Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Radhika Dhingra Date

Associations of Perfluorooctanoic Acid with Menopause and Chronic Kidney Disease

By

Radhika Dhingra
Doctor of Philosophy
Department of Environmental Health Sciences

_____
Kyle Steenland, Ph.D.
Adviser

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

_____
Lyndsey A. Darrow, Ph.D.
Committee Member

_____
Mitchel Klein, Ph.D.
Committee Member

Accepted:

_____
Lisa A. Tedesco, Ph.D.
Dean of the Graduate School

_____
Date

Associations of Perfluorooctanoic Acid with Menopause and Chronic Kidney Disease

By

Radhika Dhingra
Doctor of Philosophy
Department of Environmental Health Sciences

B.S., University of Texas at Austin, 2004
M.S., Georgia Institute of Technology, 2009
M.S.P.H., Emory University, 2011

Adviser: Kyle Steenland, Ph.D.

An abstract of
a dissertation submitted to the Faculty of the
James T. Laney School Graduate School of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in Environmental Health Sciences
2015

Abstract

Associations of Perfluorooctanoic Acid with Menopause and Chronic Kidney Disease

By Radhika Dhingra

In this dissertation I investigated the associations of perflurooctanoic acid (PFOA) with menopause and chronic kidney disease (CKD) in a mid-Ohio Valley community cohort (N=32,254), subjected to a wide range of PFOA exposures via drinking water since 1951. Persistent in the environment and the human body, PFOA is found at low concentrations in the serum of nearly all U.S. residents. Earlier menopause and CKD have been positively associated with increased serum PFOA in previous cross-sectional studies.

Using Cox proportional hazards models, I conducted longitudinal analyses of menopause among women, aged 40 years or greater, (N=8,759) and of chronic kidney disease (CKD) among adults, aged 20 years or greater (N=32,254). In prior work, year-specific serum PFOA concentrations (1951-2011) in this population were retrospectively modeled, independently of measured PFOA. Estimated glomerular filtration rate (eGFR; a marker of kidney function) and serum PFOA level were measured in blood samples collected at enrollment (2005/2006). Individual self-reported histories of menopause and CKD were collected in 2008-2011, and self-reported CKD was validated via medical records or the US Renal Data System registry. I retrospectively investigated associations of menopause and of CKD with modeled PFOA exposure, either using year-specific estimates or estimated cumulative PFOA exposure. For both outcomes, I prospectively analyzed cohort members who had not yet experienced the outcome at enrollment. Using cross-sectional analyses to assess possible reverse causation, I evaluated the associations of measured and modeled serum PFOA with menopause among women aged 30-65, and with eGFR among adults. I also assessed the impact of the number of years since menopause on measured serum PFOA concentrations.

In longitudinal analyses, neither menopause nor CKD were associated with exposure to cumulative or year-specific PFOA estimates. Measured serum PFOA was positively associated with both menopause and eGFR (trend tests $p=0.0005$ and $p=0.013$, respectively), while modeled serum PFOA was not. Measured serum PFOA concentrations appeared to increase for the first seven years after menopause (trend test, $p<0.0001$). These results suggest that earlier menopause and CKD are not caused by PFOA exposure, and that positive findings in previous cross-sectional studies may have been the result of reverse causation.

Associations of Perfluorooctanoic Acid with Menopause and Chronic Kidney Disease

By

Radhika Dhingra
Doctor of Philosophy
Department of Environmental Health Sciences

B.S., University of Texas at Austin, 2004
M.S., Georgia Institute of Technology, 2009
M.S.P.H., Emory University, 2011

Adviser: Kyle Steenland, Ph.D.

A dissertation submitted to the Faculty of the
James T. Laney School Graduate School of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in Environmental Health Sciences
2015

Acknowledgments

I would like to thank Dr. Steenland the members of my dissertation committee for their effort, wisdom, encouragement, and especially for the generous gift of their patient time. I would also specifically like to thank Andrea Winquist and Cathy Lally for their guidance in making my way into the most complicated dataset I have yet seen. I thank my colleagues and faculty for making my time at Emory University such a supportive, broad and gratifying experience. I would thank my family and friends for their support, patience and encouragement. Finally, I would like to thank my ever-constant fiancé, Andrew, for the many thousands of miles you drove just to support me through the end of this process.

Table of Contents

Tables and figures

Chapter 1: Introduction

Though subject to extended scrutiny since ~2005 in relation to diverse chronic disease outcomes, perfluorooctanoic acid (PFOA) has not been adequately examined, in relation to age at menopause and nonmalignant kidney disease. Both outcomes have been predominantly studied using cross-sectional study designs (i.e., exposure and outcome are determined simultaneously; Steenland et al., 2010) at low general-population PFOA exposure levels. However either outcome may alter the toxicokinetics of PFOA in the human body. If accumulation, distribution, metabolism or excretion are systematically different for cases and non-cases, then significant cross-sectional associations with PFOA may result from reverse causation and be misinterpreted as a result. For studying the association of PFOA and either menopause or chronic kidney disease (CKD), cross-sectional study designs are likely inadequate for causal inference.

In this dissertation, the relationship between two health outcomes, menopause and chronic kidney disease are longitudinally examined (i.e., exposure is determined before the outcome), in a mid-Ohio valley community with both high and low exposures. These two studies comprise the first and second paper. In the final paper, the possible presence of reverse causation in cross-sectional study designs is investigated for both menopause and chronic kidney disease.

Perfluorooctanoic acid (PFOA or C8)

Perflurooctanoic acid is a perfluorinated 8-carbon chain carboxylic acid that has aided in the manufacture of Teflon and Gore-Tex. As a result of a stewardship program

initiated by Environmental Protection Agency in 2006, the eight major companies using PFOA agreed to voluntarily reduce emissions of PFOA to 5% of 2000 levels by 2010 (EPA, 2012). Nonetheless, historic emissions that survive in the environment, primarily in groundwater, remain a concern for human health, as PFOA is environmentally persistent, has a long residence time in the human body and is present in low levels in the blood of most of the US population (median in U.S. = 4 μg/L, mean in U.S. = 3.9 μg/L).

PFOA has a reported half-life ranging from 2.3 to 4.7 years (Bartell et al., 2010; Brede et al., 2010; Olsen et al., 2007), and, based on rodent studies (Jensen and Leffers, 2008; Lau et al., 2007), is expected to be primarily stored in the kidney, in the liver and in serum. Neither lipophilic nor genotoxic, PFOA persists in the human body bonded to serum proteins, and does not appear to cause acute toxicity. Its known primary mode of action in animals is as a PPAR-α agonist (Lau et al., 2007), though, less clearly so in humans (DeWitt et al., 2009). Studies *in vivo* and *in vitro* suggest that PFOA acts as an endocrine disruptor, and it is suspected to alter endocrine homeostasis, as a xenoestrogen (Kjeldsen and Bonefeld-Jørgensen, 2013; Lau et al., 2007; White et al., 2011).

The mid-Ohio Valley cohort and the strength of its data

In 1951, DuPont's (E.I. du Pont de Nemours Company) Washington Works facility, a fluoropolymer plant 10 miles outside of Parkersburg, WV in the mid-Ohio Valley, began using PFOA as a surfactant in their manufacturing processes (Paustenbach et al., 2007). PFOA from the plant was emitted into the air and released in liquid and solid waste into landfills, on-site digestion ponds and the Ohio River (Paustenbach et al., 2007). In 2001, community members filed a class-action lawsuit against DuPont that

claimed adverse health effects as a result of the plant's PFOA emissions into the environment.

Settlement of the lawsuit brought by this mid-Ohio valley community funded two groups of studies, named the C8 Health Project (C8HP) and the C8 Science Panel (C8SP) studies. These cohorts drawn from this Mid-Ohio valley community, including workers at the DuPont plant, have been extensively documented and studied in several phases during 2005-2011. The initial survey of 69,030 individuals, termed the C8HP, was conducted in 2005-2006 and was accompanied by a blood draw to determine serum PFOA concentrations and other biomarkers (Frisbee et al., 2009). Second and third surveys were conducted subsequently, as part of the work of C8SP, in 2008-10 and 2010-11, respectively (Winquist et al., 2013). Both the C8HP survey and the C8SP series of studies were funded by a class action lawsuit, carried out to determine if there is a "probable link" (i.e., a link between exposure and human disease more probable than not) between PFOA and disease. The studies that comprise this dissertation are carried out in this mid-Ohio valley cohort using data collected in the course of the C8SP and the C8HP studies.

Many investigations into the chronic health effects of PFOA in adults, exposed in non-occupational settings, have relied on cross-sectional study design (Steenland et al., 2010) in low-exposed populations (e.g., Danish National Cohort and the National Health and Nutrition Examination). The contemporaneously collected exposure and outcome data used in a cross-sectional study design makes causal inference difficult. Additionally, the narrow range of low exposures investigated in these populations may limit either the detection of relationship or complete description of the dose-response relationship between PFOA exposure and outcome.

By contrast, the cohort investigated in the present dissertation was drawn from a mid-Ohio Valley community with measures of exposure that precede outcome reporting, and with a wide range of exposures to PFOA-contaminated drinking water. The highest of these exposures were several orders of magnitude larger than those found in the general population (Frisbee et al., 2009). In this population, the mean and median serum concentrations of PFOA in 2005 were 82.9 μg/L and 28.2 μg/L, respectively (Frisbee et al., 2009; Steenland et al., 2010).

In this community cohort, measures of exposure via drinking water were reconstructed through an environmental fate and transport model coupled via the residential history of each participant with a pharmacokinetic model (Shin et al., 2011a, 2011b). The product of this retrospective modeling procedure was a serum estimate for each participant in each year from birth or 1951. Participants who had a history of working at the DuPont plant, which was the source of exposure, underwent an additional retrospective modeling procedure that incorporated knowledge of chemical processes, 2,125 historical measures of PFOA in the serum of workers, and a job-exposure matrix (Woskie et al., 2012). For individuals who had worked at the plant, if their yearly serum estimate based on work-related exposure was higher than their community estimate (i.e., water ingestion exposure), the participant was assigned the work-related exposure estimate for that year (Winquist et al., 2013). The yearly serum PFOA estimates correlated reasonably (Spearman's rank correlation coefficient, $\rho = 0.71$) with the single measured serum PFOA concentration determined from a blood sample provided by the cohort's participants in 2005/2006 (Winquist et al., 2013). This environmental modeling of individual, yearly estimates of serum PFOA concentration provides a distinct

advantage over other cohorts by making available longitudinal exposure estimates that precede the outcome.

In addition to the wide range of exposures and the availability of longitudinal exposure estimates, this cohort affords a number of other advantages, not found in other cohorts. Our C8SP cohort consists of 32,256 individuals, which is much larger than other cohorts investigating PFOA (e.g., Shankar et al., 2011; Taylor et al., 2014). Additionally, self-reported chronic disease, including cancer, chronic kidney disease, thyroid disease, and liver disease and others, were confirmed through medical records validation or through matching with disease registries (i.e., SEER Cancer registry and the US Renal Data System registry). Finally, when surveyed as part of the C8SP, individuals were asked to provide an age at diagnosis for each reported chronic disease and ages for other health behaviors (e.g., smoking) or events (e.g., pregnancies). Along with the modeled yearly serum estimates, these longitudinal data are a significant strength of the present cohort and the present work.

Prior findings in the literature: Menopause and CKD

Using this rich dataset, in this dissertation we longitudinally investigate two outcomes, earlier natural menopause and chronic kidney disease. Both have been positively associated with increased serum PFOA levels in cross-sectional studies. Earlier menopause has not previously been longitudinally studied and chronic kidney disease has not been studied longitudinally in adults.

In an analysis of data from the C8HP, Knox et al. noted that menopausal women, grouped by ages 42-51 and ages 51-65, had higher odds (odds = 1.4 and odds = 1.7,

respectively) of being in the highest quintile of measured serum PFOA, as compared to the lowest quintile (2011). In the U.S. population (as studied in the National Health and Nutrition Examination Survey (NHANES)), Taylor et al. observed a positive significant association (HR = 1.36, 95%CI: (1.05, 1.75) for the highest tertile as compared to the lowest tertile of PFOA exposure) between PFOA and menopausal age (2014). In a related cross-sectional study of menarche in female children, Lopez-Espinosa et al. (2011) noted a 130 day delay of menarche for those in the highest quartile (>58 μg/L serum PFOA) of exposure as compared to the lowest (<11.4 μg/L serum PFOA).

Several studies have also made use of cross-sectional data to fit pharmacokinetic models that account for altered PFOA excretion rates as a result of blood loss. In a small sampling of 36 women who had resided in Tokyo for 10 years with background levels of PFOA exposure, Harada et al. observed that measured serum PFOA concentrations were higher among post-menopausal women than among pre-menopausal women (2005). In this study, the addition of a term for menstrual blood loss per month to a single compartment pharmacokinetic model appeared to correspond moderately well with measured serum PFOA in the 8 menopausal women. Two additional studies in adults have noted that including a term for blood loss was able to explain the difference in blood perfluoroalkyl acids concentrations between men and women. An analysis of 132 adults PFOA-exposed at low concentrations showed that menstruation blood loss (assumed to be 35 mL/month) could cause a 22% difference in serum PFOA concentrations; this agreed quite closely with the observed 24% difference in serum PFOA concentrations (Lorber et al., 2015). Though studying the related compound perfluorooctane sulfonic acid (PFOS), Wong et al. found that accounting for a menstruation rate of 6.1 mL/kg

body weight/year, accounted for 30% of the difference in elimination rates of PFOS between men and women (2014). A similar PBPK model assessed the delay in menarche that resulted from exposure to PFOA (Wu et al. 2015); though menarche was still found to be delayed in those exposed to PFOA, the delay of menarche per natural log increase in perfluoroalkyl substances that was observed in the PBPK model was 1/3 of the delay per natural log increased PFOA exposure association reported by Lopez-Espinosa et al. (2011). All of these PBPK studies made use of cross-sectionally collected data and have no recourse to external quantification of PFOA dose.

Among those with estimated glomerular filtration (eGFR) rates below 60 mL/minute/1.73m^2 (a diagnostic criteria for CKD), Shankar et al. found that the odds of being in the highest quartile of measured serum PFOA was 1.73 times that of being in the lowest (referent) quartile (2011). One occupational cohort mortality study, conducted among a cohort of workers from the same population considered in this dissertation, that estimated PFOA exposure by using a job-exposure matrix, found an increased rate of CKD mortality, both cause-specific and all-cause, with increased PFOA exposure (Steenland and Woskie, 2012). In a study of children in the C8HP, decreased kidney function, measured as eGFR, was significantly associated with increased PFOA, measured from blood samples, but was not associated with the environmentally estimated PFOA exposure (Watkins et al., 2013).

Study design of papers 1 and 2

In order to correctly assess the effect of PFOA on age at menopause and risk of CKD, a study of each outcome was carried out using modeled longitudinal exposure data that preceded the outcome and that could not be impacted by either outcome. These

longitudinal studies of natural menopause and chronic kidney disease comprise papers 1 and 2 of this dissertation, respectively.

In paper 1, the relationship between natural menopause and PFOA was modeled using Cox proportional hazards regression analyses with natural menopause as the event of interest and age as the time scale. We carried out a retrospective analysis on the complete cohort and a prospective analysis that was restricted to women who were premenopausal at time of the 2005/2006 C8HP survey, and had serum PFOA measurements at that time. Follow-up began in 1951 or at age 40 (whichever was later), in the retrospective analysis and, and at the time of the C8HP or at age 40 (whichever was later), in the prospective analysis. The retrospective analyses used time-varying, modeled cumulative exposure (the sum of all yearly serum estimates up to a given year) and yearly serum estimates. The prospective analyses used time-varying, modeled cumulative exposure or measured PFOA levels in 2005/2006. In order to assess the relevant exposure time frame, we conducted lagged analyses of cumulative exposure (5, 10, and 20 year lags) on the complete cohort. All models were stratified on birth year. Covariates in these models included time-varying smoking status (Gold, 2011; Harlow and Signorello, 2000; Jick et al., 1977), educational attainment (Bleil et al., 2012; Bromberger et al., 1997; Wise et al., 2002), time-varying alcohol consumption (Torgerson et al., 2014), time-varying parous/nulliparous status (Dvornyk et al., 2006; Stanford et al., 1987), time-varying hypertension and time-varying high cholesterol. To account for hysterectomy as a competing risk in this analysis, hysterectomies were either excluded entirely from the model or censored at the age of hysterectomy; that is, person-time for hysterectomies was either included until they exited the risk set or excluded entirely.

In paper 2, the relationship between chronic kidney disease in adults and PFOA was also modeled using Cox proportional hazards models, where in CKD was the dependent variable, PFOA exposure was the principal independent variable, and age was the time scale. We conducted both a retrospective and a prospective analysis. Follow-up in the retrospective analysis began at age 20 in any calendar year or 1951 (whichever was later) and ended at the time of failure for cases, and at the last survey or at death, whichever was earlier, for non-cases. For the prospective analysis, the population was limited to those who were alive and disease-free at the start of follow-up, which began at the time of the 2005/2006 survey. We used modeled cumulative PFOA exposure (the sum of all modeled serum PFOA estimates up to a given year) as the exposure metric in both retrospective and prospective analyses. Cumulative PFOA exposure was chosen as the exposure metric since the mechanism by which PFOA might cause CKD would likely involve chronic disease processes that resulted from repeated exposures over time. Modeled yearly serum PFOA was also considered as the exposure metric in the retrospective analysis.

Covariates included in models included gender, time-varying self-reported hypertension diagnosis (Coresh et al., 2007; Haroun et al., 2003; Saydah et al., 2007), time-varying self-reported diabetes diagnosis (Coresh et al., 2007; Saydah et al., 2007), time-varying self-reported high cholesterol diagnosis (Anavekar and Pfeffer, 2004), time-varying current/former/never smoking (Haroun et al., 2003), category of BMI (<18.5, 18.5-25, 25-30, ≥30 kg/m^2) in 2005-06 (Coresh et al., 2007; Saydah et al., 2007), and category of completed education (‘less than high school (HS),’ ‘HS diploma,’ ‘some undergraduate education,’ ‘bachelor’s degree’).

The results of these investigations of CKD and menopause were largely negative and disagreed with prior results in the literature. This dissonance between our longitudinal analyses and the prior positive results motivated an investigation into the causal direction of previous cross-sectional work. This investigation comprised the 3rd paper in this dissertation.

Recall that many of the previously mentioned cross-sectional studies of menopause or kidney disease have found significant associations. In many of the previous cross-sectional studies of PFOA in relation to these outcomes, the measured concentration of PFOA in serum was used as the exposure metric. As a result of collecting blood samples at the same time as the individual had already achieved the outcome classification (e.g. premenopausal vs. menopausal or diminished eGFR vs. normal eGFR), the causal direction of the relationship between exposure and outcome is indeterminate, as many of the authors noted.

Reverse causation and serum biomarkers of chemical exposure

Reverse causation is a well-known problem of temporality, in which the outcome influences the exposure measure or status. The Bradford Hill criteria for ascertaining causation names the requirement that the exposure precedes the outcome the condition of temporality (Hill, 1965). In writing about temporality, he states "[t]his temporal problem may not arise often, but it certainly needs to be remembered…" Sir Bradford Hill may not have foreseen in 1968 that concerns about temporality would, with the rise of use of biomarkers in environmental studies, arise more often.

Serum biomarkers are often seen as objective measures of internal dose. In studies of cross-sectional data, their use introduces the possibility of reverse causation, since the measure of exposure and outcome are concurrent. In particular, the causal order of chemical exposure and disease may be in question if the outcome can impact the absorption, distribution, metabolism or excretion of the chemical or of its biomarker (e.g., a metabolite) in the body. For example, diminished rates of excretion may cause an increased rate of accumulation in the body.

Reverse causation is a well-known concern in cross-sectional studies of biomarkers including those of chemical exposure (Engel and Wolff, 2013; Villanueva et al., 2014). In order to demonstrate that reverse causation is occurring (and thus clarify the true causal direction between exposure and outcome), both the cross-sectionally measured serum biomarker and an alternative measure of exposure unaffected by possible reverse causation are required. Here we investigate further possible reverse causation using two exposure measures, one subject to reverse causation and one that is not. We examine two specific examples in which altered excretion rates of PFOA likely resulted from the health outcomes, menopause and chronic kidney disease.

Chronic kidney disease is characterized by a progressive loss of renal function that often occurs over the span of years and greatly increases the risk of end-stage renal disease (Saydah et al., 2007) and cardiovascular mortality (Coresh et al., 2005; Sarnak et al., 2003). Obesity, hypertension and diabetes are the greatest risk factors for developing CKD (Coresh et al., 2007).

Estimated glomerular filtration rate (eGFR) is a measure of renal function and is based on serum creatinine concentration. Diagnosis of the early stages of kidney disease

requires evidence of kidney damage, often measured using albuminuria, and evidence of decreased kidney function, often quantified as estimated glomerular filtration rate (eGFR) from measured serum creatinine (Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group, 2013). In later stages of kidney disease, eGFR is greatly diminished and often sufficient for diagnosis (Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group, 2013).

As urine is thought to be a major mode of PFOA excretion, diminished kidney function may cause PFOA to be excreted from the body at a decreased rate. If this were the case, then as eGFR decreased, serum concentration of PFOA would be expected to increase, as was noted in Shankar et al.'s study (2011).

Menopause is characterized by the cessation of menstruation. Early menopause is associated with increased morbidity in later life, including osteoporosis (e.g., Qiu et al., 2013), cardiovascular disease (Shuster et al., 2010), and all-cause mortality (e.g., Jacobsen et al., 2003; Li et al., 2013). As PFOA appears to be found primarily in liver and blood across multiple sex/species combinations (Hundley et al., 2006), it is reasonable to expect that PFOA might be lost during menstruation.

Based upon the mean ± standard deviation reported weight at age 40 (152 ± 37 lbs.) in the cohort studied here, we can assume a mean ± standard deviation blood volume of 4.5 ± 1.1 L (Morgan et al., 2001). Assuming a normal blood loss of 35–50 ml per cycle (Warrilow et al., 2004) and 13 cycles per year, menstruation results in blood loss of approximately 455-650 ml/year. Therefore, menstruation may result in a blood volume loss every year of 10%-14%, though an alternative estimation found the population mean of menstrual blood loss to be to be higher at 868mL/year (Verner and Longnecker, 2015).

Additionally, menopause is also accompanied by hormonal changes that might impact kidney function, which could further affect excretion rates.

Study design of paper 3

In the third paper, two exposure formulations were used to cross-sectionally investigate whether reverse causation may have been responsible for the associations observed prior work. First we aim to recreate the positive results found in prior cross-sectional work. Then, we conducted analyses using as the exposure metric modeled serum estimates from 2005/2006 (whichever year the individual gave a blood sample); this modeled metric is not vulnerable to reverse causation. Watkins et al. (2013) used a similar approach in studying kidney function and PFOA in children in this same population; here we extend this approach by considering adults, among whom decreased kidney function is likely to be more common.

Serum concentrations of PFOA measured at the time of outcome data collection may have been impacted by the processes that characterize menopause and CKD. The environmental estimation of serum levels at the time of survey could not have been impacted by menopause or CKD and thus are unaffected by reverse causation. By comparing the associations of each outcome with the two exposure formulations, we are able to make inferences about the presence of reverse causation and its impact at various ranges of exposure.

References

Anavekar, N.S., Pfeffer, M.A., 2004. Cardiovascular risk in chronic kidney disease. Kidney Int. 66, S11–S15.

Bartell, S.M., Calafat, A.M., Lyu, C., Kato, K., Ryan, P.B., Steenland, K., 2010. Rate of decline in serum PFOA concentrations after granular activated carbon filtration at two public water systems in Ohio and West Virginia. Environ. Health Perspect. 118, 222–8. doi:10.1289/ehp.0901252

Bleil, M.E., Adler, N.E., Pasch, L. A, Sternfeld, B., Gregorich, S.E., Rosen, M.P., Cedars, M.I., 2012. Psychological stress and reproductive aging among pre-menopausal women. Hum. Reprod. 27, 2720–8. doi:10.1093/humrep/des214

Brede, E., Wilhelm, M., Göen, T., Müller, J., Rauchfuss, K., Kraft, M., Hölzer, J., 2010. Two-year follow-up biomonitoring pilot study of residents' and controls' PFC plasma levels after PFOA reduction in public water system in Arnsberg, Germany. Int. J. Hyg. Environ. Health 213, 217–223. doi:http://dx.doi.org/10.1016/j.ijheh.2010.03.007

Bromberger, J.T., Matthews, K.A., Kuller, L.H., Wing, R.R., Meilahn, E.N., Plantinga, P., 1997. Prospective study of the determinants of age at menopause. Am. J. Epidemiol. 145, 124–133. doi:10.1093/oxfordjournals.aje.a009083

Coresh, J., Byrd-Holt, D., Astor, B.C., Briggs, J.P., Eggers, P.W., Lacher, D. A, Hostetter, T.H., 2005. Chronic kidney disease awareness, prevalence, and trends

among U.S. adults, 1999 to 2000. J. Am. Soc. Nephrol. 16, 180–8. doi:10.1681/ASN.2004070539

Coresh, J., Selvin, E., Stevens, L. A, Manzi, J., Kusek, J.W., Eggers, P., Van Lente, F., Levey, A.S., 2007. Prevalence of chronic kidney disease in the United States. JAMA 298, 2038–2047. doi:10.1001/jama.298.17.2038

DeWitt, J.C., Shnyra, A., Badr, M.Z., Loveless, S.E., Hoban, D., Frame, S.R., Cunard, R., Anderson, S.E., Meade, B.J., Peden-Adams, M.M., Luebke, R.W., Luster, M.I., 2009. Immunotoxicity of Perfluorooctanoic Acid and Perfluorooctane Sulfonate and the Role of Peroxisome Proliferator-Activated Receptor Alpha. Crit. Rev. Toxicol. 39, 76–94. doi:10.1080/10408440802209804

Dvornyk, V., Long, J.-R., Liu, P.-Y., Zhao, L.-J., Shen, H., Recker, R.R., Deng, H.-W., 2006. Predictive factors for age at menopause in Caucasian females. Maturitas 54, 19–26. doi:10.1016/j.maturitas.2005.08.005

Engel, S.M., Wolff, M.S., 2013. Causal Inference Considerations for Endocrine Disruptor Research in Children's Health. Annu Rev Public Heal. 34, 139–158. doi:10.1016/j.biotechadv.2011.08.021.Secreted

EPA, 2012. PFOA Stewardship Program Description [WWW Document]. URL http://www.epa.gov/oppt/pfoa/pubs/stewardship/pfoastewardshipbasics.html

Frisbee, S.J., Brooks, A. P., Maher, A., Flensborg, P., Arnold, S., Fletcher, T., Steenland, K., Shankar, A., Knox, S.S., Pollard, C., Halverson, J. a, Vieira, V.M., Jin, C.,

Leyden, K.M., Ducatman, A.M., 2009. The C8 health project: design, methods, and participants. Environ. Health Perspect. 117, 1873–82. doi:10.1289/ehp.0800379

Gold, E.B., 2011. The timing of the age at which natural menopause occurs. Obstet. Gynecol. Clin. North Am. 38, 425–40. doi:10.1016/j.ogc.2011.05.002

Harada, K., Inoue, K., Morikawa, A., Yoshinaga, T., Saito, N., Koizumi, A., 2005. Renal clearance of perfluorooctane sulfonate and perfluorooctanoate in humans and their species-specific excretion. Environ. Res. 99, 253–261. doi:10.1016/j.envres.2004.12.003

Harlow, B.L., Signorello, L.B., 2000. Factors associated with early menopause. Maturitas 35, 3–9. doi:10.1016/s0378-5122(00)00092-x

Haroun, M.K., Bernard G. Jaar, Sandra C. Hoffman, Comstock, G.W., Michael J. Klag, Coresh, J., 2003. Risk Factors for Chronic Kidney Disease: A Prospective Study of 23,534 Men and Women in Washington County, Maryland. J. Am. Soc. Nephrol. 14, 2934–2941. doi:10.1097/01.ASN.0000095249.99803.85

Hill, A.B., 1965. The Enviroment and Disease: Association or Causation. Proc. R. Soc. Med. 58, 295–300.

