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Understanding the Health Impacts of Per- and Polyfluoroalkyl Substances (PFAS) and Air Pollution on Susceptible Populations in the US

DISSERTATION

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for the degree of

DOCTOR OF PHILOSOPHY

in Public Health

by

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DEDICATION

To

my parents and Chengyi

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ABSTRACT OF THE DISSERTATION

Understanding the Health Impacts of Per- and Polyfluoroalkyl Substances (PFAS) and Air Pollution on Susceptible Populations in the US

by

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Doctor of Philosophy in Public Health

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PFAS and air pollution are two groups of modifiable risk factors in the environment. According to the National Health and Nutrition Examination Survey, PFAS are ubiquitous in the serum of the general US population. Contaminated seafood and drinking water, packaged fast food, stain-resistant or water-repellant consumer products, dust, and air are the major exposure routes to humans. In addition, ambient air pollution remains the greatest environmental risk factor and is associated with 100,000-200,000 deaths annually in the US, although the air quality in the US has dramatically improved over the past few decades compared to its historical levels and other countries in the world. Infants, pregnant women, and elderly people are three typical populations that are especially susceptible to these environmental pollutants.

In Chapter 2, we examined the associations between serum PFAS concentrations and type of residential flooring among the general US population aged 12 years and older using

the 2005-2006 NHANES survey data. We concluded that low pile carpeting was associated with increased serum concentrations of PFHxS and MeFOSAA in the general US population.

In Chapters 3 and 4, we conducted two nationwide studies investigating the impacts of PFAS detected in drinking water on birthweight and HDP status, respectively. We used county-level aggregated PFAS data from EPA UCMR3 and county-level multiple-stratified birth/pregnancy data from CDC WONDER. We concluded that the PFAS mixture found in drinking water may contribute to low birthweight and hypertensive disorder of pregnancy in the US.

In Chapters 5 and 6, we further conducted two subgroup analyses using data from the C8 and IHS projects, respectively, focusing on two population subgroups with significantly higher environmental exposure to PFOA and O_3 in the US due to local industries. Our studies suggest PFOA is moderately associated with preeclampsia in the C8 project and O_3 is associated with a higher risk of dementia in American Indians.

Future efforts to reduce exposure to PFAS and air pollution might help lower risks of low birthweight, hypertensive disorders of pregnancy/preeclampsia, and dementia in the US.

CHAPTER I: Introduction

Due to fast urbanization and industrialization, human activities have adversely impacted the environment by polluting the air we breathe, the water and food we consume, the products we use, and the soil where plants grow (Manisalidis et al., 2020). Populations that are vulnerable to environmental pollutants include children, pregnant women, elderly people, and people with underlying conditions such as diabetes, heart diseases, and lung diseases (Hackley et al., 2007; Manisalidis et al., 2020). In this dissertation, I discussed exposure to two groups of modifiable environmental risk factors: PFAS and air pollution, and associated effects on populations that are susceptible and sensitive to these environmental pollutants. I covered three groups of susceptible populations in this dissertation: infants, pregnant women, and elderly people.

1.1 Per- and Polyfluoroalkyl Substances (PFAS) and Associated Health Effects

PFAS are a large group of human-made chemicals that consist of a fully fluorinated (perfluoro-) carbon chain (alkane). They were produced via the electrochemical fluorination (ECF) method by the 3M Company since 1949 and the telomerization method developed by the DuPont Company since the 1970s. Due to the strong single bond between carbon (C) and fluorine (F), which requires very high energy to dissociate, PFAS have unique physiochemical characteristics such as stability in extreme temperatures and water resistance (Faithfull and Weers, 1998). Strong carbon-fluorine bonds also make PFAS resistant to hydrolysis, microbial degradation, and metabolism by vertebrates (ATSDR, 2018). Therefore, they have been used in a variety of applications since the 1950s, including

stain repellents, lubricants, paints, textiles, carpeting, food packaging, nonstick cookware, and firefighting foams (Kissa, 2001). Long-chain PFAS can persist indefinitely in the environment, and bioaccumulate in humans and other organisms, they are also called "forever chemicals" (Giesy and Kannan, 2001; Buck et al., 2011; ATSDR, 2018).

Contaminated seafood and drinking water, packaged fast food, consumer products (non-stick cookware, stain-resistant carpeting, and water-repellant clothing), dust, and air are the major exposure routes to humans (Egeghy, 2011; Jian et al., 2017; ATSDR, 2020; Zhu et al., 2021). Special excretion pathways for females include menstrual blood loss (Wu et al., 2015), maternal transfer to offspring through pregnancy (Beesoon et al., 2011), and breastfeeding (Fromme et al., 2010).

According to the National Health and Nutrition Examination Survey (NHANES), PFAS are ubiquitous in the serum of the general US population (Calafat et al., 2007; Kato et al., 2011). In 2015-2016, the geometric means of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) in the serum samples of the general US population were 1.56 ng/ml, 4.72 ng/ml, 0.58 ng/ml, and 1.18 ng/ml, respectively (CDC, 2019). The average half-lives for serum or plasma elimination of PFAS in humans were reported as 2-4 years for PFOA (Bartell et al., 2010; Olsen et al., 2007; Worley et al., 2017), 3-27 years for PFOS, 5-35 years for PFHxS (Olsen et al., 2007; Worley et al., 2017; Li et al., 2018; Zhang et al., 2013), and 2-4 years for PFNA (Zhang et al., 2013) in previous studies.

Despite the known use of PFAS in stain-resistant carpet treatments, the effects of various types of carpeting on serum PFAS concentrations have been less studied. In Chapter 2 of this dissertation, I investigated the associations between serum PFAS concentrations

and the type of residential flooring among the general US population aged 12 years and older using the 2005-2006 NHANES. We found low pile carpet is associated with increased serum PFHxS and MeFOSAA concentrations, while no clear association has been observed between the other types of carpeting and serum PFAS concentrations. demonstrating that various types of carpeting may present different PFAS exposure for humans, and stain-resistance carpet treatment could explain accountable human PFAS exposure.

Among nearly 5,000 types of PFAS, PFOA and PFOS are the two most extensively produced and studied chemicals, both of which have attracted extensive attention from the global scientific and regulatory community (US EPA, 2009). In laboratory-based animal studies, PFOA and PFOS have shown the potential for developmental toxicity and reproductive effects (Negri et al., 2017), but the health effects have been inconclusive in humans (Li et al., 2017; Shi et al., 2017; Cao et al., 2018; Steenland et al., 2018; Steenland et al., 2020). In Chapters 3-5, I investigated the potential effects of various PFAS on infant and maternal health.

1.1.1 PFAS and Birthweight

Birthweight is an important marker of infant health. It is estimated that more than 80% of neonatal deaths occur in infants of small size and/or low birthweight, i.e., weight at birth of less than 2,500 g or 5.5 lbs (Lawn et al., 2014; UNICEF-WHO, 2019). Globally, low birthweight accounts for 15-20% of all births, representing over 20 million births per year; and more than half of the low birthweight cases are from Asia (WHO, 2014; Blencowe et al., 2019; UNICEF-WHO, 2019). As one of the leading risk factors for global health, low birthweight accounted for 10.6% (95% uncertainty interval: 9.9%, 11.4%) and 6.3% (5.5%,

7.3%) of attributable Disability-adjusted Life Years (DALYs) in 1990 and 2019, respectively (GBD, 2019). In children less than 10 years old, low birthweight is the top risk factor, accounting for 28.9% (95% CI: 27.3%, 30.4%) of the total DALYs in 2019 (GBD, 2019). Despite a substantial decrease of 41.4% in the number of DALYs during the last three decades, low birthweight continues to be a significant public health issue worldwide and has been linked to a wide range of short- and long-term consequences affecting human capital (Lawn et al., 2014).

Low birthweight is not only the most important determinant of prenatal mortality and morbidity, but is also associated with physical growth, poor cognitive development, stunting and lower IQ in childhood, and obesity, diabetes, and cardiovascular disease later in life (Peng, 2005; Risnes et al., 2011; Lawn et al., 2014; Larroque et al., 2001). Although the negative effects of low birthweight on children's cognitive development may be prevented through a series of experimental interventions during the neonatal period (Achenbach et al., 1993), a large number of funds and human capital could be saved had those infants been born with normal birthweights. According to the Lancet Series on Neonatal Survival, there will be 15.1 million preterm birth and 30 million small for gestational age (including 10.4 million low birthweight) infants, and subsequently 3.7 million stunted children, if no actions are taken to improve birth outcomes by 2035 (Lawn et al., 2014).

Some epidemiological studies suggest that exposure to PFAS could cause low birthweight in humans (Apelberg et al., 2007; Fei et al., 2007; Johnson et al., 2014; Kishi et al., 2015; Vélez et al., 2015; Li et al., 2017, Cao et al., 2018), while others found little association (Kim et al., 2011; Savitz et al., 2012; Shi et al., 2017). The results differed by the study population, PFAS chemicals, and infant sex. Also, the time of blood sampling is likely

to be a key determinant in the association (Steenland et al., 2018; Dzierlenga et al., 2020). Due to pregnancy-associated physiological changes, including increased glomerular filtration rate (GFR) and parallel expansion of blood volume (Gibson, 1973; Cheung and Lafayette, 2013), the observed inverse association between PFAS concentrations and birthweight could be due to reverse causality or uncontrolled confounding factors such as GFR if the serum PFAS concentrations were measured late in pregnancy or after pregnancy (Verner et al., 2015; Steenland et al., 2018). Steenland et al. (2018) summarized three possible directed acyclic graphs (DAGs) to explain the underlying mechanisms. First, it has been found that the glomerular filter rate (GFR) increases by 40-50% during 30-35 weeks of gestation, which increases the urinary excretion of PFAS, leading to decreased serum concentrations (Verner et al., 2015). Women whose GFR fails to rise sufficiently tend to have babies with lower birthweight (Gibson, 1973; Cheung and Lafayette, 2013). Therefore, the observed inverse association between prenatal PFAS exposure and low birthweight could be confounded by the GFR. Verner et al. (2015) supported this explanation and concluded that GFR accounts for a substantial proportion of the observed association between prenatal PFAS and birthweight. Second, the growth of fetal could lead to the expansion of blood volume in mothers, leading to a decrease in the serum PFAS concentrations. Thus, the observed association between PFAS concentrations and low birthweight could be due to reverse causality. Third, some unknown factors such as a healthy placenta could be associated with both maternal blood expansion/increased GFR during pregnancy and fetal growth, thus confounding the association between PFAS concentrations and birthweight (Steenland et al., 2018).

A meta-analysis of 24 studies found an inverse association between PFOA and birthweight when the blood was sampled late in the pregnancy or at birth; however, little association was found when blood was sampled at the beginning of pregnancy when the result is less susceptible to physiological confounding or reverse causation (Steenland et al., 2018). Another recent meta-analysis based on 29 studies on PFOS and birthweight further supported the finding that blood sample timing played an important role in determining the observed association (Dzierlenga et al., 2020). Additionally, maternal transfer to offspring can also lead to a reduction of PFAS serum concentration in mothers (Beesoon et al., 2011). This highlights the need to sample blood before or in early pregnancy to avoid physiological confounding and/or reverse causality.

Compared to PFOA and PFOS, only a few studies have investigated the association between birthweight and other PFAS chemicals such as PFNA and PFHxS, which are also found to be ubiquitous at detectable levels in the general US population (CDC, 2019), and their associations with birthweight warrant further research.

In Chapter 3 of this dissertation, I investigated the association between birthweight and PFAS detections in public water supplies in the US using aggregated county-level exposure data obtained from the EPA UCMR3 database. PFAS water concentrations used in this study are free of reverse causality and/or physiological confounding compared to PFAS serum measurements. Also, using county-level multiple-stratified average birthweights in weighted regression models produces effect estimates equivalent to those that would be obtained from using individual-level data on birthweight and confounders. In addition, we explore the association between PFAS and birthweight more comprehensively by including some understudied PFAS chemicals (i.e., PFHxS and PFHpA) and accounting for co-exposure

to other contaminants and the number of drinking water violations. Overall, our work is the first nationwide statistical analysis in the US on PFAS in public water supplies and birthweight.

1.1.2 PFAS and Hypertensive Disorders of Pregnancy/Preeclampsia

Hypertensive disorders of pregnancy (HDP) complicate approximately 5-10% of pregnancies in the US. HDP includes both pregnancy-induced hypertension (PIH; or gestational hypertension) and preeclampsia (PE) (C8 Science Panel, 2011; Borghese et al., 2020; Steenland et al., 2020; ATSDR, 2021), the latter of which is defined as new-onset hypertension combined with proteinuria (≥300 mg of protein excretion in a 24-hour urine collection) after 20 weeks of gestation (Milne et al., 2005).

PFAS can inhibit placental trophoblast migration and invasion (Szilagyi et al., 2020), leading to impaired remodeling of the spiral artery, and subsequent placental oxidative stress, which plays a central role in the etiology of PE/HDP (Brosens et al., 1972; Steegers et al., 2010; Aouache et al., 2018). However, the epidemiological findings on PFAS and PE/HDP have been mixed and inconclusive. A series of health studies conducted in West Virginia and Ohio in the US ("C8 Health Study") and elsewhere in the world have investigated the association between PFAS and PE/HDP. The C8 Health Study only found weak positive associations between PFOA and PE (Stein et al., 2009; Savitz et al., 2012; Avanasi et al., 2016), and significant positive associations between PFOA and PFOS and PIH (Darrow et al. 2013). The studies conducted elsewhere have found significant positive associations between PE/PIH/HDP and other PFAS chemicals, including PFOS, PFHxS, PFBS, and PFNA (Huang et

al., 2019; Wikström et al., 2019; Rylander et al., 2020; Birukov et al., 2021). Additionally, a study in Norway found an inverse association between PE and PFUnDA (Starling et al., 2014).

The C8 Health Study in the US had some important limitations. First, all three studies used self-reported PE without any validation from medical records, which may be subject to recall bias (Stein et al., 2009; Savitz et al., 2012; Avanasi et al., 2016). Second, Stein et al. (2009) is restricted to pregnancies occurring within five years before the exposure measurement, that is, their exposure assessment occurred after the outcome, which is a violation of temporality in epidemiology and may be subject to reverse causation. Because fetal transfer in pregnancy and breastfeeding after pregnancy are both important excretion pathways for PFOA in females, the measured serum PFOA concentrations in a few years after pregnancy may not reflect the body burden of the women before pregnancy. Additionally, Savitz et al. (2012) and Avanasi et al. (2016) assessed serum PFOA levels based on historical exposure reconstruction rather than actual measurements and therefore may be subject to substantial exposure measurement error. In Chapter 5 of this dissertation, we used an approximate Bayesian computation (ABC) method to calibrate the estimated serum PFOA concentrations for the participants in the C8 Studies. The ABC method combines the single biomarker measurement with the environmentally modeled exposure, which can reduce exposure measurement error (Zhu et al., 2022).

It is also important to note that the US studies only investigated one or two PFAS chemicals: PFOA and PFOS. With PFOA and PFOS being gradually phased out in the US in the last two decades, there are alternative chemicals such as PFNA and PFHxS that warrant investigation.

In Chapter 4 of this dissertation, we examined associations between the detections/concentrations of four PFAS chemicals in public water supplies and HDP in the US during 2013-2015. PFAS water concentrations used in this study are free of reverse causality and/or physiological confounding compared to PFAS serum measurements. In addition, we were able to explore the association between PFAS and HDP more comprehensively by including some understudied PFAS chemicals (i.e., PFHxS and PFHpA), and by taking co-exposures into account. We found a small but statistically significant positive association between HDP and population-weighted average concentrations of all four PFAS (PFOA, PFOS, PFHpA, and PFHxS) and the sum of PFAS concentrations in public water supplies. Overall, our work is the first nationwide statistical analysis in the US on PFAS in public water supplies and HDP.

1.2 Air Pollution and Associated Health Effects on Susceptible Populations

Besides PFAS, air pollution is another group of modifiable environmental risk factors that pose a major threat to human health across the globe. It is referred to as "the new tobacco" and "a silent public health emergency" (WHO, 2018a). It is estimated that 90% of the world's population breathes unhealthy air containing high levels of pollutants that exceed WHO guideline limits (WHO, 2020). The combined effects of ambient (outdoor) and household (indoor) air pollution cause seven million premature deaths every year (WHO, 2020). Indoor air pollution affects impoverished communities in the low- and middle-income countries the most due to the use of highly polluting cooking fuels including wood, crop wastes, charcoal, coal, dung, and kerosene, which affect health in many ways (WHO, 2018b). In developed countries such as the US, ambient air pollution remains the greatest environmental risk factor and is still associated with 100,000-200,000 deaths annually, although the air quality in the US has dramatically improved over the past few decades compared to its historical levels and other countries in the world (Burnett et al., 2018; Tessum et al., 2019).

