8, 1.3), 1.0 (0.8, 1.2), 1.0 (0.8, 1.2), 0.9 (0.7, 1.1), and 0.9 (0.7, 1.1)

and for men were 1.0 (ref), 0.9 (0.6, 1.1), 1.0 (0.7, 1.3), 1.0 (0.8, 1.4), 0.7 (0.5, 0.9), and 0.9 (0.7, 1.1). Odds ratios for adult obesity risk were similar.

Conclusions: Elevated levels of PFOA exposure in early life were not associated with overweight and obesity risk in adulthood and results did not vary by sex.

INTRODUCTION

Perfluorooctanoic acid (PFOA) is a synthetic chemical used since the late 1940's to manufacture industrial and household products (Steenland 2010). It is persistent in the environment, has an estimated human half-life of two to four years, and is found at low levels in the serum of most people living in the U.S. (Lau 2007, Olsen 2007a, Seals 2011, Calafat 2007b). Exposure sources in the general population are not well established, but likely include drinking water, diet, food packaging, and household products (Lau 2007). Although PFOA in US manufacturing has been phased out over the past five years, PFOA serum concentrations have not considerably decreased (Kato 2011).

Research from the growing fetal origins of adult disease field suggests that the body's organs and systems are malleable while developing and are particularly susceptible to the environment experienced while in utero and during the first few years of life (Barker 2004, Lynch 2005, Newbold 2007, Oken 2003). It has been hypothesized that early life PFOA exposure may affect adult weight by permanently altering weight controlling hormones, perhaps by its known activation of the peroxisome proliferator-activated receptor alpha, which is a hormone receptor that plays a role in metabolism. A relatively newer hypothesis posits that PFOA may alter hormone levels through ovarian effects and consequently women may be more susceptible to the effects than men (White 2011, Zhao 2010).

If PFOA is predominantly conveyed through drinking water, then infants and young children may be particularly vulnerable to PFOA's effects because per body weight, they consume more fluid than adults and thus have higher relative exposure for their size (Post 2012). Infants and toddlers could conceivably receive exposure from breast milk from mothers who

drink contaminated water and/or from formula prepared with contaminated drinking water. In a large mother-child pair study involving participants exposed to PFOA contaminated drinking water, PFOA serum levels in children under age 5 years were 44% higher than maternal PFOA levels (Mondal 2012). Additionally, PFOA serum levels are typically higher in children than adults, both in the general population and in highly exposed populations (Calafat 2007b, Steenland 2009a).

There are only two studies that examine possible effects of early life PFOA exposure on adult adiposity. One animal study found that mice exposed to low doses of PFOA while in utero were overweight in mid-life compared to both unexposed and highly exposed mice (Hines 2009). The same study also reported no mid-life effect when the same low-dose PFOA regimen was instead administered to mice in young adulthood.

In an epidemiologic study, researchers measured PFOA in the serum of 665 pregnant women recruited from the general population in Denmark and then measured the BMI of the offspring 20 years later (Halldorsson 2012). Twenty-year-old women who had been more highly exposed to PFOA in utero (PFOA serum concentrations of 4.8 - 19.8 ng/mL in the pregnant mother) were more likely to be overweight or obese compared to their less exposed counterparts (PFOA serum concentrations of 0.1 - 4.8 ng/mL in the pregnant mother). Adjusted relative risks and 95% confidence intervals by increasing PFOA quartile were 1.0, 1.3 (0.6 - 2.8), 1.7 (0.8 - 3.5), and 2.5 (1.3 - 5.0). There was no relationship between *in utero* PFOA exposure and adult overweight or obesity in the twenty-year-old men (all relative risks = 1.0). Participants in this study had low PFOA levels typical of general populations limiting the reported dose-response relationship to a narrow window of exposure levels.

Here we examine whether estimated PFOA exposure serum concentration levels experienced during the first 3 years of life were associated with BMI in adulthood. The participants were recruited from a mid-Ohio valley community that was exposed to PFOA through drinking water due to emissions from a chemical plant. Participants had a wide range of early life PFOA exposure estimates from exceptionally high PFOA levels to lower levels equal to the general U.S. population. For nearly half the participants, estimated early life PFOA serum concentrations were higher than typically seen in the general population and we focus here on the effects of this elevated early life PFOA exposure on adult BMI.

METHODS

Study Population

DuPont chemical plant in Washington, West Virginia began using PFOA in its manufacturing process in 1951. The plant released PFOA into the Ohio River and air beginning in the 1950s, peaking in the 1990s, and decreasing after 2001. PFOA emitted from the plant entered the groundwater which was the public drinking water source.

In 2001, residents living near the plant filed a class action lawsuit alleging health damage due to PFOA contaminated drinking water. A pretrial settlement required DuPont to provide funding for an independent community health study called the "C8 Health Project" (C8 Health Project 2012, Frisbee 2009). People were eligible to participate if they lived, worked, or attended school for at least one year in one of six contaminated water districts near the plant between 1950 and December 3rd, 2004. Participants were enrolled during 2005-2006. Participants took a survey and gave blood samples for PFOA serum concentration measurements. Subsequent

follow-up surveys were conducted during 2008-2011 on C8 Health Project participants and a group of DuPont workers. A cohort of 32,254 adult worker and resident participants who completed at least one subsequent survey was assembled and previously described (Winquist 2013). This analysis included the 8,764 participants who were between the ages of 20 and 40 years at the time of their first follow-up survey and had self-reported their height and weight at the time of the follow-up survey.

PFOA Estimates

PFOA serum concentration estimates were calculated retrospectively for each participant for each year of their life beginning on the participant's date of birth. Estimation procedure details have been published (Shin 2011a, Shin 2011b) and are briefly summarized here. The correlation between estimated and measured serum concentrations in 2005 among C8 Health Project participants from the assembled cohort (n=30,303) was 0.71 (Winquist 2013). Estimates combined two types of PFOA exposure: 1) background exposure not originating from DuPont emissions and 2) exposure originating from DuPont. Estimates for participants who had ever worked at the DuPont plant also took into account occupational exposure they may have received at their specific job.

Background exposure was based on PFOA levels observed in the general population. The National Health and Nutrition Examination Survey (NHANES) began measuring PFOA serum levels in the U.S. general population in 1999. The measurements from the NHANES periods 1999-2000 and 2003-2004 were considered background values in the estimation process for these years and were also used to generate the estimated background levels for earlier years. Specifically, background exposure for the study participants was assumed to be zero in the year

1950, and then was interpolated linearly by year to the period 1999-2000 and then again to the period 2003-2004.