Hundley, S.G., Sarrif, A. M., Kennedy, G.L., 2006. Absorption, distribution, and excretion of ammonium perfluorooctanoate (APFO) after oral administration to various species. Drug Chem. Toxicol. 29, 137–145. doi:10.1080/01480540600561361

Jacobsen, B.K., Heuch, I., Kvåle, G., 2003. Age at natural menopause and all-cause mortality: A 37-year follow-up of 19,731 Norwegian women. Am. J. Epidemiol. 157, 923–929. doi:10.1093/aje/kwg066

Jensen, A.A., Leffers, H., 2008. Emerging endocrine disrupters: perfluoroalkylated substances. Int. J. Androl. 31, 161–9. doi:10.1111/j.1365-2605.2008.00870.x

Jick, H., Porter, J., Morrison, A.S., 1977. Relation between smoking and age of natural menopause. Lancet 1354–1355. doi:0.1016/S0140-6736(77)92562-4

Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group, 2013. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney Int. Suppl. 3, 150. doi:10.1038/kisup.2012.76

Kjeldsen, L.S., Bonefeld-Jørgensen, E.C., 2013. Perfluorinated compounds affect the function of sex hormone receptors. Environ. Sci. Pollut. Res. Int. 20, 8031–44. doi:10.1007/s11356-013-1753-3

Knox, S.S., Jackson, T., Javins, B., Frisbee, S.J., Shankar, A., Ducatman, A.M., 2011. Implications of early menopause in women exposed to perfluorocarbons. J. Clin. Endocrinol. Metab. 96, 1747–53. doi:10.1210/jc.2010-2401

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol. Sci. 99, 366–94. doi:10.1093/toxsci/kfm128

Li, S., Rosenberg, L., Wise, L. A., Boggs, D. A., LaValley, M., Palmer, J.R., 2013. Age at natural menopause in relation to all-cause and cause-specific mortality in a follow-up study of US black women. Maturitas 75, 246–52. doi:10.1016/j.maturitas.2013.04.003

Lopez-Espinosa, M.-J., Fletcher, T., Armstrong, B., Genser, B., Dhatariya, K., Mondal, D., Ducatman, A., Leonardi, G., 2011. Association of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS) with age of puberty among children living near a chemical plant. Environ. Sci. Technol. 45, 8160–6. doi:10.1021/es1038694

Lorber, M., Eaglesham, G.E., Hobson, P., Toms, L.-M.L., Mueller, J.F., Thompson, J.S., 2015. The effect of ongoing blood loss on human serum concentrations of perfluorinated acids. Chemosphere 118, 170–177. doi:10.1016/j.chemosphere.2014.07.093

Morgan, G.E.J., Mikhail, M.S., Larson, C.P.J. (Eds.), 2001. Morgan and Mikhail's Clinical Anesthesiology, 3rd ed. McGraw-Hill, New York.

Olsen, G.W., Burris, J.M., Ehresman, D.J., Froehlich, J.W., Seacat, A.M., Butenhoff, J.L., Zobel, L.R., 2007. Half-life of serum elimination of perfluorooctanesulfonate,perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ. Health Perspect. 115, 1298–305. doi:10.1289/ehp.10009

Paustenbach, D.J., Panko, J.M., Scott, P.K., Unice, K.M., 2007. A methodology for estimating human exposure to perfluorooctanoic acid (PFOA): a retrospective exposure assessment of a community (1951-2003). J. Toxicol. Environ. Health. A 70, 28–57. doi:10.1080/15287390600748815

Qiu, C., Chen, H., Wen, J., Zhu, P., Lin, F., Huang, B., Wu, P., Lin, Q., Lin, Y., Rao, H., Huang, H., Liang, J., Li, L., Gong, X., Peng, S., Li, M., Chen, L., Tang, K., Chen, Z., Lin, L., Lu, J., Bi, Y., Ning, G., Chen, G., 2013. Associations between age at menarche and menopause with cardiovascular disease, diabetes, and osteoporosis in Chinese women. J. Clin. Endocrinol. Metab. 98, 1612–21. doi:10.1210/jc.2012-2919

Sarnak, M.J., Levey, A.S., Schoolwerth, A.C., Coresh, J., Culleton, B., Hamm, L.L., McCullough, P. A., Kasiske, B.L., Kelepouris, E., Klag, M.J., Parfrey, P., Pfeffer, M., Raij, L., Spinosa, D.J., Wilson, P.W., 2003. Kidney Disease as a Risk Factor for Development of Cardiovascular Disease: A Statement From the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Circulation 108, 2154–2169. doi:10.1161/01.CIR.0000095676.90936.80

Saydah, S., Rios-Burrows, M.E.N., Williams, D., Geiss, L., Dorsey, R., 2007. Prevalence of Chronic Kidney Disease and Associated Risk Factors --- United States, 1999--2004. MMWR 56, 161–165.

Shankar A, Xiao J, Ducatman A. 2011. Perfluoroalkyl chemicals and chronic kidney disease in US adults. Am. J. Epidemiol. 174:893–900; doi:10.1093/aje/kwr171.

Shin, H.-M., Vieira, V.M., Ryan, P.B., Detwiler, R., Sanders, B., Steenland, K., Bartell, S.M., 2011a. Environmental fate and transport modeling for perfluorooctanoic acid emitted from the Washington Works Facility in West Virginia. Environ. Sci. Technol. 45, 1435–42. doi:10.1021/es102769t

Shin, H.-M., Vieira, V.M., Ryan, P.B., Steenland, K., Bartell, S.M., 2011b. Retrospective exposure estimation and predicted versus observed serum perfluorooctanoic acid concentrations for participants in the C8 Health Project. Environ. Health Perspect. 119, 1760–5. doi:10.1289/ehp.1103729

Shuster, L.T., Rhodes, D.J., Gostout, B.S., Grossardt, B.R., Rocca, W. A., 2010. Premature menopause or early menopause: long-term health consequences. Maturitas 65, 161–6. doi:10.1016/j.maturitas.2009.08.003

Stanford, J.L., Hartge, P., Brinton, L. A., Hoover, R.N., Brookmeyer, R., 1987. Factors influencing the age at natural menopause. J. Chronic Dis. 40, 995–1002. doi:10.1016/0021-9681(87)90113-5

Steenland, K., Fletcher, T., Savitz, D. A., 2010. Epidemiologic evidence on the health effects of perfluorooctanoic acid (PFOA). Environ. Health Perspect. 118, 1100–8. doi:10.1289/ehp.0901827

Steenland, K., Woskie, S., 2012. Cohort mortality study of workers exposed to perfluorooctanoic acid. Am. J. Epidemiol. 176, 909–17. doi:10.1093/aje/kws171

Taylor, K.W., Hoffman, K., Thayer, K. A., Daniels, J.L., 2014. Polyfluoroalkyl Chemicals and Menopause among Women 20-65 Years of Age (NHANES). Environ. Health Perspect. 122, 145–50. doi:10.1289/ehp.1306707

Torgerson, D.J., Thomas, R.E., Campbell, M.K., Reid, D.M., 2014. Alcohol consumption and age of maternal menopause are associated with menopause onset. Maturitas 26, 21–25. doi:10.1016/S0378-5122(96)01075-4

Verner, M.-A., Longnecker, M.P., 2015. Comment on "Enhanced Elimination of Perfluorooctanesulfonic Acid by Menstruating Women: Evidence from Population-Based Pharmacokinetic Modeling." Environ. Sci. Technol. 49, 5836–5837. doi:10.1021/acs.est.5b00187

Villanueva, C.M., Kogevinas, M., Cordier, S., Templeton, M.R., Vermeulen, R., Nuckols, J.R., Nieuwenhuijsen, M.J., Levallois, P., 2014. Assessing exposure and health consequences of chemicals in drinking water: Current state of knowledge and research needs. Environ. Health Perspect. 122, 213–221. doi:10.1289/ehp.1206229

Warrilow, G., Kirkham, C., Ismail, K.M., Wyatt, K., Dimmock, P., O'Brien, S., 2004. Quantification of menstrual blood loss. Obstet. Gynaecol. 6, 88–92. doi:10.1016/S0015-0282(02)03638-5

Watkins, D.J., Josson, J., Elston, B., Bartell, S.M., Shin, H., Vieira, V.M., Savitz, D.A., Fletcher, T., Wellenius, G.A., 2013. Exposure to Perfluoroalkyl Acids and Markers of Kidney Function among Children and Adolescents Living near a Chemical Plant. Environ. Health Perspect. 121, 625–630.

White, S.S., Fenton, S.E., Hines, E.P., 2011. Endocrine disrupting properties of perfluorooctanoic acid. J. Steroid Biochem. Mol. Biol. 127, 16–26. doi:10.1016/j.jsbmb.2011.03.011

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, 893–9. doi:10.1289/ehp.1206450

Wise, L.A., Krieger, N., Zierler, S., Harlow, B.L., 2002. Lifetime Socioeconomic Position in Relation to Onset of Perimenopause. J. Epidemiol. Community Health 56, 851–860. doi:10.1136/jech.56.11.851

Wong, F., MacLeod, M., Mueller, J.F., Cousins, I.T., 2014. Enhanced Elimination of Perfluorooctane Sulfonic Acid by Menstruating Women: Evidence from Population-Based Pharmacokinetic Modeling. Environ. Sci. Technol. 48, 8807–8814. doi:dx.doi.org/10.1021/es500796y

Woskie, S.R., Gore, R., Steenland, K., 2012. Retrospective exposure assessment of perfluorooctanoic acid serum concentrations at a fluoropolymer manufacturing plant. Ann. Occup. Hyg. 56, 1025–37. doi:10.1093/annhyg/mes023

Wu, H., Yoon, M., Verner, M.-A., Xue, J., Luo, M., Andersen, M.E., Longnecker, M.P., Clewell, H.J., 2015. Can the observed association between serum perfluoroalkyl substances and delayed menarche be explained on the basis of puberty-related

changes in physiology and pharmacokinetics? Environ. Int. 82, 61–68.

doi:10.1016/j.envint.2015.05.006

Chapter 2: Perfluorooctanoic acid exposure and natural menopause: a longitudinal study in a community cohort

[Formatted for submission to *Environmental Health*]

Authors: Radhika Dhingra[a], Lyndsey A. Darrow, PhD [b], Mitch Klein, PhD[a], Andrea Winquist, , MD, PhD[a], Kyle Steenland, PhD[a]

Affiliations: [a] Department of Environmental Health, Rollins School of Public Health, Emory University, 1518 Clifton Road, Atlanta, GA 30322, USA. rdhingra@emory.edu; mklein@emory.edu; awinqui@emory.edu; nsteenl@emory.edu

[b] Department of Epidemiology, Rollins School of Public Health, Emory University, 1518 Clifton Road, Atlanta, GA 30322, USA. ldarrow@emory.edu

Abstract

Introduction: Perfluorooctanoic acid (PFOA), a suspected endocrine disruptor, is a bio-persistent chemical found at low levels in the serum of nearly all U.S. residents. Early menopause has been positively associated with serum PFOA in prior cross-sectional studies.

Methods: We conducted a longitudinal analysis of age at menopause among women, aged ≥40 years, (N = 8,759) in a Mid-Ohio Valley community cohort, exposed to high PFOA levels via contaminated drinking water. Using estimated retrospective year-specific serum PFOA concentrations (1951-2011), we examined the associations between PFOA, as cumulative exposure or year-specific serum estimates, and natural menopause using Cox proportional hazards models. As participants were initially recruited in 2005-2006, we also analyzed the cohort prospectively (i.e., from the time of enrollment), using both modeled cumulative PFOA, and PFOA serum levels measured in 2005-2006. Women with hysterectomy (a competing risk) were either censored or excluded from the analysis.

Results: Neither in the retrospective nor the prospective cohort did we find a significant (at **α**=0.05) trend between PFOA exposure and natural menopause. The non-significant, hazard ratios by quintile of increasing cumulative serum PFOA were 1.00 (referent), 1.00, 1.09, 1.05 and 1.06 (trend test for log cumulative exposure: p = 0.37) with hysterectomies censored, and 1.00 (referent), 1.06, 1.13, 1.09 and 1.11 (trend test for log cumulative exposure: p = 0.85) with hysterectomies excluded. Year-specific serum estimates were also not associated with early menopause.

Conclusion: Our data suggest that earlier age at menopause is not associated with PFOA exposure.

Introduction

Perfluorooctanoic acid (PFOA or C8) is a widely used surfactant and emulsifier and has been used in the manufacture of polymers, such as Teflon and Gore-Tex. It is found in low but measurable quantities (median in U.S. ≈4μg/L), in the serum of most of the U.S. population (Calafat et al., 2007; Kato et al., 2011). Though its industrial use in the U.S. has voluntarily been discontinued or severely reduced (EPA, 2012), PFOA remains a potential hazard domestically because it is environmentally persistent and slowly excreted from the human body. Also, PFOA continues to be used in large quantities outside the U.S. (e.g., China, India and Russia; Wang et al., 2014). PFOA, which has a reported population mean half-life ranging from 2.3 to 3.8 years (Bartell et al., 2010; Brede et al., 2010; Olsen et al., 2007), is expected to be primarily stored in the kidney, in the liver and in serum, based on rodent studies (Jensen and Leffers, 2008; Lau et al., 2007). PFOA is suspected to alter endocrine homeostasis, as a xenoestrogen (Kjeldsen and Bonefeld-Jørgensen, 2013; Lau et al., 2007; White et al., 2011).

Early menopause (sometimes defined as occurring between 40 and 45 years of age) is a known risk factor for cardiovascular disease (Shuster et al., 2010), osteoporosis (e.g., Qiu et al., 2013) and all-cause mortality (e.g., Jacobsen et al., 2003; Li et al., 2013). Early menopause is a suspected risk factor for ischemic stroke (e.g., Lisabeth et al., 2009) and other sequelae (Shuster et al., 2010).

Two previous epidemiological studies of PFOA and age at natural menopause were cross-sectional analyses in which the outcome was ascertained through an interview at the same time as blood was drawn to determine PFOA concentration. Both a study of National Health and Nutrition Examination Survey (NHANES) participants (who were exposed at background PFOA levels), and a study of a large, highly exposed community cohort in the Mid-Ohio Valley, found

positive associations between higher measured PFOA serum concentrations and early natural menopause (Knox et al., 2011; Taylor et al., 2014) using cross-sectional study designs. The NHANES study used a proportional hazards (PH) regression analysis and found that earlier age at menopause was associated with higher PFOA serum concentration, measured at the time of the NHANES survey (Taylor et al., 2014). However, these authors also found some evidence of reverse causation, in that measured PFOA serum levels increased with years since menopause, plausibly due to the cessation of PFOA excretion via menstruation. The cross-sectional study of a Mid-Ohio Valley population, which overlaps considerably with the population studied here, found similar results using logistic regression (Knox et al., 2011). Among age groups 51-65 and 42-51 years, the odds of having already experienced natural menopause at the time of the survey increased with increasing exposure quintiles, particularly in the older group.

Here, we assessed the association between modeled serum PFOA and age of natural menopause in longitudinal analyses, while accounting for surgical menopause (hereafter referred to as 'hysterectomy', though this group may include oophorectomies) in a large longitudinal cohort in the mid-Ohio valley with a wide range of PFOA exposures. This mid-Ohio Valley community is centered around a DuPont (E.I. du Pont de Nemours Company) Washington Works manufacturing facility near Parkersburg, West Virginia where releases of PFOA began in 1951 (Frisbee et al., 2009). An initial survey of 69,030 individuals in six contaminated water districts, termed the C8 Health Project (C8HP), was conducted in 2005-2006, and was accompanied by collection of a blood sample for measurement of serum concentrations of PFOA and other biomarkers (Frisbee et al., 2009). Adults drawn from this population, as well as additional DuPont workers (combined total 32,254), were later interviewed in 2008-2011 (Winquist et al., 2013), as part of the work of C8 Science Panel (C8SP). Both the C8HP survey

and the C8SP series of studies were funded by a class action lawsuit, carried out to determine if there is a "probable link" between PFOA and disease. Here we study a sub-cohort of women from the C8SP cohort.

Methods

Cohort recruitment & survey

To qualify for inclusion in the cohort assembled by the C8SP (N = 32,254 participants = 28,541 community members + 3,713 workers), a participant must have been exposed to PFOA-contaminated water from one of six affected public water districts or from a contaminated private well for at least a year (Winquist et al., 2013), or the participant must have worked at the DuPont plant. The C8SP surveys took place in 2008-2011, and many subjects were interviewed twice during this period (Winquist et al., 2013). Participants provided information about health outcomes, demographics, health behaviors (e.g., smoking or exercise) and residential history. Each participant was asked about their history of several chronic diseases. Women were also asked about their reproductive history, including menstrual and pregnancy history. Residential histories with dates at each residence were also collected for each participant (Winquist et al., 2013). Women were asked three questions concerning menopause: (1) 'Do you still have menstrual periods?'; (2) 'How old were you when your menstrual periods stopped?'; and (3) 'Did your periods stop naturally or surgically?' If participants were still menstruating at the first survey, they were asked the same questions again in the second survey. If participants at the first survey answered that they had ceased menstruating, these questions were not repeated in the second survey.

Figure 2.1 shows how the study population was formed. Of 17,360 women in the original C8SP cohort, there were 4,411 women aged less than 40 years old at last C8SP interview, and these were excluded. Another 2,562 women, reporting an age of menopause less than 40, were also excluded; of these women, most reported hysterectomy (93%), with only 184 (7%) reporting natural menopause. Because the etiology of premature menopause, defined as natural menopause age less than 40 (occurring in 1.1% of U.S. women; Luborsky et al., 2003), is unclear and likely different from early menopause (ages 40-45) (Nelson et al., 2005; Panay and Kalu, 2009), we restricted our cohort to those who were premenopausal at age 40. Among the remaining 10,387 eligible women aged 40 or greater at time of interview, 193 provided no information about their menstrual history, 414 women reported menopause but not the type of menopause (i.e., surgical or natural), and 836 reported menopause but not the age at menopause (341 women who reported hysterectomy and 495 women who reported natural menopause); these women were excluded. For reasons of report reliability, the oldest cohort members, whose birth year was before 1920 were also excluded (n=51); this follows the precedent of previous C8SP studies. Eleven participants who reported menopausal ages greater than 65 were also excluded as implausible. A total of 123 were missing analysis covariates, and these were excluded. The resulting retrospective cohort consisted of 8,759 individuals, for which data were analyzed retrospectively, i.e. from the time of first exposure, using modeled PFOA serum estimates. Because most subjects were recruited in 2005/2006 while living in the mid-Ohio valley, our retrospective cohort may not have been fully representative of the underlying cohort of people exposed to PFOA over time in the area. Hence, we also conducted a prospective analysis from 2005/2006 forward, by restricting the retrospective cohort to the subset of 3,334 women who had not experienced menopause at the time of their C8HP survey in 2005/2006 and were not missing

measured PFOA in 2005/2006. An analysis for these women was conducted that followed them through their last C8SP survey (between August 2008 and May 2011).

Serum PFOA concentrations: measured and modeled

Measured serum PFOA levels in 2005/2006 were available for 97.0% of the women studied here (N = 8,493); those with measured serum PFOA data were participants in the C8HP survey in 2005-2006. The majority of individuals who were missing measured serum PFOA values (N =266) had a history of working at the DuPont plant and were enrolled for that reason. For historical serum estimates (i.e., modeled) for the women studied here, we used estimated PFOA serum levels from the prior work of the C8SP (Shin et al., 2011b; Winquist et al., 2013). Briefly, to estimate PFOA serum levels back in time, the C8SP developed a model to predict serum levels since birth or the beginning of PFOA production in 1951 (whichever was later), for both residential and occupational exposure. For occupational exposures (i.e., DuPont workers in the C8SP cohort), job and department-specific PFOA serum levels over time were estimated based on 2,125 historical serum PFOA measurements at the DuPont plant, knowledge of changes in manufacturing processes, job-exposure matrix and the measured serum levels, which incorporates residential exposure (Woskie et al., 2012). For residential exposures, primarily due to drinking PFOA-contaminated water, historic serum PFOA concentrations were reconstructed by coupling models in three steps:

1) An environmental fate and transport model, using historic emissions rates from the Washington Works Plant, was used to estimate PFOA concentrations in air, surface water, and groundwater, and ultimately in six contaminated municipal water systems from 1951 to 2011. The results of this model showed good correlation with measured water concentrations during 2000-2007 (Spearman's $\rho = 0.87$) (Shin et al., 2011a).

2) To estimate historical residential exposures for each individual, air and water concentrations over time produced by the fate and transport model were coupled with individual residential history.
3) To estimate time-dependent serum concentrations, historical residential exposure for each individual was coupled with a single compartment toxicokinetic model (Shin et al., 2011b).

For each year in which they worked, workers had two year-specific serum estimates, the residential (community) estimate and the occupational estimate. In those cases, the year-specific serum estimates from the occupational model were used if they were higher than the residential estimates (Winquist et al., 2013). Predicted serum levels in 2005-2006 from this model for the women in our retrospective cohort had reasonable correlation (Spearman's $\rho = 0.62$) with serum levels of PFOA measured in 2005-06. Measured levels may not have reflected historical exposure patterns in this population because many began drinking bottled water after the first, widespread publicity about PFOA in drinking water, which occurred in 2001(Steenland et al., 2009a).

Data analysis

We conducted two analyses, a retrospective analysis of the complete cohort, and a prospective analysis that was restricted to women who were premenopausal at time of the 2005/2006 C8HP survey, and had serum PFOA measurements at that time. Follow-up began in 1951 or at age 40 (whichever was later) in the retrospective analysis. In the prospective analysis, follow-up began at the time of the C8HP or at age 40 (whichever was later). The retrospective analyses used time-varying, modeled cumulative exposure, while the prospective analyses used time-varying modeled cumulative exposure or measured PFOA levels in 2005/2006. In

supplemental analyses of the retrospective cohort we also used time-varying year-specific serum estimates (current serum PFOA estimate at a given age), and we conducted lagged analyses of cumulative exposure (5, 10, 15, and 20 year lags).

The motivation for the prospective analysis was three-fold. First, restriction to women who are closer to the time of menopause would be expected to increase the accuracy of self-reported data on age at menopause (reducing digit preference that can occur in reporting menopause many years ago; see Appendix Figure 2.1). Second, a prospective analysis also enabled use of measured PFOA levels in 2005/2006, which could be preferred if one assumes that recent PFOA exposure is more important than cumulative exposure, and also that measured exposure in 2005/2006 would be more accurate than modeled exposure at the time, as a reflection of true, recent exposure. Third, given that most of the women studied here had to be alive and available for the C8HP in 2005/2006 at the time of the C8HP (true for all of the women except those who worked at the plant), retrospective analyses for menopause may be vulnerable to selection biases. Those enrolled in 2005/2006 may not fully represent the unknown target population exposed to PFOA in the mid-Ohio valley back in time, and prospective analysis may avoid some potential biases of a retrospective analysis.

For both analyses, we used Cox proportional hazards regression analyses with natural menopause as the event of interest and age as the time scale. Cox proportional hazard regressions estimate age-adjusted hazard ratios (HRs) for menopause as a function of PFOA exposure; higher estimated HRs for menopause are equivalent to an earlier average age of menopause.

Women were considered to have the event at the age of reported natural menopause; participants who were still menstruating at the end of follow-up were censored at the age of their last interview. All Cox models were stratified by birth year to account for potential confounding

by temporal trends, as both age at menopause (Nichols et al., 2006) and PFOA exposure have been changing over time (Winquist et al., 2013). Quintile cutpoints (Appendix Table 2.1) were calculated from the modeled cumulative PFOA serum concentrations (or year-specific serum concentrations) at time of menopause among those who had undergone natural menopause (i.e., cases). Natural log-transformed cumulative or year-specific PFOA exposures served as tests for trend.

Hysterectomy was a competing risk in our analysis. One report in the literature (Buck Louis et al., 2012) found an association between endometriosis (a possible cause of hysterectomy) and PFOA. If women with endometriosis have a different age at natural menopause than women without endometriosis, these results could suggest that women who have hysterectomies are different with respect to age at menopause from women who do not have hysterectomies, and this difference could cause bias in estimating the effect of exposure. To determine whether hysterectomy was associated with PFOA exposure in the retrospective cohort analyses, we conducted a proportional hazards analysis in which hysterectomy was the outcome, and women who had natural menopause and those still menstruating were censored at the age of menopause and age of last interview, respectively. To examine the potential impact of this competing risk on our results, we conducted for each exposure formulation two different analyses, in which women who had hysterectomy were either included and censored, or excluded from the analysis (Kleinbaum, 1999).

A number of potential confounders were identified from the literature for inclusion in all models of natural menopause. These are time-varying smoking status (Gold, 2011; Harlow and Signorello, 2000; Jick et al., 1977), educational attainment (Bleil et al., 2012; Bromberger et al., 1997; Wise et al., 2002), time-varying alcohol consumption (Torgerson et al., 2014), time-

varying parous/nulliparous status (Dvornyk et al., 2006; Stanford et al., 1987), time-varying hypertension and time-varying high cholesterol. Of these, smoking, educational attainment and parous/nulliparous status were retained in final models either because they were significant predictors of the outcome, or substantially changed the PFOA/menopause coefficient (>10%). Since the number of non-white participants in this group was less than 3%, and the inclusion of race did not change the PFOA/menopause association, we did not include race as a covariate in models. BMI at age 40 was also selected to be the variable of interest as BMI at the time of interview may have occurred after menopause.