Similar to PFAS, ambient air pollution impact children, pregnant women, elderly people, and people with underlying diseases the most. In previous literature, maternal exposure to ambient pollution has been identified as an important risk factor for low birthweight, preterm birth, and HDP (Wang et al., 1997; Bell et al., 2007; Stieb et al., 2012; Petersen et al., 2013; Slama et al., 2008). In particular, a meta-analysis of 14 studies found increased particulate air pollution levels of PM_{10} and $PM_{2.5}$ (particulate matter $\leq$10 and 2.5 μm) across the entire pregnancy were associated with an increased risk of low birthweight at term (Dadvand et al., 2013). Another meta-analysis reported increased risks of HDP

associated with increases in $PM_{2.5}$, PM_{10}, nitrogen dioxide (NO_2), nitrogen oxides (NO_x), and ozone (O_3) (Pedersen et al., 2014). The consistently observed effects of different air pollutants on HDP can be explained by the fact that pregnant women are generally vulnerable to developing hypertensive disorders due to increased stress on the cardiovascular system (Yoder et al., 2009) and endothelium (Steegers et al., 2010).

In addition to the impacts of air pollution on infant and maternal health, there is a growing body of epidemiological studies reporting the neurological effects of air pollution as well. Long-term/chronic exposure to $PM_{2.5}$, O_3, NO_x, or NO_2, the major toxic substances in the air, can trigger local inflammation and oxidative stress in the brain (Block and Calderon-Garciduenas, 2009; Béjot et al., 2018; Manisalidis et al., 2020), which may play a role in neurodegeneration processes and lead to dementia (Peters et al., 2019; Power et al., 2016).

Calderón-Garcidueñas et al. (2002) established the first linkage between air pollution and neurodegenerative disease in 32 healthy dogs in a highly polluted urban region in Southwest Metropolitan Mexico City. Subsequent epidemiological studies in humans further investigated the association in different populations. However, the results are inconsistent, ranging from significant to weak, null, or negative effects, possibly due to the variations in study designs, populations, air pollutants, sources of exposure, exposure levels, windows of exposure assessment, follow-up periods, outcome assessment, disease subtypes, statistical methods, adjusted confounding factors, etc.

Although most of the studies found positive associations between incident dementia/dementia hospitalization/cognitive decline and exposure to $PM_{2.5}$ (Cacciottolo et al., 2017; Chen et al., 2017; Carey et al., 2018; Grande et al., 2020; Jung et al., 2015; Shaffer et al., 2021; Shi et al., 2021; Tonne et al., 2014; Weuve et al., 2012), NO_x/NO_2 (Carey et al., 2018;

Chang et al., 2014; Chen et al., 2017; Grande et al., 2020; Oudin et al., 2016; Shi et al., 2021), or O_3 (Cerza et al., 2019; Cleary et al., 2018; Jung et al., 2015; Wu et al., 2015), some found null or negative associations between the outcome and $PM_{2.5}$ (Cleary et al., 2018; Loop et al., 2013), NO_x/NO_2 (Oudin et al., 2017), or O_3 (Chen et al., 2017). Oudin et al. (2018) found the association differed by the source of $PM_{2.5}$, i.e., $PM_{2.5}$ from traffic exhaust was associated with incident dementia, while $PM_{2.5}$ from residential wood burning was not. In contrast, Tonne et al. (2014) found that $PM_{2.5}$ from traffic was not associated with cognitive change. Also, some studies reported different results for different air pollutants (Carey et al., 2018; Chen et al., 2017; Cleary et al., 2018; Cerza et al., 2019; Jung et al., 2015; Shi et al., 2021). For example, Cerza et al. (2019) found positive associations between O_3 and dementia hospitalization, yet a negative association between NO_2 and dementia hospitalization in Rome. However, Carey et al. (2018) reported positive associations between $PM_{2.5}$, NO_2 and dementia, yet a negative association between O_3 and dementia in London. In addition to the above studies, Wang et al. (2022) recently reported reduced dementia risk associated with air quality improvement with regards to $PM_{2.5}$ and NO_2 in older women in the US.

In the US, people of color experience greater exposure to air pollution than White (Tessum et al., 2021). The disparity was found in people at all income levels across states, urban, and rural areas (Tessum et al., 2021). As a distinct population with "historical trauma" caused by forced colonization (Grayshield et al., 2015), Native Americans who live in tribes face disproportionate health impacts from air pollution due to the oil and gas industry that emits volatile organic compounds (VOCs) and NO_x, which react in sunlight to form ground-level O_3 pollution that is harmful to human health (CATF, 2018; US EPA, 2021). Also, it is noted that Native American communities have less insurance coverage, and worse access

and utilization of health services than Whites (Zuckerman et al., 2004), and thus have long been disproportionately affected by the high burden of dementia and closely related cardiovascular diseases (CVD) (Browne et al., 2016; Galloway, 2005). However, no study has investigated the effects of air pollution on the risk of developing dementia in American Indians specifically. Chapter 6 of this dissertation aims to address the important knowledge gap.

CHAPTER 2

Household low pile carpet usage was associated with increased serum PFAS concentrations in 2005-2006

Contents

2.1 Abstract

Background: Per- and polyfluoroalkyl substances (PFAS) are ubiquitous in the serum of the general US population. Food, drinking water, consumer products, dust, and air have been assessed as PFAS exposure sources for humans. The effects of various types of carpeting on serum PFAS concentrations have been less studied, despite the known use of PFAS in stain-resistant carpet treatments.

Objective: This study aimed to examine the associations between serum PFAS concentrations and type of residential flooring among the general US population aged 12 years and older using the 2005-2006 National Health and Nutrition Examination Survey (NHANES).

Methods: We used multiple linear regressions adjusted for complex survey design and relevant covariates to analyze the relations between serum PFAS concentrations and type of floor covering (smooth surface, low pile carpet, medium to high pile carpet, and combination of carpet and smooth surface), as well as other potential exposure factors. We used multiple imputation to address missing values.

Results: We found significantly higher serum concentrations of perfluorohexane sulfonic acid (PFHxS) and 2-(N-methyl-perfluorooctane sulfonamido) acetic acid (MeFOSAA) in US residents residing in homes with low pile carpeting compared with those residing in homes with smooth surface. We concluded that among US residents aged 12 years and older residing in homes with low pile carpeting in the home in 2005-2006, on average 24% and 19% of the PFHxS and MeFOSAA body burdens, respectively, could be attributed to carpeting. We found associations between other types of floor covering (medium to high pile carpet, combination of carpet and smooth surface) and some PFAS concentrations compared with

the smooth surface, but these results were less consistent and generally not statistically significant. Additionally, a group Wald Chi-squared test showed a significant result for PFOS, indicating different contributions of various types of flooring to PFOS serum concentration.

Significance: Our results are representative of the general US population at the time of the survey, and potentially informative regarding ongoing PFAS exposure from a variety of sources including carpeting.

Keywords: PFAS exposure, carpeting, serum, sampling survey

2.2 Introduction

Per- and polyfluoroalkyl substances (PFAS) are a group of human-made chemicals that widely exist in the environment, biota, and humans. Due to the strong single bond between carbon (C) and fluorine (F), which requires very high energy to dissociate, PFAS have unique physiochemical characteristics such as stability in extreme temperatures and water resistance (Faithfull and Weers, 1998). Strong carbon-fluorine bonds also make PFAS resistant to hydrolysis, microbial degradation, and metabolism by vertebrates (ATSDR, 2018). Therefore, they have been used in a variety of applications, including textiles, carpeting, food packaging, nonstick cookware, and firefighting foams (Kissa, 2001). They persist indefinitely in the environment and bioaccumulate in humans and organisms (Buck et al., 2011). For example, the average half-lives for serum or plasma elimination of PFAS in humans were reported as about 2-4 years for perfluorooctanoic acid (PFOA) (Bartell et al., 2010; Olsen et al., 2007; Worley et al., 2017), 3-27 years for perfluorooctane sulfonic acid (PFOS), 5-35 years for PFHxS (Olsen et al., 2007; Worley et al., 2017; Li et al., 2018; Zhang et al., 2013), and 2-4 years for perfluorononanoic acid (PFNA) (Zhang et al., 2013) in previous studies. Some studies have found associations between PFAS and a series of adverse health effects such as reduced fecundity (Vélez et al., 2015), low birthweight (Johnson et al., 2014), and cancers (Vieira et al., 2013).

Perfluoroalkyl carboxylic acids (PFCA, e.g., PFOA and PFNA) and perfluoroalkane sulfonic acid (PFSA, e.g., PFOS and PFHxS) both belong to perfluorinated acids (PFA) in the PFAS family (Buck et al., 2011; D'eon and Mabury, 2011). Long-chain PFAS (PFCA with $\geq$8 carbons and PFSA with $\geq$6 carbons) have been shown to be more bioaccumulative than their short-chain analogues (Martin et al., 2003; Olsen et al., 2009), and therefore have attracted

more attentions from the global scientific and regulatory community (Buck et al., 2011; US EPA, 2009). There are two main methods to produce PFAS: electrochemical fluorination (ECF) and telomerization. The ECF method was used by the 3M Company from 1949 to 2001 to manufacture perfluorooctanesulfonyl fluoride (POSF)-based materials (including PFOS, MeFOSAA, EtFOSAA, and high-molecular-weight fluorinated polymers), PFCA (such as PFOA and PFNA) and their ammonium salts (Beesoon et al., 2011; Buck et al., 2011; D'eon and Mabury, 2011). The ECF technique resulted in a mixture of linear (70-80%) and branched (20-30%) isomers, while the telomerization method initially developed by the DuPont Company in the 1970s produced almost completely linear isomers. Starting from 2002, the 3M Company, the major global manufacturer of PFOA, PFOS and related perfluorooctanesulfonyl fluoride compounds, ceased its production of these substances using the ECF technique due to health concerns, and has since introduced short-chain perfluoroalkane sulfonates and products such as perfluorobutane sulfonic acid (PFBS) (Zushi et al., 2012). In 2006, EPA initiated a global stewardship program to achieve a 95% reduction in PFOA and its precursors by 2010, and work towards the elimination of these chemicals by 2015. During this time the manufacture of PFOA, PFOS and their precursors was largely relocated to Asia, especially China (UNEP, 2015; Yue, 2008), where other manufacturers continued to use the ECF process to develop these substances (Buck et al., 2011). Unlike PFOS, which was almost solely produced using the ECF technique, PFOA could be manufactured through not only the ECF method, but also telomerization, which was still in use after 2002 to produce fluorotelomer-based chemicals including polyfluoroalkyl phosphate esters (PAPs) and PFOA linear isomers. Additionally, some fluorotelomer-based materials such as PAP diesters (diPAPs) can be enzymatically hydrolyzed to produce 8:2

fluorotelomer alcohol (8:2 FTOH), an intermediate metabolite that is subsequently transformed into perfluoroalkyl carboxylic acids (PFCA) such as PFNA, PFOA, and PFHpA (Buck et al., 2011; D'eon and Mabury, 2007; D'eon and Mabury, 2011). NHANES data have accordingly shown a significant downward trend in serum concentrations of PFOS and PFHxS in the general US population since 1999 (Calafat et al., 2007; Kato et al., 2011). However, PFOA did not decline as much as expected after the phase-out by 3M (Olsen et al., 2008; Beesoon et al., 2011) and remained essentially unchanged, and may have increased during 2003-2008 in the US (Kato et al., 2011) before declining slowly since 2008 (CDC, 2019). The percentage of linear isomer for PFOA was also found to be increasing during 1997-2012 in Sweden, indicating the ongoing production of fluorotelomer-based chemicals (Gebbink et al., 2015).

According to the Carpet and Rug Institute, most commercial and residential carpets and rugs in the US have been treated with perfluorooctane sulfonyl fluoride (POSF)-based materials in the manufacturing process to achieve stain resistance (DTSC, 2018). For example, sulfonamido-ethanol (MeFOSE) is a raw material used in textile and carpet products, and can be metabolized into MeFOSAA (aka M570), a precursor of PFOS; PFOS was a key ingredient in Scotchgard and had been extensively produced for use in carpet treatment until the phase-out. Perfluorohexane sulfonyl fluoride (PHxSF)-based derivatives such as PFHxS had also been produced by the 3M Company until the phase-out parallel to the phase-out of POSF-based products, and was used in specific postmarket carpet treatment products (Wang et al., 2014; Olsen et al., 2003). In 2009, PFOS and its precursors were added to Annex B of Stockholm Convention on Persistent Organic Pollutants (POPs). While this is an important step to restrict the production and use of PFOS and its precursors, a list of

specific exemptions associated with this treaty still allows the mass production and almost all the historic uses of PFOS and its precursors, including the use in carpets, leather and apparel, textiles and upholstery, paper and packaging, coating and coating additives, and so forth (UNEP, 2010).

Dust ingestion has been associated with stain-resistant treatment of carpets or rugs, which is an important pathway for PFAS exposure (D'Hollander et al., 2010; Beesoon et al., 2012; Harris et al., 2017; Hu et al., 2018; Hurley et al., 2018; Karásková et al., 2016). Previous studies have indicated the ubiquitous presence of diPAPs, 8:2 FTOH, PFCA, and some POSF-based materials in indoor dust or air, which could come from consumer products such as carpets, upholstery, and textiles (De Silva et al., 2012; Fraser et al., 2012; Kato et al., 2009; Strynar et al., 2008; Winkens et al., 2018; Kubwabo et al., 2005). In a Canadian study conducted by Shoeib et al. (2005), MeFOSE and N-ethylperfluorooctane sulfonamidoethanol (EtFOSE) were detected at the median concentrations of 110 ng/g and 120 ng/g in indoor air, respectively, 10-20 times higher than their outdoor concentrations. EtFOSE is a POSF-based raw material used in paper and packaging products, and it can be metabolized into EtFOSAA (Buck et al., 2011). Both EtFOSAA and MeFOSAA are precursors of perfluorooctane sulfonamidoacetic acid (FOSAA, aka M556), perfluorooctane sulfonamide (FOSA), and PFOS. A US study by Strynar and Lindstorm (2008) found ubiquitous existence of PFOS and PFOA in house dust, with median concentrations of 201 and 142 ng/g, respectively. 8:2 FTOH, an important precursor for PFOA, was also detected with a maximum concentration of 1660 ng/g in the house dust. These results from previous studies highlight the potential of house dust and air as important pathways for human PFAS exposure.

Despite efforts to remove PFOA, PFOS, and PFOS precursors (MeFOSAA and EtFOSAA) produced using the ECF method, telomerization continued to be used to manufacture PFOA and related chemicals after the 2002 phase-out. A recent review paper revealed the large uncertainty about the amount of PFAS manufactured and imported due to the fact that a large part of the amounts has been claimed as confidential business information (CBI) and only substances manufactured or imported at above 11.34 tonnage per year at a single site have been reported (Glüge et al., 2020); also, PFAS that can break down into PFOA and PFOS are still in use in the US (BloombergLaw, 2020). Additionally, commercial and residential carpets treated with POSF-based materials such as MeFOSAA, PFOS, and PFHxS may not be replaced as frequently as other products. Thus, the PFAS-treated carpets before the phase-out may continue to be used in households and commercial settings. Besides, some short-chain PFAS have been introduced as alternative chemicals to long-chain PFAS (Birnbaum et al., 2015; Gomis et al., 2018). For example, PFBS has been extensively produced and used as a replacement of PFOS in the ScotchGard formulas, and perfluorobutanoic acid (PFBA) has also been introduced as an alternative to PFOA in recent years, which may also have adverse health effects despite their relatively shorter half-lives (Buck et al., 2011; Olsen et al., 2009; Eschauzier et al., 2010; Liu et al., 2020).

Some studies have reported positive associations between PFAS concentrations in serum or indoor dust and carpet (Beesoon et al., 2012; Harris et al., 2017; Hu et al., 2018; Hurley et al., 2018; Karásková et al., 2016). However, none of these studies distinguished between different types of carpets. Because different types of carpets were designed for different social settings and frequency of foot traffic, they may present distinct patterns of PFAS exposure contribution. For example, low pile carpets are designed for and more

common in places with larger traffic than medium to high pile carpets, and may have different PFAS treatment. We here extend the previous work by investigating and quantifying the contribution of PFAS exposure from various types of floor coverings using the NHANES public-use database. In this study, we also accounted for other previously reported exposure pathways to PFAS, including dietary intake of fish and shellfish (Christensen et al., 2017), fast food consumption (Susmann et al., 2019), water contamination by firefighting foams near military sites (Hu et al., 2016), and tap water sources (Shin et al., 2011a; Shin et al., 2011b). We also accounted for kidney function (Jain et al., 2019) and special PFAS excretion pathways for females, including menstrual blood loss (Wu et al., 2015), maternal transfer to offspring through pregnancy (Beesoon et al., 2011) and breastfeeding (Fromme et al., 2010). To our knowledge, this is the first analysis that distinguishes low pile carpet from medium/high pile carpet, and focuses specifically on the association between carpet type and PFAS, which can inform PFAS exposure assessment from carpet more comprehensively.