Exposure from DuPont emissions was estimated using historical regional data including the amount of PFOA emitted by the DuPont facility over time, wind patterns, river flow, and groundwater flow. Estimates based on DuPont emissions also took into account the participant's reported residential history, drinking water source, and tap water consumption. Additionally, a PFOA absorption, distribution, metabolism, and excretion model that incorporated age and gender of each participant was used to estimate the PFOA concentration in serum over time.

Because PFOA serum concentration in young children is strongly correlated with maternal serum concentration, additional procedures were used to estimate PFOA exposure in early life (Mondal 2012, Shin 2011b). Seven previous studies reported measured cord blood PFOA concentrations in relation to maternal serum concentrations in order to approximate the PFOA exposure an infant can receive both prenatally across the placental barrier and after birth through breastfeeding (Fei 2007, Fromme 2010, Hanssen 2010, Kim 2011, Midasch 2007, Monroy 2008, Needham 2010). A previous study also calculated the ratio of maternal-infant PFOA serum concentrations by measuring PFOA serum concentrations of 40 infants and their mothers who had participated in the C8 Health Project (Shin 2011b). Because water district was the primary driver of exposure variation in this population, based on these previous studies serum concentration estimates for newborns and one-year-old infants were assumed to be 78.5% and 127% of the estimated median female serum concentration for the water district of the child's birth, respectively (Shin 2011b). By age 2, estimated PFOA concentration was based solely on the child's individual information.

We considered early life exposure as the estimated PFOA serum concentrations for each individual beginning at birth, ending at their third birthday, and averaged over the first three years of life. Every participant was estimated to have background exposure in early life but not all participants were estimated to experience DuPont emissions exposure in early life. We considered a participant to have elevated exposure if their early life estimate included exposure from the chemical plant.

BMI Measurements

BMI was self-reported. Participants were asked "how tall are you, to the nearest inch, without shoes?" and "how much do you weigh now, to the nearest pound, without shoes?" Pregnant women were asked to report pre-pregnancy weight. BMI was calculated by dividing weight in pounds by height in inches squared multiplied by 703. We considered a participant overweight if their reported height and weight corresponded to a BMI greater than or equal to 25 kg/m^2 and obese if BMI was greater than or equal to 30 kg/m^2. By definition, the overweight definition included participants who reported a BMI indicating obesity. These were used as two alternative outcomes. Most participants reported height and weight during their survey (8,764 of 8,859).

Statistical Analysis

We used logistic regression models to calculate odds ratios describing the association between early life PFOA exposure and adult overweight and obesity. We used separate models for men and women since previous hypotheses indicated that PFOA could influence men and women differently. We considered participants who received only background exposure as the

referent category. We then created quintiles or deciles of PFOA exposure above the referent group among levels for participants who received both background and DuPont emissions exposures. Indicator variables representing these categories were included in the regression models. All models were adjusted for age at interview in two-year increments (20-22, 23-24, 25-26, 27-28, 29-30, 31-32, 33-34, 35-36, 37-38, 39-40), cigarette smoking (current, former, never), education (less than highschool, highschool, some college, bachelor's degree or more), and average walking pace (slow, normal, brisk) using indicator variables. Age, smoking status, education, and walking pace were self-reported at the time of the survey. All of these variables were associated with both early life exposure and adult BMI in the data; we assumed that they were confounders. Separate regression models considered the log of PFOA exposure as a continuous variable. All analyses were done using SAS version 9.3 (SAS Institute, Cary, North Carolina).

Sensitivity Analyses

We conducted sensitivity analyses using different early life PFOA exposure definitions to explore the degree to which exposure estimates varied with age. Sensitivity analyses included models with PFOA exposure defined 5 different ways, as estimated PFOA received during the: 1) single first year of life 2) single second year of life 3) single third year of life 4) cumulative first ten years of life and 5) cumulative first twenty years of life. Models using these exposure definitions also controlled for the potential confounders listed above.

Participants who did not experience DuPont exposure in early life may be different from participants who did because they were likely not yet living in the area yet. Also, there may not be significant differences in early life exposure estimates between participants who did not

experience DuPont exposure and participants who experienced only low levels of DuPont exposure. For these reasons, we ran models that excluded participants who did not experience DuPont exposure in early life. We created quintiles of DuPont early life exposure in these models and the referent category was the first quintile of participants who experienced DuPont early life exposure.

We used walking pace as a proxy for physical activity level since the surveys did not ask specifically about physical activity. However the participants who had participated in the C8 Health Project (8,429 of 8,764) several years earlier were asked "do you engage in an exercise program?" and if so "how many times a week do you engage in an exercise program?" A third of participants (n=2,784) reported engaging in an exercise program in 2005. We considered analyses using exercise program in 2005 (yes/no) as an adjusting variable instead of walking pace.

Finally, because our overweight category (BMI $\geq$ 25 kg/m^2) by definition included participants with BMI $\geq$ 30 kg/m^2, we also considered models that re-defined overweight as a BMI between 25 and 30 kg/m^2. In these models, we excluded participants with BMI $\geq$ 30 kg/m^2.

RESULTS

Cohort Characteristics

Most of the 8,764 participants completed a first follow-up survey in 2008 or 2009 (Table 7.1). All were between the ages of 20 and 40 years old at the time of survey. Nearly all the participants reported being white race and more than half were women. Median BMI for the

cohort was 27.2 kg/m^2 and ranged from 10.9 to 76.7 kg/m^2. Median BMI was slightly higher for men compared to women (28 vs 27 kg/m^2) and increased with age (25 kg/m^2 among twenty-year old participants to 28 kg/m^2 among forty-year old participants). Median BMI was correlated with walking pace: it was lowest among participants who reported a brisk walking pace (26 kg/m^2), higher among those who reported a normal walking pace (28 kg/m^2), and highest among those who reported a slow walking pace (31 kg/m^2).

Nearly half the participants (45%) were estimated to experience elevated early life PFOA exposure (i.e. some exposure attributed to DuPont emissions). Elevated early life PFOA exposure was more common among young adults compared to older adults (Figure 7.1). Median estimated total PFOA serum concentration averaged over the first three years of life was 3.8 ng/mL (geometric mean=6.6 ng/mL) and ranged from 1.3 ng/ml to 2272.7 ng/mL. Median PFOA exposure estimates increased over time (Table 7.2). Median total exposure was higher among participants with DuPont exposure compared to participants without (12.7 ng/mL vs. 3.1 ng/mL).