Approximately 1,000 individuals failed to report BMI at age 40, and would have been excluded from our analysis as a result of including BMI at age 40 as a covariate. Hence, we chose to exclude this variable in our primary analyses. BMI was potentially an important confounder, given that BMI may be risk factor for early menopause (Akahoshi et al., 2002; Morris et al., 2012) and may be related to PFOA exposure (e.g., Eriksen et al., 2011; Steenland et al., 2009a). Other studies, however, have not found this association (e.g., Nelson et al., 2010) between BMI and early menopause. Furthermore, BMI has been postulated as an intermediate variable (Taylor et al., 2014) in analyses of PFOA and menopause, which would indicate it should not be included in models. In separate sensitivity analyses on a subset of women (N = 7,702) who have complete information about BMI at age 40, we included and excluded BMI at age 40 as a covariate in the model used in the primary analysis.

All pair-wise interactions between the covariates and the exposure were also examined in the course of model building. Where interaction was found between exposure and a covariate, separate proportional hazards regressions were carried out for the population in each category of the interacting covariate. We assessed if the proportional hazard assumption for PFOA exposure

was violated by assessing the statistical significance of an interaction term of age (the time variable) with exposure (Allison, 2010).

Results

Cohort characteristics & hysterectomy survival analysis results

Demographic statistics for the retrospective and prospective cohort are presented in Table 2.1. At the end of follow-up, 2,458 were still menstruating, 3,925 reported natural menopause, and 2,376 reported hysterectomy. Figure 2.2 shows the reported ages of natural menopause and hysterectomy in the retrospective cohort. Within the retrospective cohort, the median age at menopause was 45 and 50 for hysterectomy and natural menopause, respectively.

A modest positive association was found between hysterectomy and increasing cumulative exposure to PFOA in the retrospective cohort (Appendix Table 2.2) and a nearly statistically significant trend was found with logged cumulative exposure (p=0.07). The HR for the highest quintile of cumulative exposure vs. the lowest quintile was 1.23. A similar association was found for year-specific serum PFOA and hysterectomy in the retrospective cohort (logged serum PFOA estimate, p = 0.04). In prospective analyses, a nearly statistically significant trend was found for logged cumulative exposure (p=0.07), but we found no association between hysterectomy and measured serum PFOA concentration (Appendix Table 2.2)

Menopause survival analysis results

Retrospective cohort. Retrospective cohort analyses of the association between natural menopause and estimated cumulative PFOA exposure, in which women with hysterectomies were either excluded or censored, showed no significant association at the 0.05 level between

PFOA and natural menopause (Table 2.2). In the analyses of the overall model (i.e., main effects only and no interactions), in which hysterectomies were excluded, the estimated HRs by PFOA quintile were 1.00 (ref.), 1.06, 1.13, 1.09, and 1.11 (log-linear trend test for log cumulative exposure: $p = 0.25$). Similarly, in the hysterectomy-censored analysis (Table 2.2), the estimated HRs were 1.00 (ref.), 1.00, 1.09, 1.05 and 1.06 (log-linear trend test for cumulative exposure: $p = 0.37$).

In hysterectomy-excluded analyses, we found significant interactions between log-transformed cumulative exposure and individual categories of education, without any consistent pattern. In hysterectomy-censored analyses, significant interactions were found between log-transformed cumulative exposure and education level, and between log-transformed cumulative and smoking. Stratified analyses, however, yielded little evidence of an association between cumulative PFOA exposure and menopause in any subgroup of education, smoking, or smoking and education together.

In analyses using estimated year-specific serum PFOA estimates, we also found no marked trends between PFOA exposure and natural menopause and individual HR estimates were uniformly non-significant (results not shown). In the analyses in which hysterectomies were excluded, the non-significant, estimated HRs by year-specific serum PFOA quintile were 1.00 (ref.), 1.04, 1.04, 1.07, and 1.10 (log-linear trend test for log year-specific serum estimates: $p = 0.06$). Similarly, in the hysterectomy-censored analysis, the non-significant, estimated HRs were 1.00 (ref.), 1.02, 0.99, 1.05 and 1.07 (log-linear trend test for year-specific serum estimates: $p = 0.12$).

In hysterectomy-excluded analyses of 5-year lagged cumulative PFOA exposure, several quintiles above the reference (1^{st}) quintile were significantly associated with increased risk of

early menopause, but there was no evidence of a trend (Table 2.3; log-transformed exposure, p=0.42). All other lagged analyses of cumulative PFOA exposure and of year-specific serum PFOA in the retrospective cohort did not show evidence of positive trends at the p=0.05 level (results only for cumulative PFOA exposure in the retrospective cohort are shown in Table 2.3).

Sensitivity analyses of the subset of women who had reported BMI at age 40 showed no significant association between cumulative PFOA exposure and menopause (Appendix Table 2.3) when controlling for BMI at age 40. Excluding BMI at age 40 as a covariate from these supplemental analyses did not appreciably change the estimated HRs for cumulative PFOA exposure (results not shown). Thus, BMI at age 40 appears neither to confound the association nor to be an intermediate in the association between PFOA and menopause.

Prospective cohort. In prospective analyses, neither modeled cumulative exposure nor measured serum PFOA showed associations with natural menopause in quintile analyses (Table 2.4). Also, tests for trend were not statistically significant (log-linear trend test for cumulative exposure: $p \approx 0.6$; log-linear trend test for measured serum exposure: $p \approx 0.2$). Lagged cumulative exposure estimates were also not associated with risk of natural menopause (results not shown) in prospective analyses.

Discussion

Overall we found no association between natural menopause and PFOA in either the retrospective or the prospective analyses, i.e., women in our cohort with higher PFOA exposure did not have a higher hazard of menopause at a given age than women with lower PFOA exposure, adjusting for covariates. A modest positive association between hysterectomy and

cumulative PFOA exposure and between hysterectomy and year-specific serum PFOA estimates was found in retrospective analyses.

Having not had a child and current smoking have been shown in previously published work to be predictors of earlier menopause (Gold et al., 2001; Harlow and Signorello, 2000; Parente et al., 2008).We found the same associations in our data, lending support to the validity of our models. In our data, higher BMI at age 40 was associated with later natural menopause in the hysterectomy-censored analysis, conforming to some earlier findings (Akahoshi et al., 2002; Morris et al., 2012); however, other studies have not found an association between BMI and age at menopause (Harlow and Signorello, 2000). We found evidence of an association between low education level and early menopause. While some previous findings (Stanford et al., 1987; Wise et al., 2002) have also shown that low educational attainment is associated with earlier menopausal age, this association is not consistent in the literature (Canavez et al., 2011; Gold et al., 2001).

The availabilities of longitudinal data for menopause, estimates of internal PFOA dose, and of time-varying covariates, are significant strengths of our analyses. The C8SP cohort is unique in its large size and its wide range of exposures; many PFOA studies are restricted to general populations with low exposures. This work presents the largest study of PFOA and natural menopause to date. In previous cross-sectional analyses of PFOA and menopausal age in which serum PFOA was measured at the time of interview, exposure in post-menopausal women was determined subsequent to menopause and may have been influenced by the outcome. Because menopausal status in our study could not affect the exposure estimates generated through the modeling procedure, longitudinal estimates of serum PFOA in the present study are

not subject to reverse causation, which could affect cross-sectional analyses using measured PFOA at time of menopause.

Cohort weaknesses include the requirement of most of the cohort to have survived until 2005-2006 (all except workers) and migration into and out of the study area during the study period. It was not possible to identify and include all individuals residing in the study area during the exposure period, for reasons including migration and mortality. Eligible individuals who left the study area were less likely to be aware of and participate in the study, although prior residents were eligible and many did participate (Winquist et al., 2013). Nonetheless, neither death nor migration is likely to bias the associations observed here, as neither is likely to be strongly associated with either age at menopause or exposure to PFOA.

Measurement error may have affected both exposure and outcome. Modeled serum PFOA estimates are subject to a Berkson-type error resulting from assumptions of the modeling process (Armstrong, 1998). Though it produces little or no bias, Berskson-type error can affect the ability to detect an association, which may account for our observed null results.We observed some digit preference in the reported age of menopause (Appendix Figure 2.1), suggesting that women tended to round off their age at menopause to 40, 45, or 55, particularly in the retrospective cohort analyses, where women were asked to recall an event that averaged 14.4 years prior to the interview. Such digit preference would have reduced the accuracy of reported ages, and may have biased our results, presumably towards the null, as we have no reason to suspect recall varied by exposure. However, we also found no relationship between menopause and PFOA in prospective analyses, which showed a reduced tendency to digit preference.

While we found a modest positive trend in incidence of hysterectomy with increasing PFOA exposure, interpretation of this finding is limited. There is one prior report in the literature

showing a positive association between PFOA and endometriosis, which is one of the conditions which can lead to hysterectomy (Buck Louis et al., 2012). Hysterectomy can be undertaken for either medical or non-medical reasons. While medical conditions, such as endometriosis and uterine fibroids, may be the cause of hysterectomy, the timing of the surgery event is often substantially after the onset of the precipitating health condition. Other reasons for hysterectomy include birth control, cancer, menorrhagia and uterine fibroids. While we had some data on whether women had the hysterectomy due to endometriosis or uterine fibroids, we had no data on the date of diagnosis of these conditions, and hence were unable to conduct survival analyses for these conditions directly or for any cause-specific subset of hysterectomies. Future studies may further investigate this suggestive association by collecting more specific data on the reasons and timing of conditions leading to hysterectomy.

Conclusion

Previous cross-sectional studies of PFOA and age at natural menopause have found positive associations between measured PFOA and early menopause, but have acknowledged the potential for reverse causation (Knox et al., 2011; Taylor et al., 2014). Using longitudinal analyses with estimated time-varying PFOA exposure, we have found little evidence of an association between PFOA and menopause.

References

Akahoshi, M., Soda, M., Nakashima, E., Tominaga, T., Ichimaru, S., Seto, S., Yano, K., 2002. The effects of body mass index on age at menopause. Int. J. Obes. Relat. Metab. Disord. 26, 961–968. doi:10.1038/sj.ijo.0802039

Allison, P.D., 2010. Survival Analysis Using the SAS System: A Practical Guide, 2nd ed. SAS Institute, Cary, NC.

Armstrong, B.G., 1998. Effect of measurement error on epidemiological studies of environmental and occupational exposures. Occup. Environ. Med. 55, 651–656.

Bartell, S.M., Calafat, A.M., Lyu, C., Kato, K., Ryan, P.B., Steenland, K., 2010. Rate of decline in serum PFOA concentrations after granular activated carbon filtration at two public water systems in Ohio and West Virginia. Environ. Health Perspect. 118, 222–8. doi:10.1289/ehp.0901252

Bleil, M.E., Adler, N.E., Pasch, L. a, Sternfeld, B., Gregorich, S.E., Rosen, M.P., Cedars, M.I., 2012. Psychological stress and reproductive aging among pre-menopausal women. Hum. Reprod. 27, 2720–8. doi:10.1093/humrep/des214

Brede, E., Wilhelm, M., Göen, T., Müller, J., Rauchfuss, K., Kraft, M., Hölzer, J., 2010. Two-year follow-up biomonitoring pilot study of residents' and controls' PFC plasma levels after PFOA reduction in public water system in Arnsberg, Germany. Int. J. Hyg. Environ. Health 213, 217–223. doi:http://dx.doi.org/10.1016/j.ijheh.2010.03.007

Bromberger, J.T., Matthews, K.A., Kuller, L.H., Wing, R.R., Meilahn, E.N., Plantinga, P., 1997. Prospective study of the determinants of age at menopause. Am. J. Epidemiol. 145, 124–133. doi:10.1093/oxfordjournals.aje.a009083

Buck Louis, G.M., Peterson, C.M., Chen, Z., Hediger, M.L., Croughan, M.S., Sundaram, R., Stanford, J.B., Fujimoto, V.Y., Varner, M.W., Giudice, L.C., Kennedy, A., Sun, L., Wu, Q., Kannan, K., 2012. Perfluorochemicals and endometriosis: the ENDO study. Epidemiology 23, 799–805. doi:10.1097/EDE.0b013e31826cc0cf

Calafat, A.M., Wong, L.-Y., Kuklenyik, Z., Reidy, J.A., Needham, L.L., 2007. Polyfluoroalkyl chemicals in the U.S. population: data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. Environ. Health Perspect. 115, 1596–602. doi:10.1289/ehp.10598

Canavez, F.S., Werneck, G.L., Parente, R.C.M., Celeste, R.K., Faerstein, E., 2011. The association between educational level and age at the menopause: a systematic review. Arch. Gynecol. Obstet. 283, 83–90. doi:10.1007/s00404-009-1323-6

Dvornyk, V., Long, J.-R., Liu, P.-Y., Zhao, L.-J., Shen, H., Recker, R.R., Deng, H.-W., 2006. Predictive factors for age at menopause in Caucasian females. Maturitas 54, 19–26. doi:10.1016/j.maturitas.2005.08.005

EPA, 2012. PFOA Stewardship Program Description [WWW Document]. URL http://www.epa.gov/oppt/pfoa/pubs/stewardship/pfoastewardshipbasics.html

Eriksen, K.T., Sørensen, M., McLaughlin, J.K., Tjønneland, A., Overvad, K., Raaschou-Nielsen, O., 2011. Determinants of plasma PFOA and PFOS levels among 652 Danish men. Environ. Sci. Technol. 45, 8137–8143. doi:10.1021/es100626h

Frisbee, S.J., Brooks, A. P., Maher, A., Flensborg, P., Arnold, S., Fletcher, T., Steenland, K., Shankar, A., Knox, S.S., Pollard, C., Halverson, J. a, Vieira, V.M., Jin, C., Leyden, K.M., Ducatman, A.M., 2009. The C8 health project: design, methods, and participants. Environ. Health Perspect. 117, 1873–82. doi:10.1289/ehp.0800379

Gold, E.B., 2011. The timing of the age at which natural menopause occurs. Obstet. Gynecol. Clin. North Am. 38, 425–40. doi:10.1016/j.ogc.2011.05.002

Gold, E.B., Bromberger, J., Crawford, S., Samuels, S., Greendale, G. A., Harlow, S.D., Skurnick, J., 2001. Factors associated with age at natural menopause in a multiethnic sample of midlife women. Am. J. Epidemiol. 153, 865–74. doi:10.1093/aje/153.9.865

Harlow, B.L., Signorello, L.B., 2000. Factors associated with early menopause. Maturitas 35, 3–9. doi:10.1016/s0378-5122(00)00092-x

Jacobsen, B.K., Heuch, I., Kvåle, G., 2003. Age at natural menopause and all-cause mortality: A 37-year follow-up of 19,731 Norwegian women. Am. J. Epidemiol. 157, 923–929. doi:10.1093/aje/kwg066

Jensen, A.A., Leffers, H., 2008. Emerging endocrine disrupters: perfluoroalkylated substances. Int. J. Androl. 31, 161–9. doi:10.1111/j.1365-2605.2008.00870.x

Jick, H., Porter, J., Morrison, A.S., 1977. Relation between smoking and age of natural menopause. Lancet 1354–1355. doi:0.1016/S0140-6736(77)92562-4

Kato, K., Wong, L.Y., Jia, L.T., Kuklenyik, Z., Calafat, A.M., 2011. Trends in exposure to polyfluoroalkyl chemicals in the U.S. population: 1999-2008. Environ. Sci. Technol. 45, 8037–8045. doi:10.1021/es1043613

Kjeldsen, L.S., Bonefeld-Jørgensen, E.C., 2013. Perfluorinated compounds affect the function of sex hormone receptors. Environ. Sci. Pollut. Res. Int. 20, 8031–44. doi:10.1007/s11356-013-1753-3

Kleinbaum, D., 1999. Survival Analsis: a self-learning text. Springer-Verlag, New York.

Knox, S.S., Jackson, T., Javins, B., Frisbee, S.J., Shankar, A., Ducatman, A.M., 2011. Implications of early menopause in women exposed to perfluorocarbons. J. Clin. Endocrinol. Metab. 96, 1747–53. doi:10.1210/jc.2010-2401

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol. Sci. 99, 366–94. doi:10.1093/toxsci/kfm128

Li, S., Rosenberg, L., Wise, L. a, Boggs, D. A., LaValley, M., Palmer, J.R., 2013. Age at natural menopause in relation to all-cause and cause-specific mortality in a follow-up study of US black women. Maturitas 75, 246–52. doi:10.1016/j.maturitas.2013.04.003

Lisabeth, L.D., Beiser, A.S., Brown, D.L., Murabito, J.M., Kelly-Hayes, M., Wolf, P. a, 2009. Age at natural menopause and risk of ischemic stroke: the Framingham heart study. Stroke. 40, 1044–9. doi:10.1161/STROKEAHA.108.542993

Luborsky, J.L., Meyer, P., Sowers, M.F., Gold, E.B., Santoro, N., 2003. Premature menopause in a multi-ethnic population study of the menopause transition. Hum. Reprod. 18, 199–206. doi:10.1093/humrep/deg005

Morris, D.H., Jones, M.E., Schoemaker, M.J., McFadden, E., Ashworth, A., Swerdlow, A.J., 2012. Body mass index, exercise, and other lifestyle factors in relation to age at natural menopause: analyses from the breakthrough generations study. Am. J. Epidemiol. 175, 998–1005. doi:10.1093/aje/kwr447

Nelson, J.W., Hatch, E.E., Webster, T.F., 2010. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population. Environ. Health Perspect. 118, 197–202. doi:10.1289/ehp.0901165

Nelson, L.M., Covington, S.N., Rebar, R.W., 2005. An update: spontaneous premature ovarian failure is not an early menopause. Fertil. Steril. 83, 1327–32. doi:10.1016/j.fertnstert.2004.11.059

Nichols, H.B., Trentham-Dietz, A., Hampton, J.M., Titus-Ernstoff, L., Egan, K.M., Willett, W.C., Newcomb, P. a, 2006. From menarche to menopause: trends among US Women born from 1912 to 1969. Am. J. Epidemiol. 164, 1003–11. doi:10.1093/aje/kwj282

Olsen, G.W., Burris, J.M., Ehresman, D.J., Froehlich, J.W., Seacat, A.M., Butenhoff, J.L., Zobel, L.R., 2007. Half-life of serum elimination of perfluorooctanesulfonate,perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ. Health Perspect. 115, 1298–305. doi:10.1289/ehp.10009

Panay, N., Kalu, E., 2009. Management of premature ovarian failure. Best Pract. Res. Clin. Obstet. Gynaecol. 23, 129–40. doi:10.1016/j.bpobgyn.2008.10.008

Parente, R.C., Faerstein, E., Celeste, R.K., Werneck, G.L., 2008. The relationship between smoking and age at the menopause: A systematic review. Maturitas 61, 287–98. doi:10.1016/j.maturitas.2008.09.021

Qiu, C., Chen, H., Wen, J., Zhu, P., Lin, F., Huang, B., Wu, P., Lin, Q., Lin, Y., Rao, H., Huang, H., Liang, J., Li, L., Gong, X., Peng, S., Li, M., Chen, L., Tang, K., Chen, Z., Lin, L., Lu, J., Bi, Y., Ning, G., Chen, G., 2013. Associations between age at menarche and menopause with cardiovascular disease, diabetes, and osteoporosis in Chinese women. J. Clin. Endocrinol. Metab. 98, 1612–21. doi:10.1210/jc.2012-2919

Shin, H.-M., Vieira, V.M., Ryan, P.B., Detwiler, R., Sanders, B., Steenland, K., Bartell, S.M., 2011a. Environmental fate and transport modeling for perfluorooctanoic acid emitted from the Washington Works Facility in West Virginia. Environ. Sci. Technol. 45, 1435–42. doi:10.1021/es102769t

Shin, H.-M., Vieira, V.M., Ryan, P.B., Steenland, K., Bartell, S.M., 2011b. Retrospective exposure estimation and predicted versus observed serum perfluorooctanoic acid

concentrations for participants in the C8 Health Project. Environ. Health Perspect. 119, 1760–5. doi:10.1289/ehp.1103729

Shuster, L.T., Rhodes, D.J., Gostout, B.S., Grossardt, B.R., Rocca, W. A., 2010. Premature menopause or early menopause: long-term health consequences. Maturitas 65, 161–6. doi:10.1016/j.maturitas.2009.08.003

Stanford, J.L., Hartge, P., Brinton, L. A., Hoover, R.N., Brookmeyer, R., 1987. Factors influencing the age at natural menopause. J. Chronic Dis. 40, 995–1002. doi:10.1016/0021-9681(87)90113-5

Steenland, K., Jin, C., MacNeil, J., Lally, C., Ducatman, A., Vieira, V., Fletcher, T., 2009. Predictors of PFOA levels in a community surrounding a chemical plant. Environ. Health Perspect. 117, 1083–8. doi:10.1289/ehp.0800294

Taylor, K.W., Hoffman, K., Thayer, K. A., Daniels, J.L., 2014. Polyfluoroalkyl Chemicals and Menopause among Women 20-65 Years of Age (NHANES). Environ. Health Perspect. 122, 145–50. doi:10.1289/ehp.1306707

Torgerson, D.J., Thomas, R.E., Campbell, M.K., Reid, D.M., 2014. Alcohol consumption and age of maternal menopause are associated with menopause onset. Maturitas 26, 21–25. doi:10.1016/S0378-5122(96)01075-4

Wang, Z., Cousins, I.T., Scheringer, M., Buck, R.C., Hungerbühler, K., 2014. Global emission inventories for C4 – C14 per fluoroalkyl carboxylic acid (PFCA) homologues from 1951 to

2030, Part I: production and emissions from quantifiable sources. Environ. Int. 70, 62–75. doi:10.1016/j.envint.2014.04.013

White, S.S., Fenton, S.E., Hines, E.P., 2011. Endocrine disrupting properties of perfluorooctanoic acid. J. Steroid Biochem. Mol. Biol. 127, 16–26. doi:10.1016/j.jsbmb.2011.03.011

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, 893–9. doi:10.1289/ehp.1206450

Wise, L.A., Krieger, N., Zierler, S., Harlow, B.L., 2002. Lifetime Socioeconomic Position in Relation to Onset of Perimenopause. J. Epidemiol. Community Health 56, 851–860. doi:10.1136/jech.56.11.851

Woskie, S.R., Gore, R., Steenland, K., 2012. Retrospective exposure assessment of perfluorooctanoic acid serum concentrations at a fluoropolymer manufacturing plant. Ann. Occup. Hyg. 56, 1025–37. doi:10.1093/annhyg/mes023

Tables and Figures

Table 2.1. Cohort characteristics

	Retrospective cohort (N = 8,759)		Prospective cohort (N = 3,334)	
Variable	**Count**	**%**	**Count**	**%**
RACE				
Missing	35	0.4	35	1.1
American Indian	43	0.5	14	0.4
Asian/Pacific Islander	29	0.3	13	0.4
Black	77	0.9	35	1.1
Other	43	0.5	24	0.7
White	8,532	97.4	3,213	96.4
REPORTED BMI AT AGE 40 (kg/m^2)				
Missing	1,058	12.1	511	15.3
BMI ≤ 18.5	143	1.6	50	1.5
18.5 < BMI ≤ 25	3,893	44.5	1,176	35.3
25 < BMI ≤ 30	1,981	22.6	745	22.4
BMI > 30	1,684	19.2	852	25.6
EDUCATION LEVEL				
Less than High school diploma	708	8.1	134	4.0
High school diploma	3,482	39.8	1,057	31.7
Some College	3,022	34.5	1,292	38.8
Undergraduate degree or greater	1,547	17.7	851	25.5
EVER PREGNANT?				
No	881	10.1	399	12.0
Yes	7,878	89.9	2,935	88.0
DIABETIC?				
No	8,167	93.2	3,041	91.2
Yes	592	6.8	293	8.8
ALCOHOL USE				
Never used alcohol	6,540	74.7	2,215	66.4
Formerly used alcohol	617	7.0	320	9.6
Currently use alcohol	1,602	18.3	799	24.0
SMOKING				
Never smoker	5,098	58.2	1,878	56.3
Former smoker	1,533	17.5	726	21.8
Current smoker	2,128	24.3	730	21.9
HYPERTENSION				
No	6,671	76.2	2,401	72.0
Yes	2,088	23.8	933	28.0
HIGH CHOLESTEROL				
No	7,009	80.0	2,302	69.1
Yes	1,750	20.0	1,032	31.0
MENOPAUSAL OUTCOME				
Still menstruating at end of follow-up	2,458	28.1	2,458	73.7

Hysterectomy	2,376	27.1	267	8.0
Natural menopause	3,925	44.8	609	18.3
	Retrospective cohort (N = 8,759)		**Prospective cohort** (N = 3,334)	
BIRTHYEAR				
25th quantile	1944		1959	
Median	1953		1963	
75th quantile	1961		1966	
AGE AT NATURAL MENOPAUSE				
25th quantile	46		48	
Median	50		50	
Mean (SD)	49.3 (4.4)		50.3 (3.6)	
75th quantile	52		53	
AGE AT HYSTERECTOMY				
25th quantile	42		42	
Median	45		45	
Mean (SD)	45.4 (4.5)		45.3 (3.8)	
75th quantile	48		48	
LENGTH OF FOLLOW-UP (YEARS)				
25th quantile	4		4	
Median	8		7	
Mean (SD)	8.1 (4.7)		7.2 (4.2)	
75th quantile	11		10	

Table 2.2. Analyses of natural menopause for cumulative PFOA exposure in the retrospective cohort.

	Hysterectomy excluded		Hysterectomy censored	
Variable	**HR (95% C.I.)**	**p-value**	**HR (95% C.I.)**	**p-value**
Cumulative exposure quintile (reference: 1st)				
2nd	1.06 (0.93, 1.21)	0.37	1.00 (0.88, 1.13)	0.96
3rd	1.13 (0.99, 1.29)	0.07	1.09 (0.96, 1.25)	0.18
4th	1.09 (0.96, 1.25)	0.18	1.05 (0.92, 1.19)	0.49
5th	1.11 (0.97, 1.26)	0.14	1.06 (0.93, 1.21)	0.40
Educational Category (reference: *'less than HS diploma'*)				
'HS diploma'	0.90 (0.80, 1.01)	0.07	0.94 (0.84, 1.06)	0.30
'Some undergraduate'	0.84 (0.74, 0.95)	0.005	0.87 (0.77, 0.98)	0.02
'At least Bachelor's degree'	0.83 (0.72, 0.95)	0.01	0.88 (0.77, 1.01)	0.07
Parity (reference = Nulliparous)	0.83 (0.75, 0.92)	0.0005	0.80 (0.72, 0.89)	<.0001
Smoking status (reference: Never smoker)				
Current smoker	1.36 (1.26, 1.47)	<.0001	1.40 (1.30, 1.51)	<.0001
Former smoker	0.99 (0.90, 1.09)	0.85	0.99 (0.90, 1.08)	0.76
Log-transformed cumulative exposure, trend test	1.01 (0.99, 1.04)	0.25	1.01 (0.99, 1.03)	0.37

Table 2.3. Hazard ratio by exposure quintile for analyses of natural menopause and lagged cumulative PFOA exposure in the retrospective cohort where hysterectomies were excluded or censored.