2.3 Methods

We obtained data from the 2005-2006 NHANES data files. NHANES uses a complex, multistage, stratified, clustered, probability sampling design to select participants representative of the civilian, non-institutionalized US population (NCHS, 2012). It is a cross-sectional survey designed to monitor the health and nutritional status of adults and children in the US. NHANES survey includes interviews, physical examinations for every participant and laboratory tests for a subsample (NCHS, 2013). Sampling weights were created in the sample and subsamples in each 2-year cycle to account for oversampling, non-response, and poststratification in the complex survey design (NCHS, 2012). Our analyses were restricted to the 2005-2006 cycle based on the availability of the key variable of interest: type of floor covering. This variable was not included in subsequent cycles of NHANES.

Serum PFAS Measurements. In the 2005-2006 NHANES data, 12 PFAS were measured in a one-third subsample of eligible participants aged 12 years and older using tandem mass spectrometry, including PFOA, PFOS, PFHxS, 2-(N-ethyl-perfluorooctane sulfonamido) acetic acid (EtFOSAA), MeFOSAA, perfluorodecanoic acid (PFDA), PFBS, perfluoroheptanoic acid (PFHpA), PFNA, perfluorooctane sulfonamide (PFOSA), perfluoroundecanoic acid (PFUnDA), and perfluorododecanoic acid (PFDoA). Procedures for collecting, storing, and handling specimens, and quality control have been described elsewhere (CDC, 2013). PFAS serum concentrations below the limit of detection (LOD) were substituted with a value of LOD divided by $\sqrt{2}$ (CDC, 2013). We restricted our analyses to the six PFAS (i.e., PFOA, PFOS, PFHxS, MeFOSAA, PFDA, and PFNA) with detection rates of >70% in the sample to avoid bias from the substitutions. The LOD was 0.1 ng/ml for PFOA, PFHxS, and PFNA; and 0.2 ng/ml for PFOS, PFDA, and MeFOSAA (CDC, 2012).

Explanatory Variables. We obtained NHANES variables relevant to known or suspected PFAS exposure pathways, including the type of floor covering (collected using household interview questionnaires) from the dust allergen subsample in the laboratory data; tap water source, self-reported fish and shellfish consumption in the past 30 days from the dietary interview component; the frequency of eating out per week from the dietary behavior questionnaire; serum creatinine (an indicator of kidney function) from the standard biochemistry profile; whether or not had at least one menstrual period in the past 12 months, number of pregnancies, and number of children breastfed for at least one month from the reproductive health questionnaire data. We also obtained age, sex, race/ethnicity, education, family poverty income ratio (PIR), veteran/military status, country of birth, and body mass index (BMI) from the demographic component of the survey. These have been established as predictors for serum PFAS concentrations in previous studies (Calafat et al., 2007; Kato et al., 2011; Christensen et al., 2017; Susmann et al., 2019; Hu et al., 2016; Zhang et al., 2010; Jain, 2014). We recategorized race/ethnicity as Hispanic (including Mexican American and other Hispanic), non-Hispanic white, non-Hispanic black, and the others. We categorized education as less than college, some college, and above college.

The type of floor covering was categorized as smooth surface, low pile carpet, medium to high pile carpet, and combination of carpet and smooth surface. We recategorized the frequency of eating out per week as “Yes” and “No” based on the provided combination of numbers (1-21 times/week) and categories (never, more than 21 times per week, less than weekly) in the original variable, because this variable is not fully numeric. We checked the linearity of the relations between serum PFAS concentrations and age, family PIR, BMI, serum creatinine, number of pregnancies, and number of children breastfed for at least one

month by examining both the scatter plots and plots of residuals against each of the predictor variables, and included these predictors as continuous variables in the regression models. We treated all "Refused" and "Don't know" answers in the data as missing values. We checked data on sex, menstrual period, pregnancy, and breastfeeding against each other. For males, missing values in reproductive health information were substituted with "No" (menstrual period) or 0 (number of pregnancies and number of children breastfed). For females aged 55 and older, missing values in "at least menstrual period in the last 12 months" were substituted with "No". For females who were never pregnant, missing values in the number of pregnancies and the number of children breastfed for at least one month were substituted with 0. For females who never breastfed, the number of children breastfed for at least one month were also substituted with 0. After these substitutions, most variables have missing rates less than 10% except for type of floor covering (26.9%) and veteran/military status (19.4%). We compared the missing percentage of these variables with respect to other variables, and found the type of floor covering is missing at random (MAR) with respect to race/ethnicity and education; and veteran/military status is MAR with respect to country of birth, race/ethnicity, and education.

Statistical Analysis. We fit multiple linear regression models adjusted for covariates using log-transformed serum PFAS concentrations while accounting for complex survey design (R package *survey*). We adjusted for the sampling weights of the serum PFAS subsample, the smallest analysis subpopulation in this study, for parameter estimates in regression models to reflect the probability of selection, nonresponse, and post-stratification (NHANES, 2020a). We also accounted for the pseudo-stratum and pseudo-PSU variables in order to produce asymptotically unbiased variance estimation in our regression models

(NHANES, 2020b). Because of the dispersed distribution of missing values in different variables, listwise deletion of missing data that is performed as default in most statistical software packages including R would substantially reduce the sample size by 55%. Although listwise deletion is acceptable when some observations are "missing completely at random" (MCAR) without respect to any of the other variables, multiple imputation performs better when missingness is covariate-dependent (Little and Rubin, 2020). We used multivariate imputation by chained equations (MICE) to create k=100 imputed datasets (R package *mice*). We applied Rubin's rules (Rubin, 1987) to pool the results from k=100 analyses accounting for complex survey design based on the imputed datasets, averaging the estimates, and computing the total variance over the repeated analyses. We also compared the results from regression analyses with those of complete case analysis (analysis of data after listwise deletion). We exponentiated the regression coefficients, subtracted by one, and multiplied by 100% to estimate the percent difference in PFAS concentrations associated with each predictor. For variables with more than two categories/levels, Wald Chi-squared tests of equivalence on multiple parameters can be used to test their effects. For example, to test the hypothesis that multiple types of flooring have no effect on serum PFAS concentrations, we conducted a simultaneous Wald Chi-squared test of equivalence for all parameters related to type of flooring. Because the education levels of adolescents are mostly determined by their age rather than family socioeconomic status, in sensitivity analyses, we ran the analysis for adults (aged > 19) and adolescents (aged 12-19) separately.

Because PFAS do not tend to accumulate in the fat tissues (lipophobic property) (Benford et al., 2008), we did not include BMI (an indicator of body fatness) in the primary multiple regression models. PFAS has been suggested as a potential "cause" of body weight

change (Liu et al., 2018), not the other way around, in which case, BMI would not confound the associations between type of flooring and serum PFAS; thus adjustment for BMI would not be necessary and could even induce selection bias under certain conditions (Rothman, 2012). In sensitivity analyses, we also fit multiple regression models with additional adjustment for BMI, following the practice in some previous studies (Harris et al., 2017; Christensen et al., 2017; Susmann et al., 2019). We used R 4.0.0 for statistical analyses.

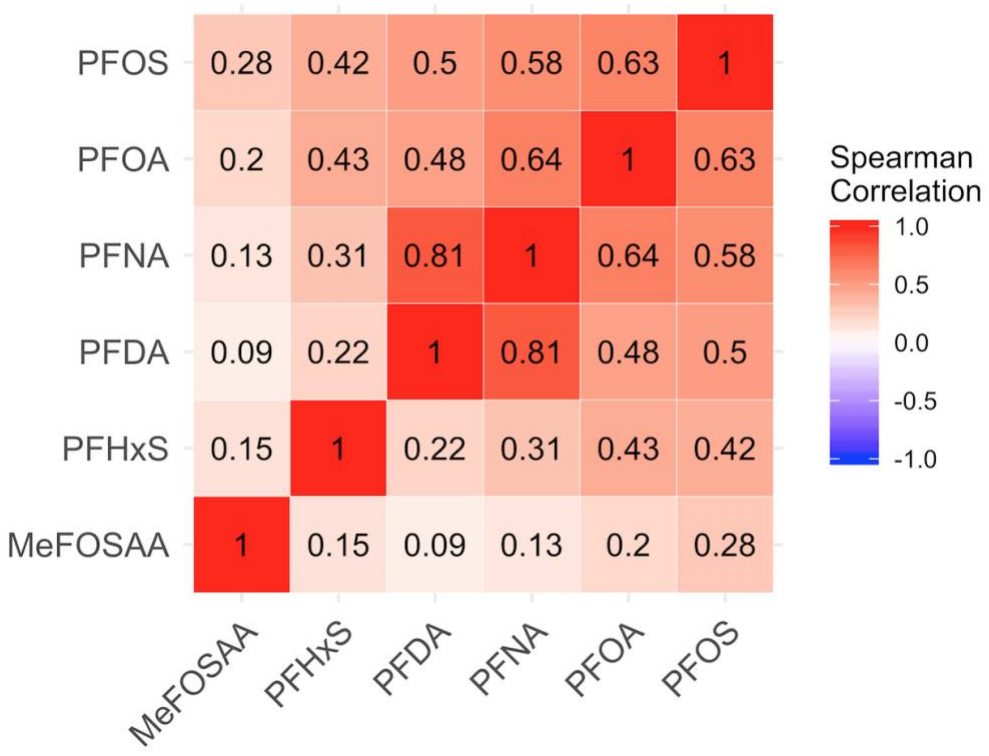

Figure 2.1. Pairwise Spearman Correlation Matrix among Different PFAS in Serum

2.4 Results

Characteristics of study participants by type of residential floor covering are shown in Table 2.1. Accounting for the complex survey design, we estimated the geometric means of PFAS serum concentrations by type of floor covering in the general US population during 2005-2006 shown in Table 2.2. In general, we found higher geometric means of PFAS in people residing in homes with low pile carpets compared to those residing in homes with smooth surfaces and medium to high pile carpets. The pairwise Spearman correlation matrix among different serum PFAS concentrations is shown in Figure 2.1. The correlations among perfluoroalkyl carboxylic acids, including PFNA, PFDA, and PFOA are moderate to high, which is likely due to the fact that they have a common precursor 8:2 FTOH (Buck et al., 2011). The high correlation between PFOA and PFOS has been reported elsewhere previously (Haug et al., 2009), indicating the likelihood of common exposure sources for these two legacy PFAS, such as food, dust, and air. However, the correlations among PFOS and its precursors MeFOSAA, PFHxS were not as high as expected, indicating the likelihood of other commercial sources of PFOS (e.g., di-SAmPAP) (Yeung et al., 2013).

After imputation, the study participants (n = 2,323) represent 244 million general US population, half of whom resided in homes with low pile carpets. Adjusting for potential confounders, we found low pile carpets were associated with 32% (95% CI: 3%-70%) increase in serum PFHxS concentration and 25% (95% CI: 7%-45%) increase in serum MeFOSAA concentration compared to smooth surfaces (Table 2.3). Given the geometric means of 1.29 ng/ml and 0.31 ng/ml for PFHxS and MeFOSAA, respectively, for people residing in homes with smooth surfaces (Table 2.2), on average low pile carpets were associated with 0.41 ng/ml increase in PFHxS and 0.08 ng/ml increase in MeFOSAA,

accounting for 24% and 19% of the geometric means of serum PFHxS and MeFOSAA concentrations, respectively, in people residing in homes with low pile carpets in the US.

The Wald Chi-squared test of equivalence for multiple parameters showed significant differences in serum PFOS and MeFOSAA concentrations among people who used different types of floor covering (p-value = 0.02 and 0.04, respectively; Table 2.3). Overall, multiple imputation and complete case analysis created similar results in regression analyses with the exception of PFHxS, for which the association with low pile carpets was not significant using complete case analysis (20%, 95% CI: [-14%, 67%], see Table S2.1 in the Supplementary material) but significant using multiple imputation (32%, 95% CI: [3%, 70%], Table 2.3). Complete case analysis only included 1,044 observations in the adjusted model, losing 55% of the information, which may bias the results (Little and Rubin, 2020). Separate analysis for adults (n=1,593) produced similar results to the analysis using all participants (n=2,323) (Table S2.2), while the results for adolescents (n=730) were different, i.e., the effects of low pile carpet on PFHxS (29%, 95% CI: [-14%, 92%], Table S2.3) and MeFOSAA (13%, 95% CI: [-9%, 41%], Table S2.3) were not significant, which is likely due to the smaller sample size of adolescents and the less statistical power. Additional adjustment for BMI in the sensitivity analyses also produced similar results to the primary analysis (see Table S2.4 in the Supplementary material).

Other important predictors for PFAS in this study include race/ethnicity, country of birth, family PIR, shellfish consumption, tap water sources, menstruation period, similar to previous findings reported elsewhere (Calafat et al., 2007; Christensen et al. 2017; Haug et al., 2010; Hurley et al., 2016; Kato et al. 2011; Suominen et al., 2011; Yamaguchi et al., 2013).

Table 2.1. Characteristics of Study Participants

Characteristics	n (%) or mean ± SD	Type of floor covering			
		Smooth surface (n = 183)	Low pile carpet (n = 1276)	Medium/high pile carpet (n = 172)	Combination of carpet and smooth surface (n = 67)
Age	38.2 ± 22.2	32.5 ± 20.0	37.3 ± 22.0	41.1 ± 23.8	45.0 ± 22.7
Gender					
Female	1,180 (50.8%)	81 (44.3%)	647 (50.7%)	77 (44.8%)	38 (56.7%)
Male	1,143 (49.2%)	102 (55.7%)	629 (49.3%)	95 (55.2%)	29 (43.3%)
Race/ethnicity					
Hispanic	623 (26.8%)	78 (42.6%)	332 (26.0%)	49 (28.5%)	11 (16.4%)
Non-Hispanic black	613 (26.4%)	49 (26.8%)	388 (30.4%)	24 (14.0%)	18 (26.9%)
Non-Hispanic white	996 (42.9%)	48 (26.2%)	503 (39.4%)	88 (51.2%)	34 (50.7%)
Others	91 (3.9%)	8 (4.4%)	53 (4.2%)	11 (6.4%)	4 (6.0%)
Education					
< College	1,503 (64.7%)	127 (69.4%)	846 (66.3%)	120 (69.8%)	38 (56.7%)
Some college	513 (22.1%)	41 (22.4%)	279 (21.9%)	34 (19.8%)	16 (23.9%)
> College	305 (13.1%)	15 (8.2%)	150 (11.8%)	18 (10.5%)	13 (19.4%)
Country of birth					
US	1,875 (80.7%)	141 (77.0%)	1028 (80.6%)	136 (79.1%)	60 (89.6%)
Foreign	447 (19.2%)	42 (23.0%)	248 (19.4%)	36 (20.9%)	7 (10.4%)
Veteran/military status					
No	1,625 (70.0%)	130 (71.0%)	876 (68.7%)	115 (66.9%)	46 (68.7%)
Yes	247 (10.6%)	8 (4.4%)	135 (10.6%)	28 (16.3%)	15 (22.4%)
Family PIR	2.5 ± 1.6	2.0 ± 1.5	2.4 ± 1.5	2.4 ± 1.4	2.5 ± 1.8
BMI	27.3 ± 7.0	27.3 ± 7.4	27.1 ± 6.9	27.7 ± 6.9	28.1 ± 8.6
Tap water source					
Don’t drink tap water	419 (18.0%)	40 (21.9%)	224 (17.6%)	35 (20.3%)	17 (25.4%)
Community supply	1,335 (57.5%)	103 (56.3%)	739 (57.9%)	91 (52.9%)	38 (56.7%)
Other	338 (0.1%)	22 (12.0%)	190 (14.9%)	30 (17.4%)	9 (13.4%)