Most participants participated in the C8 Health Project (8,387 of 8,764) during 2005-2006 and gave a blood sample for a PFOA serum concentration measurement. The geometric mean PFOA serum concentration in 2005-2006 for these adult participants was high compared to the NHANES 2005-2006 reported geometric mean (21.7 ng/mL vs. 3.9 ng/mL) (Kato 2011).

Early Life PFOA Exposure In Relation To Adult BMI

Participants estimated to have elevated PFOA exposure during the first three years of life were not at increased risk of being overweight or obese in adulthood compared to less exposed participants. Results did not vary by sex. Using those who were exposed to only background

PFOA levels in early life as the referent category with quintiles above, adjusted odds ratios (and 95% confidence intervals) for overweight or obesity risk by increasing exposure quintile for women were 1.0 (ref), 1.0 (0.8, 1.3), 1.0 (0.8, 1.2), 1.0 (0.8, 1.2), 0.9 (0.7, 1.1), and 0.9 (0.7, 1.1) and for men were 1.0 (ref), 0.9 (0.6, 1.1), 1.0 (0.7, 1.3), 1.0 (0.8, 1.4), 0.7 (0.5, 0.9), and 0.9 (0.7, 1.1) (Table 7.3). Odds ratios describing obesity risk for men and women using quintiles were similar (Table 7.3). Covariates in these adjusted models showed the expected patterns of association with BMI (data not shown). Specifically, current smokers had lower overweight or obesity risk compared to former or never smokers, those reporting slow walking paces had higher overweight or obesity risk compared to those with normal or brisk walking paces, and participants reporting a college education had lower overweight/obesity risk compared to those reporting less education.

We also considered models categorizing participants with DuPont exposure into deciles (Table 7.4). Compared to those with low exposure, there was no increased risk of adult overweight or obesity with increasing elevated PFOA exposure for men or women.

Results from adjusted models that used continuous early life PFOA exposure showed no significant trend or increased risk of overweightness or obesity by increasing PFOA unit in ng/mL (Table 7.5).

Sensitivity Analyses

We considered different definitions of early life exposure to see whether exposure estimates varied across the first three years of life and also to see whether specific years within the first three years of life were important in determining the association with adult BMI. Results

using different definitions of early life exposure were similar to reported results (Figure 7.2). Results from models that examined PFOA exposure experienced during both the first ten and twenty years of life were also similar and showed no increased risk of overweightness or obesity in adulthood for those with elevated exposure compared to those with less exposure.

There were no significant differences between participants without early life DuPont exposure (n=4,784) and participants with DuPont exposure (n=3,976) with respect to sex, race/ethnicity, or walking pace. Participants with DuPont exposure in early life were more likely to have a Bachelor's degree (31% vs. 25%), more likely to be 30 years or less at the time of interview (47% vs 35%), and less likely to report a BMI indicating obesity (32% vs. 36%). Models run that excluded participants without early life DuPont exposure were similar to reported models that included these participants.

When we replaced the walking pace covariate with physical activity level reported several years earlier, results did not change. We ran models that excluded any participant with a reported BMI ≥ 30 and results also did not change from the reported results.

DISCUSSION

We found that both male and female participants who were highly exposed to PFOA in the first three years of life were not at increased risk of being overweight or obese in adulthood compared to participants who were less exposed.

One strength of this analysis is the ability to examine the potential effects of elevated PFOA exposure since many participants had early life exposure estimates that were higher than

those seen in the general population. The wide range of exposure levels in this cohort also hopefully mitigated the effects of exposure misclassification: even if exposure estimates are misclassified, the rankings of participants within exposure category are likely still accurate. We did not have a group of completely unexposed participants since everyone in the U.S. is exposed at some level. Instead we considered those who did not receive any exposure from the DuPont emissions as the referent group. These participants born between 1968 and 1988 had a median total PFOA exposure estimate of 3.1 ng/mL (range= 1.3 - 5.2 ng/mL) while the PFOA level in the general population in 2005-2006 was estimated as 3.9 ng/mL in 2005-2006 (Kato 2011). Most results in the current literature are based on information from participants recruited from the general population who experience exposure levels similar to our referent group.

Our results are not directly comparable to what Halldorsson et al. report from a cohort of participants recruited from the general Danish population. In that study, pregnant women had PFOA measured during their gestational week 30 and their offspring were then followed up twenty years later. They found that higher in-utero PFOA exposure was positively and significantly associated with the risk of being overweight at age twenty years among the female offspring but not the male offspring. The median PFOA serum concentration in the Danish participants was 3.7 ng/mL, as expected from a population without any local contamination sources. Although the estimated median serum concentration among the participants in our study was 3.8 ng/mL, the estimates ranged from 1.2 ng/mL to 1161.6 ng/mL.

We could have looked at the PFOA effect only in the referent group to compare to Halldorsson et al results but we did not believe our exposure estimates in this category were sensitive enough to divide up into even smaller categories. The participants in our referent group were not estimated to have received exposure from DuPont emissions during early life and

consequently their corresponding exposure estimates were based only on the participant's age, gender, and residential location. The exposure estimation process was targeted at estimating PFOA exposure due to DuPont emissions. Detailed residential histories, drinking water consumption, and water district information were collected for this purpose. Although the estimation process involved several assumptions that may have led to exposure misclassification, we believe an infant living in a highly exposed water district likely had higher PFOA levels than infants living in areas not affected by the contamination. Additionally, exposure estimates in our study did not rely on biomarkers that could be subject to bias driven by slight differences in pharmacokinetics and metabolism of PFOA across individuals predisposed or not predisposed to the outcome.

Previous studies have reported associations between prenatal PFOA exposure and low birth weight (Maisonet 2012, Apelberg 2007, Fei 2007). These relationships could also explain observed relationships between early-life PFOA exposure and adult BMI as low birth weight is associated with an increased risk of overweight and obesity in later life (Te Velde 2003). However, in studies of birth outcomes conducted in the same region as our study, with subjects exposed to a wide range of PFOA levels, we have observed little evidence of associations between measured or modeled maternal PFOA levels and low birth weight (Darrow 2013, Stein 2009, Savitz 2012a, Savitz 2012b). For adult BMI as well as birth outcomes, it is possible that all of the variation in risk is at the low end of the exposure spectrum where exposure measurement error in our study is greatest.