		5 year lag		10 year lag		15 year lag		20 year lag	
	Variable	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Cumulative exposure, with hysterectomies excluded	**PFOA Quintile (ref. = 1st)**								
	2nd	1.17 (1.02, 1.36)	0.03	1.13 (0.96, 1.33)	0.16	1.16 (0.98, 1.37)	0.09	1.07 (0.77, 1.48)	0.69
	3rd	1.15 (0.99, 1.33)	0.07	1.13 (0.95, 1.36)	0.18	1.13 (0.94, 1.37)	0.20	1.08 (0.76, 1.54)	0.67
	4th	1.17 (1.01, 1.35)	0.04	1.11 (0.93, 1.32)	0.27	1.13 (0.93, 1.36)	0.22	1.13 (0.79, 1.62)	0.49
	5th	1.18 (1.01, 1.37)	0.03	1.13 (0.95, 1.35)	0.18	1.15 (0.95, 1.38)	0.16	1.15 (0.81, 1.63)	0.45
	Log-linear, test for trend	1.01 (0.99, 1.03)	0.45	1.01 (0.98, 1.03)	0.58	1.01 (0.98, 1.04)	0.57	1.03 (0.99, 1.07)	0.20
Cumulative exposure, with hysterectomies censored	**PFOA Quintile (ref. = 1st)**								
	2nd	1.14 (0.99, 1.32)	0.07	1.11 (0.94, 1.31)	0.21	1.14 (0.97, 1.35)	0.12	1.02 (0.74, 1.42)	0.90
	3rd	1.12 (0.96, 1.30)	0.15	1.10 (0.92, 1.31)	0.3	1.09 (0.91, 1.31)	0.36	1.01 (0.71, 1.44)	0.95
	4th	1.13 (0.97, 1.30)	0.12	1.06 (0.89, 1.27)	0.5	1.08 (0.90, 1.31)	0.4	1.05 (0.74, 1.50)	0.78
	5th	1.14 (0.98, 1.33)	0.08	1.10 (0.92, 1.32)	0.28	1.11 (0.92, 1.34)	0.26	1.08 (0.76, 1.53)	0.67
	Log-linear, test for trend	1.01 (0.98, 1.03)	0.58	1.01 (0.98, 1.03)	0.66	1.01 (0.98, 1.04)	0.6	1.03 (0.99, 1.07)	0.17

Table 2.4. Analyses of natural menopause for various PFOA exposures in the prospective cohorts.

	Variable	# of cases	HR (95% CI)	p-value
Cumulative exposure, hysterectomies excluded	**PFOA Quintile (ref. = 1st)**	122		
	2nd	121	1.10 (0.85, 1.42)	0.49
	3rd	122	0.99 (0.77, 1.29)	0.96
	4th	122	0.99 (0.76, 1.28)	0.92
	5th	122	1.10 (0.84, 1.43)	0.51
	Log-linear trend test	609	1.02 (0.96, 1.08)	0.63
Cumulative exposure, hysterectomies censored	**PFOA Quintile (ref. = 1st)**	122		
	2nd	121	1.15 (0.89, 1.48)	0.30
	3rd	122	1.03 (0.80, 1.33)	0.81
	4th	122	0.99 (0.77, 1.29)	0.95
	5th	122	1.10 (0.85, 1.44)	0.47
	Log-linear trend test	609	1.02 (0.96, 1.08)	0.61
Measured serum PFOA, hysterectomies excluded	**PFOA Quintile (ref. = 1st)**	106		
	2nd	107	0.89 (0.69, 1.16)	0.39
	3rd	107	1.04 (0.80, 1.35)	0.76
	4th	107	1.03 (0.80, 1.33)	0.82
	5th	107	1.12 (0.86, 1.45)	0.40
	Log-linear trend test	534	1.04 (0.98, 1.12)	0.20
Measured serum PFOA, hysterectomies censored	**PFOA Quintile (ref. = 1st)**	106		
	2nd	107	0.88 (0.68, 1.13)	0.31
	3rd	107	1.08 (0.84, 1.40)	0.54
	4th	107	1.02 (0.79, 1.32)	0.87
	5th	107	1.14 (0.88, 1.47)	0.33
	Log-linear trend test	534	1.05 (0.98, 1.12)	0.19

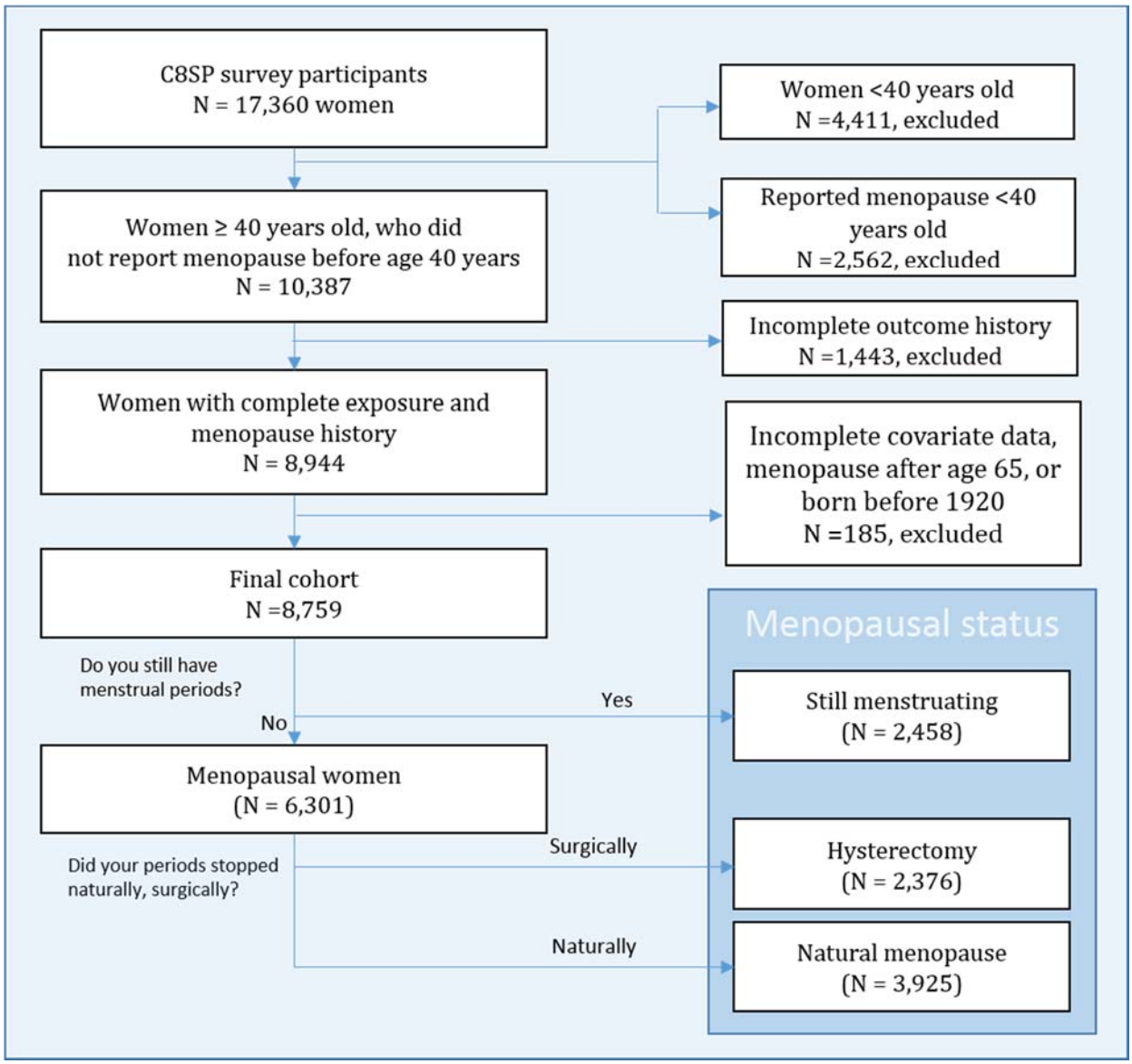


Figure 2.1. Retrospective cohort construction. C8 Science Panel (C8SP) questions were used to assign respondents to analytical categories, 'Still menstruating,' 'Hysterectomy,' and 'Natural menopause,' at the end of their follow-up.

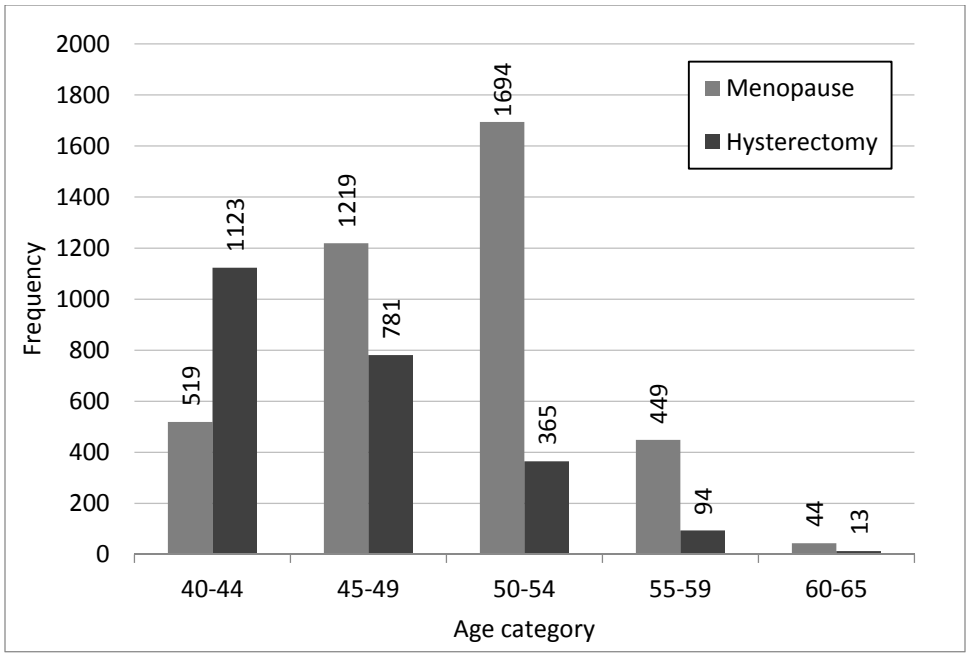


Figure 2.2. Reported ages of natural menopause and hysterectomy, by 5 year. age categories.

Appendix Tables and Figures

Appendix Table 2.1. Mean, Minimum, Maximum and upper quintile cutpoints for values of cumulative exposure, year-specific serum estimates and measured serum for each analysis°.

	Minimum observed value	Upper cutpoint for quintile				
		1st	2nd	3rd	4th	5th*
Retrospective cohort, outcome=hysterectomy						
Cumulative Exposure (ng/L*yr)	0.007	0.09	0.16	0.35	1.76	29.4
Year-specific Serum estimate (ug/L)	1.17	4.56	8.37	20.90	112.90	3,169.80
Retrospective cohort, outcome=natural menopause						
Cumulative Exposure (ng/L*yr)	0.007	0.11	0.19	0.40	2.13	34.22
Year-specific Serum estimate (ug/L)	1.27	4.83	9.43	24.60	124.30	4,267.80
Prospective cohort, outcome=hysterectomy						
Cumulative Exposure (ng/L*yr)	0.01	0.20	0.45	1.43	4.81	29.42
Measured serum (ug/L)**	0.6	7.4	12.3	27.4	69.0	820.8
Prospective cohort, outcome=natural menopause						
Cumulative Exposure (ng/L*yr)	0.05	0.23	0.42	1.27	4.67	34.22
Measured serum (ug/L)**	1.7	8.4	17.8	33.6	80.8	1,131.8

° The quintile cut points were determined among cases at the time of failure.

* Note that the upper cutpoint of the 5th quintiles is also the maximum observed value.

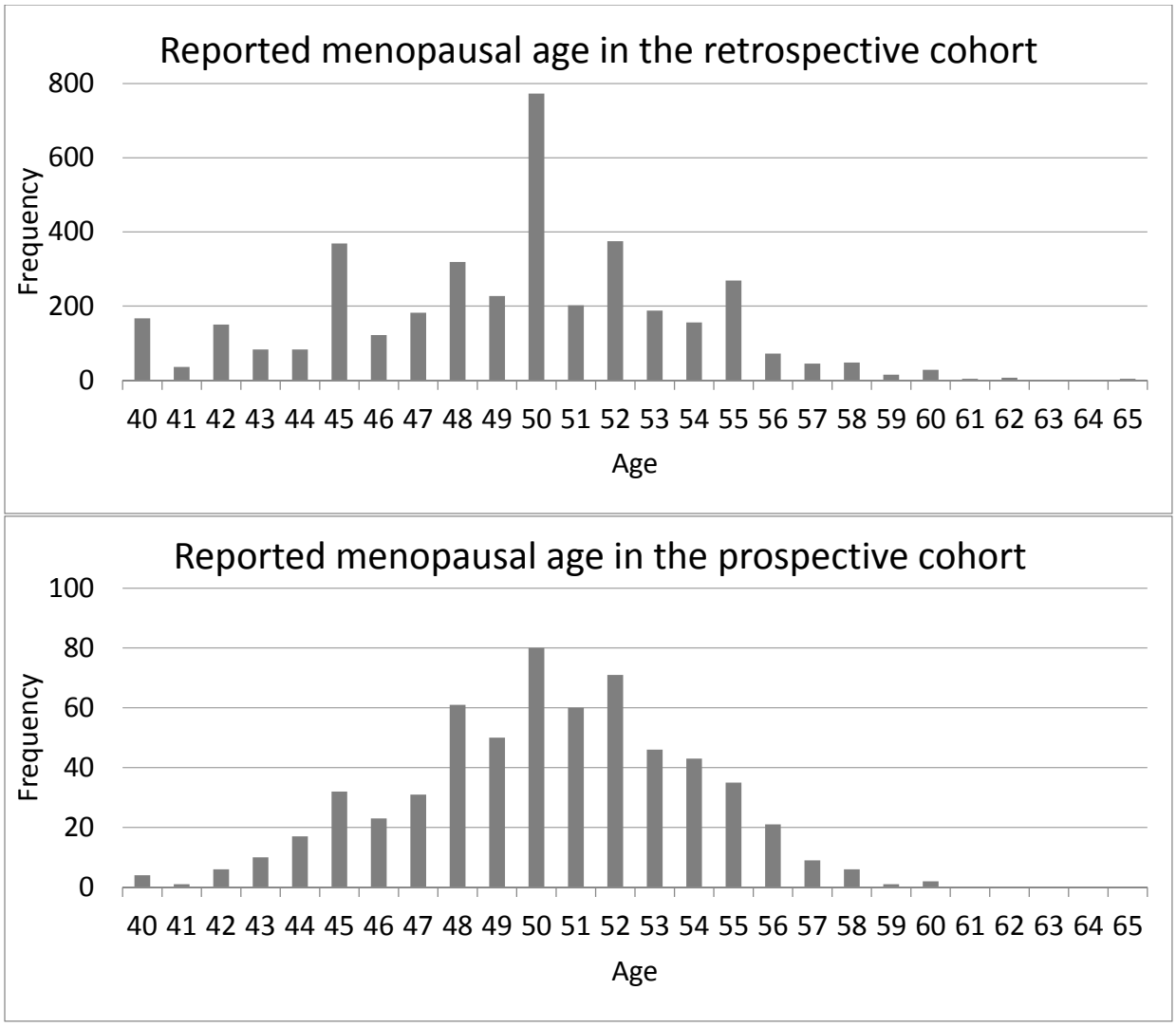
** Laboratory measurements of serum PFOA were reported to one significant digit.

Appendix Table 2.2. Overall analyses of the association between hysterectomy and PFOA exposure (in an analysis censoring women at the time of natural menopause), either as estimated cumulative exposure and year-specific serum concentrations in the retrospective analytic cohort, or as cumulative exposure and measured serum PFOA in the prospective cohort.

	Variable		# of cases	HR (95% CI)	p-value
Cum. Exposure + retrospective cohort	**PFOA Quintile (ref. = 1st)**		585		
		2nd	513	1.16 (0.98, 1.37)	0.09
		3rd	376	1.07 (0.90, 1.28)	0.44
		4th	471	1.20 (1.01, 1.43)	0.04
		5th	431	1.23 (1.03, 1.47)	0.02
	Log-linear, test for trend		2,376	1.03 (1.00, 1.06)	0.07
Year-specific Serum Estimate + retrospective cohort	**PFOA Quintile (ref. = 1st)**		551		
		2nd	465	1.04 (0.90, 1.20)	0.58
		3rd	461	1.11 (0.96, 1.28)	0.16
		4th	442	1.07 (0.92, 1.23)	0.39
		5th	457	1.15 (1.00, 1.33)	0.06
	Log-linear, test for trend		2,376	1.03 (1.00, 1.06)	0.04
Cum. Exposure + prospective cohort	**PFOA Quintile (ref. = 1st)**		65		
		2nd	37	0.69 (0.47, 1.02)	0.06
		3rd	48	0.85 (0.57, 1.25)	0.40
		4th	63	0.95 (0.64, 1.41)	0.79
		5th	54	1.12 (0.75, 1.68)	0.57
	Log-linear, test for trend		267	1.09 (1.00, 1.19)	0.07
Measured serum + prospective cohort	**PFOA Quintile (ref. = 1st)**		57		
		2nd	55	1.00 (0.68, 1.47)	0.99
		3rd	33	0.66 (0.45, 0.97)	0.03
		4th	45	0.75 (0.51, 1.11)	0.15
		5th	39	0.87 (0.59, 1.28)	0.48
	Log-linear, test for trend		229	0.95 (0.85, 1.05)	0.31

Appendix Table 2.3. Sensitivity analyses of cumulative exposure and menopause, on the subset of women (N = 7,702) with complete information on BMI at age 40.

	Hysterectomy excluded		Hysterectomy censored	
Variable	**HR (95% C.I.)**	**p-value**	**HR (95% C.I.)**	**p-value**
Cumulative exposure quintile (reference: 1st)				
2nd	1.11 (0.97, 1.27)	0.14	1.06 (0.92, 1.21)	0.31
3rd	1.16 (1.01, 1.34)	0.04	1.14 (0.99, 1.30)	0.07
4th	1.13 (0.98, 1.30)	0.09	1.11 (0.97, 1.27)	0.14
5th	1.15 (1.00, 1.32)	0.06	1.11 (0.97, 1.28)	0.14
Educational category (reference: *'less than HS diploma'*)				
'HS diploma'	0.89 (0.79, 1.01)	0.06	0.92 (0.81, 1.04)	0.16
'Some undergraduate'	0.85 (0.75, 0.97)	0.01	0.86 (0.76, 0.98)	0.02
'At least Bachelor's degree'	0.83 (0.71, 0.96)	0.01	0.86 (0.74, 1.00)	0.04
BMI category at age 40 (reference: 18.5<BMI≤25)				
BMI≤18.5	1.02 (0.80, 1.31)	0.85	0.99 (0.77, 1.27)	0.92
25<BMI≤30	1.02 (0.93, 1.12)	0.67	1.03 (0.94, 1.13)	0.55
BMI>30	0.93 (0.84, 1.04)	0.20	0.89 (0.80, 0.99)	0.04
Parity (reference. = Nulliparous)	0.83 (0.74, 0.93)	0.001	0.82 (0.73, 0.92)	0.0004
Smoking status (reference: Never smoker)				
Current smoker	1.32 (1.21, 1.43)	<.0001	1.36 (1.25, 1.47)	<.0001
Former smoker	0.98 (0.89, 1.08)	0.74	1.00 (0.90, 1.10)	0.91
Log-transformed cumulative exposure, trend test	1.01 (0.99, 1.04)	0.27	1.01 (0.99, 1.04)	0.37



Appendix Figure 2.1. Frequency of reported ages at menopause in the retrospective and prospective cohorts.

Chapter 3: Perfluorooctanoic acid and chronic kidney disease: longitudinal analysis of a Mid-Ohio Valley community

Authors: Radhika Dhingra[a], Cathy Lally, MPH[b], Lyndsey A. Darrow, PhD[c], Mitch Klein, PhD[a], Andrea Winquist, MD, PhD[a], Kyle Steenland, PhD[a]

Affiliations: [a] Department of Environmental Health, Rollins School of Public Health, Emory University, 1518 Clifton Road, Atlanta, GA 30322, USA. rdhingra@emory.edu; mklein@emory.edu; awinqui@emory.edu; nsteenl@emory.edu

[b] Department of Health Policy and Management , Rollins School of Public Health, Emory University, 1518 Clifton Road, Atlanta, GA 30322, USA. clally@emory.edu

[c] Department of Epidemiology, Rollins School of Public Health, Emory University, 1518 Clifton Road, Atlanta, GA 30322, USA. ldarrow@emory.edu

Abstract

Introduction: Perfluorooctanoic acid (PFOA) is an environmentally persistent chemical found at low-levels in the serum of almost all U.S. residents. Chronic kidney disease (CKD) has been positively associated with serum PFOA in prior cross-sectional studies and in one occupational mortality study. One longitudinal study in children found no association between kidney function and PFOA.

Methods: We conducted a longitudinal analysis of chronic kidney disease among adults, aged ≥20 years, (N = 32,254) in a Mid-Ohio Valley community cohort, exposed to high PFOA levels from contaminated drinking water. Estimated retrospective yearly serum PFOA concentrations (1951-2011) were previously modeled in this population. Information about lifetime history of CKD diagnosis was collected during surveys in 2008-2011; self-reported CKD diagnoses were validated through medical record review. Using a Cox proportional hazards model, we retrospectively examined the association between validated adult onset CKD, and modeled PFOA exposure. We also analyzed data for the cohort prospectively, among people with no CKD diagnosis prior to enrollment in a baseline survey in 2005-2006. Both the full cohort and a non-diabetic subset were analyzed, retrospectively and prospectively.

Results: Neither in retrospective nor in prospective analyses did we find a significant (α=0.05) trend between PFOA exposure and CKD. In the full cohort, estimated hazard ratios by quintile of cumulative serum PFOA in the retrospective analysis were 1.00 (referent), 1.26, 1.12, 1.12 and 1.24 (trend test for log cumulative exposure: p=0.80).

Conclusion: Our analyses suggest that CKD is not associated with exposure to PFOA.

Introduction

Perfluorooctanoic acid (PFOA) is an industrial byproduct of the manufacture of commercial polymers such as Teflon and Gore-Tex. PFOA is found in small, measurable quantities (median in U.S. ≈ 4 μg/L, mean in U.S. = 3.9 μg/L) in the serum of more than 95% the U.S. population (Calafat et al., 2007; Kato et al., 2011). Though its use in the U.S. has been substantially reduced (EPA, 2012), PFOA continues to be used at high levels internationally, in countries such as China and Russia (Wang et al., 2014). Even if eliminated from use, PFOA remains a potential hazard; it is environmentally persistent and is slowly excreted from the human body, with a reported half-life ranging from 2.3 to 3.8 years (Bartell et al., 2010; Brede et al., 2010; Olsen et al., 2007).

Chronic kidney disease (CKD) is characterized by diminishing kidney function. CKD is frequently asymptomatic and undiagnosed in the early disease stages (Coresh et al., 2005), progresses slowly, and greatly increases the risk of end stage renal disease (Saydah et al., 2007) or cardiovascular mortality (Sarnak et al., 2003). In 2007-2012, the prevalence of CKD in U.S. adults (age ≥ 20 years) based on data from the national health and nutrition survey (NHANES) was reported to be 13.6%, when adjusted for age, sex, race/ethnicity and risk-factor categories (United States Renal Data System, 2014).

Toxicological studies, *in vitro and in vivo*, suggest that exposure to PFOA may increase the risk either of chronic kidney disease or of predictors of chronic kidney disease. In addition to the liver and serum, the kidney is expected to be a primary storage site for PFOA, based on rodent studies (Cui et al., 2009; Jensen and Leffers, 2008; Lau et al., 2007). Its primary, known mode of action is as a PPAR-α agonist in animals (Lau et al., 2007) - though, perhaps less clearly so in humans (DeWitt et al., 2009). PPAR-α, in humans, is expressed in the proximal tubule of

the kidney. Under a chronic dosing regimen, renal hypertrophy as well as cell and tissue changes indicative of renal disease (Cui et al., 2009) were observed in highly exposed, but not in low-exposed, rats; this finding offers a potential mechanism by which PFOA might cause the diminished kidney function that characterizes CKD.

Though few epidemiological studies are available, two cross-sectional studies and one mortality study have shown an association between measured serum PFOA concentrations and reduced kidney function. One study examined the association between PFOA and CKD (defined as estimated Glomerular Filtration Rate (eGFR) < 60 mL/min/1.73m^2) in adults (Shankar et al., 2011a) and the second examined the association between PFOA and eGFR in children (Watkins et al., 2013). The authors of both studies cautioned that the observed associations might not have been causal. In both studies, the outcome and the PFOA serum concentrations were determined from the same blood sample. In the study of PFOA and eGFR in children, the authors also demonstrated that modeled serum PFOA concentrations (modeled independently of measured PFOA) did not show any association with eGFR (Watkins et al., 2013). Additionally, a cohort mortality study of a highly exposed worker cohort found a positive association between CKD mortality and modeled PFOA exposure, as defined via a job-exposure matrix based on over 2000 measured PFOA blood levels (Steenland and Woskie, 2012).

At various levels of chronic exposure in humans, PFOA has also been linked in some studies to a number of risk factors for (or potential biomarkers of) chronic kidney disease, including, increased homocysteine levels (Min et al., 2012), and increased uric acid levels (Costa et al., 2009; Shankar et al., 2011b; Steenland et al., 2009b). An increased risk of hypertension, a strong predictor of CKD, was found to be associated with higher PFOA in a the low-exposed U.S. population (i.e., NHANES) (Min et al., 2012). However, this finding was not confirmed in

a highly exposed population (Winquist et al., 2013). While increased rates of diabetes (a strong risk factor for CKD) mortality have been found in PFOA-exposed workers (Leonard et al., 2008), an association between PFOA exposure and diabetes was not found in studies of community populations, including two studies in the highly exposed population considered here (Karnes et al., 2014; Lin et al., 2009; MacNeil et al., 2009).