Eat out per week					
No	225 (9.7%)	18 (9.8%)	133 (10.4%)	17 (9.9%)	7 (10.4%)
Yes	2,064 (88.9%)	161 (88.0%)	1126 (88.2%)	154 (89.5%)	60 (89.6%)
Eating shellfish in the past 1 month					
No	1,109 (47.7%)	90 (49.2%)	615 (48.2%)	80 (46.5%)	35 (52.2%)
Yes	1,114 (48.0%)	80 (43.7%)	618 (48.4%)	84 (48.9%)	29 (43.3%)
Eating fish in the past 1 month					
No	793 (34.1%)	68 (37.2%)	445 (34.9%)	48 (27.9%)	17 (25.4%)
Yes	1,431 (61.6%)	103 (56.3%)	788 (61.8%)	116 (67.4%)	47 (70.1%)
Serum Creatinine (mg/dL)	0.9 ± 0.3	0.8 ± 0.3	0.8 ± 0.3	0.9 ± 0.3	0.9 ± 0.7
Had at least one period in the past 1 year					
No	1,506 (64.8%)	120 (65.6%)	820 (64.3%)	118 (68.6%)	44 (65.7%)
Yes	706 (30.4%)	51 (27.9%)	396 (31.0%)	49 (28.5%)	23 (34.3%)
Number of pregnancies	1.0 ± 1.9	0.8 ± 1.7	1.0 ± 1.9	1.0 ± 1.9	0.9 ± 2.0
Number of children breastfed at least 1 month	0.4 ± 1.1	0.3 ± 1.0	0.3 ± 1.0	0.4 ± 1.1	0.3 ± 0.9

Table 2.2. Geometric Means of Serum PFAS Concentrations by Type of Floor Covering

Type of Floor Covering	Geometric Mean, 95% CI (ng/ml)					
	PFOA	PFOS	PFHxS	MeFOSAA	PFDA	PFNA
Smooth surface	3.45, (2.95, 4.04)	13.67, (10.93, 17.08)	1.29, (0.94, 1.78)	0.31, (0.25, 0.39)	0.30, (0.25, 0.36)	0.94, (0.81, 1.08)
Low pile carpet	3.85, (3.41, 4.35)	17.34, (16.20, 18.55)	1.74, (1.46, 2.08)	0.41, (0.38, 0.45)	0.35, (0.29, 0.41)	1.05, (0.88, 1.25)
Medium to high pile carpet	3.71, (3.00, 4.58)	15.31, (13.22, 17.73)	1.63, (1.20, 2.22)	0.41, (0.31, 0.56)	0.29, (0.25, 0.34)	0.94, (0.83, 1.05)
Combination of carpet and smooth surface	3.75, (3.16, 4.45)	16.66, (13.53, 20.53)	1.56, (1.24, 1.97)	0.44, (0.35, 0.56)	0.33, (0.27, 0.42)	1.08, (0.86, 1.35)

Table 2.3. Adjusted Associations* from Multiple Regression Models with Multiple Imputation (k=100) (n=2,323)

% difference in PFAS concentrations**, 95% CI	PFOA	PFOS	PFHxS	MeFOSAA	PFDA	PFNA
Type of floor covering						
Smooth surface	ref	ref	ref	ref	ref	ref
Low pile carpet	7, (-8, 23)	14, (-2, 33)	32, (3, 70)	25, (7, 45)	12, (-6, 34)	9, (-7, 29)
Medium to high pile carpet	-2, (-19, 17)	-2, (-19, 19)	22, (-15, 75)	23, (-5, 59)	-6, (-22, 12)	-6, (-21, 13)
Combination of carpet and smooth surface	-2, (-21, 22)	2, (-20, 31)	13, (-18, 55)	20, (-8, 57)	3, (-19, 31)	4, (-17, 30)
p-value of Wald Chi-squared test	*0.5*	*0.02*	*0.1*	*0.04*	*0.1*	*0.3*

* We adjusted for age, gender (female/male), race/ethnicity (Hispanic/non-Hispanic black/non-Hispanic white/others), education (less than college/some college/college graduate or above), country of birth (foreign/US), veteran/military status (yes/no), family PIR, tap water source (don't drink tap water/community supply/others), eating out per week (yes/no), eating shellfish during past 30 days (yes/no), eating fish during past 30 days (yes/no), serum creatine, had at least one period in the past 12 months (yes/no), number of pregnancies, and number of children breastfed at least 1 month in the regression models.

** We exponentiated the regression coefficients, subtracted by one, and multiplied by 100% to estimate the percent difference in PFAS concentrations associated with each predictor.

2.5 Discussion

In this study, we examined the associations between type of floor covering and serum PFAS concentrations. Descriptive statistics in Table 2.2 also showed higher geometric means of serum PFAS concentrations in people residing in homes with low pile carpets than people residing in homes with smooth surfaces and medium to high pile carpets. After adjusting for potential confounding variables in the multiple regression model, we found significantly higher serum PFHxS and MeFOSAA concentrations in people residing in homes with low pile carpets compared with those residing in homes with smooth surfaces, especially adults; while the effects of medium to high pile carpets and combination of carpet and smooth surface on serum PFAS concentrations were less clear, suggesting that low pile carpet is a more important source of exposure compared with other types of flooring. Because PFHxS and MeFOSAA are both key ingredients in carpet treatment products that are intended for stain resistance, our results could be explained by the fact that low pile carpets frequently used in highly-trafficked spaces were more likely to have more extensive PFAS treatment. In addition, we concluded that among US residents using low pile carpeting in the home in 2005-2006, on average 24% and 19% of the PFHxS and MeFOSAA body burdens, respectively, could be attributed solely to the carpeting.

A previous study by Harris et al. (2017) found higher serum PFOS, PFHxS, and MeFOSAA concentrations in US children aged 6-10 years who slept in a bedroom with carpeting or a rug, indicating hand-to-mouth transfer from treated carpets or inhalation of volatile precursors as important exposure pathways for children. Our study does not include children less than 12 years old, who are more likely than adolescents and adults to crawl on the ground and have their hands contact the carpets. Our results derived from the NHANES

data are more representative of the general US population aged 12 and older, and can potentially inform the PFAS exposure from various types of flooring, especially low pile carpeting. Previous studies have indicated carpets as both a source and sink of PFAS chemicals. Given that the dust ingestion of children is about two times that of adults (Shoeib et al., 2005; Strynar et al., 2008), the PFAS exposure coming from house carpets for children less than 12 years old is likely to be higher.

Similar to the previous studies (Beesoon et al., 2012; Hurley et al., 2018; Goosey and Harrad, 2011), we found that other long-chain PFAS such as PFHxS and MeFOSAA were elevated in individuals residing in homes with carpets. We also found somewhat different PFOS concentrations among people using various types of flooring, which may suggest distinct PFAS treatment or formulations for different types of carpets, and the potential for other PFAS to degrade or be metabolized to PFOS (ATSDR, 2018). However, the conclusion is tempered by the limitation of using *p-value*<0.05 to represent statistically significance in the Wald Chi-squared tests, especially with multiple testing (Wasserstein and Lazar, 2016). Our estimates with 95% confidence intervals in Table 2.3 more directly address effect sizes and their associated uncertainties.

This study has several noteworthy features. Among them is the use of multiple imputation to address missing values in the dataset, which appropriately accounted for the uncertainty of imputation. Additionally, we used statistical methods accounting for the complex survey design to obtain unbiased estimates of regression parameters and accurate variance estimation. Hu et al. (2018) have used the same data as us to assess the associations between PFAS and carpet. Based on the 2005-2006 NHANES data, they concluded that fully or partially carpet covered floors were associated with serum concentrations of PFOS, PFHxS,

and MeFOSAA (p-value<0.05). However, they did not distinguish between the contributions of various types of carpeting. Although they may have applied sampling weights to obtain the regression parameter estimates that are representative of the general US population, their analysis did not appear to incorporate other attributes of the complex survey design (cluster and stratification) to obtain the correct variance estimation, given this limitation of the gam package in R that they used. Failure to account for the homogeneity of individuals within a cluster would lead to inaccurate lower variance estimates that bias the results of statistical hypothesis testing (NHANES, 2020b). Although Hu et al. (2018) applied statistical methods to deal with left-censored data on serum PFAS concentrations, it is not clear how they addressed missing values in the explanatory variables.

Our study has several limitations. First, our analyses were restricted to the 2005-2006 cycle based on the unique availability of the key variable interest, type of floor covering in the NHANES datasets. Future research collecting and/or using more recent data from other sources would be a valuable addition to the present study. Second, although similar compositional patterns have been found in carpets and dust previously (Wu et al., 2020), there is a lack of linkage between PFAS in air and/or dust samples and PFAS in the house carpets in our study due to the limitation of the NHANES datasets. Also, lack of information on other furniture and upholstery, which may also be treated with PFAS, may also impact our inference due to the potential uncontrolled confounding. Third, although we tried to obtain a crude estimate of PFAS exposure through selection of several key dietary recall variables, there are no direct measurements of PFAS in the NHANES participants' individual diets and drinking water. Although dietary seafood intake has been found to be a strong predictor of serum PFAS concentrations (Christensen et al., 2007), our use of seafood

consumption in the past 30 days is limited in its ability to predict long-term seafood consumption. Previous studies have implicated consumption of PFAS-contaminated tap water as a significant predictor for serum PFAS concentrations (Shin et al., 2011a; Shin et al., 2011b) and published exposure-pharmacokinetic models predict that tap water contributions can be dominant at fairly low PFAS water concentrations (Bartell, 2017; Lu and Bartell, 2019). We did not adjust for the amount of tap water consumed due to the spatial heterogeneity of PFAS contamination in US public water supplies, the removal of geographical identifiers from public use NHANES data sets, and the limitations of using short-term recall data to represent long-term water consumption. Future research linking individual-level data from NHANES to the nationwide PFAS detection data in public water supplies (UCMR 3) would help assess the contributions of drinking water to serum PFAS.

2.6 Conclusions

This study found that low pile carpeting was associated with increased serum concentrations of PFHxS and MeFOSAA in the general US population, while no clear association has been observed between the other types of carpeting and serum PFAS concentrations. Further studies would be needed to fully understand PFAS formulations in different types of carpeting.

2.7 Supplemental Material

Table S2.1. Adjusted Associations* from Multiple Regression Models with Complete Case Analysis (N=1,044)

% difference in PFAS concentrations**, 95% CI	PFOA	PFOS	PFHxS	MeFOSAA	PFDA	PFNA
Type of floor covering						
Smooth surface	ref	ref	ref	ref	ref	ref
Low pile carpet	9, (-10, 31)	17, (-6, 47)	20, (-14, 67)	27, (3, 56)	8, (-17, 40)	7, (-17, 37)
Medium to high pile carpet	2, (-20, 30)	-2, (-23, 26)	17, (-18, 66)	16, (-11, 52)	-8, (-26, 15)	-7, (-26, 17)
Mixed surface	1, (-22, 31)	8, (-23, 51)	11, (-25, 63)	33, (-6, 88)	5, (-21, 39)	3, (-20, 33)
p-value of Wald Chi-squared test	*0.6*	*0.03*	*0.7*	*0.07*	*0.6*	*0.3*

* We adjusted for age, gender (female/male), race/ethnicity (Hispanic/non-Hispanic black/non-Hispanic white/others), education (less than college/some college/college graduate or above), country of birth (foreign/US), veteran/military status (yes/no), family PIR, tap water source (don't drink tap water/community supply/others), eating out per week (yes/no), eating shellfish during past 30 days (yes/no), eating fish during past 30 days (yes/no), serum creatine, had at least one period in the past 12 months (yes/no), number of pregnancies, and number of children breastfed at least 1 month in the regression models.

** We exponentiated the regression coefficients, subtracted by one, and multiplied by 100% to estimate the percent difference in PFAS concentrations associated with each predictor.

Table S2.2. Adjusted Associations* from Multiple Regression Models with Multiple Imputation (k=100) for Adults (n=1,593)

% difference in PFAS concentrations**, 95% CI	PFOA	PFOS	PFHxS	MeFOSAA	PFDA	PFNA
Type of floor covering						
Smooth surface	ref	ref	ref	ref	ref	ref
Low pile carpet	7, (-9, 27)	15, (-4, 37)	33, (2, 73)	27, (6, 52)	13, (-8, 38)	8, (-11, 32)
Medium to high pile carpet	-3, (-23, 21)	-2, (-20, 20)	22, (-15, 73)	23, (-6, 61)	-8, (-25, 14)	-8, (-26, 14)
Mixed surface	1, (-23, 30)	5, (-21, 39)	15, (-17, 60)	22, (-9, 65)	5, (-20, 39)	6, (-18, 38)
p-value of Wald Chi-squared test	*0.5*	*0.03*	*0.2*	*0.06*	*0.1*	*0.3*

* We adjusted for age, gender (female/male), race/ethnicity (Hispanic/non-Hispanic black/non-Hispanic white/others), education (less than college/some college/college graduate or above), country of birth (foreign/US), veteran/military status (yes/no), family PIR, tap water source (don't drink tap water/community supply/others), eating out per week (yes/no), eating shellfish during past 30 days (yes/no), eating fish during past 30 days (yes/no), serum creatine, had at least one period in the past 12 months (yes/no), number of pregnancies, and number of children breastfed at least 1 month in the regression models.

** We exponentiated the regression coefficients, subtracted by one, and multiplied by 100% to estimate the percent difference in PFAS concentrations associated with each predictor.

Table S2.3. Adjusted Associations* from Multiple Regression Models with Multiple Imputation (k=100) for Adolescents (n=730)

% difference in PFAS concentrations**, 95% CI	PFOA	PFOS	PFHxS	MeFOSAA	PFDA	PFNA
Type of floor covering						
Smooth surface	ref	ref	ref	ref	ref	ref
Low pile carpet	5, (-11, 24)	11, (-7, 33)	29, (-14, 92)	13, (-9, 41)	11, (-12, 42)	15, (-9, 45)
Medium to high pile carpet	6, (-16, 35)	5, (-26, 49)	32, (-33, 158)	33, (-7, 91)	3, (-25, 42)	13, (-19, 59)
Mixed surface	-27, (-59, 30)	-10, (-43, 44)	8, (-56, 167)	19, (-28, 97)	-16, (-54, 51)	-32, (-75, 83)
p-value of Wald Chi-squared test	*0.5*	*0.6*	*0.6*	*0.4*	*0.6*	*0.4*

* We adjusted for age, gender (female/male), race/ethnicity (Hispanic/non-Hispanic black/non-Hispanic white/others), education (less than college/some college/college graduate or above), country of birth (foreign/US), veteran/military status (yes/no), family PIR, tap water source (don't drink tap water/community supply/others), eating out per week (yes/no), eating shellfish during past 30 days (yes/no), eating fish during past 30 days (yes/no), serum creatine, had at least one period in the past 12 months (yes/no), number of pregnancies, and number of children breastfed at least 1 month in the regression models.

** We exponentiated the regression coefficients, subtracted by one, and multiplied by 100% to estimate the percent difference in PFAS concentrations associated with each predictor.

Table S2.4. Adjusted Associations* from Multiple Regression Models with Multiple Imputation and Additional Adjustment for BMI (k=100) (N=2,323)

% difference in PFAS concentrations**, 95% CI	PFOA	PFOS	PFHxS	MeFOSAA	PFDA	PFNA
Type of floor covering						
Smooth surface	ref	ref	ref	ref	ref	ref
Low pile carpet	6, (-8, 23)	14, (-2, 32)	31, (2, 68)	23, (7, 43)	12, (-7, 34)	9, (-8, 29)
Medium to high pile carpet	-2, (-18, 17)	-2, (-19, 19)	22, (-15, 74)	22, (-5, 58)	-7, (-22, 12)	-6, (-21, 12)
Mixed surface	-3, (-22, 21)	2, (-20, 30)	12, (-19, 53)	18, (-9, 54)	2, (-19, 30)	4, (-17, 30)
p-value of Wald Chi-squared test	*0.6*	*0.02*	*0.1*	*0.04*	*0.2*	*0.3*

* We adjusted for age, gender (female/male), race/ethnicity (Hispanic/non-Hispanic black/non-Hispanic white/others), education (less than college/some college/college graduate or above), country of birth (foreign/US), veteran/military status (yes/no), family PIR, tap water source (don't drink tap water/community supply/others), eating out per week (yes/no), eating shellfish during past 30 days (yes/no), eating fish during past 30 days (yes/no), serum creatine, had at least one period in the past 12 months (yes/no), number of pregnancies, and number of children breastfed at least 1 month in the regression models.

** We exponentiated the regression coefficients, subtracted by one, and multiplied by 100% to estimate the percent difference in PFAS concentrations associated with each predictor.

CHAPTER 3

PFAS in Drinking Water and Birthweight in the US

Contents

3.1 Abstract

Background: Per- and polyfluoroalkyl substances (PFAS) are ubiquitous in the serum of the general US population, and were detected in public water systems serving approximately 16.5 million US residents during 2013-2015. Low birthweight was associated with PFAS exposures in previous studies.