We limited the analysis to adults aged 20 through 40 years at the time of interview for a few reasons. First, we thought early life exposure estimates would be most accurate for those ages since early life for these participants was relatively recent. Second, adults aged 20 through

40 at the time of interview had birth years in the mid-1960's through mid-1980's. This means we had a wide range of exposure levels corresponding with DuPont emission patterns. Finally, we hoped that limiting the analysis cohort to young adults made it more likely that self-reported BMI was not yet caused or influenced by co-morbidities or illnesses that tend to affect adults of older ages.

BMI was self-reported by participants. Previous studies have shown that study participants often under-report weight and over-report height (Nawaz 2001). The bias towards reporting a lower weight may be greater in participants who are obese (Shirley 2013). We report results where we grouped both participants with an overweight BMI and an obese BMI together. We expect BMI misclassification would be non-differential with respect to early life PFOA exposure and could bias results toward the null if misclassification is large.

We found that elevated PFOA exposure in early life was not associated with overweight or obesity risk in adulthood compared to low exposure. Our results can not address whether risk varies within the category of low exposure.

Table 7.1 Participant demographic characteristics

Demographic characteristic	N (%)	Median BMI (range)
Participants	8,764	27.2 (11-77)
Year of survey		
2008	4,324 (49.3)	27.4 (14-78)
2009	3,886 (44.3)	27.1 (11-75)
2010	489 (5.6)	26.6 (17-77)
2011	65 (0.7)	26.1 (13-45)
Sex		
Male	3,685 (42.1)	27.7 (13-77)
Female	5,079 (58.0)	26.6 (11-77)
Age in years at time of survey		
20-22	78 (0.89)	25.2 (16-57)
23-24	789 (9.0)	25.5 (15-58)
25-26	863 (9.9)	25.7 (14-56)
27-28	920 (10.5)	26.6 (14-57)
29-30	924 (10.5)	27.1 (17-73)
31-32	911 (10.4)	27.1 (16-77)
33-34	933 (10.7)	27.5 (17-68)
35-36	993 (11.3)	27.6 (17-75)
37-38	1203 (13.7)	28.2 (16-69)
39-40	1150 (13.1)	28.2 (11-77)
Race/Ethnicity*		
White	8,483 (97.3)	27.2 (11-77)
Non-white	232 (2.7)	27.4 (17-52)
Education at interview*		
Less than highschool education	464 (5.3)	26.9 (17-57)
Highschool education or GED	2,431 (27.8)	28.0 (13-77)
Some college	3,414 (39.0)	27.8 (11-75)
Bachelor's degree or more	2,445 (27.9)	25.8 (15-77)
Smoking at interview*		
Current	2,366 (27.1)	26.4 (14-77)
Former	1,448 (16.6)	27.7 (16-69)
Never	4,914 (56.3)	27.4 (11-77)
Walking Pace*		
Slowly	471 (5.4)	31.0 (13-77)
Normally - between slow and brisk	5,679 (64.8)	27.8 (11-73)
Briskly (always in a hurry)	2,597 (29.6)	25.6 (16-55)
Unable to walk	11 (0.1)	27.3 (22-32)
BMI at interview		
<18.5	157 (1.8)	17.8 (11-18)
18.5 – 24.9	2,886 (32.9)	22.5 (19-25)
25.0 – 29.9	2,748 (31.4)	27.3 (25-30)
≥30	2,973 (33.9)	34.5 (30-77)
Estimated early life^ PFOA exposure attributed to DuPont emissions (ng/mL)		

0	4,784 (54.6)	27.4 (11-77)
0.0002 - 8.0	1,899 (21.7)	27.4 (15-69)
8.1 - 100.0	1,544 (17.6)	26.5 (14-73)
100.1 - 2266.2	533 (6.1)	25.8 (16-56)

*49 missing race, 10 missing education, 36 missing smoking (7 of 36 said yes to ever smoked so they could be former or current smokers), 6 missing walking

^Early life defined as average annual exposure from birth to the 3rd birthday

Table 7.2 Median estimated total early life PFOA exposure by birth year (n=8,760)

Birth year	Total #	Median early life PFOA exposure (ng/mL)	Interquartile Range	Range
1967 - 1969	1,059	2.5	2.2, 3.0	1.3 - 280.4
1970 - 1971	1,211	2.7	2.4, 4.3	1.5 - 251.0
1972 - 1973	1,019	3.0	2.7, 9.2	1.6 - 245.4
1974 - 1975	917	3.3	3.0, 10.4	1.7 - 409.8
1976 - 1977	909	3.7	3.2, 12.6	2.0 - 644.9
1978 - 1979	930	4.0	3.5, 14.2	2.2 - 1065.7
1980 - 1981	928	4.3	3.8, 16.7	2.2 - 1121.2
1982 - 1983	836	4.7	4.1, 22.8	3.4 - 1750.0
1984 - 1985	877	5.1	4.4, 23.8	3.6 - 2272.7
1986 - 1988	74	9.7	4.2, 21.6	3.8 - 1383.1
TOTAL	8,760	3.8	2.9, 11.4	1.3 - 2272.7
Total exposure in participants without DuPont exposure	4,784	3.1	2.6, 3.7	1.3 - 5.2
Total exposure in participants with DuPont exposure	3,976	12.7	5.4, 24.9	1.5 - 2272.7

Table 7.3 Associations between early life PFOA exposure and risk of adult overweightness (BMI≥25) and obesity (BMI≥30) by PFOA categories^

Median above background early life PFOA (range) in ng/mL	Total #	# overweight	ADJUSTED* OR (95% CI) Overweight	# obese	ADJUSTED* OR (95% CI) Obese
FEMALES (n=5,079)					
Category 1: 0 (---)	2780	1687	Referent	981	Referent
Category 2: 0.3 (0.01 – 1.1)	460	274	1.0 (0.8, 1.3)	145	0.9 (0.7, 1.1)
Category 3: 5.0 (1.1 – 6.7)	460	281	1.0 (0.8, 1.2)	163	1.0 (0.8, 1.2)
Category 4: 8.5 (6.7 – 10.5)	458	277	1.0 (0.8, 1.2)	161	1.0 (0.8, 1.3)
Category 5: 14.5 (10.5 - 32.2)	461	250	0.9 (0.7, 1.1)	150	1.0 (0.8, 1.3)
Category 6: 194.3 (32.3-2125.0)	459	237	0.9 (0.7, 1.1)	114	0.8 (0.6, 1.0)
MALES (n=3,685)					
Category 1: 0 (---)	2004	1519	Referent	723	Referent
Category 2: 0.3 (0.01 – 0.8)	336	246	0.9 (0.6, 1.1)	107	0.8 (0.6, 1.1)
Category 3: 3.5 (0.8 – 6.1)	335	258	1.0 (0.7, 1.3)	115	0.9 (0.7, 1.1)
Category 4: 8.2 (6.1 – 10.3)	336	260	1.0 (0.8, 1.4)	129	1.1 (0.8, 1.3)
Category 5: 13.6 (10.3 - 31.0)	335	206	0.7 (0.5, 0.9)	89	0.8 (0.6, 1.0)
Category 6: 164.6 (31.0-2266.2)	336	225	0.9 (0.7, 1.1)	96	0.9 (0.7, 1.2)

^ The first category consists of participants who received only background PFOA exposure. The next 5 categories are quintiles of those who received DuPont exposure.