Here, we assessed, retrospectively and prospectively, the association between modeled serum PFOA and risk of chronic kidney disease, in a large longitudinal cohort in the mid-Ohio valley with a wide range of PFOA exposures, largely above background levels. This mid-Ohio valley community was centered around DuPont's Washington Works manufacturing facility (near Parkersburg, West Virginia), which began releasing PFOA into the environment in 1951 (Frisbee et al., 2009). The C8 Health Project (C8HP), a survey of 69,030 individuals in the six contaminated water districts surrounding the plant, was conducted in 2005-2006. Survey participants provided a blood sample to determine serum PFOA concentrations and to measure other biomarkers (Frisbee et al., 2009).

In a follow-up to the C8HP, adults drawn from this C8HP cohort and workers from the DuPont facility (combined total of 32,254 individuals), were again interviewed in 2008-2011 (Winquist et al., 2013), as part of the work of C8 Science Panel (C8SP). The C8SP was created in 2006 to study chronic disease in relation to PFOA in this highly exposed population in the mid-Ohio valley. A class action lawsuit funded both the C8HP and the C8SP. The mandate of the C8SP was to determine if there was a "probable link" (i.e., 'more probably than not linked') between PFOA and several chronic diseases. The C8SP concluded that PFOA was not probably linked to kidney disease in unpublished work in 2012, available on the C8SP website (http://www.c8sciencepanel.org/prob_link.html). The present paper presents in detail and

extends some of the analyses on which the C8SP probable link determination for kidney disease was based.

Methods

Study cohort & survey data

The Mid-Ohio valley community was exposed to PFOA concentrations far above background levels observed in the general U.S. population. The mean and median serum PFOA concentrations in 2005/2006 among participants in the C8HP were 82.9 μg/L and 28.2 μg/L, respectively (Frisbee et al., 2009; Steenland et al., 2010). To qualify for inclusion into the cohort assembled by the C8SP (N = 32,254 = 28,541 community members + 3,713 workers), a participant must have been exposed for at least one year either to water from one of six PFOA-contaminated public water districts or from a contaminated private well and participated in the C8HP, or the participant must have worked at the DuPont plant (Winquist et al., 2013).

During 2008-2011, follow up surveys were conducted in which participants provided information about health outcomes, demographics, health behaviors (e.g., smoking or exercise) and residential history (including dates at each residence; Winquist et al., 2013). Many subjects completed two follow-up surveys during this time period (Winquist et al., 2013). Each participant was asked about their history of several chronic diseases (including CKD, coronary artery disease, congestive heart failure, high cholesterol, diabetes and hypertension among others), as well as the specific disease type where applicable, and age(s) of diagnosis. Selected self-reported chronic diseases were validated through review of medical records and/or through matching with a disease registry, such as the United States Renal Data System (USRDS) or a cancer registry (Winquist et al., 2013).

Case definition and cohort construction

In the follow up surveys, participants were asked the following question about kidney disease: “Have you ever been told by a doctor or other health professional that you have any kidney disease such as kidney infection, kidney stones, chronic kidney disease or kidney failure (not including bladder infections or incontinence)?” If the participant responded “yes” to this question, they were asked whether they had kidney infection, kidney stones, chronic kidney disease (not including kidney failure), kidney failure (either on dialysis or transplanted) or other kidney disease, with participants being allowed to report multiple conditions. If the participant reported “other kidney disease”, they were asked to specify the type of disease. The participant was also asked their age at first diagnosis for each reported type of kidney disease.

A self-report of either ‘chronic kidney disease,’ or ‘kidney failure’ was considered a self-reported CKD case. If the participant reported ‘other kidney disease,’ their free response entry specifying the condition was examined for a report of a condition indicating CKD. Responses considered to have indicated CKD (primarily those indicating renal insufficiency or kidney failure) are listed in the Appendix Table 3.1. A self-reported CKD case was considered a validated CKD case if it was confirmed either through medical record review or through matching with the USRDS, which is a registry of end-stage renal disease patients. For a match with the USRDS to be considered validation of CKD, the diagnosis in the USRDS had to indicate a non-neoplastic, non-genetic condition. People who had end-stage renal disease identified through matching with the USRDS registry but not through self-report were also considered to have validated CKD. If multiple ages of CKD diagnosis were reported (e.g., age at diagnosis was reported through both the USRDS registry and survey) the earliest age was used as the age of CKD diagnosis. Analyses here are restricted to validated cases of CKD.

Figure 3.1 shows how cases and non-cases were defined. If either the participant self-reported CKD or matched to the USRDS disease registry or had a death certificate diagnosis of CKD, they were defined a case (n = 762). Of those, 35 were excluded because disease diagnosis was obtained only from the death certificate. Of the remaining 727, medical records or a USRDS match were obtained for 80%. Of those whose medical records were available, 75% were confirmed for CKD. Of the 435 confirmed cases, 23 were excluded from the analysis because their CKD diagnosis was made before age 20 (to consider only adult CKD) or because they were born before 1920 (due to uncertain quality of disease diagnosis and reporting among this group). An additional 15 cases were missing either age at diagnosis, covariate data, or exposure data, and were excluded. Of the 397 people with validated cases that were included in the analysis, 187 were self-reported non-diabetic. Of the 397 people with validated cases in the analysis, 212 were diagnosed with CKD after the baseline (i.e., C8HP) interview in 2005/2006 and were included in the prospective analysis, of which 106 were self-reported non-diabetic.

Of the 32,254 available adults in the C8SP cohort, 31,492 did not self-report CKD and did not match with the USRDS registry. Of these, 159 were excluded because they were born before 1920 (as is consistent with prior C8SP work). An additional 1.2% (374) had incomplete covariate information and they were excluded. Some individuals indicated kidney conditions in the absence of a self-report of CKD; these 9.7% (3,116) were excluded to avoid potential case misclassification. The resulting full cohort of 28,240 consisted of 397 cases and 27,843 non-cases. The prospective cohort, which included only people who had not been diagnosed with CKD before 2005/2006 and who were still alive at the time of the C8HP, included 212 cases and 27,740 non-cases.

Modeled PFOA exposure

Yearly modeled PFOA serum concentrations and cumulative exposure (the sum of all modeled serum concentrations up to a given year) have been previously estimated for each individual in the C8SP cohort (Shin et al., 2011a, 2011b). For community participants, whose exposure was primarily through drinking water, historic serum PFOA concentrations were reconstructed by sequential coupling of an environmental fate and transport model, an exposure estimation model, and a pharmacokinetic model for each individual. This sequence of models is detailed elsewhere, but presented briefly here.

First, historic records of emissions rates from the Washington Works Plant were used in an environmental fate and transport model. This model provided estimated longitudinal (1951-2011) PFOA concentrations in air, surface water, groundwater and six municipal water systems. Most exposure to mid-Ohio residents came from drinking water. The modeled predictions showed good correlation (Spearman's correlation ρ=0.87) with measured PFOA concentrations in water samples (Shin et al., 2011a). Air and water PFOA concentrations over time produced by the fate and transport model were used in conjunction with individual residential histories, and maps of public water supply networks to estimate historical exposures for each individual in the community (Shin et al., 2011b). Finally, each individual's historical exposure was input into a single compartment toxicokinetic model, to determine year-specific serum concentrations for each individual. These estimates were found to have satisfactory correlation (Spearman's ρ = 0.67) with serum PFOA levels measured in 2005-06 as part of the C8HP (Shin et al., 2011b).

For those with occupational history at the DuPont facility, job and department-specific occupational exposures were estimated based on approximately 2,000 historical serum PFOA measurements (Woskie et al., 2012). For the years in which they worked, workers could have

had two yearly serum estimates, a residential (community) estimate and an occupational estimate; in that case, the higher of the two estimates was chosen for that year (Winquist et al., 2013). After inclusion of worker exposure estimates, the Spearman's rank correlation coefficient increased to $\rho = 0.71$ (Winquist et al., 2013).

Data analyses

We conducted two analyses, a retrospective analysis and a prospective analysis. Follow-up in the retrospective analysis began at age 20 in any calendar year or 1951, whichever was later, and ended at the time of failure for cases, and at the last survey or at death, whichever was earlier, for non-cases. Because data collection began in 2005/2006 during the follow-up period, this is not a purely retrospective analysis, but hereafter, we refer to it as such in the interest of brevity. In the prospective analysis, the population was restricted to those who were alive and disease-free at the start of follow-up, and follow-up began at the time of the 2005/2006 survey and continued until age of failure for cases, or at last survey or death, whichever was earlier, for non-cases.

Our primary analyses, both retrospective and prospective, used modeled cumulative PFOA exposure (the sum of all modeled serum PFOA estimates up to a given year) as the exposure metric. Cumulative PFOA exposure was modeled both without any lag and with lags of 5, 10 and 20 years. Cumulative PFOA exposure was deemed the ideal exposure metric; the mechanism through which PFOA might cause CKD would likely involve chronic damage to the kidneys as a result of chronic exposure over time rather than acute exposure in a given year. In addition to cumulative serum levels, we also considered modeled yearly serum PFOA as the exposure metric in the retrospective analysis of CKD.

A Cox proportional hazards model, with CKD as the dependent variable, PFOA exposure as the principal independent variable, and age as the time scale, was used for all analyses. PFOA exposure was included in the model as a time-varying variable. Within risk sets formed for each case, the exposure, either cumulative exposure or year-specific serum estimate, was evaluated at the age of case failure, for both the case and non-cases. The Cox proportional hazards models were stratified on single birth year, to account for changing PFOA exposure over time (Winquist et al., 2013) and for increasing CKD risk over time. We modeled exposures, both lagged and not lagged, as quintile categories. Exposure was also modeled as a log-transformed continuous variable as a test for trend. Quintile category cutpoints (Appendix Table 3.2) were determined from modeled PFOA exposure among cases at the time of diagnosis.

Potential confounders identified from the literature were *a priori* included in models, and included gender, time-varying self-reported hypertension diagnosis (Coresh et al., 2007; Haroun et al., 2003; Saydah et al., 2007), time-varying self-reported diabetes diagnosis (Coresh et al., 2007; Saydah et al., 2007), time-varying self-reported high cholesterol diagnosis (Anavekar and Pfeffer, 2004), time-varying current/former/never smoking (Haroun et al., 2003), category of BMI (<18.5, 18.5-25, 25-30, ≥30 kg/m^2) in 2005-06 (Coresh et al., 2007; Saydah et al., 2007), and category of completed education ('less than high school (HS),' 'HS diploma,' 'some undergraduate education,' 'bachelor's degree') which may be understood as a proxy variable for SES (Bello et al., 2008; Saydah et al., 2007). The proportional hazards assumption was assessed and confirmed for all proportional hazards models by assessing the statistical significance of an interaction term between age and exposure (Allison, 2010). Diabetes is a well-known strong risk factor for CKD, and thus diabetics may constitute a group with an alternate disease etiology with

respect to PFOA. Thus, analyses also were also carried out on the non-diabetic subset in both the retrospective and prospective analyses.

Results

Cohort characteristics

Demographic characteristics of the full cohort and of people with validated CKD cases are presented in Table 3.1. As compared to all people with validated CKD, non-diabetic people with validated CKD included a lower proportion of people with hypertension, high cholesterol, and obesity. The median age at which CKD was diagnosed was 61 years among all people with validated CKD and 60 years among all non-diabetic people with validated CKD.

Survival analysis: Cumulative PFOA exposure

In retrospective analyses of the full cohort (Table 3.2), the estimated hazard ratios (HRs) by cumulative PFOA quintile were 1.0 (reference), 1.26, 1.12, 1.12, and 1.24, with no statistical significance found for the test for trend ($p = 0.80$ for log PFOA). Retrospective analysis of the non-diabetic subset also did not show evidence of increasing CKD with increasing cumulative exposure (Table 3.2). In the prospective analysis, the HR estimates by PFOA quintiles were 1.00 (reference), 1.36, 0.94, 1.08, and 1.12, with no significant trend ($p = 0.77$). In prospective analyses of the non-diabetic subset, the estimated HRs by quintiles were 1.00 (reference), 0.93, 1.04, 0.70, and 1.24, with no significant trend ($p = 0.81$).

In the analysis using lagged cumulative PFOA exposure estimates, HR estimates for the higher PFOA exposure quintiles were largely non-significant for both the retrospective (Table 3.3) and prospective analyses (results not shown). Retrospective (Table 3.3) and prospective

analyses (not shown) of lagged exposure also showed no trends. The same was true in analyses of the non-diabetic subsets (results not shown).

In the primary retrospective and prospective analyses of the full cohort, diabetes ($HR_{retrospective}$=3.2, $HR_{prospective.}$=2.4; $p<0.001$) and hypertension ($HR_{retrospective}$=3.6, $HR_{prospective.}$=3.6; $p<0.001$), were highly significant positive predictors of CKD. Hypertension remained a very strong predictor ($p<0.001$) of CKD in retrospective and prospective analyses for the non-diabetic subsets. Gender and education level were not found to be significant predictors of CKD.

Survival analysis: Serum PFOA estimates

In the retrospective analyses of modeled yearly serum PFOA concentrations (Table 3.4), non-significant HR estimates for the quintiles were 1.00 (reference), 1.14, 1.12, 1.35, and 1.02. The test for trend using log-transformed yearly serum PFOA was non-significant ($p = 0.50$). Similarly, in retrospective analyses of the non-diabetic subset, HR estimates did not increase monotonically with increasing exposure quintiles and the trend test was not significant ($p = 0.36$).

Discussion

In both the overall cohort and in the non-diabetic subsets, we found no association between PFOA exposure and CKD in either the retrospective or the prospective analyses. Significant trends of increasing CKD risk with increased exposure to PFOA were not found in any analyses.

Hypertension (Haroun et al., 2003), and diabetes (Saydah et al., 2007) have been shown in previous work to be strongly and significantly associated with increased CKD risk; these findings are replicated here, lending support to the validity of the present analyses.

Many PFOA studies published to date are restricted to general populations with low exposures. Our cohort is unique in its large size and wide range of exposures. The availability of longitudinal data on disease diagnoses, time-varying estimates of internal PFOA dose, and time-varying covariates, is a significant strength of the present analyses. As a consequence of both the longitudinal construction of our analyses and the use of modeled time-varying exposure estimates, our findings are not subject to reverse causation which has likely affected findings in some cross-sectional studies using measured PFOA serum concentrations (e.g., Shankar et al., 2011a).

Another strength of this study is the consideration of CKD morbidity, rather than CKD mortality. As the recording of cause of death is often not standardized, CKD may not be accurately reported as the cause of death or CKD may not be reported at all as a comorbid condition at death, in studies of cause-specific or multiple-cause mortality, respectively. While case validation through medical record review and matching with the USRDS kidney disease registry is also a substantial strength of this study, this validation was limited by a failure to obtain medical records for approximately 20% of self-reported cases and the lack of data on kidney disease severity for most cases.

For most of the cohort (all except workers), participation was dependent on survival until the C8HP survey in 2005-2006. In the context of an analysis that includes time before the C8HP survey, this exclusion is of some concern in examining a disease, such as CKD, that substantially

increases the rate of mortality. CKD is often fatal, and is furthermore associated with chronic diseases that have substantial mortality rates (e.g., congestive heart failure; Sarnak et al., 2003).

If individual susceptibility to CKD as a result of PFOA exposure varies in the population, overall cohort susceptibility may change with time, as those who are most vulnerable may acquire the CKD early in the exposure history. In our cohort, this could result in a downward bias if those who are most susceptible to developing disease as a result of exposure develop disease and die before the time of enrollment (Applebaum et al., 2011). In this scenario, Applebaum et al. demonstrated that for left truncated cohorts in which the cohort is defined cross sectionally (cohort members must have survived to a given point in time) and no person time nor disease before that time is counted, there may be a modest downward bias in the estimated exposure-response coefficient, on the order of 10-15% (2011). However, while entry into our cohort largely required survival to 2005-2006, we included person-time and CKD from time of first exposure, before 2005-2006. Hence our cohort was only partially left truncated (only deceased cases were missing), which is likely to result in less bias.

On the other hand, if all exposed subjects are uniformly susceptible to disease, the survivorship requirement in the present cohort may not bias the results of the survivor cohort analysis. Barry et al. demonstrated that, in the presence of uniform susceptibility to the effects of exposure on disease, the survivorship requirement produces bias in the effect estimate of a fatal disease only when survival after disease differed by exposure status (Barry et al., 2015). When exposure is independent of case fatality, the survivorship requirement only reduces power for fatal outcomes, but causes no bias (Barry et al., 2015).

Because of migration, it was not possible to identify and include all individuals residing in the study area during the entire exposure period from 1951-2011. Eligible individuals who

emigrated from the study area were less likely to be aware of and to participate in the study, although prior residents were eligible and many did participate (Winquist et al., 2013). However, migration patterns are unlikely to be related to exposure, and thus, would not be expected, *a priori*, to cause any bias in our results.

Conclusion

Previous cross-sectional studies of PFOA and CKD have found positive associations between CKD and measured PFOA (Shankar et al., 2011a; Watkins et al., 2013), and one mortality study found an association between PFOA exposure estimated from a job exposure matrix and chronic kidney disease mortality (Steenland and Woskie, 2012). The cross-sectional studies may have suffered from reverse causation (Shankar et al., 2011a; Watkins et al., 2013), while mortality studies are subject to more disease misclassification than morbidity studies for non-fatal diseases. Using longitudinal analyses with estimated time-varying PFOA exposure, we have found little evidence of an association between PFOA and CKD in either retrospective or prospective analysis.

References

Allison, P.D., 2010. Survival Analysis Using the SAS System: A Practical Guide, 2nd ed. SAS Institute, Cary, NC.

Anavekar, N.S., Pfeffer, M.A., 2004. Cardiovascular risk in chronic kidney disease. Kidney Int. 66, S11–S15.

Applebaum, K.M., Elizabeth J. Malloy, Ellen A. Eisen, 2011. Left Truncation, Susceptibility, and Bias in Occupational Cohort Studies. Epidemiology 22, 599–606. doi:10.1097/EDE.0b013e31821d0879

Barry, V., Klein, M., Winquist, A., Darrow, L.A., Steenland, K., 2015. Conditions for bias in survivor cohorts. Environ. Res. 140, 275–281.

Bartell, S.M., Calafat, A.M., Lyu, C., Kato, K., Ryan, P.B., Steenland, K., 2010. Rate of decline in serum PFOA concentrations after granular activated carbon filtration at two public water systems in Ohio and West Virginia. Environ. Health Perspect. 118, 222–8. doi:10.1289/ehp.0901252

Bello, A.K., Peters, J., Rigby, J., Rahman, A. A., El Nahas, M., 2008. Socioeconomic status and chronic kidney disease at presentation to a renal service in the United Kingdom. Clin. J. Am. Soc. Nephrol. 3, 1316–1323. doi:10.2215/CJN.00680208

Brede, E., Wilhelm, M., Göen, T., Müller, J., Rauchfuss, K., Kraft, M., Hölzer, J., 2010. Two-year follow-up biomonitoring pilot study of residents' and controls' PFC plasma levels after

PFOA reduction in public water system in Arnsberg, Germany. Int. J. Hyg. Environ. Health 213, 217–223. doi:http://dx.doi.org/10.1016/j.ijheh.2010.03.007

Calafat, A.M., Wong, L.-Y., Kuklenyik, Z., Reidy, J.A., Needham, L.L., 2007. Polyfluoroalkyl chemicals in the U.S. population: data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. Environ. Health Perspect. 115, 1596–602. doi:10.1289/ehp.10598

Coresh, J., Byrd-Holt, D., Astor, B.C., Briggs, J.P., Eggers, P.W., Lacher, D. A, Hostetter, T.H., 2005. Chronic kidney disease awareness, prevalence, and trends among U.S. adults, 1999 to 2000. J. Am. Soc. Nephrol. 16, 180–8. doi:10.1681/ASN.2004070539

Coresh, J., Selvin, E., Stevens, L. A., Manzi, J., Kusek, J.W., Eggers, P., Van Lente, F., Levey, A.S., 2007. Prevalence of chronic kidney disease in the United States. JAMA 298, 2038–2047. doi:10.1001/jama.298.17.2038

Costa, G., Sartori, S., Consonni, D., 2009. Thirty Years of Medical Surveillance in Perfluorooctanoic Acid Production Workers. J. Occup. Environ. Med. 51.

Cui, L., Zhou, Q., Liao, C., Fu, J., Jiang, G., 2009. Studies on the toxicological effects of PFOA and PFOS on rats using histological observation and chemical analysis. Arch. Environ. Contam. Toxicol. 56, 338–49. doi:10.1007/s00244-008-9194-6

DeWitt, J.C., Shnyra, A., Badr, M.Z., Loveless, S.E., Hoban, D., Frame, S.R., Cunard, R., Anderson, S.E., Meade, B.J., Peden-Adams, M.M., Luebke, R.W., Luster, M.I., 2009. Immunotoxicity of Perfluorooctanoic Acid and Perfluorooctane Sulfonate and the Role of

Peroxisome Proliferator-Activated Receptor Alpha. Crit. Rev. Toxicol. 39, 76–94. doi:10.1080/10408440802209804

EPA, 2012. PFOA Stewardship Program Description [WWW Document]. URL http://www.epa.gov/oppt/pfoa/pubs/stewardship/pfoastewardshipbasics.html

Frisbee, S.J., Brooks, A. P., Maher, A., Flensborg, P., Arnold, S., Fletcher, T., Steenland, K., Shankar, A., Knox, S.S., Pollard, C., Halverson, J. A., Vieira, V.M., Jin, C., Leyden, K.M., Ducatman, A.M., 2009. The C8 health project: design, methods, and participants. Environ. Health Perspect. 117, 1873–82. doi:10.1289/ehp.0800379

Haroun, M.K., Bernard G. Jaar, Sandra C. Hoffman, Comstock, G.W., Michael J. Klag, Coresh, J., 2003. Risk Factors for Chronic Kidney Disease: A Prospective Study of 23,534 Men and Women in Washington County, Maryland. J. Am. Soc. Nephrol. 14, 2934–2941. doi:10.1097/01.ASN.0000095249.99803.85

Jensen, A.A., Leffers, H., 2008. Emerging endocrine disrupters: perfluoroalkylated substances. Int. J. Androl. 31, 161–9. doi:10.1111/j.1365-2605.2008.00870.x

Karnes, C., Winquist, A., Steenland, K., 2014. Incidence of type II diabetes in a cohort with substantial exposure to perfluorooctanoic acid. Environ. Res. 128, 78–83. doi:10.1016/j.envres.2013.11.003

Kato, K., Wong, L.Y., Jia, L.T., Kuklenyik, Z., Calafat, A.M., 2011. Trends in exposure to polyfluoroalkyl chemicals in the U.S. population: 1999-2008. Environ. Sci. Technol. 45, 8037–8045. doi:10.1021/es1043613

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol. Sci. 99, 366–94. doi:10.1093/toxsci/kfm128

Leonard, R.C., Kreckmann, K.H., Sakr, C.J., Symons, J.M., 2008. Retrospective cohort mortality study of workers in a polymer production plant including a reference population of regional workers. Ann. Epidemiol. 18, 15–22. doi:10.1016/j.annepidem.2007.06.011

Lin, C.-Y., Chen, P.-C., Lin, Y.-C., Lin, L.-Y., 2009. Association among serum perfluoroalkyl chemicals, glucose homeostasis, and metabolic syndrome in adolescents and adults. Diabetes Care 32, 702–7. doi:10.2337/dc08-1816

MacNeil, J., Steenland, N.K., Shankar, A., Ducatman, A., 2009. A cross-sectional analysis of type II diabetes in a community with exposure to perfluorooctanoic acid (PFOA). Environ. Res. 109, 997–1003. doi:10.1016/j.envres.2009.08.002

Min, J.-Y., Lee, K.-J., Park, J.-B., Min, K.-B., 2012. Perfluorooctanoic acid exposure is associated with elevated homocysteine and hypertension in US adults. Occup. Environ. Med. 69, 658–62. doi:10.1136/oemed-2011-100288

Olsen, G.W., Burris, J.M., Ehresman, D.J., Froehlich, J.W., Seacat, A.M., Butenhoff, J.L., Zobel, L.R., 2007. Half-life of serum elimination of perfluorooctanesulfonate,perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ. Health Perspect. 115, 1298–305. doi:10.1289/ehp.10009

Sarnak, M.J., Levey, A.S., Schoolwerth, A.C., Coresh, J., Culleton, B., Hamm, L.L., McCullough, P. A., Kasiske, B.L., Kelepouris, E., Klag, M.J., Parfrey, P., Pfeffer, M., Raij, L., Spinosa, D.J., Wilson, P.W., 2003. Kidney Disease as a Risk Factor for Development of Cardiovascular Disease: A Statement From the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Circulation 108, 2154–2169. doi:10.1161/01.CIR.0000095676.90936.80

Saydah, S., Rios-Burrows, M.E.N., Williams, D., Geiss, L., Dorsey, R., 2007. Prevalence of Chronic Kidney Disease and Associated Risk Factors --- United States, 1999--2004. MMWR 56, 161–165.

Shankar, A., Xiao, J., Ducatman, A., 2011a. Perfluoroalkyl chemicals and chronic kidney disease in US adults. Am. J. Epidemiol. 174, 893–900. doi:10.1093/aje/kwr171

Shankar, A., Xiao, J., Ducatman, A., 2011b. Perfluoroalkyl chemicals and elevated serum uric acid in US adults. Clin. Epidemiol. 3, 251–258.

Shin, H.-M., Vieira, V.M., Ryan, P.B., Detwiler, R., Sanders, B., Steenland, K., Bartell, S.M., 2011a. Environmental fate and transport modeling for perfluorooctanoic acid emitted from the Washington Works Facility in West Virginia. Environ. Sci. Technol. 45, 1435–42. doi:10.1021/es102769t

Shin, H.-M., Vieira, V.M., Ryan, P.B., Steenland, K., Bartell, S.M., 2011b. Retrospective exposure estimation and predicted versus observed serum perfluorooctanoic acid

concentrations for participants in the C8 Health Project. Environ. Health Perspect. 119, 1760–5. doi:10.1289/ehp.1103729

Steenland, K., Fletcher, T., Savitz, D. A, 2010. Epidemiologic evidence on the health effects of perfluorooctanoic acid (PFOA). Environ. Health Perspect. 118, 1100–8. doi:10.1289/ehp.0901827

Steenland, K., Tinker, S., Frisbee, S., Ducatman, A., Vaccarino, V., 2009. Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. Am. J. Epidemiol. 170, 1268–78. doi:10.1093/aje/kwp279

Steenland, K., Woskie, S., 2012. Cohort mortality study of workers exposed to perfluorooctanoic acid. Am. J. Epidemiol. 176, 909–17. doi:10.1093/aje/kws171

United States Renal Data System, 2014. 2014 Annual Data Report: Epidemiology of Kidney Disease in the United States, Chapter 1 : CKD in the General Population. Bethesda, MD.