Methods: Birthweights for singleton births during 2013-2015 were obtained from CDC WONDER, multiply stratified by county, maternal age, race, education, smoking status, and parity. PFAS water concentrations were obtained from EPA UCMR3 database and aggregated by county. Multiple regression weighted by inverse variance was used to produce effect estimates equivalent to those that would be obtained from individual-level data on birthweight and confounders.

Results: Adjusting for stratification demographic confounders (maternal age, race, education, smoking status, and parity), we found an average change in birthweight of 0.9 g (95% CI: -0.02, 1.7), -1.3 g (-1.5, -1.0), -3.8 g (-4.5, -3.1), -3.8 g (-4.1, -3.5) per ng/L increase in the population-weighted average PFOA, PFOS, PFHpA, and PFHxS in public water supplies by county, respectively. We found an average change in birthweight of -1.0 g (95% CI: -1.1, -0.9) per ng/L increase in the sum of PFOA, PFOS, PFHpA, and PFHxS concentrations in public water supplies.

Conclusions: The direction and magnitude of association between PFAS and birthweight varied by PFAS chemical in this study. Conclusions are tempered by inherent limitations of the two public-use datasets, and by the sensitivity of our results to alternative methods such as mutual adjustment for co-exposures.

KEY WORDS: PFAS, geographic, pregnancy, birth outcomes

3.2 Introduction

PFAS are synthetic chemicals consisting of a fully fluorinated carbon chain. Due to their chemical and thermal stability, PFAS have been widely used in consumer products and industrial process since the 1940s, such as stain repellents, lubricants, paints, textiles, firefighting foams, non-stick cookware, and food packaging (Kissa, 2001). Long-chain PFAS can persist indefinitely in the environment, and bioaccumulate in humans and other organisms (Giesy and Kannan, 2001; Buck et al., 2011; ATSDR, 2018). Food sources, drinking water, dust, and air are the main exposure routes to humans (Egeghy and Lorber, 2011; Jian et al., 2017).

According to the National Health and Nutrition Examination Survey (NHANES) in 2015-2016, the geometric means of perfluorooctanoic acid (PFOA), perfluorooctane sulfonate (PFOS), perfluorononanoic acid (PFNA), and perfluorohexane sulfonic acid (PFHxS) in the serum samples of the general US population were 1.56 ng/ml, 4.72 ng/ml, 0.58 ng/ml, and 1.18 ng/ml, respectively (CDC, 2019). The half-lives for serum or plasma elimination of PFAS in humans range from 2 to more than 10 years in previous studies (Olsen et al., 2007; Bartell et al., 2010; Zhang et al., 2013; Li et al., 2018). For pregnant women, the PFAS concentrations in maternal serum, umbilical cord serum, and breast milk are strongly associated with each other (Kim et al., 2011).

Some studies suggest that exposure to PFAS could cause adverse reproductive health effects in humans (Apelberg et al., 2007; Fei et al., 2007; Li et al., 2017; Cao et al., 2018; Kishi et al., 2015), while others found little association (Kim et al., 2011; Savitz et al., 2012; Shi et al., 2017). Due to physiological changes during pregnancy, including increased glomerular filtration rate (GFR) and parallel expansion of blood volume (Gibson, 1973; Cheung and

Lafayette, 2013), the observed inverse association between PFAS concentrations and birthweight could be due to reverse causality or uncontrolled confounding factors such as GFR (Verner et al., 2015; Steenland et al., 2018). A recent meta-analysis found an inverse association between PFOA and birthweight when the blood was sampled late in the pregnancy; however, little association was found when blood was sampled at times less susceptible to physiological confounding or reverse causation (i.e., shortly before conception or early in pregnancy) (Steenland et al., 2018).

Under the third Unregulated Contaminant Monitoring Rule (UCMR3), the US Environmental Protection Agency (US EPA) tested thirty contaminants, including six PFAS, in public water systems (PWSs) during 2013-2015 (US EPA, 2012). Based on samples collected from multiple points in a PWS, UCMR3 provides scientifically valid data on the occurrence of unregulated contaminants. It is the most comprehensive dataset of PFAS occurrence in public drinking water in the US (Hu et al., 2016; Hurley et al., 2016).

Few epidemiological studies on PFAS and birthweight have accounted for or use study designs that are resistant to reverse causality/physiological confounding, or have considered co-exposure to PFAS other than PFOA and PFOS, or co-exposure with other pollutants. Based on UCMR3 and the birthweight data from CDC WONDER, we conducted a county-level study of PFAS and birthweight in the US while adjusting for maternal age, race, education, smoking status, and parity, a similar set of adjustment variables to previous studies (Savitz et al., 2012; Thompson et al., 2010; Kato et al., 2011). In addition, we were able to investigate co-exposures to other UCMR3 contaminants and overall water quality when examining the association between a specific PFAS and birthweight. Although use of county-level exposure measures likely introduces some degree of measurement error, this

study is free of reverse causality/physiological confounding due to our use of an external exposure metric, PFAS concentrations in public water, rather than an exposure metric potentially influenced by physiological processes, i.e., serum concentrations during pregnancy (Weisskopf and Webster, 2017). In addition, we show that our use of county-level multiple-stratified average birthweights in weighted regression models produces effect estimates equivalent to those that would be obtained from using individual-level data on birthweight and the stratification variables.

3.3 Methods

3.3.1 Data Collection

Multiple-stratified Birthweight Data. We obtained the average birthweight from singleton births from 2013-2015, multiple-stratified by county, maternal age, bridged race (race), education, tobacco use (smoking status), and live birth order (parity) from CDC WONDER (CDC, 2020). CDC suppresses data for groups with less than 10 births. Within each state, counties with less than the population of 100,000 persons were de-identified and combined under the label of "Unidentified Counties" in the dataset, thus we excluded them from our analysis as they could not be linked with the UCMR3 data. Equivalent subdivisions included "Parish" in Louisiana, "Borough" in Alaska, and "Independent City" in Virginia, Maryland, and Missouri. Overall, there were 580 US counties with populations greater than 100,000. In the states and years that applied the 1989 US Standard Certificate of Live Birth (Alabama, Arizona, Arkansas, Hawaii, Maine, Michigan, and West Virginia in 2013; Rhode Island during 2013-2014; Connecticut and New Jersey during 2013-2015), education and smoking status of the mothers were recoded by CDC as "Excluded" and "Not Reported", respectively, as they were not comparable to the data that used the 2003 revision of the birth certificate. We excluded the groups with "Unknown or Not Stated", "Excluded", or "Not Reported" information in education, smoking status, and parity, which accounted for 9.6% of the singleton births in the 580 large US counties (Figure S3.1, Supplemental Digital Content I). After the exclusion, the birthweight data covered 552 counties in the US. Overall, these counties could represent the US counties with populations greater than 100,000 that applied the 2003 revision of the birth certificate. For crude (unadjusted) epidemiological analysis, we obtained the average birthweight from singleton births from 2013-2015

stratified by county only, excluding the "Unknown or Not Stated", "Excluded", and "Not Reported" categories in education, smoking status, and parity.

PFAS and Other Water Quality Indicators. Under UCMR3, thirty contaminants, including six PFAS (PFOA, PFOS, PFBS, PFNA, PFHpA, and PFHxS) were monitored using analytical methods developed by the US EPA (US EPA, 2020). PFAS were monitored using EPA Method 537 at 4,908 US PWSs during 2013-2015, including almost all PWSs serving >10,000 people and a representative sample of around 800 PWSs serving ≤10,000 people. The number of UCMR3 water samples collected at each PWS during 2013-2015 ranged from 1 to 484. In total, 1,928 counties were monitored in UCMR3 over the 3 years, covering the 50 US states, District of Columbia (DC), and some of the other US territories. Multiple PWSs could serve the same county, and different counties could also share a common PWS. In our analyses, the number of water samples taken within a county was the sum of water samples from all the PWSs that serve this county; if a PWS served two counties, then the water samples were counted in the number of water samples for both counties. The distribution of the number of water samples taken per county is shown in Table S3.1 in Supplemental Digital Content I. The minimum reporting level (MRL) was 10 ng/L for PFHpA, 20 ng/L for PFOA and PFNA, 30 ng/L for PFHxS, 40 ng/L for PFOS, and 90 ng/L for PFBS. Overall, around 16.5 million people in the US were served by PWSs containing at least one of the six PFAS at concentrations exceeding the MRLs. Proxy indicators for PFAS exposure in this study include the percentage of water measurements with PFAS detection by county and the population-weighted average PFAS water concentrations by county. We merged the UCMR3 PFAS data with the CDC birthweight data by county, and excluded Hampton City in Virginia as it was not monitored under UCMR3. The final datasets include 551 counties in

the US (see Figure S3.1 and Table S3.2 in Supplemental Digital Content I), covering 47 US states and District of Columbia.

Because only a limited number of contaminants were monitored under UCMR3, and might not be indicative of general water quality, we also obtained the count of violations by PWS for the 551 counties during 2013-2015 from the US EPA (SDWIS Federal Reports Advanced Search) and used the average count of violations per PWS by county under the US EPA rules (US EPA, 2020) as an indicator of the overall water quality. Because only four counties had detections for PFBS and only ten counties had detections for PFNA, we excluded both PFBS and PFNA from the analysis. PFOA, PFOS, PFHpA, and PFHxS are all moderately or highly correlated with each other (correlation coefficients range from 0.49 to 0.74), which is expected as they often have shared sources. Other water quality indicators including the number of violations and other UCMR3 contaminants except for 1,4-dioxane are weakly associated with PFAS ($r<0.3$), and thus were not adjusted for in our analyses (see Figure S3.2). Among the 551 counties in the data, 87 counties had detection for at least one of PFOA, PFOS, PFHpA, and PFHxS. In comparison, among the 1,928 counties monitored under UCMR3 during 2013-2015, 162 counties had detection for at least one of the four PFAS.

For analyses using continuous PFAS concentrations, we only used data for the 87 counties with at least one detection of PFOA, PFOS, PFHpA, or PFHxS. For each UCMR3 water measurement in these 87 counties, we substituted values that were below the MRL with $\frac{MRL}{\sqrt{2}}$. After the substitution, for each PFAS chemical we first averaged the concentrations by PWS, and then averaged across PWSs by county, weighting by the average population served by each PWS during 2013-2015. The distribution of the number of water samples taken from the 87 counties is shown in Table S3.3 in Supplemental Digital Content I.

Descriptive statistics for the percentage of water measurements with detection for PFOA, PFOS, PFHpA, and PFHxS are shown in Table 3.1, and the population-weighted average water concentrations of PFAS in the counties with detection of at least one of the four PFAS are shown in Table 3.2. The average percentages in Table 3.1 are all less than 1% due to the fact that over 80% of the counties did not have detection for PFAS. Because our study only includes a small part of the counties monitored by UCMR3, we also show the descriptive statistics of water measurements for all 1,928 counties in UCMR3 and the 162 counties with detection of at least one of the four PFAS in UCMR3 in Table S3.1.1 and Table S3.1.2 in Supplemental Digital Content I. The average values of the two proxy indicators for PFAS exposure in all UCMR3 counties are similar to those of the counties covered by our study.

Table 3.1. Percentage of Water Measurements with Detection (%) of PFAS in 551 Counties in the US, 2013-2015

Contaminant	Mean	SD	Min	Max
PFOA	0.68%	3.35%	0.00%	42.98%
PFOS	0.59%	2.87%	0.00%	37.19%
PFHpA	0.65%	4.26%	0.00%	58.33%
PFHxS	0.40%	2.60%	0.00%	47.11%

Table 3.2. Population-weighted Average of UCMR3 Water Concentrations (ng/L) of PFAS in 87 Counties in the US with Detection of at least one of PFOA, PFOS, PFHpA, or PFHxS, 2013-2015. Values that were below the MRL were Substituted with $\frac{MRL}{\sqrt{2}}$.

Contaminant	Mean	SD	Min	Max
PFOA	14.84	1.33	14.14	20.53
PFOS	30.58	4.17	28.28	54.31
PFHpA	7.74	1.88	7.07	20.18
PFHxS	22.60	2.92	21.21	35.08

3.3.2 Weighted Linear Regression Models

We obtained multiple-stratified county-level birthweight statistics (mean and standard deviation) from the CDC. We calculated the aggregated county-level birthweight statistics (Table 3.3) for the 551 counties, for which the distributions of age, race, education, smoking status, and parity are similar to that of the entire US population (Table S3.4, Supplemental Digital Content I). In the Supplemental Digital Content II, we show that using county-level multiple-stratified average birthweights in weighted regression models produces equivalent effect estimates to those that would be obtained from individual-level data on birthweight and confounders. In particular, using the number of births in each stratum for the weights produces the same effect estimate that would be obtained from unweighted multiple linear regression with the individual-level data, and using the inverse variance (Neter et al., 1996) of the average birthweight for the weights produces the same effect estimate that would be obtained from individual-level weighted regression allowing for heteroscedasticity.

This is a very useful result for avoiding aggregation bias (also known as ecological fallacy) for analysis of public-use birthweight data and other data sets that multiply stratify on key confounding variables. However, results will only be identical for covariates that are available with multiple stratification at the county-level; adjustment for covariates that are not multiply stratified (e.g. US Census poverty rates) could result in different parameter estimates than those that would be obtained using individual-level data (e.g. personal socioeconomic status). Because of this mathematical result and the strong negative correlation between poverty and education level based on 2013, 2014, and 2015 American Community Survey (ACS) 1-year estimates (r=-0.82), we did not include the county-level

percentage of poverty in the primary analyses. Additional adjustment for the county-level poverty percentage was only conducted as sensitivity analyses. In addition, analyses using individual-level exposure measurements (e.g., tap water PFAS concentrations measured at each participant's home) may produce different results than analyses using group-level exposure assignments.

However, the estimated variances for the regression coefficients produced by statistical software packages are not identical for individual-level and group-level analyses. Fortunately, we were able to derive formulas for obtaining the correct variance estimates for the regression coefficients from individual-level analysis based on the multiple stratified data and group-level regression output. For our analyses relating PFAS to birthweight, the corrected variance formulas produce smaller estimated variances and confidence intervals than we had originally reported. Here we present variance correction formulas.

We derived the correct estimated variance and standard error (SE) for the individual-level inverse-variance weighted regression parameters based on the group-level inverse-variance weighted regression as follows. The detailed derivation and explanations have been added to the Supplemental Digital Content II of the publication.

$$\widehat{var}(\hat{\beta}_{individual}) = \frac{(n\ + WSSE_{group})(m-p-1)}{(n-p-1)WSSE_{group}} \cdot \widehat{var}(\hat{\beta}_{group}),$$

$$\widehat{se}(\hat{\beta}_{individual}) = \sqrt{\frac{(n\ + WSSE_{group})(m-p-1)}{(n-p-1)WSSE_{group}}} \cdot \widehat{se}(\hat{\beta}_{group}).$$

In the above formulas, n is the total number of births (observations in individual-level analysis), m is the number of groups (observations in group-level analysis), and p is the number of regression parameters not including the intercept. $WSSE_{group}$ is the weighted

sum of squared errors of the group-level analysis. The standard error $\widehat{se}(\hat{\beta}_{group})$ can be obtained easily from the output of group-level weighted regression.

In secondary analyses, we employed lasso regression to account for exposure mixtures, penalizing the coefficients for each PFAS chemical and 1,4-dioxane.

We used statistical software R, version 3.6.0 for statistical analyses.

Table 3.3. Predictors of Birthweight* among Singleton Pregnancies in 551 Counties in the US, 2013-2015

Categories	No. of Births (%)	Average Birthweight (gram) (SD)
Total	8,128,278 (100%)	3,311 (537)
Maternal Age (years)		
<15	2,443 (0.03%)	3,072 (538)
15-19	473,761 (5.8%)	3,176 (536)
20-24	1,715,641 (21.1%)	3,244 (532)
25-29	2,346,570 (28.9%)	3,322 (531)
30-34	2,318,108 (28.5%)	3,358 (535)
35-39	1,076,339 (13.2%)	3,350 (557)
40-44	192,050 (2.4%)	3,312 (579)
45-49	3,343 (0.04%)	3,273 (606)
≥50	23 (0.0%)	3,346 (607)
Race		
American Indian or Alaska Native	30,786 (0.4%)	3,339 (552)
Asian or Pacific Islander	612,625 (7.5%)	3,215 (500)
Black or African American	1,351,695 (16.6%)	3,140 (593)
White	6,133,172 (75.5%)	3,358 (528)

Education		
8th grade or less	267,655 (3.3%)	3,316 (537)
9th through 12th grade with no diploma	878,382 (10.8%)	3,217 (548)
High school graduate or GED completed	1,908,856 (23.5%)	3,260 (552)
Some college credit, but not a degree	1,665,300 (20.5%)	3,302 (551)
Associate degree	571,644 (7.0%)	3,347 (538)
Bachelor's degree	1,635,478 (20.1%)	3,380 (515)
Master's degree	739,914 (9.1%)	3,381 (509)
Doctorate or professional degree	198,879 (2.5%)	3,356 (495)
Unknown or not stated	94,496 (1.2%)	3,297 (572)
Excluded	167,674 (2.1%)	3,304 (544)
Smoking Status		
No	7,437,775 (91.5%)	3,320 (536)
Yes	366,430 (4.5%)	3,123 (559)
Not reported	262,122 (3.2%)	3,306 (545)
Unknown or not stated	61,951 (0.8%)	3,313 (538)
Parity		
1st	3,276,032 (40.3%)	3,258 (547)
2nd	2,573,346 (31.7%)	3,355 (517)

3rd and over	2,254,966 (27.7%)	3,337 (546)
Unknown or not stated	23,934 (0.3%)	3,274 (555)

* This table summarizes birthweights singly stratified by one predictor at a time, but regression models used multiply stratified birthweight statistics.