* Adjusted for age in 2-year increments, smoking (current, former, never), education (less than highschool, highschool, some college, bachelor's degree or more) and walking pace (slow, normal, brisk) using indicator variables

Table 7.4 Associations between early life PFOA exposure and risk of adult overweightness (BMI≥25) and obesity (BMI≥30) by PFOA categories^

Median above background early life PFOA (range) in ng/mL	Total #	# overweight	ADJUSTED* OR (95% CI) Overweight	# obese	ADJUSTED* OR (95% CI) Obese
FEMALES (n=5,079)					
Category 1: 0 (---)	2780	1687	Referent	981	Referent
Category 2: 0.1 (0.01 – 0.3)	229	126	0.9 (0.7, 1.2)	69	0.9 (0.6, 1.2)
Category 3: 0.6 (0.3 – 1.1)	231	148	1.2 (0.9, 1.6)	76	1.0 (0.7, 1.3)
Category 4: 2.4 (1.1 – 5.0)	229	135	1.0 (0.8, 1.3)	73	0.9 (0.7, 1.3)
Category 5: 5.8 (5.0 – 6.7)	231	146	1.0 (0.7, 1.3)	90	1.0 (0.8, 1.4)
Category 6: 7.6 (6.7 – 8.5)	229	143	1.0 (0.8, 1.4)	86	1.1 (0.8, 1.4)
Category 7: 9.4 (8.5 – 10.5)	229	134	1.0 (0.7, 1.3)	75	0.9 (0.7, 1.3)
Category 8: 11.6 (10.5 – 14.5)	230	135	1.1 (0.8, 1.5)	82	1.2 (0.9, 1.6)
Category 9: 20.2 (14.5 – 32.2)	231	115	0.7 (0.6, 1.0)	68	0.8 (0.6, 1.2)
Category 10: 84.6 (32.3 – 192.0)	229	128	1.0 (0.7, 1.3)	62	0.8 (0.6, 1.1)
Category 11:412.6 (194.3–2125)	230	109	0.8 (0.6, 1.1)	52	0.7 (0.5, 1.0)
MALES (n=3,685)					
Category 1: 0 (---)	2004	1519	Referent	723	Referent
Category 2: 0.1 (0.01 – 0.3)	168	129	0.9 (0.6, 1.4)	52	0.7 (0.5, 1.0)
Category 3: 0.5 (0.3 – 0.8)	168	117	0.8 (0.6, 1.1)	55	1.0 (0.7, 1.4)
Category 4: 1.6 (0.8 – 3.5)	168	121	0.9 (0.6, 1.3)	57	1.0 (0.7, 1.4)
Category 5: 5.2 (3.5 – 6.1)	167	137	1.1 (0.7, 1.7)	58	0.8 (0.6, 1.1)
Category 6: 7.2 (6.1 – 8.2)	168	139	1.3 (0.8, 2.0)	68	1.0 (0.7, 1.5)
Category 7: 9.2 (8.2 – 10.3)	168	121	0.9 (0.6, 1.2)	61	1.1 (0.8, 1.5)
Category 8: 11.6 (10.3 – 13.6)	168	103	0.7 (0.5, 0.9)	46	0.8 (0.6, 1.2)
Category 9: 19.7 (13.8 – 31.0)	167	103	0.7 (0.5, 1.0)	43	0.8 (0.5, 1.1)
Category 10: 67.3 (31.0 – 166.1)	169	116	0.9 (0.6, 1.3)	52	1.0 (0.7, 1.4)
Category 11:451.6 (167.1–2266)	167	109	0.8 (0.6, 1.2)	44	0.9 (0.6, 1.3)

^ The first category consists of participants who received only background PFOA exposure. The next 10 categories are deciles of those who received DuPont exposure.

* Adjusted for age in 2-year increments, smoking (current, former, never), education (less than highschool, highschool, some college, bachelor's degree or more) and walking pace (slow, normal, brisk) using indicator variables

Table 7.5 Associations between early life PFOA exposure and risk of being overweight (BMI≥25) and obese (BMI≥30) in young adulthood by logged continuous PFOA exposure

Risk of overweight	Total #	# overweight	ADJUSTED* OR (95% CI)	p-value
Female	5079	2991	1.00 (0.99, 1.00)	0.37
Male	3685	2701	0.99 (0.98, 1.00)	0.07
Risk of obesity	Total #	# obese	ADJUSTED* OR (95% CI)	p-value
Female	5079	1706	0.99 (0.99, 1.00)	0.23
Male	3685	1255	0.99 (0.98, 1.00)	0.15

* Adjusted for age in 2-year increments, smoking (current, former, never), education (less than highschool, highschool, some college, bachelor's degree or more) and walking pace (slow, normal, brisk) using indicator variables

Figure 7.1 Estimated average early life PFOA exposure by birth year (n=8,764)