Wang, Z., Cousins, I.T., Scheringer, M., Buck, R.C., Hungerbühler, K., 2014. Global emission inventories for C4 – C14 per fluoroalkyl carboxylic acid (PFCA) homologues from 1951 to 2030, Part I: production and emissions from quantifiable sources. Environ. Int. 70, 62–75. doi:10.1016/j.envint.2014.04.013

Watkins, D.J., Josson, J., Elston, B., Bartell, S.M., Shin, H., Vieira, V.M., Savitz, D.A., Fletcher, T., Wellenius, G.A., 2013. Exposure to Perfluoroalkyl Acids and Markers of Kidney Function among Children and Adolescents Living near a Chemical Plant. Environ. Health Perspect. 121, 625–630.

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, 893–9. doi:10.1289/ehp.1206450

Woskie, S.R., Gore, R., Steenland, K., 2012. Retrospective exposure assessment of perfluorooctanoic acid serum concentrations at a fluoropolymer manufacturing plant. Ann. Occup. Hyg. 56, 1025–37. doi:10.1093/annhyg/mes023

Tables and Figures

Table 3.1. Cohort characteristics.

	Full Cohort (N = 28,240)		All validated cases (N = 397)		Non-diabetic, validated cases (N = 187)	
	Frequency	%	Frequency	%	Frequency	%
GENDER						
Female	14,486	51.3	194	48.9	94	50.3
Male	13,754	48.7	203	51.1	93	49.7
RACE						
White	27,333	96.8	381	956.0	180	96.3
Non-white	907	3.2	16	4.0	7	3.7
HYPERTENSION						
No	16,172	57.3	99	24.9	57	30.5
Yes	12,068	42.7	298	75.1	130	69.5
DIABETES						
No	4,027	14.5	210	52.9	0	0.00
Yes	23,816	85.5	187	47.1	187	100.00
HIGH CHOLESTEROL						
No	19,955	70.7	195	49.1	112	59.89
Yes	8,285	29.3	202	50.9	75	40.11
BMI CATEGORY						
<18.5	7,603	26.9	79	19.9	49	26.2
18.5-25	370	1.3	9	2.3	7	3.74
25-30	10,044	35.6	115	29.0	65	34.76
≥30	10,223	36.2	194	48.9	66	35.29
EDUCATION CATEGORY						
Less than high school	2,438	8.6	72	18.1	27	14.44
High school diploma	11,328	40.1	163	41.1	81	43.32
Some undergraduate	9,229	32.7	116	29.2	52	27.81
Bachelor's degree	5,245	18.6	46	11.6	27	14.44
WORKER						
No	24,869	88.1	353	88.9	161	86.1
Yes	3,371	11.9	44	11.1	26	13.9
SMOKING						
Never smoker	13,791	48.8	165	41.6	86	46.0
Former smoker	8,778	31.1	142	35.8	56	30.0
Current smoker	5,671	20.1	90	22.7	45	24.1
AGE						
Median	52		61		60	
Mean (SD)	51.8 (15.2)		59.5 (14.0)		58.5 (15.1)	
CUMULATIVE PFOA EXPOSURE (ng/mL x yr.) AT FAILURE OR END OF FOLLOW-UP						
Median	0.63		0.55		0.67	
Mean (SD)	3.32 (7.26)		3.32 (6.40)		4.08 (7.86)	

Table 3.2. Estimated hazard ratios (HR) for chronic kidney disease by cumulative PFOA exposure quintile, in prospective and retrospective analyses.

		Full cohort		Non-diabetic population	
	Variable	**HR (95%CI)**	**p**	**HR (95%CI)**	**p**
		397 cases and 27,843 non-cases		187 cases and 23,816 non-cases	
Full cohort	**Cumulative exposure quintile (ref. = 1st quintile) *,****				
	2nd quintile	1.26 (0.90, 1.75)	0.18	0.91 (0.56, 1.49)	0.72
	3rd quintile	1.12 (0.80, 1.55)	0.52	0.90 (0.55, 1.47)	0.67
	4th quintile	1.12 (0.81, 1.56)	0.49	0.87 (0.53, 1.43)	0.59
	5th quintile	1.24 (0.88, 1.75)	0.21	1.23 (0.74, 2.05)	0.43
	Log (Cum. Exp.), trend test		0.80		0.26
		212 cases and 27,740 non-cases		106 cases and 23,736 non-cases	
Prospective cohort	**Cumulative exposure quintile (ref. – 1st quintile) °, †**				
	2nd quintile	1.36 (0.89, 2.09)	0.16	0.93 (0.51, 1.71)	0.82
	3rd quintile	0.94 (0.62, 1.45)	0.79	1.04 (0.57, 1.92)	0.89
	4th quintile	1.08 (0.70, 1.66)	0.74	0.70 (0.38, 1.29)	0.25
	5th quintile	1.12 (0.72, 1.75)	0.60	1.24 (0.67, 2.31)	0.50
	Log (Cum. Exp.), trend test		0.77		0.81

* For the full cohort, the upper cutpoints of the 1st, 2nd, 3rd and 4th quintiles are 0.20, 0.34, 1.07, and 6.16 ng/L x yr, respectively.

** For the non-diabetic subcohort, the upper cutpoints of the 1st, 2nd, 3rd and 4th quintiles are 0.19, 0.36, 1.23, and 7.21 ng/L x yr, respectively.

° For the full cohort, the upper cutpoints of the 1st, 2nd, 3rd and 4th quintiles are 0.25, 0.40, 1.37, and 6.82 ng/L x yr, respectively.

† For the non-diabetic subcohort, the upper cutpoints of the 1st, 2nd, 3rd and 4th quintiles are 0.25, 0.50, 1.28, and 8.70 ng/L x yr, respectively.

Table 3.3. Estimated hazard ratios (HR) for chronic kidney disease in relation to lagged cumulative PFOA exposure quintiles in retrospective analyses (397 cases and 27,843 non-cases).

	5 year lag		10 year lag		20 year lag	
Parameter	**HR (95%CI)**	**p**	**HR (95%CI)**	**p**	**HR (95%CI)**	**p**
Cumulative Exposure quintile (ref = 1st quintile)						
2nd quintile	0.92 (0.65, 1.31)	0.66	0.89 (0.61, 1.29)	0.53	0.71 (0.44, 1.14)	0.16
3rd quintile	1.00 (0.71, 1.41)	0.98	1.10 (0.76, 1.58)	0.62	0.79 (0.50, 1.27)	0.34
4th quintile	0.97 (0.69, 1.37)	0.87	0.97 (0.67, 1.40)	0.88	0.75 (0.47, 1.20)	0.23
5th quintile	1.05 (0.73, 1.49)	0.81	0.97 (0.67, 1.42)	0.88	0.78 (0.49, 1.25)	0.30
Log (Cum. Exp.), trend test		0.81		0.85		0.68

Table 3.4. Hazard ratios for chronic kidney disease in relation to yearly PFOA serum concentration quintiles in retrospective analyses.

	Full cohort*		Non-diabetic subcohort**	
	HR (95%CI)	**p**	**HR (95%CI)**	**p**
ariable	397 cases and 27,843 non-cases		397 cases and 27,843 non-cases	
Serum PFOA, quintile (ref. = 1st quintile)				
2nd quintile	1.14 (0.83, 1.57)	0.43	0.76 (0.48, 1.22)	0.25
3rd quintile	1.12 (0.81, 1.54)	0.49	1.05 (0.65, 1.68)	0.85
4th quintile	1.35 (0.97, 1.86)	0.07	0.79 (0.49, 1.28)	0.34
5th quintile	1.02 (0.74, 1.41)	0.91	1.13 (0.70, 1.82)	0.62
Log (Serum PFOA), trend test		0.50		0.36

* For the full cohort, the upper cutpoints of the 1st, 2nd, 3rd and 4th quintiles 6.18, 12.50, 31.62, 85.68 µg/L, respectively.
** For the non-diabetic subcohort, the upper cutpoints of the 1st, 2nd, 3rd and 4th quintiles 5.21, 13.00, 29.50, 133.7 µg/mL x yr, respectively.

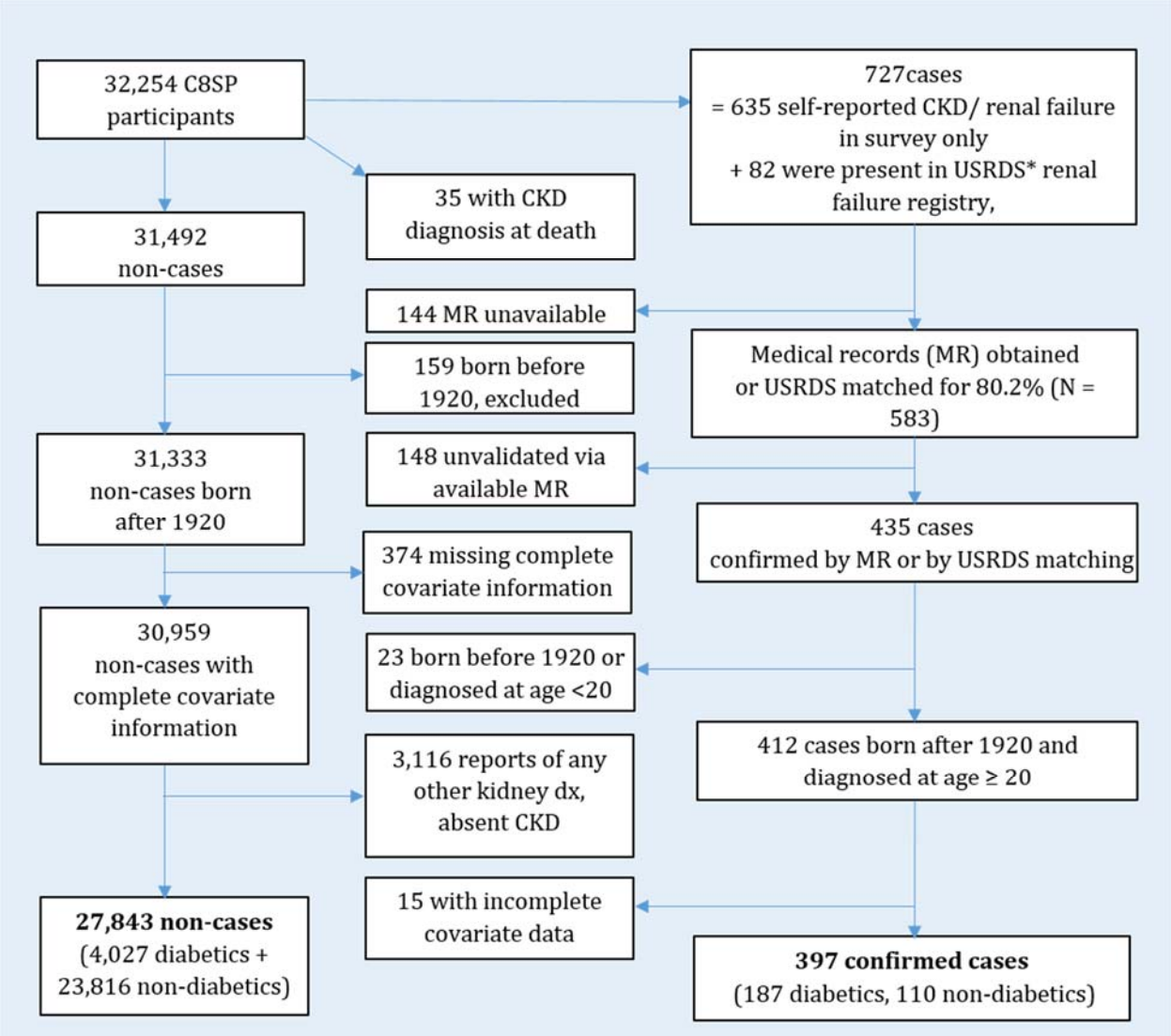


Figure 3.1. Cohort construction. An individual was classified as diabetic if they were diagnosed in any year up to and including the year in which follow-up ended, which was the year of diagnosis for cases and the year of the last survey for non-cases.

Appendix Tables

Appendix Table 3.1. Free response entries for individuals reporting "Other Kidney Disease"

Conditions considered chronic kidney disease	**NOT considered chronic kidney disease**
Atrophy, not otherwise specified (NOS)	Alport Syndrome
Decreased renal function-CKD	Autoimmune kidney disease
Decreased renal function-no level given	Bartters syndrome
Kidney Failure-dialysis or transplant	Blockage/Stenosis NOS
Kidney failure NOS	Decr RF-Stage 1
Kidney failure- no dialysis	Decr RF-less than half lost
Glomerulonephritis	Fabrys disease
Focal segmental glomerulosclerosis	Lupus
Membranous Nephropathy	Polycystic Kidney Disease
Nephritis NOS	Renovascular disease
Nephrotic Syndrome	Wegener granulomatosis
Renal Tubular Acidosis	Amyloidosis
Protein in urine	Enlarged/swollen kidney
Kidney disease NOS	Medullary Sponge Kidney (genetic)

*NOS = not otherwise specified

Appendix Table 3.2. PFOA quintile cutpoints. The 20th, 40th, 60th, & 80th percentiles are the upper cutpoint for the 1st, 2nd, 3rd and 4th quintiles.

	Cutpoints				
	20th percentile	**40th percentile**	**60th percentile**	**80th percentile**	**Maximum value**
CUMULATIVE EXPOSURE (ng/L x yr.) + RETROSPECTIVE ANALYSIS					
All validated cases	0.20	0.34	1.07	6.16	63.18
Non-diabetic population	0.19	0.36	1.23	7.21	63.18
CUMULATIVE EXPOSURE (ng/L x yr.) + PROSPECTIVE ANALYSIS					
All validated cases	0.25	0.40	1.37	6.82	28.39
Non-diabetic population	0.25	0.50	1.28	8.70	28.39
YEARLY SERUM CONC. (µg/L) + RETROSPECTIVEANALYSIS					
All validated cases	6.18	12.50	31.62	85.68	1631.20
Non-diabetic population	5.21	13.00	29.5	133.7	1547

Chapter 4: A study of reverse causation: Examining the associations of perfluorooctanoic acid serum levels with two outcomes

Authors: Radhika Dhingra[a], Andrea Winquist, MD, PhD [a], Lyndsey A. Darrow, PhD [b], Mitch Klein, PhD [a], Kyle Steenland, PhD [a]

Affiliations: [a] Department of Environmental Health, Rollins School of Public Health, Emory University, 1518 Clifton Road, Atlanta, GA 30322, USA. rdhingra@emory.edu; mklein@emory.edu; awinqui@emory.edu; nsteenl@emory.edu

[b] Department of Epidemiology, Rollins School of Public Health, Emory University, 1518 Clifton Road, Atlanta, GA 30322, USA. ldarrow@emory.edu

Abstract

Background: Kidney disease and early menopause have been positively associated with perfluorooctanoic acid (PFOA) in prior cross-sectional studies. Reverse causation, whereby health outcomes increase serum PFOA, may underlie these observed associations.

Objective: We compared measured (i.e., subject to reverse causation) vs. modeled (i.e., unaffected by reverse causation) serum PFOA in association with these outcomes, to examine the possible role of reverse causation in these associations.

Methods: We analyzed PFOA in relation to self-reported menopause among women (N=9,192) aged 30-65, and in relation to kidney function among adults aged 20+ (N=29,499), in a Mid-Ohio Valley cohort, using cross-sectional analyses. Estimated glomerular filtration rate (eGFR, a marker of kidney function) and serum PFOA level were measured in blood samples collected during 2005-2006. Retrospective year-specific serum PFOA estimates were modeled independently of measured PFOA. Cross-sectional associations with measured and modeled PFOA were assessed for eGFR and menopause. We also analyzed measured PFOA in relation to the number of years since menopause.

Results: Decreased kidney function and menopause were both positively associated with measured PFOA (trend tests p=0.013 and 0.0005, respectively) but not modeled serum PFOA (p=0.50 and 0.76, respectively). Measured PFOA levels increased for the first seven years after menopause (trend test, $p<0.0001$), suggesting reverse causation due to decreased PFOA excretion after menopause.

Conclusion: Our results support the conjecture that in prior studies, earlier menopause and impaired kidney function may have been the cause rather than the result of increased measured serum PFOA.

Introduction

Reverse causation, refers to a well-known problem of temporality in which the outcome influences an individual's exposure status or measure (Rothman et al. 2008). Though serum biomarkers of chemical exposure are often seen as objective measures of internal dose, their use in cross-sectional studies introduces a possibility of reverse causation, since measurement of both disease and the biomarker are concurrent. In particular, causal relationships between chemical exposure and disease may be questionable if the outcome of interest can impact an aspect of a chemical's pharmacokinetics, such as through decreasing excretion and consequently increasing accumulation in the body.

Though reverse causation is a well-known concern in cross-sectional studies of biomarkers including those of chemical exposure, demonstration of this problem is not common due to characteristics of biological processes or study design. For many chemical exposures, changes in accumulation at the population level may be difficult to observe, especially as not all exposures are chronic or have a long-residence time in the body. Further, many processes may impact accumulation of a chemical or its metabolites, including sequestration in other tissues, changes in metabolism and changes in excretion rate. In particular, case alterations in excretion rate may be small compared to population variation in the excretion rate; thus, altered excretion may remain unobservable in most cross-sectional sampling schemes unless carefully timed after exposure. Even if altered accumulation is effectively captured at the population level in a cross-sectional study, demonstration of causal direction between exposure and outcome requires an alternative measure of exposure, (i.e., a measure unaffected by possible reverse causation), which can be used instead of the potentially affected biomarker. Here, we show two specific examples of likely altered excretion rates of perfluorooctanoic acid (PFOA) resulting from health

outcomes, menopause and chronic kidney disease (CKD), and discuss the potential impact of reverse causation on epidemiologic analyses of each health outcome in cross-sectional analyses.

PFOA was a widely used surfactant and emulsifier in the manufacture of polymers, such as Teflon and Gore-Tex. It is currently used in large quantities in China, India and Russia (Wang et al. 2014). Though its industrial use in the U.S. has severely declined (EPA 2012), PFOA remains a potential hazard domestically because it resists environmental deterioration and can accumulate in water sources (Post et al. 2012). PFOA is found at low but measurable levels (median in U.S. = 4 μg/L) in the serum of almost all of the U.S. population (Calafat et al. 2007), and is slowly excreted with a reported half-life in humans ranging from 2.3 to 3.8 years (Bartell et al. 2010; Brede et al. 2010; Olsen et al. 2007). PFOA is not metabolized in the human body (Post et al. 2012), and is expected to be primarily stored in the kidney, in the liver and in serum, based on rodent studies (Lau et al. 2007).

Blood loss and urine have been shown to be routes of PFOA elimination. Decreased levels of PFOA have been shown in a population of men experiencing regular blood withdrawals in the course of medical treatment (Lorber et al. 2015). Harada et al. also noted higher serum PFOA concentrations in post-menopausal women than in pre-menopausal women (2005), clearly suggesting a role for menstruation in the observed differences. Menstruation could affect PFOA excretion as a result of blood loss. In addition, the cessation of menstruation is accompanied by hormonal changes that might impact kidney function. In animal models for PFOA (Post et al., 2012), renal clearance of PFOA mediated in rodents by Organic Anion Transporters (OAT) has been shown to be affected by sex hormones (Kudo et al., 2002)..

At menopause, menstruation ceases. Without this mode of recurring excretion in chronically exposed women, the rate of PFOA accumulation in blood may be greater than before

menopause. Both a cross-sectional study of National Health and Nutrition Examination Survey (NHANES) data, which considered people exposed at background PFOA levels, and a cross-sectional study of a large, chronically and highly exposed mid-Ohio Valley cohort, found positive associations between high measured serum concentrations and early menopause (Knox et al. 2011; Taylor et al. 2014). In an exploration of possible reverse causation in the NHANES study Taylor et al. also found a positive linear association (β= 0.07, 95%CI: 0.013-0.13) between the number of years after natural menopause and the log-transformed measured serum PFOA concentration (2014), suggesting that decreased excretion after menstruation led to higher serum levels (i.e., reverse causation).

Analogously, the diminished renal function (calculated as estimated glomerular filtration rate (eGFR)) that characterizes CKD, may result in decreased PFOA excretion and a consequent increased rate of serum PFOA accumulation. A cross-sectional study of the adult NHANES population found a positive association between higher measured serum PFOA and CKD, as determined by the eGFR (Shankar et al. 2011). Watkins et al. (2013) examined this association further in children, aged 0-18 years, and found that increased serum PFOA measured cross-sectionally in 2005/06 was associated with diminished kidney function (i.e., lower eGFR), determined from the same blood sample. This finding was not, however, confirmed when using modeled serum PFOA concentrations, which were estimated independently of measured PFOA. Similarly, an analysis conducted as part of a court-ordered series of studies (termed the C8 Science Panel (C8SP) studies) that were conducted to study chronic disease in the same population considered here, for which results were previously available in an abbreviated form, [http://www.c8sciencepanel.org/pdfs/Probable_Link_C8_Kidney_29Oct2012.pdf], found no link between modeled cumulative PFOA exposure and medically confirmed CKD.

Our study population is the same community in the Mid-Ohio Valley that was studied by Watkins et al. for eGFR (2013), by the C8SP study for CKD, and by Knox et al. for menopause (2011). This community was exposed to PFOA (median measured serum concentration in 2005/06 was 28.2 μg/L) above background exposures observed in the general U.S. population (Frisbee et al. 2009). The community is centered around the DuPont Washington Works manufacturing facilities near Parkersburg, WV, where releases of PFOA began in 1951 (Frisbee et al. 2009).

Here, we investigate the potential for reverse causation in associations of PFOA with menopause and with CKD, in the Mid-Ohio Valley cohort. To explore reverse causation with respect to CKD, we compare measured and modeled serum PFOA cross-sectionally at the time of blood draw (2005/06), as predictors of eGFR; this extends the work of Watkins et al. (2013) in children, to adults in the mid-Ohio Valley population. If there is a true relationship of PFOA exposure and kidney function, then we might expect that both the modeled and measured metrics of PFOA exposures would be associated cross-sectionally with eGFR. To explore reverse causation in relation to menopause, we compare the association of measured serum PFOA and modeled serum PFOA as predictors of menopause in cross-sectional models in 2005/06. Here, the etiologic exposure of interest would be PFOA exposure prior to the occurrence of menopause. In our population of menopausal women, serum PFOA modeled at the time menopause is highly correlated with serum PFOA, modeled in 2005/06 (ρ=0.86), and moderately correlated with measured serum PFOA in 2005/06 (ρ=0.64). In the context of these correlations, if there is a true relationship of PFOA exposure to natural menopause, then we might expect that modeled PFOA would be more strongly associated with reported past menopause in 2005/06

than measured PFOA, when comparing levels between post-menopausal women with age matched pre-menopausal women

To further describe the relationship between PFOA and menopause, we also examined the yearly accumulation of PFOA after menopause, which might be expected to increase due to the loss of excretion through menstrual blood. Post-menopausal women would be expected to have higher PFOA than premenopausal women, suggesting that reverse causation might be responsible for prior findings of an association of measured PFOA and early menopause.

In separate work we have analyzed the relationship between modeled PFOA and both outcomes, (clinical) CKD and menopause, longitudinally (Dhingra, Darrow, et al., *submitted*; Dhingra, Lally, et al., *submitted*). In neither case did we find evidence of an association. In this context, we now consider the possibility of reverse causation in prior cross-sectional analyses of measured PFOA and these outcomes, which showed an association.

Methods

The population studied here was drawn from a cohort of 69,030 individuals, studied cross-sectionally in the C8 Health Project (C8HP). To be included in the C8HP individuals must have been exposed to PFOA-contaminated water from one of six affected public water districts or from a contaminated private well for at least one year (Winquist et al. 2013). The C8HP was conducted in 2005-06 and included collection of a blood sample for measurement of serum PFOA concentrations and other biomarkers, including creatinine (Frisbee et al. 2009). Interviews with a sub-set of the C8HP participants were conducted in 2008-11, as part of the work of the C8SP (Winquist et al. 2013).

The present cohort (N = 30,303) consists of those in the C8HP population who consented to follow-up by the C8SP, were subsequently interviewed, and also had both serum measurements of PFOA from the C8HP and PFOA serum levels modeled for each year based (1951-2011) on residential history (Shin et al. 2011a, 2011b) and/or occupational exposure (Winquist et al. 2013; Woskie et al. 2012). Figure 4.1 shows the construction of the population studied here.

Data: Surveys, PFOA and eGFR

In C8SP surveys (2008-11), participants provided information on demographics, health behaviors (e.g., smoking, exercise), and their history of several chronic diseases. Residential history, including dates at each residence, was also collected for each participant. Women were also asked about their reproductive history, including menstrual and pregnancy history. (Winquist et al. 2013)

Year-specific (1951-2011) estimated PFOA serum concentrations have been previously estimated for each individual in the cohort studied here. The methods for generating these estimates have been described previously (Shin et al. 2011a, 2011b). Briefly, historic serum PFOA concentrations were reconstructed for community participants using (1) an environmental fate and transport model to determine water source concentrations through time, (2) historical reconstruction of each individual's exposure, through both air and water, based on residence history and reported water source, and (3) a single-compartment, toxicokinetic model for each individual. For DuPont workers in the C8SP cohort (11.5%), job and department-specific occupational exposures were estimated based on ~2,000 historical serum PFOA measurements and participant work history (Woskie et al. 2012). All the estimation was done independently of the measured PFOA values in 2005/06. Concurrent (i.e., modeled in the year of the blood

sample) serum PFOA estimates have a Spearman correlation with serum PFOA levels measured in the blood sample (2005/06) of 0.71 (Winquist et al. 2013).

Serum PFOA (μg/L) and serum creatinine concentrations (mg/dL) were measured in the blood collected during the C8HP interview in 2005/06. Creatinine levels were entered into the Modification of Diet and Renal Disease study equations (MDRD) to calculate eGFR in adults. MDRD is a validated equation for determining eGFR in Caucasians and African Americans, aged 18-70 (National Kidney Disease Education Program 2014).

Analyses: eGFR and PFOA in adults

Of the 30,303 adults available from the C8HP for this analysis, 590 were excluded because they were born before 1920 (consistent with all prior C8SP analyses), were missing covariates in regression models, were missing creatinine for eGFR determination, or were younger than 20 in 2005/2006. An additional 72 people with implausibly high eGFR values (>150) were excluded; eGFR was normally distributed after this exclusion. 29,641 subjects remained for analysis.