3.4 Results

In Figure 3.1 and Figure 3.2, we display the relation between average birthweight and the two proxies for PFAS exposure while using inverse-variance weights in the regression models.

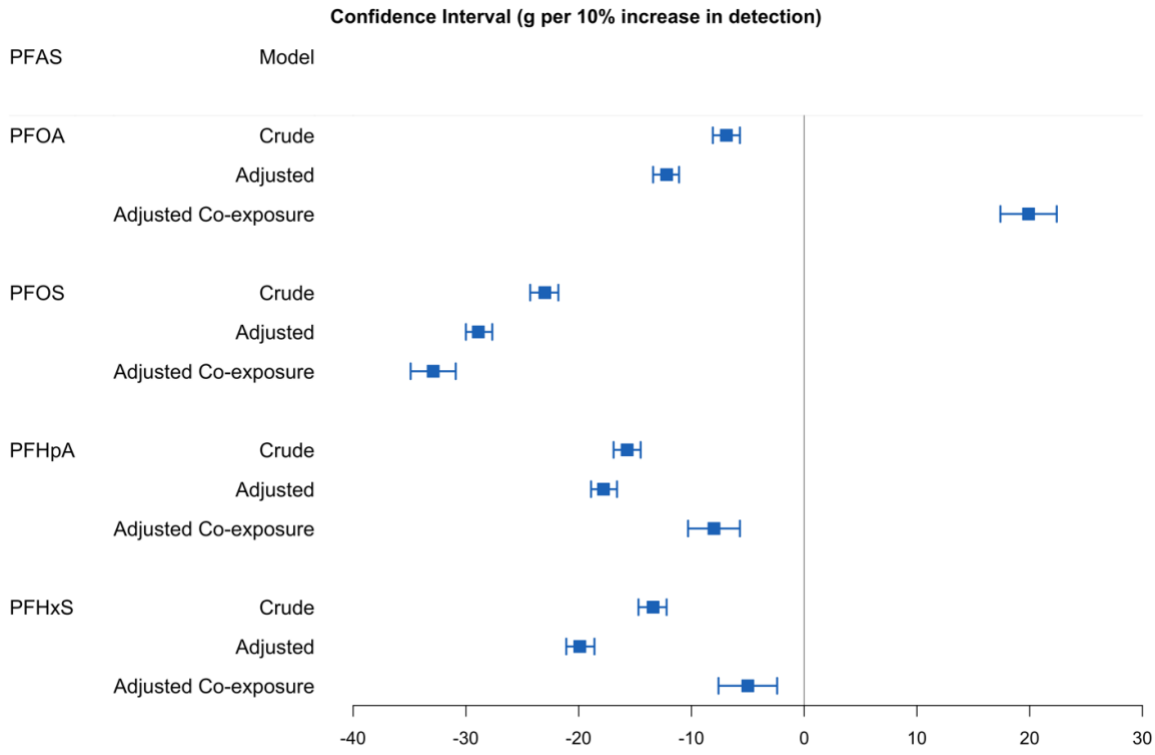

Figure 3.1. The change of average birthweight (g) for 10% increase in the detection of PFAS: MLE, 95% CI. Using regressions weighted by inverse variance of average birthweight. Crude model: association between PFAS and birthweight only. Adjusted model: adjusted for maternal age (<15, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (8th grade or less; 9th through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (Yes, No), and parity (1st, 2nd, 3rd and over). Adjusted co-exposure model: adjusted for the other three PFAS, 1,4-dioxane, and all covariates in the adjusted model.

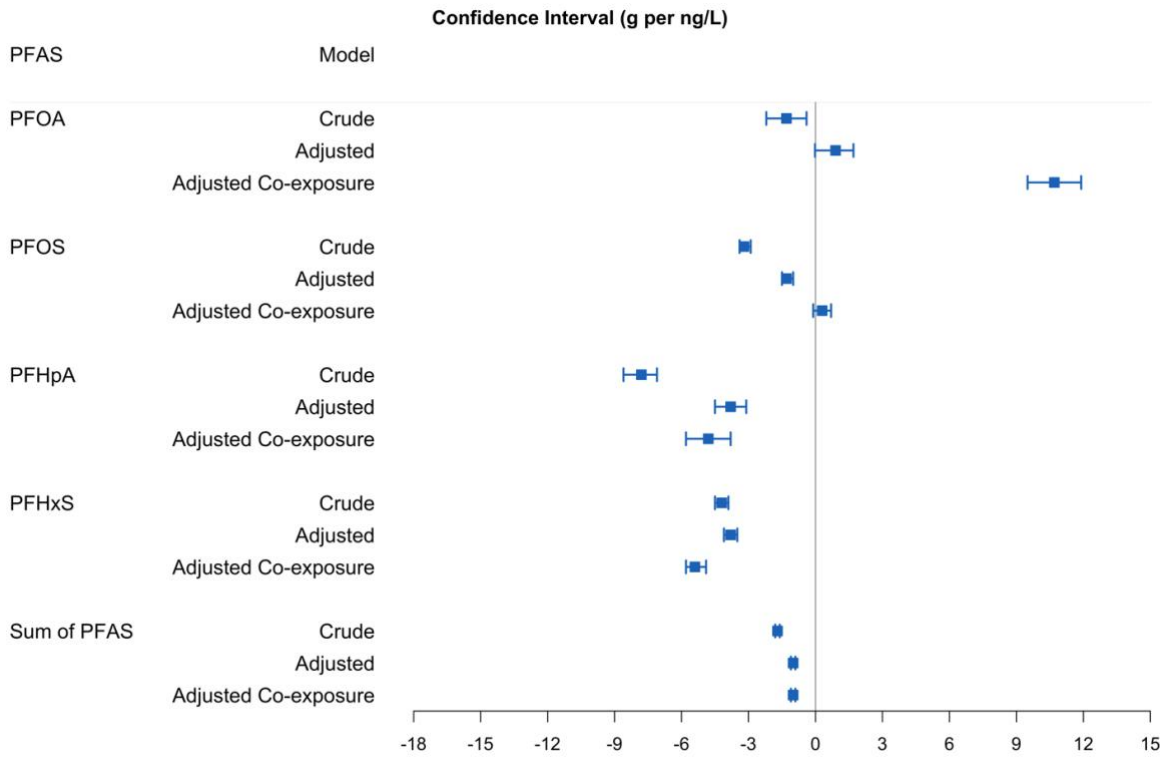

Figure 3.2. The change of average birthweight (g) for 1ng/L increase in the population-weighted average PFAS water concentration: MLE, 95% CI (1 g per ng/L = 1 g per ppt = 1,000 g per ng/ml). Using $\frac{MRL}{\sqrt{2}}$ substitution for the non-detections and regressions weighted by inverse variance of average birthweight. Crude model: association between PFAS and birthweight only. Adjusted model: adjusted for maternal age (<15, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (8th grade or less; 9th through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (Yes, No), and parity (1st, 2nd, 3rd and over). Adjusted co-exposure model: adjusted for the co-exposures (the other three PFAS and 1,4-dioxane; or 1,4-dioxane only for the model includes the sum of PFAS), and all covariates in the adjusted model.

Using inverse-variance weights and covariate-adjusted models, we found for the 551 counties significant negative associations between birthweight and PFAS detection; while adjusting for co-exposures to other PFAS and 1,4-dioxane reversed the effect estimate for PFOA (Figure 3.1). In addition, we compared two different weights in regression: group size (number of births), and inverse variance of average birthweight. The results of analyses using these two sets of weights in different models are similar, but adjustment for confounders produces somewhat different results from the crude analyses (Table S3.5 - Table S3.8, Supplemental Digital Content I).

For the 87 counties with detection of at least one of the four PFAS, we examined the association between the population-weighted average PFAS water concentrations and birthweight, using inverse-variances as regression weights (Table S3.9 - Table S3.12, Supplemental Digital Content I). We also summed the population-weighted average water concentrations of the four PFAS to determine the overall association of PFAS with birthweight (Table S3.13, Supplemental Digital Content I). Using inverse-variance weights and $\frac{MRL}{\sqrt{2}}$ substitution in the covariate-adjusted models, we found no association between birthweight and PFOA concentration (0.9, [-0.02, 1.7] g per ng/L, Table S3.9), and significant negative associations between birthweight and PFOS (-1.3, [-1.5, -1.0] g per ng/L, Table S3.10), PFHpA (-3.8, [-4.5, -3.1] g per ng/L, Table S3.11), and PFHxS (-3.8, [-4.1, -3.5] g per ng/L, Table S3.12) concentrations. Additionally adjusting for co-exposures to other PFAS and 1,4-dioxane greatly impacted the effect estimate for PFOA. Overall, the sum of four PFAS was negatively associated with birthweight (-1.0, [-1.1, -0.9] g per ng/L, Table S3.13). We also conducted sensitivity analyses with zero substitution and MRL substitution to compare to the results from $\frac{MRL}{\sqrt{2}}$ substitution. For PFOA, PFHpA, PFHxS, and the sum of four PFAS, the

results were consistent regardless of the substitution methods (Table S3.9, Table S3.11, Table S3.12, and Table S3.13). For PFOS, the three substitution methods produced noticeably different results when adjusting for other PFAS (Table S3.10).

3.5 Discussion

We used the public-use dataset of UCMR3 and Natality data from CDC WONDER to conduct a county-level study of birthweight and PFAS concentrations in drinking water in the US, with multiple stratification by key confounding variables to yield equivalent estimation of the individual-level associations. There are several advantages of using the two datasets in the study. First, epidemiological associations using PFAS water concentrations are free of reverse causality and/or physiological confounding, which may have biased the epidemiological associations reported by other studies using PFAS serum measurements, especially when collected late in pregnancy. Second, we explore the association between PFAS and birthweight more comprehensively by including some understudied PFAS chemicals, i.e., PFHxS and PFHpA. Third, the availability of multiple-stratified birthweight data from CDC allows us to control for maternal age, education, race, smoking status, and parity, producing equivalent results to those that would be obtained from individual-level data on birthweight and these confounding variables. In this study, weighting by group size (equivalent to ordinary multiple regression using individual-level data) or inverse variance (equivalent to weighted multiple regression for heteroscedasticity using individual-level data) produced similar results.

Continued exposure to relatively low PFAS concentrations in drinking water can substantially increase serum concentrations, with reported steady-state serum:drinking

water ratios of about 114:1 for PFOA, 125:1 for PFOS, and 194:1 for PFHxS (Bartell, 2003; Hoffman et al., 2011; Bartell, 2020; Lu and Bartell, 2020). After long-term consumption of contaminated drinking water, the population-weighted average water concentrations of 14.84 ng/L, 30.58 ng/L, and 22.60 ng/L (1 ng/L = 10-3 ng/ml) for the 87 counties in the US during 2013-2015 (Table 3.2) are expected to increase serum concentrations by about 1.7 ng/ml, 3.8 ng/ml, and 4.4 ng/ml for PFOA, PFOS, and PFHxS, respectively. The estimates are similar to the medians (interquartile range) of 1.6 (1.1-2.5) ng/ml and 4.8 (2.8-8.1) ng/ml for PFOA and PFOS, respectively, and higher than the median (interquartile range) of 1.2 (0.7-2.1) ng/ml for PFHxS for the general US population, suggesting that these exposures may have had measurable impacts on serum PFAS concentrations in these communities. The estimated effect sizes for PFAS serum concentrations would be 1/114, 1/125, and 1/194 of that of the water concentrations for PFOA, PFOS, and PFHxS, respectively. So, after adjusting for potential demographic confounders and correcting the standard errors, our estimated effects are equivalent to an average change in birthweight of 7.6 g (95% CI: -0.2, 15.3) per ng/ml increase in serum PFOA; -10.1 g (95% CI: -12.0, -8.2) per ng/ml increase in serum PFOS; and -19.5 g (95% CI: -21.1, -17.9) per ng/ml increase in serum PFHxS. In comparison, a recent meta-analysis by Steenland et al. (2018) (Steenland et al., 2018) reported a change in birthweight of -10.5 g (-16.7, -4.4) per ng/ml increase of PFOA in maternal or cord blood; and -3.3 g (-9.6, 3.0) per ng/ml when restricting to studies where blood was sampled early in pregnancy or shortly before conception, similar to the null association we found between PFOA and birthweight while adjusting for demographic confounders. Nevertheless, this is just a preliminary comparison without accounting for any uncertainty for the water to serum conversion factor, which is beyond the scope of this paper and should be addressed in future

research. In addition, with the above estimates after water to serum conversions, we can expect a change in birthweight of 10.6 g (95% CI: -0.3, 21.4) for an interquartile range (IQR) change of serum PFOA (1.4 ng/ml); -53.5 g (95% CI: -63.6, -43.5), for an IQR change of serum PFOS (5.3 ng/ml), and -27.3 g (95% CI: -29.5, -25.1) for an IQR change of serum PFHxS (1.4 ng/ml) in the general US population.

Our study also has a number of limitations. First, we attempted to identify the causal effects of PFAS on birthweight, but our interpretations are limited by the observational nature of the data and limited availability of multiple-stratified variables that had been collected on birth certificates at the individual level, which makes it difficult to rule out measurement error and uncontrolled confounding. Although effect estimates for the percentage of water measurements with detection for each PFAS became slightly larger in the negative direction after adjustment for known multiple-stratified confounders, suggesting that further adjustment using more accurate confounder measures would only increase the absolute effect sizes, we cannot guarantee the absence of an unidentified confounder strong enough to reverse the association. In sensitivity analyses, additional adjustment for the county-level percentage of poverty did not substantially change the results in Figure 3.1 and Figure 3.2 (see Figure S3.3, Figure S3.4, Table S3.14, and Table S3.15 in Supplemental Digital Content I), with the exception of population-weighted average PFOA water concentration, for which the effect was changed from null (0.9 g per ng/L, 95% CI: [-0.02, 1.7]; Table S3.9) to negative (-2.4 g per ng/L, 95% CI: [-3.3, -1.5]; Table S3.15) in the model adjusted for demographic confounders. Nevertheless, the percentage of poverty was barely correlated with the two proxy indicators for PFAS exposure on the county-level (Table S3.16 and Table S3.17, Supplemental Digital Content I); therefore, it was unlikely to confound

the associations observed in Figure 3.1 and Figure 3.2. The change in effect estimate for PFOA from Figure 3.2 to Figure S3.4 by additionally adjusting for the county-level percentage of poverty highlights the difficulty in interpreting multi-level studies, and potential cross level bias in ecological inference when including a county-level variable (i.e., the percentage of poverty) that is highly correlated with an individual-level variable (i.e., education level) (Blakely and Woodward, 2000). The increases in precision from crude models to adjusted models in Figure 3.1 and Figure 3.2 can be explained by the well-established result of decreased residual standard error for multiple regression after adjusting for strong predictors of the outcome (Fisher, 1934). This setting is quite different from logistic regression, for which adjustment for covariates can result in a loss or at best no gain of precision (Robinson and Jewell, 1991). Adjustment for co-exposure to other PFAS and 1,4-dioxane changed the effect estimates differently for the percentage of water measurements with detection for each PFAS (Figure 3.1), reversing the effect from negative to positive for PFOA, increasing the effect size in the negative direction for PFOS, and attenuating the negative associations towards the null for the other two PFAS. This highlights some of the difficulties in fitting and interpreting statistical models with correlated exposure mixtures, even using a large dataset. In particular, bias amplification could occur due to residual confounding while including co-exposures with a common source (Weisskopf et al., 2018), which could explain why the adjustment for the other PFAS had such a strong effect on the regression parameter for PFOA. However, the direction and magnitude of bias amplification are not readily predictable in this setting. Overall, we believe that the results from adjusted models that do not include co-exposures are more reliable because they are less susceptible to bias amplification (Weisskopf et al., 2018). In secondary analysis, we used lasso regression

(with 10-fold cross-validation to obtain the optimal shrinkage parameter) to penalize the coefficients for four PFAS and 1,4-dioxane in inverse-variance weighted models. In this analysis, the coefficients for PFOA, PFHpA, and PFHxS are attenuated towards the null; while the coefficient for PFOS does not change significantly and the coefficient for 1,4-dioxane is zeroed out. In the models using population-weighted average water concentrations of the chemicals (PFOA, PFOS, PFHpA, PFHxS, and 1,4-dioxane) as proxy exposure for the 87 counties with at least one detection for PFOA, PFOS, PFHpA, or PFHxS, adjustment for co-exposure increases the effect size in the positive direction for PFOA, attenuates the negative association towards the null for PFOS, increases the effect size in the negative directions for PFHpA and PFHxS (Figure 3.2). In the lasso regression using inverse-variance weights and $\frac{MRL}{\sqrt{2}}$ substitution, the coefficient for population-weighted average water concentration of PFOS is zeroed out, and the coefficients for population-weighted average water concentrations of the other PFAS are attenuated towards the null.