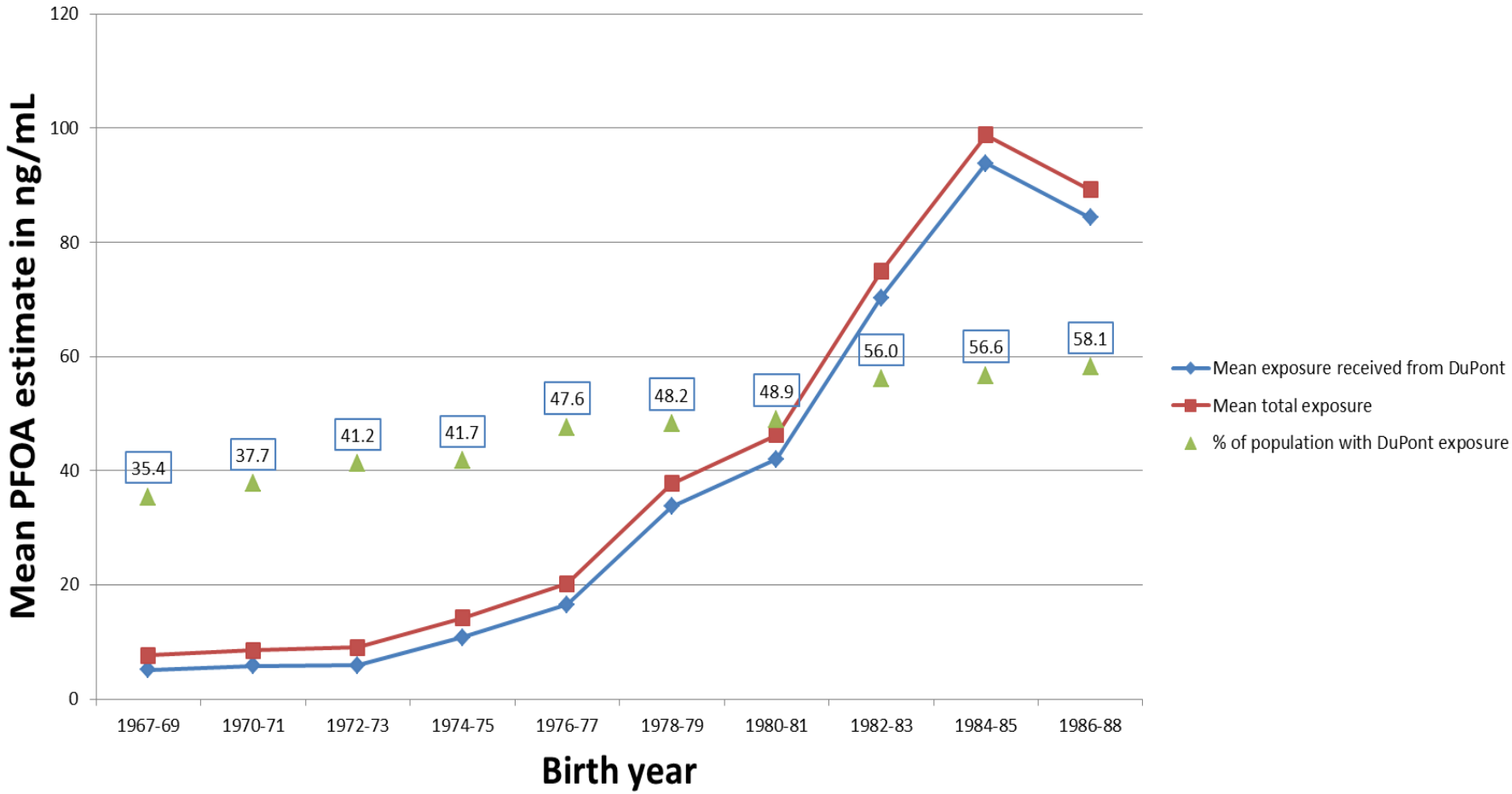
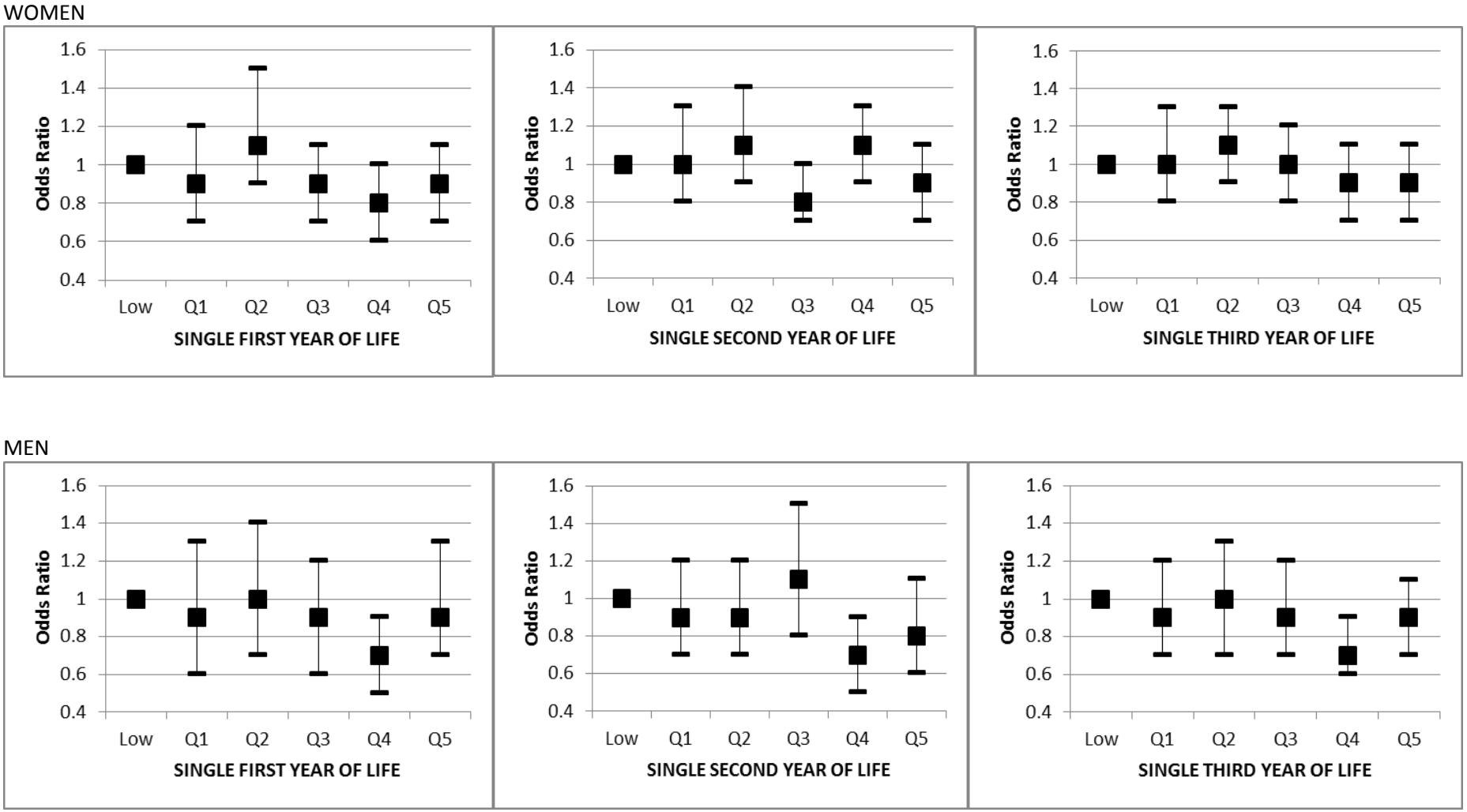


Figure 7.2 Associations between early life PFOA exposure* and adult overweight risk (BMI ≥ 25) by different early life exposure time periods.