Using linear regression in cross-sectional analyses, eGFR was regressed in separate models on three PFOA exposure metrics: serum concentrations measured in blood samples (2005/06); estimated serum concentrations in 2005/06 from the serum prediction model; and cumulative exposure in 2005/06 (the sum of all year-specific, modeled serum PFOA concentrations up to a given year). Each exposure variable was categorized into quintiles, while the log-transformed continuous exposure served as a test for trend. In order to clarify the dose-response relationship between measured serum PFOA and eGFR, measured serum PFOA was additionally categorized into deciles. Potential confounders were chosen *a priori*, from the literature and included reported clinically diagnosed hypertension (Anderson et al. 2009; Haroun

et al. 2003), reported clinically diagnosed high cholesterol, smoking status (current/former/never; Haroun et al. 2003), BMI (<18.5, 18.5-25, 25-30, ≥30 kg/m^2), education level ('less than high school (HS),' 'HS diploma,' 'some undergraduate education,' 'bachelor's degree or higher') which was included as a measure of socioeconomic status (Saydah et al. 2007), gender (Haroun et al. 2003) and race (white vs. non-white; Anderson et al. 2009; Haroun et al. 2003; Saydah et al. 2007). Because age was strongly related both to eGFR and exposure, we controlled for birth year as a categorical variable.

Regression coefficients and p-values for models of modeled and measured PFOA were compared in order to assess the potential for reverse causation. Assuming PFOA does not cause changes in kidney function, measured PFOA might still show an inverse association with eGFR if pre-existing decreased renal function impacts excretion and subsequent accumulation of serum PFOA. On the other hand, if PFOA does not cause changes in kidney function, no association of eGFR with either modeled serum PFOA in 2005/06 or with estimated cumulative serum PFOA up to (2005/06) would be expected, under the same assumption.

Analyses: Menopause and PFOA

In our target population of 30,303 adults, 16,870 were women. Of these, we excluded 4,455 women who were less than 30 or greater than 65 years of age in 2005/2006 (similar to the approach in Taylor et al. 2014). We also excluded an additional 3,223 women, who had incomplete menopausal history, reported menopause before age 30, or were missing covariates. Of the remaining 9,192 women, 2,355 and 2,850 had experienced natural menopause and hysterectomy, respectively.

Cross-sectional logistic regression. Following prior cross-sectional analyses by Knox et al. (2011), we conducted logistic regression in which reported natural menopause as of 2005/06 was the outcome and the predictor of interest was either measured serum PFOA in 2005/06, modeled serum PFOA in 2005/06 or modeled cumulative exposure in 2005/06. PFOA exposure was categorized in quintiles, for which cutpoints were determined among menopausal women in the analysis. The log-transformed continuous exposure functioned as a test for trend. To clarify the dose-response relationship between measured serum PFOA and natural menopause, measured serum PFOA was additionally categorized into deciles.

For this analysis, we further restricted the cohort to women aged 40-60 in 2005/06 to ensure that every age had both post-menopausal and pre-menopausal women, and excluded women with hysterectomies, following Knox et al. (2011); 6,342 women remained after these exclusions. Covariates in the model were included *a priori*, based on a review of factors associated with menopause by Gold et al. (2011). Covariates (categories as above unless otherwise specified) included age in 2005/06 (linear term); parity status (parous/nulli-parous); smoking status; education category; and BMI category in 2005/06. We included age as a linear term, after observing a monotonic linear trend of increasing log odds of menopause with increasing age when controlling for age in 2-year age categories. Using a linear age variable in place of the 2-year age categories produced no change in the results, and resulted in a more parsimonious model.

PFOA and years since menopause. In the cohort of 9,192 women, cross-sectional linear regression analyses were conducted with log-transformed measured serum PFOA as the outcome variable and the number of years elapsed since menopause at the time of blood draw in 2005/06 (hereafter, referred to as 'years since menopause') as the main predictor of interest. In primary

analyses, we used either a linear variable or a 2-year categorical variable for years since menopause. We hypothesized, as in the NHANES analyses (Taylor et al. 2014), that we would observe a trend of increasing measured serum PFOA for each year after menopause, if reverse causation was present. The referent group (years since menopause = 0) was composed of pre-menopausal women and those reporting that menopause occurred in the year in which their blood sample was collected (newly menopausal). In order to capture any non-linear relationship between years since menopause and log-transformed measured serum levels, we used a restricted cubic spline with three knots, as described in Harrell et al. (1988) and a linear spline with one knot. Knots were chosen to maximize fit, judged via R^2.

Covariates (categories as above unless otherwise specified) included known predictors of measured PFOA in 2005/06 (Steenland et al. 2009): smoking status; education; BMI (in 2005/06); growing one's own vegetables (yes/no); reported, clinically-diagnosed high cholesterol; reported, clinically diagnosed diabetes; current water district of residence in 2005/06 (indicator variables for six water districts); having previously lived or worked in a contaminated water district (indicator variables for the six water districts); bottled water consumption (yes/no); well water consumption (yes/no); birthyear (2-yr. categorical variable); evidence of having worked at the DuPont plant (yes/no); and month of blood sample collection (categorical variables representing two month intervals). We also included, as a covariate, modeled serum PFOA in 2005/2006 to account for variable levels of external exposure within the different water districts.

Results

eGFR analyses

Cohort characteristics for the analysis of eGFR in relation to PFOA are shown in Table 4.1. Estimated glomerular filtration rate was approximately normally distributed.

There was a negative trend in eGFR across measured serum PFOA quintiles (Table 4.2; β=-0.66, -1.07, -0.94, -1.07 for the 2nd-5th quintiles as compared to the 1st quintile). Log-transformed continuous measured serum PFOA concentration was negatively associated with eGFR (p=0.013). Neither year-specific nor cumulative modeled exposure showed any association with eGFR (Table 4.2). Examination of eGFR and measured serum PFOA, as deciles, gave a dose-response curve that decreased until the 4th decile and remained approximately flat thereafter (Figure 4.2).

Menopause analyses

Table 4.3 shows characteristics of the women in the menopause analyses. We found a modest, significant, increasing trend of reported menopause as of 2005/06 with increasing measured PFOA category (Table 4.4), after adjustment for age, similar to the findings of Knox et al. (2011). When using modeled serum PFOA instead of measured, this trend disappeared, decile categorization of measured serum PFOA as a predictor of menopause showed a dose response curve that increased up to the 4th decile and then, with the exception of a drop at the 5th decile, remained approximately level thereafter (Figure 4.3).

All regression models of log-transformed measured serum PFOA in relation to 'years since menopause', which was included in the model in various forms, showed positive and significant associations between more years since menopause and measured serum PFOA (Figure 4.4). Although all exposure metrics for years since menopause had similar fit as judged by R-square, varying only slightly from 0.679 to 0.681 among metrics, the two-piece linear

spline model appeared to follow the 2-year categorization of years since menopause best. The partial R-square for 'years since menopause' in all of these models was low (range: 0.02, 0.04). The two-piece linear spline showed a steady increase of approximately 4% per year in measured PFOA with each additional year since menopause until year 7, after which point no further increase was observed.

Discussion

Our analyses demonstrate substantial evidence of reverse causation in cross-sectional analyses of PFOA in relation to CKD (as measured by eGFR) and early menopause. Measured serum PFOA concentrations showed a significant negative association with eGFR, but neither modeled exposure metric showed this association. The negative association between eGFR and measured PFOA is consistent with an increase in serum PFOA that might be expected to result from decreased clearance of PFOA in the kidneys.

Similarly, cross-sectional logistic regression models of menopause showed that measured serum PFOA in 2005/06 was positively associated with increased risk of menopause, while modeled year-specific PFOA and modeled cumulative exposure were not. Furthermore, measured serum PFOA increased approximately 4% per year for the first 7 years after menopause, as compared to pre-menopausal and newly menopausal women (i.e., those reporting menopause in the year of blood sample collection), after controlling for other factors that are known to influence serum PFOA levels in this cohort. This result is consistent with the elimination of a route of PFOA excretion at menopause (Harada et al. 2007; Zhang et al. 2013). After 7 post-menopausal years, this increased rate of accumulation appears to stop in our population, perhaps as a result of establishment of a new steady state between intake and excretion, or more error in the reported menopausal age further from the event.

Figure 4.5 shows the possible causal relationships between different metrics of PFOA exposure and the outcomes, menopause and eGFR, in cross-sectional analyses. Figure 4.5a for PFOA and kidney function is fairly straightforward, as described above. If cross-sectional associations between measured serum PFOA and kidney function were causal (and not due to reverse causation), one would expect to replicate the association using modeled serum PFOA. As noted, longitudinal studies of CKD found no association with PFOA exposure in this population (Dhingra et al., *submitted*).

The situation is more complicated with menopause (Figure 4.5b). Neither measured nor modeled PFOA in 2005/06 is the relevant exposure for menopause, which often occurred years earlier. However, among menopausal women, serum PFOA modeled at the time of menopause is correlated with serum PFOA modeled in 2005/06 (Spearman's correlation ρ=0.86) and with measured serum PFOA in 2005/2006 (Spearman's correlation ρ=0.64). Given these associations, if there was true causal relationship from PFOA to menopause absent reverse causation, one might expect to see both measured and modeled PFOA in 2005/06 associated with prior menopause. That the measured has a positive relationship to the early menopause while the modeled does not, can be considered evidence of reverse causation. Again, our findings are consistent with longitudinal studies of natural menopause, which found no association with PFOA exposure in this population (Dhingra et al., *submitted*).

The amount of PFOA excreted is might be expected to be proportional to the body burden, as represented by serum concentrations. Given that reverse causation is operating in the observed relationship between odds of natural menopause and exposure decile, our observation that the strength of association does not increase monotonically beyond the 4th decile (Figure 4.3) may not support this assumption. A number of studies have noted that, at higher doses of PFOA in

various animal species (e.g., rodents, monkeys), the pharmacokinetics of PFOA do not conform to a single compartment first-order model; that is, serum levels do not increase proportionally with dose, except in some studies of low doses (Post et al. 2012). Thus, the amount of PFOA subsequently accumulated as a result of diminished or ceased excretion might not increase linearly at higher exposures.

For several reasons the PFOA-exposed community in the mid-Ohio valley, studied by C8SP and C8HP, offers an opportunity to examine the extent to which reverse causation impacts the observed associations between PFOA exposure and disease. First, historical estimates of serum PFOA levels are available from an extensive, longitudinal exposure reconstruction (Shin et al. 2011a, 2011b). The availability of cotemporaneous modeled and measured exposure metrics in the same cohort allows the comparison of observed associations with the outcome for an exposure metric potentially affected by the outcome of interest (i.e., measured), and one unaffected by the outcome of interest (i.e., modeled). The historical modeled exposure estimates also allow analyses using a cumulative exposure metric, which may be more relevant to chronic health outcomes than current exposure. In our analyses, neither modeled exposure metric showed associations with either health outcome.

Characteristics of PFOA and our cohort's exposure pattern facilitate observation of alterations in excretion. PFOA is not metabolized in the body, is not lipophilic and, is likely stored primarily in the liver, kidney and serum (Lau et al. 2007). Though new exposure had decreased drastically by 2005/06, the Mid-Ohio valley cohort was chronically exposed to elevated (above background) doses of a chemical with a biological half-life of 2-4 years. This exposure scenario and absence of metabolism allow an easier detection of altered excretion.

The expected accumulation of PFOA, when menstruation ends, can be estimated. We can assume a mean blood volume of 4.5 ± 1.1 L (Morgan et al. 2001), approximated from the mean±S.D. observed weight at age 40 (152±37 lbs.) in the Mid-Ohio Valley cohort. Assuming a normal blood loss volume of 35–50 ml (Warrilow et al. 2004) per cycle, menstruation results in a yearly blood loss (13 cycles) of approximately 455-650 ml/year, though an alternative calculation estimates greater blood loss (Verner and Longnecker 2015). Menstruation represents a significant source of blood loss every year (10%-14%), which conforms reasonably to our finding that post-menopausal women had a 4% increase in measured serum PFOA per post-menopausal year.

Other studies have pointed to the role of menstruation in perfluoroalkyl acid blood levels. Two prior pharmacokinetic modeling studies at background perfluoroalkyl acid levels have noted that including a term for menstruation partially explained the higher serum PFOA concentrations observed in men versus women. An analysis of 132 individuals with low PFOA concentrations showed that menstruation could be expected to lead to a 22% difference in PFOA concentrations between males and females, which was close to the observed 24% difference between male and female PFOA concentrations (Lorber et al. 2015). In modeling the pharmacokinetics of perfluorooctanesulfonic acid, another perfluoroalkyl acid with somewhat similar excretion kinetics to PFOA, Wong et al. estimated that menstruation accounts for 30% of the estimated difference in perfluorooctanesulfonic acid elimination between males and females in NHANES (2014). Menstruation may also be responsible, in part, for the reduction in blood serum PFOA observed in post-pubertal girls (Wu et al. 2015). These data strongly suggest that blood loss is a substantial means of PFOA elimination.

Our analyses have several limitations, including possible measurement error, an inability to capture intra-individual variation in eGFR and reporting imprecision for menopausal age. Modeled PFOA serum estimates likely have some exposure measurement error. While we believe this to be a minor Berkson-type error that produces little bias but reduces our ability to detect an association (Armstrong 1998), it may partially explain null results in cross-sectional models using modeled exposure estimates. There is also substantial intra-individual variation in creatinine clearance and thus in eGFR (Levey et al. 2015), which could bias results of analyses between PFOA and eGFR toward the null.

Self-reports of menopausal age are subject to recall bias (Rodstrom et al. 2005) and possible digit preference (Crawford 2002). This may introduce measurement error into the estimate of years since menopause, particularly if the participant is much older than their menopausal age, although differential error by predictor status is not expected. Such error might affect our analyses of measured PFOA and years since menopause, resulting in more error at the highest levels of years since menopause.

Nonetheless, even if elevated measured serum PFOA concentrations are truly a product of reverse causation, those concentrations do reflect the true internal dose at a given moment. Increased internal doses of PFOA due to menopause or diminished GFR may thus represent an increased health risk for a variety of other health endpoints that are associated with PFOA.

Conclusions

Internal measures of dose may not be appropriate for some cross-sectional epidemiological studies, due to reverse causation. If a modeled exposure metric can derived independently of

measured internal exposure which is susceptible to individual toxicokinetics, it may be preferable.

References

Anderson, S., Halter, J. B., Hazzard, W. R., Himmelfarb, J., Horne, F. M., Kaysen, G. A., et al. 2009. Prediction, progression, and outcomes of chronic kidney disease in older adults. J. Am. Soc. Nephrol. 20:1199–209; doi:10.1681/ASN.2008080860.

Armstrong, B. G. 1998. Effect of measurement error on epidemiological studies of environmental and occupational exposures. Occup. Environ. Med. 55: 651–656.

Bartell, S. M., Calafat, A. M., Lyu, C., Kato, K., Ryan, P. B., Steenland K. 2010. Rate of decline in serum PFOA concentrations after granular activated carbon filtration at two public water systems in Ohio and West Virginia. Environ. Health Perspect. 118:222–8; doi:10.1289/ehp.0901252.

Brede, E., Wilhelm, M., Göen, T., Müller, J., Rauchfuss, K., Kraft, M., et al. 2010. Two-year follow-up biomonitoring pilot study of residents' and controls' PFC plasma levels after PFOA reduction in public water system in Arnsberg, Germany. Int. J. Hyg. Environ. Health 213:217–223; doi:http://dx.doi.org/10.1016/j.ijheh.2010.03.007.

Calafat, A. M., Wong, L.-Y., Kuklenyik, Z., Reidy, J. A., Needham, L. L. 2007. Polyfluoroalkyl chemicals in the U.S. population: data from the National Health and Nutrition Examination Survey (NHANES) 2003-2004 and comparisons with NHANES 1999-2000. Environ. Health Perspect. 115:1596–602; doi:10.1289/ehp.10598.

Crawford, S. L. 2002. Assessment of Digit Preference in Self-reported Year at Menopause: Choice of an Appropriate Reference Distribution. Am. J. Epidemiol. 156:676–683; doi:10.1093/aje/kwf059.

Dhingra, R., Darrow, L. A., Klein, M., Winquist, A., Steenland, K. Perfluorooctanoic acid exposure and natural menopause: a longitudinal study in a community cohort.

Dhingra, R., Lally, C., Darrow, L., Klein, M., Winquist, A., Steenland, K. Perfluorooctanoic acid and chronic kidney disease: longitudinal analysis of a Mid-Ohio Valley community.

EPA. 2012. PFOA Stewardship Program Description. Available: http://www.epa.gov/oppt/pfoa/pubs/stewardship/pfoastewardshipbasics.html.

Frisbee, S. J., Brooks, A. P., Maher, A., Flensborg, P., Arnold, S., Fletcher, T., et al. 2009. The C8 health project: design, methods, and participants. Environ. Health Perspect. 117:1873–82; doi:10.1289/ehp.0800379.

Gold, E. B. 2011. The timing of the age at which natural menopause occurs. Obstet. Gynecol. Clin. North Am. 38:425–40; doi:10.1016/j.ogc.2011.05.002.

Harada, K., Inoue, K., Morikawa, A., Yoshinaga, T., Saito, N., Koizumi, A. 2005. Renal clearance of perfluorooctane sulfonate and perfluorooctanoate in humans and their species-specific excretion. Environ. Res. 99:253–261; doi:10.1016/j.envres.2004.12.003.

Harada, K., Koizumi, A., Saito, N., Inoue, K., Yoshinaga, T., Date, C., et al. 2007. Historical and geographical aspects of the increasing perfluorooctanoate and perfluorooctane sulfonate

contamination in human serum in Japan. Chemosphere 66:293–301; doi:10.1016/j.chemosphere.2006.05.010.

Haroun, M. K., Jaar, B. G., Hoffman, S. C., Comstock, G. W., Klag, M. J., Coresh, J. 2003. Risk Factors for Chronic Kidney Disease: A Prospective Study of 23,534 Men and Women in Washington County, Maryland. J. Am. Soc. Nephrol. 14:2934–2941; doi:10.1097/01.ASN.0000095249.99803.85.

Harrell, F. E. J., Lee, K. L., Pollock, B. G. 1988. Regression models in clinical studies: determining relationships between predictors and response. J Natl Cancer Inst 80: 1198–202.

Knox, S. S., Jackson, T., Javins, B., Frisbee, S. J., Shankar, A., Ducatman, A. M. 2011. Implications of early menopause in women exposed to perfluorocarbons. J. Clin. Endocrinol. Metab. 96:1747–53; doi:10.1210/jc.2010-2401.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J. 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. Toxicol. Sci. 99:366–94; doi:10.1093/toxsci/kfm128.

Levey, A. S., Becker, C., Inker, L. A. 2015. Glomerular Filtration Rate and Albuminuria for Detection and Staging of Acute and Chronic Kidney Disease in Adults. JAMA 313:837; doi:10.1001/jama.2015.0602.

Lorber, M., Eaglesham, G. E., Hobson, P., Toms, L.-M.L., Mueller, J. F., Thompson J.S. 2015. The effect of ongoing blood loss on human serum concentrations of perfluorinated acids. Chemosphere 118:170–177; doi:10.1016/j.chemosphere.2014.07.093.

Morgan, G. E. J., Mikhail, M. S., Larson, C. P. J., eds. 2001. Morgan and Mikhail's Clinical Anesthesiology. 3rd ed. McGraw-Hill, New York.

National Kidney Disease Education Program. 2014. Estimating GFR. 6. Available: http://nkdep.nih.gov/lab-evaluation/gfr/estimating.shtml.

Olsen, G. W., Burris, J. M., Ehresman, D. J., Froehlich, J. W., Seacat, A. M., Butenhoff, J. L., et al. 2007. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environ. Health Perspect. 115:1298–305; doi:10.1289/ehp.10009.

Post, G.B., Cohn, P. D., Cooper, K. R. 2012. Perfluorooctanoic acid (PFOA), an emerging drinking water contaminant: a critical review of recent literature. Environ. Res. 116:93–117; doi:10.1016/j.envres.2012.03.007.

Rodstrom, K., Bengtsson, C., Lissner, L., Bjorkelund, C. 2005. Reproducibility of self-reported menopause age at the 24-year follow-up of a population study of women in Göteborg, Sweden. Menopause 12:275–280; doi:10.1097/01.GME.0000135247.11972.B3.

Rothman, K. J., Greenland, S., Lash T. L. 2008. Modern Epidemiology. Third. Lippincott Williams & Wilkins, Philadelphia.

Saydah, S., Rios-Burrows, M. E. N., Williams, D., Geiss, L., Dorsey, R. 2007. Prevalence of Chronic Kidney Disease and Associated Risk Factors --- United States, 1999--2004. MMWR 56: 161–165.

Shankar, A., Xiao, J., Ducatman, A. 2011. Perfluoroalkyl chemicals and chronic kidney disease in US adults. Am. J. Epidemiol. 174:893–900; doi:10.1093/aje/kwr171.

Shin, H.-M., Vieira, V. M., Ryan, P. B., Detwiler, R., Sanders, B., Steenland K., et al. 2011a. Environmental fate and transport modeling for perfluorooctanoic acid emitted from the Washington Works Facility in West Virginia. Environ. Sci. Technol. 45:1435–42; doi:10.1021/es102769t.

Shin, H.-M., Vieira, V. M., Ryan, P. B., Steenland, K., Bartell, S. M. 2011b. Retrospective exposure estimation and predicted versus observed serum perfluorooctanoic acid concentrations for participants in the C8 Health Project. Environ. Health Perspect. 119:1760–5; doi:10.1289/ehp.1103729.

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. 117:1083–8; doi:10.1289/ehp.0800294.

Taylor, K. W., Hoffman, K., Thayer, K. A., Daniels, J. L. 2014. Polyfluoroalkyl Chemicals and Menopause among Women 20-65 Years of Age (NHANES). Environ. Health Perspect. 122:145–50; doi:10.1289/ehp.1306707.

Verner, M.-A., Longnecker, M. P. 2015. Comment on "Enhanced Elimination of Perfluorooctanesulfonic Acid by Menstruating Women: Evidence from Population-Based Pharmacokinetic Modeling." Environ. Sci. Technol. 49:5836–5837; doi:10.1021/acs.est.5b00187.

Wang, Z., Cousins, I. T., Scheringer, M., Buck, R. C., Hungerbühler, K.. 2014. Global emission inventories for C4 – C14 per fluoroalkyl carboxylic acid (PFCA) homologues from 1951 to 2030, Part I: production and emissions from quantifiable sources. Environ. Int. 70:62–75; doi:10.1016/j.envint.2014.04.013.

Warrilow, G., Kirkham, C., Ismail, K. M., Wyatt, K., Dimmock, P., O'Brien S. 2004. Quantification of menstrual blood loss. Obstet. Gynaecol. 6:88–92; doi:10.1016/S0015-0282(02)03638-5.

Watkins, D. J., Josson, J., Elston, B., Bartell, S. M., Shin, H., Vieira, V. M., et al. 2013. Exposure to Perfluoroalkyl Acids and Markers of Kidney Function among Children and Adolescents Living near a Chemical Plant. Environ. Health Perspect. 121: 625–630.

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. 121:893–9; doi:10.1289/ehp.1206450.

Wong, F., MacLeod, M., Mueller, J. F., Cousins, I. T. 2014. Enhanced Elimination of Perfluorooctane Sulfonic Acid by Menstruating Women: Evidence from Population-Based Pharmacokinetic Modeling. Environ. Sci. Technol. 48:8807–8814; doi:dx.doi.org/10.1021/es500796y.

Woskie, S. R., Gore, R., Steenland, K. 2012. Retrospective exposure assessment of perfluorooctanoic acid serum concentrations at a fluoropolymer manufacturing plant. Ann. Occup. Hyg. 56:1025–37; doi:10.1093/annhyg/mes023.

Wu, H., Yoon, M., Verner, M.-A., Xue, J., Luo, M., Andersen, M. E., et al. 2015. Can the observed association between serum perfluoroalkyl substances and delayed menarche be explained on the basis of puberty-related changes in physiology and pharmacokinetics? Environ. Int. 82:61–68; doi:10.1016/j.envint.2015.05.006.

Zhang, Y., Beesoon, S., Zhu, L., Martin, J. W. 2013. Biomonitoring of perfluoroalkyl acids in human urine and estimates of biological half-life. Environ. Sci. Technol. 47:10619–10627; doi:10.1021/es401905e.

Tables and Figures

Table 4.1. Characteristics of people included in the analysis of eGFR (N = 29,641).

GENDER		
	Female	55.6%
	Male	44.4%
HYPERTENSION		
	No	66.3%
	Yes	33.7%
HIGH CHOLESTEROL		
	No	76.5%
	Yes	23.5%
SMOKING		
	Never smoker	47.4%
	Former smoker	27.4%
	Current smoker	25.2%
EDUCATION CATEGORY		
	Less than HS education	8.8%
	HS diploma	38.2%
	Some undergraduate	34.8%
	Bachelor's degree	18.3%
BODY MASS INDEX (BMI)		
	BMI ≤ 18.5	1.4%
	18.5 < BMI ≤ 25	26.6%
	25 < BMI ≤ 30	34.9%
	BMI > 30	37.2%
AGE AT THE TIME OF BLOOD SAMPLE COLLECTION IN 2005/2006		
	Mean (SD)	48.2 (15.2)
	Median	48.0
MEASURED PFOA SERUM CONCENTRATION IN 2005/2006(µg/mL)		
	Mean (SD)	87.0 (281.3)
	Median	26.1
MODELED PFOA SERUM CONCENTRATION IN THE YEAR OF THE BLOOD SAMPLE COLLECTION(µg /mL)		
	Mean (SD)	85.2 (189.7)
	Median	16.5
MODELED CUMULATIVE PFOA EXPOSURE IN THE YEAR OF THE BLOOD SAMPLE COLLECTION (ng/mL yrs)		
	Mean (SD)	2801.6 (6346)
	Median	481.9
ESTIMATED GLOMERULAR FILTRATION RATE (mL/min/1.73m^2)		
	Mean (SD)	77.1 (16.8)
	Median	76.3

Table 4.2. Cross-sectional regression of eGFR on modeled or measured PFOA exposure. (N = 29,641).

PFOA Exposure variable	Parameter Estimate	S.E.	P
Measured serum concentration in 2005/06, quintiles (ref. = 1st quintile)*			
2nd	-0.66	0.268	0.013
3rd	-1.07	0.269	<.0001
4th	-0.94	0.271	0.0005
5th	-1.07	0.274	<.0001
Measured serum concentration, log-transformed	-0.17	0.07	0.013
Modeled serum concentration in 2005/2006, quintiles (ref. = 1st quintile)**			
2nd	-0.06	0.267	0.83
3rd	0.34	0.268	0.20
4th	0.18	0.269	0.49
5th	0.21	0.270	0.44
Modeled serum exposure in 2005/2006, log-transformed	0.04	0.058	0.50
Modeled cumulative exposure as of 2005/2006, quintiles (ref. = 1st quintile) °			
2nd	0.07	0.269	0.80
3rd	-0.03	0.269	0.91
4th	-0.17	0.270	0.52
5th	-0.14	0.274	0.60
Modeled cumulative exposure, log-transformed	-0.02	0.06	0.66

* For measured serum concentration, the upper cutpoints of the 1st, 2nd, 3rd and 4th quintiles are 11.1, 19.4, 36.3, & 88.0 µg/mL, respectively.
** For modeled serum concentration in 2005/06, the upper cutpoints of the 1st, 2nd, 3rd and 4th quintiles are 5.8, 11.4, 26.8, & 82.4 µg/mL, respectively.
° For modeled cumulative exposure, the upper cutpoints of the 1st, 2nd, 3rd and 4th quintiles are 190.4, 310.8, 799.1, & 4,061.1 µg/mL x yr, respectively.