Second, the public-use birthweight data from CDC WONDER has several notable drawbacks, as shown by the flow chart of data processing in Figure S3.1. (A) CDC suppresses data for the groups with less than 10 births; when we multiply stratified the data by county, maternal age, race, education, smoking status, and parity, we lost 5.3% of births compared to the data that was only stratified by county. (B) Counties with less than 100,000 population are de-identified in the dataset, which cannot be linked to the UCMR3 dataset, thus restricting the scope of the study to less than 580 counties. (C) There is likely some underreporting of maternal smoking status, which is difficult to obtain reliable data from birth certificate (Northam and Knapp, 2006). (D) We excluded the missing values from the data, which accounted for 9.6% of the singleton births in the 580 counties. The “Excluded”

category in education and "Not Reported" category in smoking status were missing at random, dependent only on the version of birth certificate used in the state in a specific year, rather than the value of the variables (education and smoking status) that are missing; and conditional on the version of birth certificate, the probability of missingness does not depend on the value of the variables. However, the "Unknown or Not Stated" in parity, education, and smoking status could depend on the actual values of these variables thus could be missing not at random. We lost all the births from 28 counties and 5.6% of the remaining 552 counties due to the exclusion. In all, the suppression, de-identification, and missingness reduced the number of births in our analysis by 32.9%; therefore, our results based on the 551 counties may not be generalizable to the whole US. For the 551 counties, the missingness rate is 5.3%. With CDC permission and security clearances it is possible to obtain access to unsuppressed and fully identified birthweight data at secure federal facilities; we are currently taking steps to apply for access.

Third, our estimates for PFAS exposure solely relied on the UCMR3 data, which has several limitations: (1) We were unable to account for other sources of exposure to PFAS and other chemicals, such as food, dust, air pollution, non-UCMR3 chemicals in water, and/or unmeasured PFAS chemicals may be associated with both UCMR3 PFAS exposure and birthweight, in which case they may contribute uncontrolled confounding to our results. (2) The detection thresholds (MRLs) for the six PFAS measured in UCMR3 were as much as 16 times higher than the detection limits for the current standard testing method (Method 537), so only the highest levels of PFAS contamination were reflected in the data; thus, it is likely that the percentages of water measurements with PFAS detection for each county underestimate the true extent of exposure. (3) Because UCMR3 only provides the PFAS

concentrations in drinking water during 2013-2015 and the PFAS concentrations assigned for each county in our study may not reflect historical water exposure or total body burden for each individual, likely adding some degree of nondifferential exposure measurement error. (4) The number of samples taken from each PWS varied substantially across different counties but was often small, limiting the precision of the average concentrations at the PWS level. (5) One PWS could serve several counties, but we only know the total population served by each PWS rather than the population served by each PWS within each county, thus the population-weighted average water concentrations of PFAS by county could also be inaccurate. (6) UCMR3 was designed to monitor water quality by PWS, not to measure county-level exposure or exposure to specific subgroups defined by race, education or other characteristics that might be associated with residential location and water supply within a county; however, we assumed that everyone within a county had the same average PFAS exposure level and assign the population-weighted average water concentrations of PFAS to all the groups within the same county. (7) We also did not account for the fact that some people in these counties may be using private wells or smaller water systems not included in UCMR3, and thus may have had different exposures than their neighbors. We understand that USGS is developing information on PFAS in private wells in the US; incorporating that information into birthweight analyses would be a valuable future direction.

3.6 Supplemental Digital Content I

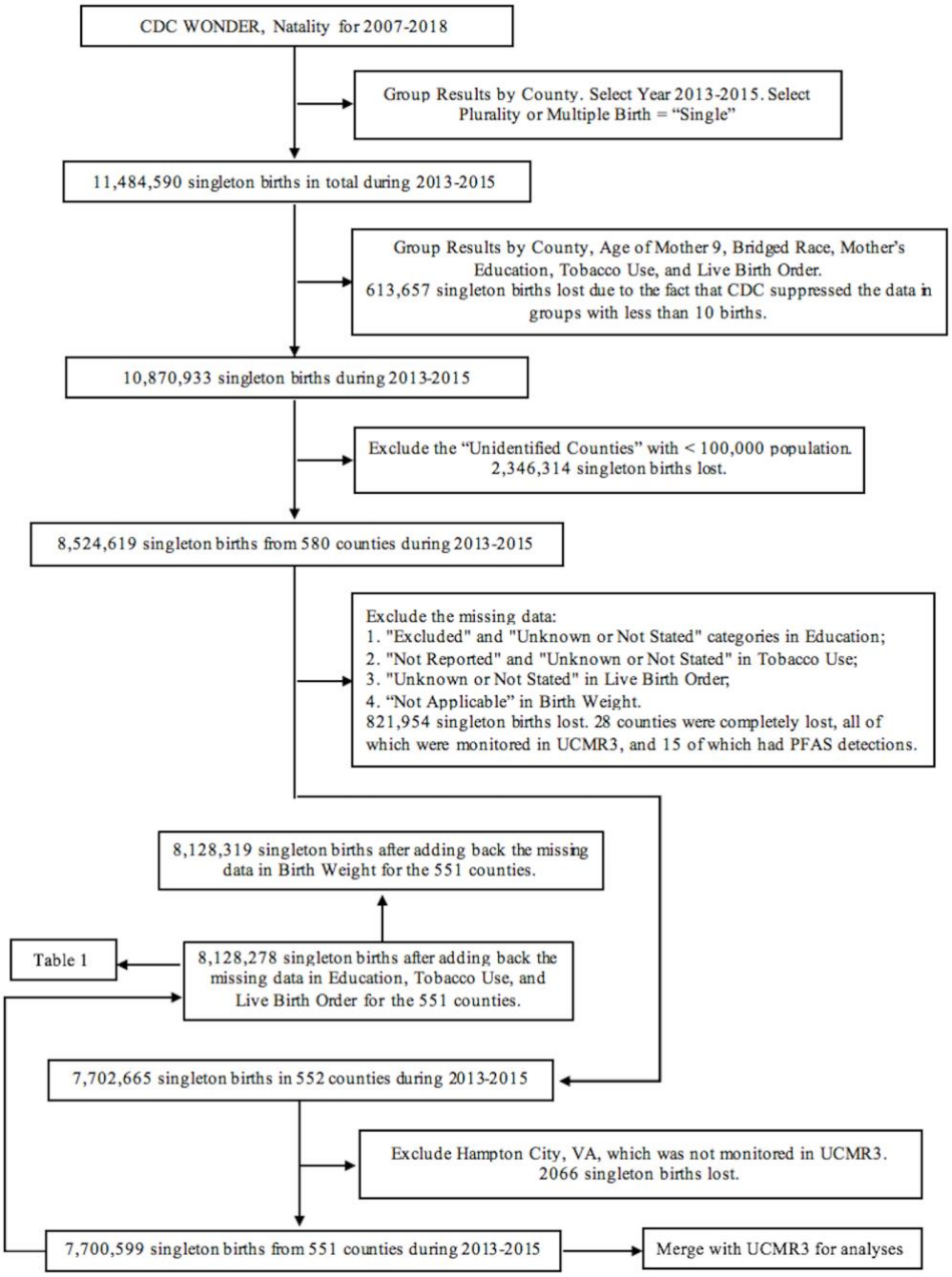

Figure S3.1. Study Profile

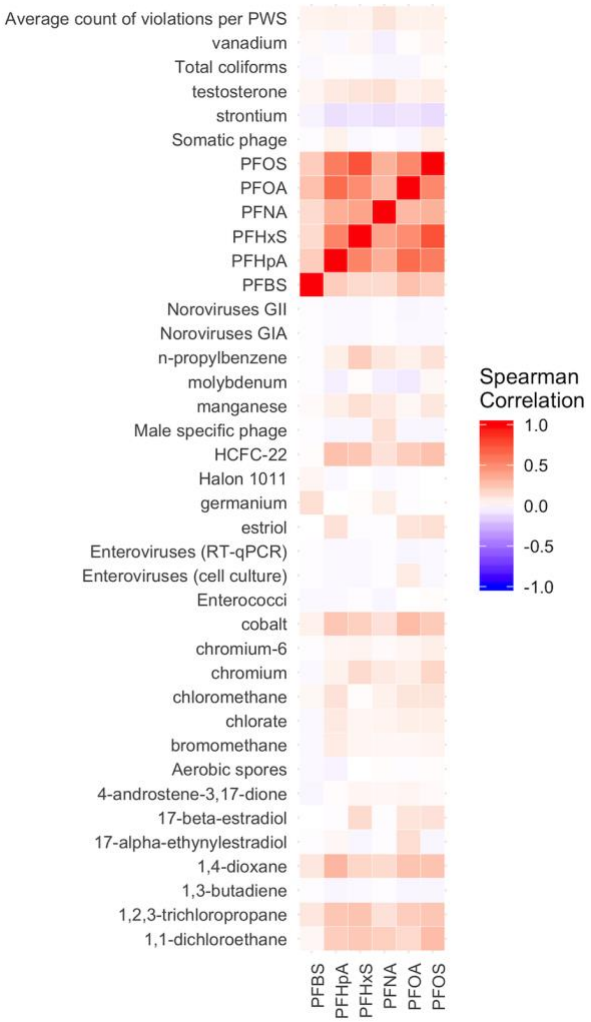

Figure S3.2. Spearman correlation heatmap between PFAS and other water quality indicators under the US EPA rules in 551 counties in the US, 2013-2015. Noroviruses GIA: Noroviruses genogroup I with RT-qPCR primer set A; Noroviruses GII: Noroviruses genogroup II. E. coli, equilin, estrone, Noroviruses GIB (genogroup I with RT-qPCR primer set B), sec-butylbenzene, and tellurium had no detections under UCMR3 and were excluded from the correlation heatmap.

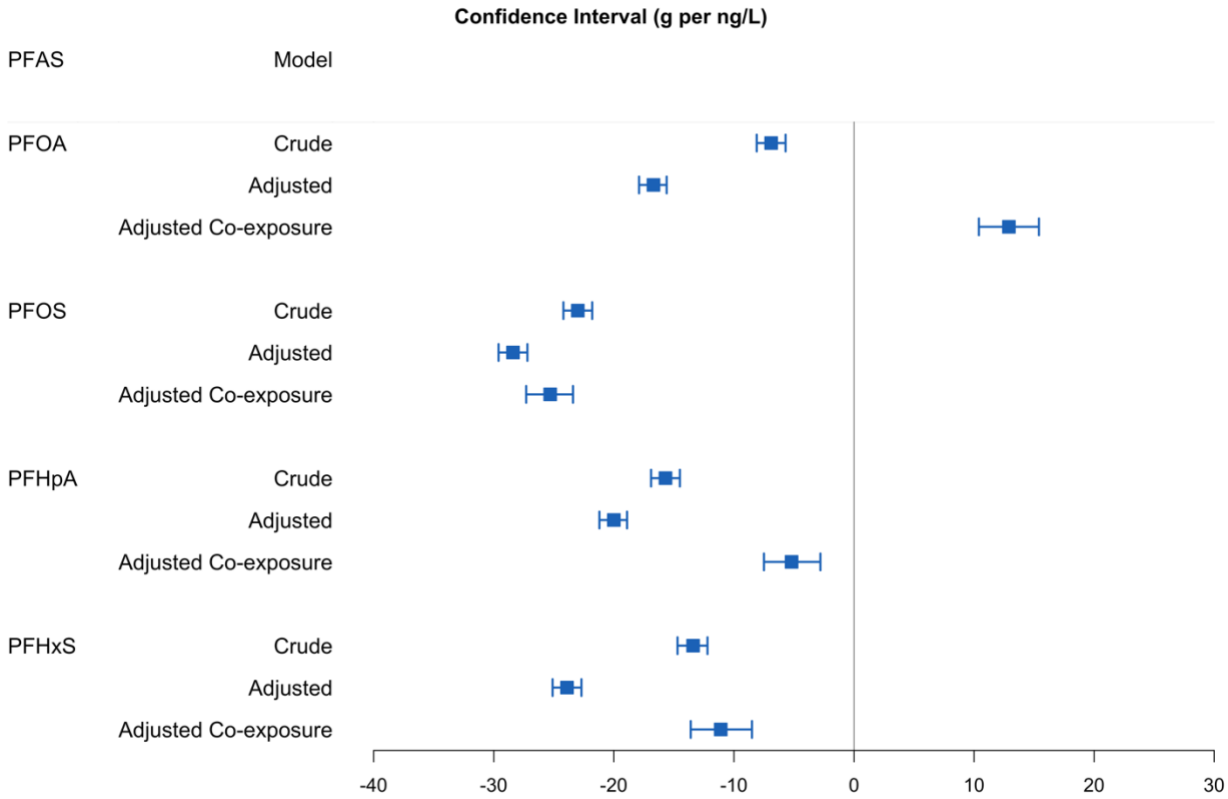

Figure S3.3. The change of average birthweight (g) for 10% increase in the detection of PFAS: MLE, 95% CI. Using regressions weighted by inverse variance of average birthweight. Crude model: association between PFAS and birthweight only. Adjusted model: adjusted for maternal age (<15, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (8th grade or less; 9th through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (Yes, No), parity (1st, 2nd, 3rd and over), and county-level percentage of poverty. Adjusted co-exposure model: adjusted for the other three PFAS, 1,4-dioxane, and all covariates in the adjusted model. Adjusted and adjusted co-exposure models in Figure S3.3 additionally adjusted for the county-level percentage of poverty compared with those in Figure 3.1 in the main text. Detailed effect estimates for the models in Figure S3.3 can be found in Table S3.14.