*The first category consists of participants who received only background PFOA exposure. The next 5 categories are quintiles of those who received DuPont exposure.

CHAPTER 8: CONCLUSIONS AND FUTURE DIRECTIONS

We found that PFOA exposure was positively associated with testicular, kidney, and thyroid cancer in this mid-Ohio valley population. PFOA was not associated with the 18 other cancers we examined. PFOA caused liver, testicular, and pancreatic tumors in male rats fed PFOA in their diet over a two year period and thus, these three cancers were of a priori interest (Biegel et al. 2001). It is difficult to know exactly how to interpret our results for the more fatal cancers. There was no relationship between PFOA exposure and any of the most fatal cancers. If PFOA exposure were to cause a specific cancer to be more or less fatal than it usually is without PFOA exposure, results may be biased since this is a survivor cohort. However, as our simulation demonstrated, if cancer fatality did not differ by PFOA exposure level then PFOA-cancer estimates would not be biased, assuming no other biases or confounding. Future studies could genotype tumor tissue in participants highly exposed to PFOA to see if a different type of tumor is created with PFOA exposure.

We also found that high levels of PFOA exposure experienced during the first three years of life were not associated with overweight and obesity risk in adulthood. Results did not vary by sex. The largest strength of this analysis was the ability to examine the effects of elevated PFOA exposure since many participants had early life exposure estimates that were higher than those seen in the general population. Future research is needed to corroborate or dispute these findings since there is concern that exposure to environmental chemicals in early life could be playing a role in the obesity epidemic.

Previous research on PFOA and health outcomes has been primarily restricted to animal experiments, mortality studies of male workers with occupational exposure, and community studies of populations with low exposure levels. Human studies have been limited by small numbers of cases of illness. This dissertation examined the relationship between PFOA and

human health in a large community of residents and workers encompassing a range of exposure levels.

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APPENDIX

Table A1 Number of reported and validated[a] primary cancer cases among community (n=28,541) and occupational (n=3,713) groups

Group	Community		Occupational	
Cancer	# reported	# validated (% validated)	# reported	# validated (% validated)
Bladder	83	80 (96.4)	32	31 (96.9)
Brain	26	18 (69.2)	7	5 (71.4)
Breast	589	566 (96.1)	19	15 (79.0)
Cervical	369	21 (5.7)	14	1 (7.1)
Colorectal	264	232 (87.9)	47	44 (93.6)
Esophagus	16	12 (75.0)	5	3 (60.0)
Kidney	102	94 (92.2)	22	19 (86.4)
Leukemia	62	55 (88.7)	17	14 (82.4)
Liver	16	9 (56.3)	2	1 (50.0)
Lung	113	97 (85.8)	20	16 (80.0)
Lymphoma	145	126 (86.9)	19	16 (84.2)
Melanoma	444	204 (46.0)	75	41 (54.7)
Oral	32	19 (59.4)	3	1 (33.3)
Ovarian	85	43 (50.6)	2	0 (0)
Pancreatic	26	22 (84.6)	9	4 (44.4)
Prostate	354	322 (91.0)	161	136 (84.5)
Soft Tissue	20	14 (70.0)	5	3 (60.0)
Stomach	26	11 (42.3)	3	1 (33.3)
Testicular	27	17 (63.0)	5	2 (40.0)
Thyroid	87	79 (90.8)	11	8 (72.7)
Uterine	213	98 (46.0)	12	7 (58.3)
TOTAL	3099[b]	2139 (69.0)	490[c]	368 (75.1)

[a]Validated cases were limited to participants who reported the cancer and were subsequently confirmed either by Ohio/West Virginia cancer registry or medical record review; participants reported whether a doctor had ever told them they had a cancer or malignancy of any kind

[b]These 3,099 cancers were self-reported by 2,851 participants; some participants reported more than 1 cancer type

[c]These 490 cancers were self-reported by 441 participants; some participants reported more than 1 cancer type

Table A2 Hazard ratios and 95% confidence intervals assessing the effect of logged estimated cumulative PFOA serum concentration on cancer risk in the community (n=28,541) and occupational (n=3,713) groups