Table 4.3. Characteristics of women included in the menopause analyses.

	Years since menopause (N =9,192) %	Logistic analysis (N = 6,342) %
EDUCATION CATEGORY		
Less than high school education	6.4	5.4
High school diploma	35.9	33.8
Some undergraduate	38.9	38.7
Bachelor's degree	18.9	22.1
QUALIFYING WATER SOURCE		
City of Belpre, OH	13.4	13.5
Little Hocking Water Association	19.8	20.4
Lubeck Public Service District	22.0	22.1
Mason County	19.2	18.6
Tested Private Well	0.1	0.1
Tuppers Plains	19.4	19.4
Village of Pomeroy	6.1	5.9
PAROUS/NULLI-PAROUS		
Nulli-parous	10.0	11.2
Parous	90.0	88.8
DIABETES		
No	89.2	91.0
Yes	10.8	9.0
HIGH CHOLESTEROL		
No	84.9	82.7
Yes	15.1	17.3
BMI CATEGORY		
BMI<18.5	1.2	1.4
18.5≤BMI<25	31.4	33.1
25≤BMI<30	29.3	28.7
BMI≥30	39.1	36.8
ALCOHOL USE		
Never	72.0	70.1
Current alcohol use	19.1	20.9
Former alcohol use	8.9	9.0
SMOKING		
Never Smoker	54.6	55.0
Current Smoker	24.3	24.5
Former smoker	21.1	20.6
WORKER AT DUPONT		
No	97.6	97.7
Yes	2.4	2.3
AGE CATEGORY IN 2005/2006		
30 to 34	11.9	16.4
35 to 39	13.8	17.2
40 to 44	14.5	15.8
45 to 49	15.2	13.8
50 to 54	15.9	14.2
55 to 59	14.9	12.2
60 to 65	13.85	10.4
CURRENT WATER SOURCE		
City of Belpre, OH	7.5	7.1
Tuppers Plains	13.6	13.5

Little Hocking Water Association	13.0	13.0
Lubeck Public Service District	11.0	10.8
Mason County	15.4	14.8
Village of Pomeroy	1.9	1.6
Study Area without a qualifying water source	33.4	34.7
Well	4.3	4.5
CONSUMED ALCOHOL WITHIN THE LAST 3 DAYS		
No	88.6	87.8
Yes	11.4	12.2
VEGETARIAN		
No	99.0	99.1
Yes	1.0	0.9
GROWS THEIR OWN VEGETABLES		
No	73.4	73.1
Yes	26.7	26.9
DRINKS BOTTLED WATER		
No	94.3	93.5
Yes	5.7	6.5
MONTH OF BLOOD DRAW DURING SURVEY PERIOD		
First 2 months	11.8	10.9
Second 2 months	14.8	14.1
Third 2 months	26.4	26.8
Fourth 2 months	25.7	25.7
Fifth 2 months	13.1	13.6
Sixth 2 months	8.2	8.8
MENOPAUSAL STATUS IN 2005/2006		
Still Menstruating	43.4	62.9
Natural Menopause	25.6	37.1
Hysterectomy	31.0	-
MODELED SERUM PFOA CONCENTRATION (µg/mL) IN 2005/2006		
Mean (Std. Dev.)	84.8 (179.7)	81.8 (175.0)
Median	16.7	16.3
MEASURED SERUM PFOA CONCENTRATION (µg/mL)		
Mean (Std. Dev.)	74.6 (215.6)	69.2 (195.6)
Median	23.0	21.4
MODELED CUMULATIVE EXPOSURE (µg/mL yrs) IN 2005/2006		
Mean (Std. Dev.)	2,256.1 (3,999.6)	2,216.4 (3,999.6)
Median	471.8	456.8

Table 4.4. Odds ratios (OR) and 95% confidence intervals (CI) for logistic regression of PFOA and Menopause.

		OR (95%CI)	p
Measured serum, quintiles (ref. = 1st) *			
	2nd	1.68 (1.21, 2.35)	0.002
	3rd	1.45 (1.04, 2.02)	0.03
	4th	1.39 (1.00, 1.93)	0.05
	5th	1.58 (1.14, 2.19)	0.006
Measured serum, log-transformed		1.19 (1.08, 1.31)	1.09 (1.002, 1.18)
Modeled serum in 2005/2006, quintiles (ref. = 1st) **			
	2nd	0.98 (0.70, 1.37)	0.90
	3rd	1.05 (0.75, 1.45)	0.78
	4th	0.78 (0.56, 1.08)	0.14
	5th	0.92 (0.65, 1.30)	0.62
Modeled serum, log-transformed		0.98 (0.70, 1.37)	0.90
Modeled cum. Exposure in 2005/2006, quintiles (ref. = 1st) °			
	2nd	0.88 (0.63, 1.23)	0.46
	3rd	0.89 (0.64, 1.25)	0.50
	4th	0.75 (0.53, 1.05)	0.09
	5th	0.89 (0.64, 1.24)	0.50
Modeled cum. exposure, log-transformed		0.98 (0.91, 1.05)	0.48

* For measured serum concentration, the upper cutpoints of the 1st, 2nd, 3rd and 4th quintiles are 9.7, 17.2, 31.9, & 78.8 µg/mL, respectively.

** For modeled serum concentration in 2005/06, the upper cutpoints of the 1st, 2nd, 3rd and 4th quintiles are 6.1, 11.8, 26.8, & 78.0 µg/mL, respectively.

° For modeled cumulative exposure, the upper cutpoints of the 1st, 2nd, 3rd and 4th quintiles are 198.0, 315.7, 751.9, & 3,347.2 µg/mL x yr, respectively.

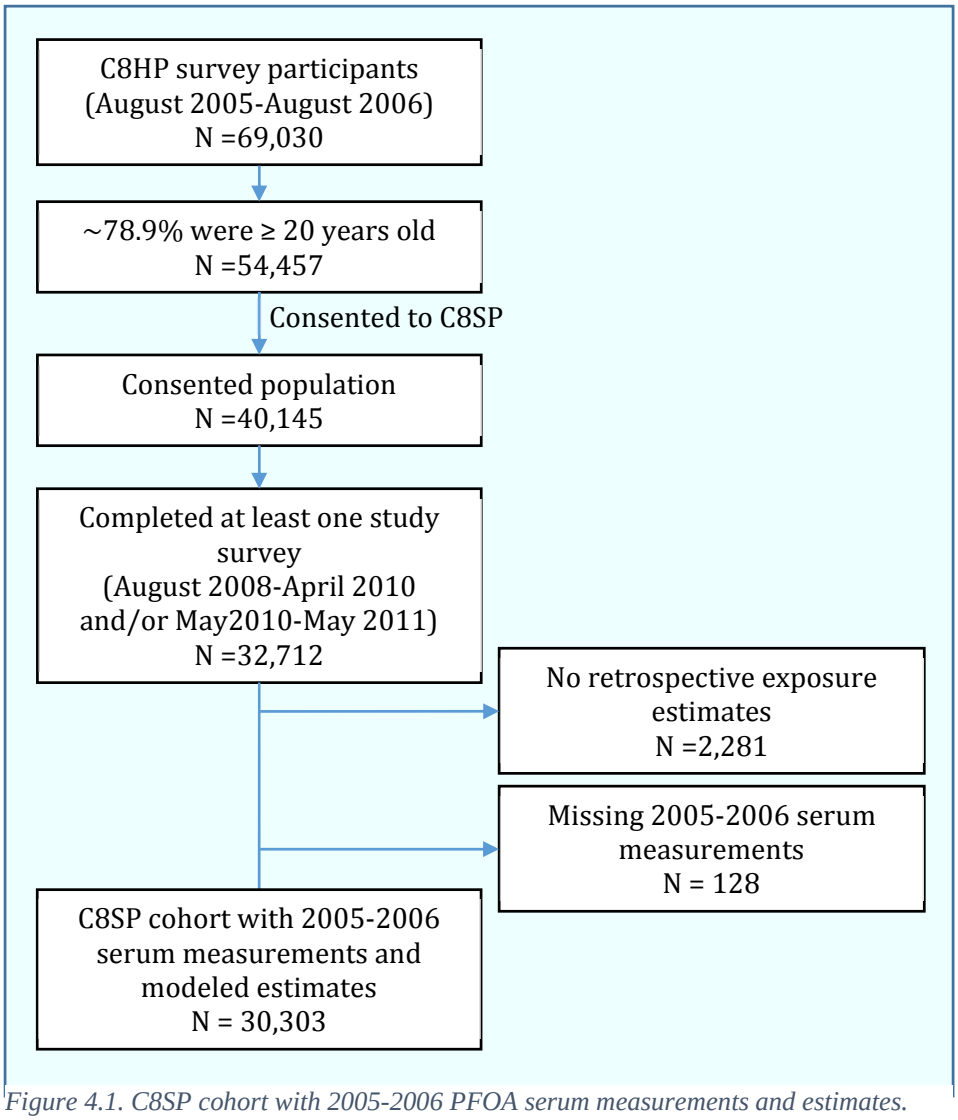


Figure 4.1. C8SP cohort with 2005-2006 PFOA serum measurements and estimates.

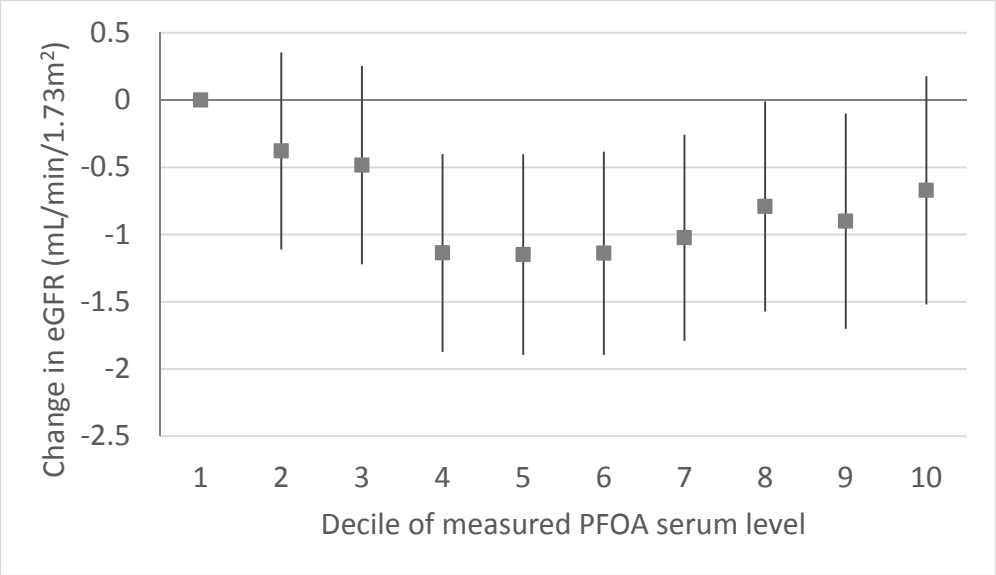


Figure 4.2. Dose response curve showing the change in eGFR with increasing decile of measured serum PFOA level.

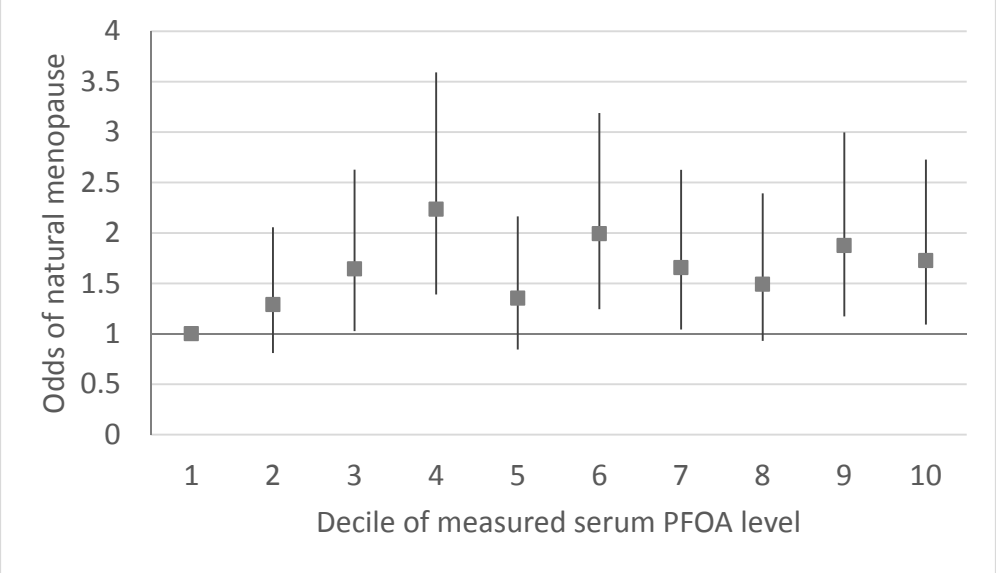


Figure 4.3. Dose response curve showing the odds of natural menopause with increasing decile of measured serum PFOA level.

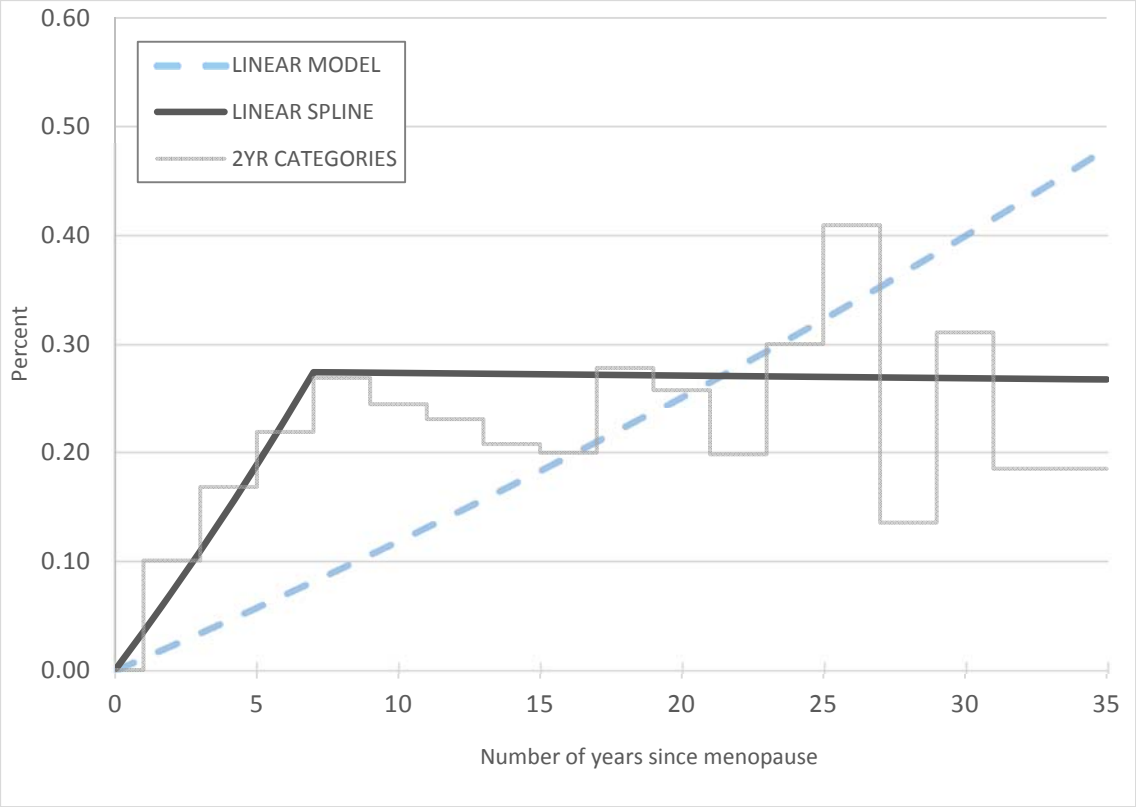


Figure 4.4. Percent increase in measured serum PFOA level as a function of 'Years since Menopause.

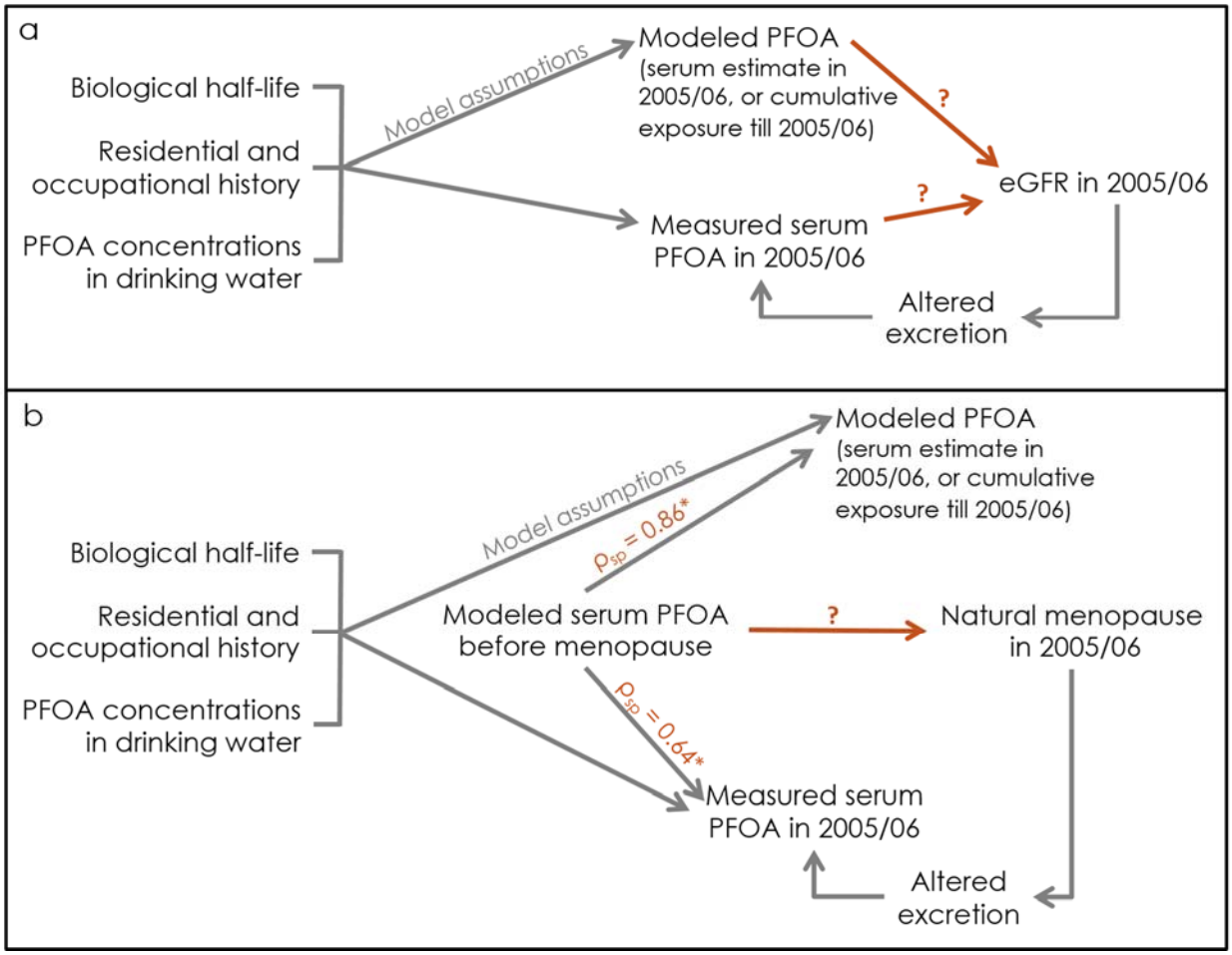


Figure 4.5. Proposed causal diagram for associations of exposure metrics with (a) eGFR and (b) natural Menopause. Correlations in (b) are calculated among cases only.*

Chapter 5: Summary and Conclusion

In the present work, neither longitudinal analysis produced evidence of an association between perfluorooctanoic acid (PFOA) and chronic kidney disease (CKD) or of an association between PFOA and menopause. The absence of a longitudinal association between menopause and PFOA in the present work differs from prior, positive, cross-sectional studies of menopause and PFOA (Knox et al., 2011; Taylor et al., 2014). Similarly, results of the present longitudinal analysis of PFOA and CKD diverge from the prior, significant cross-sectional association found between kidney function and PFOA (e.g., Shankar et al., 2011b). The authors of these three cross-sectional papers do acknowledge the possibility of reverse causation, and our longitudinal findings lend support to this possibility.

The third paper, herein, provided additional support to the hypothesis that reverse causation may have led to the observed cross-sectional associations of PFOA with menopause and PFOA with CKD. This conclusion was based on cross-sectional analyses of each outcome, with comparison of the associations observed using two metrics of PFOA exposure, one potentially susceptible to reverse causation (serum PFOA measured contemporaneously with outcome data) and one that is not (modeled PFOA). The exposure metric that is susceptible to reverse causation, measured serum FPOA, was significantly associated with both menopause and with CKD (replicating earlier findings), while the metric that is invulnerable to reverse causation, environmentally modeled serum PFOA, was not significantly associated with either outcome. This dissonance further supports the hypothesis that reverse causation impacted results from prior studies, in which measured serum PFOA was used in a cross-sectional study design.

Should reverse causation be operative, both menopause and CKD would be expected to cause decreased excretion and consequently increase accumulation of PFOA in the blood. Investigation of trends in the association between measured serum PFOA and the number of years since menopause and the pattern of changes in eGFR across deciles of measured PFOA levels indicates that the relationship between PFOA excretion changes and PFOA accumulation may not be linear.

Our results show that women who are between 1 and 7 years post menopause have 4% higher serum PFOA levels for each year they are post-menopausal. Thus, at 7 years, their serum levels are approximately 28% higher than pre-menopausal women. Women who are at least 7 years post-menopausal maintain serum PFOA levels that are approximately 28% higher than pre-menopausal women. These results suggest that the increased rate of serum PFOA accumulation that women experience after menopause ends at approximately 7 years.

Initially, decreased eGFR was associated with progressively increased measured serum PFOA level up to a decrease in eGFR of approximately 1 mL/min/1.73m^2. After that point, there was no further increase in PFOA levels with decreasing eGFR. These results suggest that, while decreased eGFR increases serum PFOA at lower levels of exposure, the effect of decreased eGFR on serum PFOA reaches a threshold.

The results of these three papers clearly demonstrate the need for longitudinal study design, as compared to cross-sectional. However, the two longitudinal studies of PFOA also have weaknesses that may produce bias or may impact our power to detect an association. One concern is the survivorship requirement for inclusion into the present cohort. Individuals were required to be alive at the time of recruitment (2005/2006),

which was well after the exposure began for most people. Though many people moved into the area after 1951, this survivorship requirement is unlikely to have biased the results of our analysis of menopause, an outcome which is not fatal. In our analysis of chronic kidney disease, which often increases likelihood of death, the survivorship requirement could have produced a downward bias under certain circumstances (Applebaum et al. 2011; a true positive relationship, less than 100% susceptibility), but we believe such bias would have been modest.

Migration into and out of the research area prevented identification of all individuals eligible for the cohort. As migration patterns are unlikely to be related to exposure levels or to either outcome (menopause or CKD), it is unlikely that migration patterns caused any bias in our results. Another limitation in our analysis of menopause is a digit-preference in reporting menopause that could potentially result in bias due to erroneous reporting of the year of menopause. Finally, measurement error in modeled estimates of exposure may have biased results of analyses using those estimates; the direction of this bias is likely towards the null and exposure measurement error may be an alternative explanation for our results indicating no association.

The present cohort provides significant advantages in the study of the health effects of PFOA. The large cohort size and wide range of exposures are not found in any other cohort exposed to PFOA (Winquist et al., 2013). Also, the time-varying reconstruction of each individual's exposure to PFOA is remarkable and consistent with individual serum PFOA levels measured in 2005/2006 (Shin et al., 2011a, 2011b). Furthermore, the longitudinal data on health outcomes, and their validation through medical record review and registry matching provides a particularly robust dataset.

Future work might include toxicological research as well as epidemiologic research. Toxicological work might focus on determining the altered rate of PFOA accumulation that occurs when a mode of excretion is lost (e.g., menopause), reduced (e.g., CKD) or gained (e.g., menarche, childbirth or lactation) under various levels of PFOA exposure. With respect to epidemiologic work, it is possible that outcomes that alter excretion rates, such as menopause and CKD, could define sub-populations, vulnerable to higher levels of PFOA, and thus, also more vulnerable to other PFOA – related outcomes. Further study of such vulnerable sub-populations might require either repeated samples of serum PFOA that precede the event of interest or serum PFOA estimates that account for the various potential routes of PFOA excretion that include renal excretion and blood loss.

References

Applebaum, K.M., Elizabeth J. Malloy, Ellen A. Eisen, 2011. Left Truncation, Susceptibility, and Bias in Occupational Cohort Studies. Epidemiology 22, 599–606. doi:10.1097/EDE.0b013e31821d0879

Knox, S.S., Jackson, T., Javins, B., Frisbee, S.J., Shankar, A., Ducatman, A.M., 2011. Implications of early menopause in women exposed to perfluorocarbons. J. Clin. Endocrinol. Metab. 96, 1747–53. doi:10.1210/jc.2010-2401

Shankar A, Xiao J, Ducatman A. 2011. Perfluoroalkyl chemicals and chronic kidney disease in US adults. Am. J. Epidemiol. 174:893–900; doi:10.1093/aje/kwr171.

Shin, H.-M., Vieira, V.M., Ryan, P.B., Detwiler, R., Sanders, B., Steenland, K., Bartell, S.M., 2011a. Environmental fate and transport modeling for perfluorooctanoic acid emitted from the Washington Works Facility in West Virginia. Environ. Sci. Technol. 45, 1435–42. doi:10.1021/es102769t

Shin, H.-M., Vieira, V.M., Ryan, P.B., Steenland, K., Bartell, S.M., 2011b. Retrospective exposure estimation and predicted versus observed serum perfluorooctanoic acid concentrations for participants in the C8 Health Project. Environ. Health Perspect. 119, 1760–5. doi:10.1289/ehp.1103729

Taylor, K.W., Hoffman, K., Thayer, K. A., Daniels, J.L., 2014. Polyfluoroalkyl Chemicals and Menopause among Women 20-65 Years of Age (NHANES). Environ. Health Perspect. 122, 145–50. doi:10.1289/ehp.1306707

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, 893–9. doi:10.1289/ehp.1206450