		NO LAG			10 YEAR LAG		
		# cases	HR (95% CI)[a]	p-value	# cases	HR (95% CI) [a]	p-value
Group	Cancer[b]						
Community	Bladder	76	0.96 (0.81, 1.14)	0.65	76	0.90 (0.75, 1.09)	0.29
Occupational		29	0.65 (0.44, 0.95)	0.02	29	0.73 (0.55, 0.98)	0.04
Community	Brain	13	1.14 (0.78, 1.65)	0.50	13	1.02 (0.68, 1.52)	0.94
Occupational		4	0.82 (0.26, 2.59)	0.74	4	0.73 (0.32, 1.67)	0.46
Community	Breast	546	0.96 (0.90, 1.02)	0.16	546	0.95 (0.89, 1.01)	0.11
Occupational		13	1.01 (0.59, 1.74)	0.97	13	1.03 (0.59, 1.79)	0.92
Community	Cervical	21	0.94 (0.67, 1.32)	0.74	21	1.02 (0.72, 1.43)	0.92
Occupational		1	---	---	1	---	---
Community	Colorectal	223	0.98 (0.89, 1.08)	0.75	223	0.98 (0.89, 1.09)	0.77
Occupational		41	1.12 (0.81, 1.54)	0.50	41	1.08 (0.84, 1.39)	0.55
Community	Esophagus	12	1.00 (0.66, 1.51)	0.99	12	1.01 (0.67, 1.52)	0.96
Occupational		3	1.42 (0.21, 9.74)	0.72	3	1.17 (0.19, 7.36)	0.86
Community	Kidney	87	1.14 (0.99, 1.32)	0.07	87	1.11 (0.96, 1.29)	0.17
Occupational		18	0.95 (0.59, 1.52)	0.82	18	0.99 (0.67, 1.46)	0.97
Community	Leukemia	53	0.92 (0.76, 1.13)	0.43	53	0.92 (0.75, 1.13)	0.41
Occupational		13	1.30 (0.73, 2.33)	0.37	13	1.30 (0.78, 2.18)	0.31
Community	Liver	8	0.62 (0.29, 1.29)	0.20	8	0.53 (0.21, 1.34)	0.18
Occupational		1	---	---	1	---	---
Community	Lung	95	0.85 (0.73, 1.00)	0.05	95	0.89 (0.76, 1.05)	0.17
Occupational		13	0.87 (0.51, 1.47)	0.59	13	1.04 (0.68, 1.58)	0.86
Community	Lymphoma	121	1.05 (0.92, 1.19)	0.48	121	1.02 (0.89, 1.17)	0.80
Occupational		15	1.24 (0.72, 2.14)	0.45	15	1.10 (0.73, 1.65)	0.66
Community	Melanoma	200	0.99 (0.89, 1.10)	0.82	200	1.02 (0.92, 1.14)	0.66
Occupational		41	0.80 (0.59, 1.08)	0.15	41	0.93 (0.73, 1.18)	0.53
Community	Oral	17	0.96 (0.65, 1.40)	0.82	17	0.77 (0.47, 1.27)	0.31
Occupational		1	---	---	1	0.70 (0.19, 2.62)	0.60
Community	Ovarian	43	1.00 (0.79, 1.25)	0.97	43	0.94 (0.73, 1.22)	0.66
Occupational		0	---	---	0	---	---
Community	Pancreatic	21	1.06 (0.79, 1.43)	0.68	21	0.98 (0.72, 1.34)	0.92
Occupational		3	0.98 (0.21, 4.65)	0.98	3	1.14 (0.33, 3.89)	0.84
Community	Prostate	317	0.97 (0.90, 1.05)	0.50	317	0.98 (0.90, 1.06)	0.58
Occupational		129	0.94 (0.77, 1.17)	0.59	129	0.98 (0.83, 1.16)	0.83
Community	Soft Tissue	13	0.68 (0.40, 1.14)	0.14	13	0.64 (0.36, 1.13)	0.12
Occupational		2	1.20 (0.30, 4.76)	0.80	2	0.91 (0.25, 3.33)	0.89
Community	Stomach	11	0.70 (0.40, 1.23)	0.22	11	0.74 (0.41, 1.31)	0.30

Occupational		1	---	---	1	---	---
Community	Testicular	15	1.73 (1.24, 2.40)	0.01	15	1.53 (1.09, 2.15)	0.01
Occupational		2	0.85 (0.04, 19.7)	0.92	2	1.61 (0.21, 12.20)	0.65
Community	Thyroid	78	1.04 (0.89, 1.23)	0.61	78	1.00 (0.84, 1.20)	0.96
Occupational		8	1.93 (1.00, 3.71)	0.05	8	1.12 (0.61, 2.05)	0.71
Community	Uterine	96	1.02 (0.88, 1.18)	0.79	96	0.99 (0.84, 1.16)	0.88
Occupational		7	1.05 (0.56, 1.97)	0.88	7	0.96 (0.42, 2.18)	0.92

[a] per unit of log estimated cumulative PFOA serum concentration

[b] A proportional hazards regression model was run for each cancer. Each model was adjusted for time-dependent smoking, time-dependent alcohol consumption, gender, education, and stratified by 5 year period of birth year. Time began at age 20 if the person's 20th birthday was in 1952 or later. Otherwise time began at the age the person was in 1952. Time ended at age of cancer diagnosis, age at last follow-up survey, or age on December 31st 2011, whichever came first.

--- model did not converge

Table A3 Hazard ratios and 95% confidence intervals by PFOA quartile[a] for thyroid, kidney, and testicular cancer cases among the community (n=28,541) and occupational (n=3,713) groups

			Hazard Ratio (95% CI)[b]				
		#cases	Quartile 1 (Reference)	Quartile 2	Quartile 3	Quartile 4	p-value[c]
	Cancer						
Community	Kidney–no lag	87	1.00	**1.34** (0.71, 2.52)	**1.95** (1.03, 3.70)	**2.04** (1.07, 3.88)	0.20
	Kidney –10 yr lag	87	1.00	**0.94** (0.45, 1.99)	**1.08** (0.52, 2.25)	**1.50** (0.72, 3.13)	0.02
Occupational	Kidney–no lag	18	1.00	**0.84** (0.21, 3.4)	**4.20** (1.07, 16.44)	**0.83** (0.20, 3.55)	0.54
	Kidney –10 yr lag	18	1.00	**1.22** (0.28, 5.3)	**3.27** (0.76, 14.10)	**0.99** (0.21, 4.68)	0.42
Community	Testicular–no lag	15	1.00	**0.80** (0.16, 3.97)	**3.07** (0.61, 15.36)	**5.80** (0.97, 34.58)	0.05
	Testicular–10 yr lag	15	1.00	**0.98** (0.13, 7.14)	**1.54** (0.19, 12.21)	**4.66** (0.52, 41.63)	0.02
Occupational	Testicular–no lag	2	---	---	---	---	---
	Testicular–10 yr lag	2	---	---	---	---	---
Community	Thyroid–no lag	78	1.00	**1.54** (0.73, 3.26)	**1.71** (0.81, 3.59)	**1.40** (0.66, 2.97)	0.46
	Thyroid–10 yr lag	78	1.00	**2.09** (0.91, 4.82)	**1.92** (0.82, 4.50)	**1.42** (0.60, 3.37)	0.56
Occupational	Thyroid–no lag	8	1.00	**4.64** (0.42, 50.8)	**9.70** (0.67, 141.2)	**14.72** (0.85, 253.9)	0.04
	Thyroid–10 yr lag	8	1.00	**1.65** (0.09, 31.5)	**4.52** (0.10, 198.4)	**5.85** (0.13, 257.1)	0.01

[a] Quartiles were defined by the estimated cumulative PFOA serum concentration among the thyroid, kidney, or testicular cancer cases at the time of cancer diagnosis

[b] A proportional hazards regression model was run for each cancer. Each model was adjusted for time-dependent smoking, time-dependent alcohol consumption, gender, education, and stratified by 5 year period of birth year. Time began at age 20 if the person's 20th birthday was in 1952 or later. Otherwise time began at the age the person was in 1952. Time ended at age of cancer diagnosis, age at last follow-up survey, or age on December 31st 2011, whichever came first.

[c] P-value is for linear trend test in log rate ratios across quartiles. P-values were calculated using exposure category mid-points and inverse variance weighting in a no-intercept linear regression model.

--- Not enough cases for quartile analysis