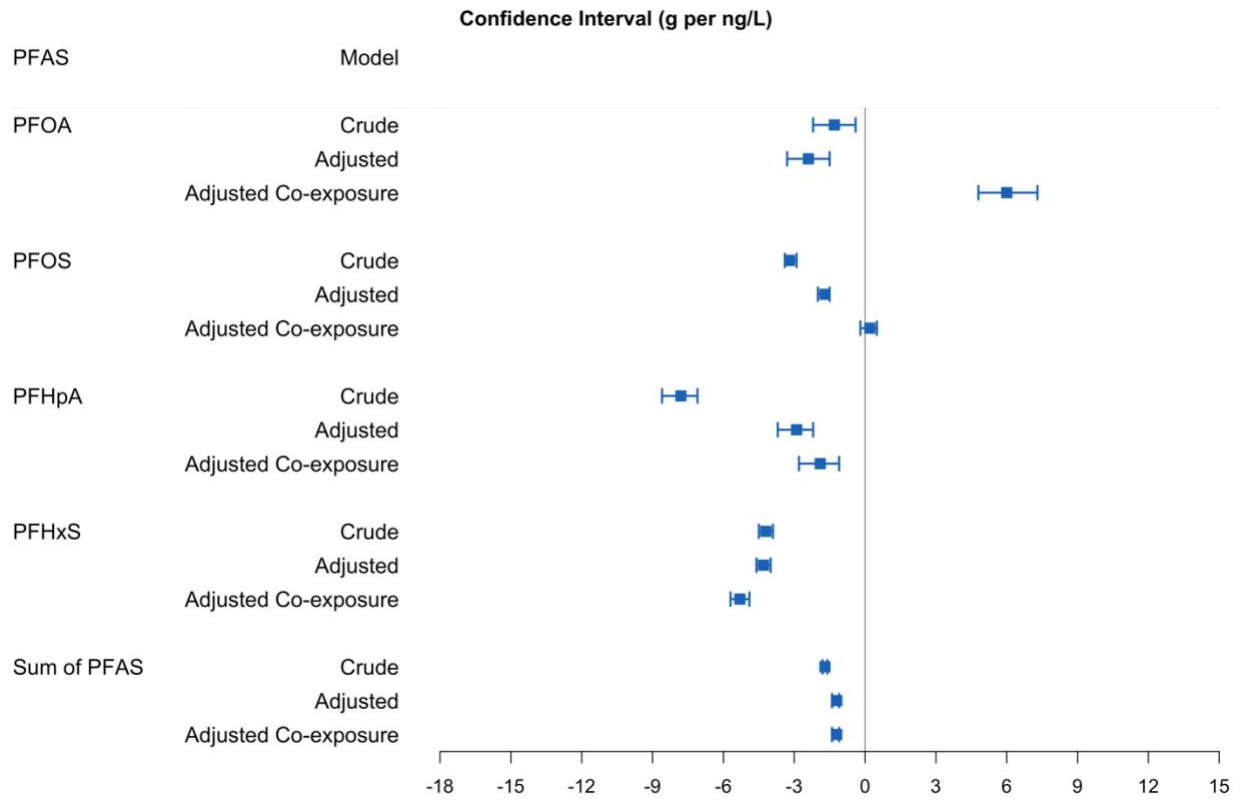

Figure S3.4. The change of average birthweight (g) for 1ng/L increase in the population-weighted average PFAS water concentration: MLE, 95% CI. Using $\frac{MRL}{\sqrt{2}}$ substitution for the non-detections and regressions weighted by inverse variance of average birthweight. Crude model: association between PFAS and birthweight only. Adjusted model: adjusted for maternal age (<15, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (8th grade or less; 9th through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (Yes, No), parity (1st, 2nd, 3rd and over), and county-level percentage of poverty. Adjusted co-exposure model: adjusted for the co-exposures (the other three PFAS and 1,4-dioxane; or 1,4-dioxane only for the model includes the sum of PFAS), and all covariates in the adjusted model. Adjusted and adjusted co-exposure models in Figure S3.4 additionally adjusted for the county-level percentage of poverty compared with those in Figure 3.2 in the main text. Detailed effect estimates for the models in Figure S3.4 can be found in Table S3.15.

Table S3.1. Number of Water Samples in 1,928 Counties Covered by UCMR3 in the US, 2013-2015

Min	1st Quantile	Median	Mean	3rd Quantile	Max	SD
1.00	4.00	10.00	24.55	22.00	1018.00	56.29

Table S3.1.1. Percentage of Water Measurements with Detection (%) of PFAS in 1,928 Counties Covered by UCMR3 in the US, 2013-2015

Contaminant	Mean	SD	Min	Max
PFOA	0.68%	4.27%	0.00%	100.00%
PFOS	0.36%	3.24%	0.00%	100.00%
PFHpA	0.35%	2.98%	0.00%	58.33%
PFHxS	0.19%	1.97%	0.00%	50.00%

Table S3.1.2. Population-weighted Average of UCMR3 Water Concentrations (ng/L) of PFAS in 162 Counties Covered by UCMR3 in the US with Detection of at least one of PFOA, PFOS, PFHpA, or PFHxS, 2013-2015. Values that were below the MRL were Substituted with $\frac{MRL}{\sqrt{2}}$.

Contaminant	Mean	SD	Min	Max
PFOA	15.64	4.25	14.14	63.60
PFOS	30.63	6.01	28.28	80.64
PFHpA	7.76	2.30	7.07	24.38
PFHxS	22.76	7.06	21.21	104.11

Table S3.2. Number of Water Samples in the 551 Counties that can be Merged with CDC Birthweight Data in the US, 2013-2015

Min	1st Quantile	Median	Mean	3rd Quantile	Max	SD
2.00	15.50	29.00	56.19	58.00	1018.00	94.32

Table S3.3. Number of Water Samples in 87 Counties in the US with Detection of at least one of PFOA, PFOS, PFHpA or PFHxS, 2013-2015

Min	1st Quantile	Median	Mean	3rd Quantile	Max	SD
4.00	28.00	50.00	127.00	136.50	1018.00	187.73

Table S3.4. Predictors of Birthweight among Singleton Pregnancies in the US, 2013-2015.

Categories	No. of Births (%)	Average Birthweight (gram) (SD)
Total	11,484,590 (100%)	3,306 (561)
Maternal Age (years)		
<15	8,276 (0.07%)	3,076 (591)
15-19	739,856 (6.4%)	3,182 (550)
20-24	2,568,226 (22.4%)	3,246 (549)
25-29	3,311,229 (28.8%)	3,321 (551)
30-34	3,079,890 (26.8%)	3,354 (559)
35-39	1,444,763 (12.6%)	3,341 (585)
40-44	311,894 (2.7%)	3,292 (615)
45-49	19,046 (0.17%)	3,238 (648)
≥50	1,410 (0.01%)	3,188 (661)
Race		
American Indian or Alaska Native	131,714 (1.1%)	3,342 (582)
Asian or Pacific Islander	803,171 (7.0%)	3,215 (516)
Black or African American	1,839,887 (16.0%)	3,131 (610)
White	8,709,818 (75.8%)	3,350 (546)
Education		
8th grade or less	399,353 (3.5%)	3,306 (557)
9th through 12th grade with no diploma	1,242,484 (10.8%)	3,205 (571)
High school graduate or GED completed	2,703,542 (23.5%)	3,256 (571)
Some college credit, but not a degree	2,295,104 (20.0%)	3,301 (569)

Associate degree	862,375 (7.5%)	3,346 (558)
Bachelor's degree	2,034,035 (17.7%)	3,384 (533)
Master's degree	898,457 (7.8%)	3,384 (530)
Doctorate or professional degree	256,032 (2.2%)	3,357 (518)
Unknown or not stated	146,362 (1.3%)	3,256 (631)
Excluded	646,846 (5.6%)	3,305 (558)
Smoking Status		
No	9,672,851 (84.2%)	3,323 (556)
Yes	870,235 (7.6%)	3,121 (578)
Not reported	774,075 (6.7%)	3,307 (560)
Unknown or not stated	167,429 (1.5%)	3,266 (604)
Parity		
1st	4,519,520 (39.4%)	3,253 (566)
2nd	3,638,902 (31.7%)	3,348 (540)
3rd and over	3,269,850 (28.5%)	3,331 (571)
Unknown or not stated	56,318 (0.5%)	3,245 (628)

Table S3.5. Crude and Adjusted Associations between Percentage of Water Measurements with PFOA Detection and Average Birthweight among Singleton Live Births in 551 Counties, 2013-2015

Model	The Change of Average Birthweight (g) for 10% Increase in the Detection of PFOA: MLE, 95% CI	
	Weighted by group size	Weighed by inverse variance of average birthweight
Crude Model	-5.1, [-6.3, -3.9]	-6.9, [-8.1, -5.7]
Adjusted Model*	-11.9, [-13.1, -10.7]	-12.2, [-13.4, -11.1]
Adjusted Co-exposure Model**	20.0, [17.4, 22.6]	19.9, [17.4, 22.4]

* Adjusted for maternal age (<15, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (8th grade or less; 9th through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (Yes, No), and parity (1st, 2nd, 3rd and over).

** Adjusted for PFOS, PFHpA, PFHxS, 1,4-dioxane, and all covariates in the adjusted model.

Table S3.6. Crude and Adjusted Associations between Percentage of Water Measurements with PFOS Detection and Average Birthweight among Singleton Live Births in 551 Counties, 2013-2015

Model	The Change of Average Birthweight (g) for 10% Increase in the Detection of PFOS: MLE, 95% CI	
	Weighted by group size	Weighted by inverse variance of average birthweight
Crude Model	-22.1, [-23.3, -20.8]	-23.0, [-24.2, -21.8]
Adjusted Model*	-27.8, [-29.1, -26.6]	-28.9, [-30.0, -27.7]
Adjusted Co-exposure Model**	-29.8, [-31.9, -27.7]	-32.9, [-34.9, -30.9]

* Adjusted for maternal age (<15, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (8th grade or less; 9th through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (Yes, No), and parity (1st, 2nd, 3rd and over).

** Adjusted for PFOA, PFHpA, PFHxS, 1,4-dioxane, and all covariates in the adjusted model.

Table S3.7. Crude and Adjusted Associations between Percentage of Water Measurements with PFHpA Detection and Average Birthweight among Singleton Live Births in 551 Counties, 2013-2015

Model	The Change of Average Birthweight (g) for 10% Increase in the Detection of PFHpA: MLE, 95% CI	
	Weighted by group size	Weighted by inverse variance of average birthweight
Crude Model	-14.3, [-15.5, -13.1]	-15.7, [-16.9, -14.5]
Adjusted Model*	-18.3, [-19.5, -17.0]	-17.8, [-18.9, -16.6]
Adjusted Co-exposure Model**	-9.6, [-12.1, -7.0]	-8.0, [-10.3, -5.7]

* Adjusted for maternal age (<15, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (8th grade or less; 9th through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (Yes, No), and parity (1st, 2nd, 3rd and over).

** Adjusted for PFOA, PFOS, PFHxS, 1,4-dioxane, and all covariates in the adjusted model.

Table S3.8. Crude and Adjusted Associations between Percentage of Water Measurements with PFHxS Detection and Average Birthweight among Singleton Live Births in 551 Counties, 2013-2015

Model	The Change of Average Birthweight (g) for 10% Increase in the Detection of PFHxS: MLE, 95% CI	
	Weighted by group size	Weighted by inverse variance of average birthweight
Crude Model	-12.2, [-13.5, -10.9]	-13.4, [-14.7, -12.2]
Adjusted Model*	-19.7, [-21.0, -18.3]	-19.9, [-21.1, -18.6]
Adjusted Co-exposure Model**	-6.0, [-8.8, -3.3]	-5.0, [-7.6, -2.4]

* Adjusted for maternal age (<15, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (8th grade or less; 9th through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (Yes, No), and parity (1st, 2nd, 3rd and over).

** Adjusted for PFOA, PFOS, PFHpA, 1,4-dioxane, and all covariates in the adjusted model.

Table S3.9. Crude and Adjusted Associations between Population-weighted Average PFOA Water Concentration and Average Birthweight among Singleton Live Births in 87 Counties, 2013-2015

Model	The Change of Average Birthweight (g) for 1ng/L Increase in the Population-weighted Average PFOA Water Concentration: MLE, 95% CI*		
	$\frac{MRL}{\sqrt{2}}$ substitution	Zero substitution	MRL substitution
Crude Model	-1.3, [-2.2, -0.4]	-1.1, [-1.7, -0.5]	-1.1, [-2.2, 0.1]
Adjusted Model**	0.9, [-0.02, 1.7]	1.0, [0.4, 1.5]	0.4, [-0.7, 1.5]
Adjusted Co-exposure Model***	10.7, [9.5, 11.9]	6.7, [5.9, 7.4]	9.5, [7.9, 11.1]

* Weighted by inverse variance of average birthweight.

**Adjusted for maternal age (<15, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (8th grade or less; 9th through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (Yes, No), and parity (1st, 2nd, 3rd and over).

***Adjusted for PFOS, PFHpA, PFHxS, 1,4-dioxane, and all covariates in the adjusted model.

Table S3.10. Crude and Adjusted Associations between Population-weighted Average PFOS Water Concentration and Average Birthweight among Singleton Live Births in 87 Counties, 2013-2015

Model	The Change of Average Birthweight (g) for 1ng/L Increase in the Population-weighted Average PFOS Water Concentration: MLE, 95% CI*		
	$\frac{MRL}{\sqrt{2}}$ substitution	Zero substitution	MRL substitution
Crude Model	-3.2, [-3.4, -2.9]	-3.4, [-3.6, -3.2]	-2.4, [-2.7, -2.2]
Adjusted Model**	-1.3, [-1.5, -1.0]	-1.9, [-2.1, -1.7]	-0.6, [-0.8, -0.3]
Adjusted Co-exposure Model***	0.3, [-0.1, 0.7]	-1.0, [-1.3, -0.7]	1.7, [1.3, 2.1]

* Weighted by inverse variance of average birthweight.

** Adjusted for maternal age (<15, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (8th grade or less; 9th through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (Yes, No), and parity (1st, 2nd, 3rd and over).

*** Adjusted for PFOA, PFHpA, PFHxS, 1,4-dioxane, and all covariates in the adjusted model.

Table S3.11. Crude and Adjusted Associations between Population-weighted Average PFHpA Water Concentration and Average Birthweight among Singleton Live Births in 87 Counties, 2013-2015

Model	The Change of Average Birthweight (g) for 1ng/L Increase in the Population-weighted Average PFHpA Water Concentration: MLE, 95% CI*		
	$\frac{MRL}{\sqrt{2}}$ substitution	Zero substitution	MRL substitution
Crude Model	-7.8, [-8.6, -7.1]	-6.2, [-6.7, -5.7]	-8.3, [-9.3, -7.4]
Adjusted Model**	-3.8, [-4.5, -3.1]	-3.3, [-3.8, -2.8]	-3.6, [-4.5, -2.7]
Adjusted Co-exposure Model***	-4.8, [-5.8, -3.8]	-3.4, [-4.1, -2.6]	-3.9, [-5.1, -2.8]

* Weighted by inverse variance of average birthweight.

** Adjusted for maternal age (<15, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (8th grade or less; 9th through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (Yes, No), and parity (1st, 2nd, 3rd and over).

*** Adjusted for PFOA, PFOS, PFHxS, 1,4-dioxane, and all covariates in the adjusted model.

Table S3.12. Crude and Adjusted Associations between Population-weighted Average PFHxS Water Concentration and Average Birthweight among Singleton Live Births in 87 Counties, 2013-2015

Model	The Change of Average Birthweight (g) for 1ng/L Increase in the Population-weighted Average PFHxS Water Concentration: MLE, 95% CI*		
	$\frac{MRL}{\sqrt{2}}$ substitution	Zero substitution	MRL substitution
Crude Model	-4.2, [-4.5, -3.9]	-3.1, [-3.4, -2.9]	-4.7, [-5.1, -4.4]
Adjusted Model**	-3.8, [-4.1, -3.5]	-2.7, [-2.9, -2.4]	-4.4, [-4.7, -4.0]
Adjusted Co-exposure Model***	-5.4, [-5.8, -4.9]	-2.6, [-2.9, -2.3]	-6.9, [-7.4, -6.5]

* Weighted by inverse variance of average birthweight.

** Adjusted for maternal age (<15, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (8th grade or less; 9th through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (Yes, No), and parity (1st, 2nd, 3rd and over).

*** Adjusted for PFOA, PFOS, PFHpA, 1,4-dioxane, and all covariates in the adjusted model.

Table S3.13. Crude and Adjusted Associations between the Sum of Population-weighted Average Water Concentrations of Four PFAS and Average Birthweight among Singleton Live Births in 87 Counties, 2013-2015

Model	The Change of Average Birthweight (g) for 1ng/L Increase in the Population-weighted Average PFHxS Water Concentration: MLE, 95% CI*		
	$\frac{MRL}{\sqrt{2}}$ substitution	Zero substitution	MRL substitution
Crude Model	-1.7, [-1.8, -1.6]	-1.6, [-1.7, -1.5]	-1.6, [-1.7, -1.5]
Adjusted Model**	-1.0, [-1.1, -0.9]	-1.0, [-1.1, -0.9]	-0.9, [-1.0, -0.8]
Adjusted Co-exposure Model***	-1.0, [-1.1, -0.9]	-1.0, [-1.1, -0.9]	-0.9, [-1.0, -0.8]

* Weighted by inverse variance of average birthweight.

** Adjusted for maternal age (<15, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (8th grade or less; 9th through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (Yes, No), and parity (1st, 2nd, 3rd and over).

*** Adjusted for 1,4-dioxane and all covariates in the adjusted model.

Table S3.14. Crude and Adjusted Associations between Percentage of Water Measurements with PFAS Detection and Average Birthweight among Singleton Live Births in 551 Counties, 2013-2015[1]

Model	The Change of Average Birthweight (g) for 10% Increase in the Detection of PFAS: MLE, 95% CI[2]			
	PFOA	PFOS	PFHpA	PFHxS
Crude Model	-6.9, [-8.1, -5.7]	-23, [-24.2, -21.8]	-15.7, [-16.9, -14.5]	-13.4, [-14.7, -12.2]
Adjusted Model[3]	-16.7, [-17.9, -15.6]	-28.4, [-29.6, -27.2]	-20, [-21.2, -18.9]	-23.9, [-25.1, -22.7]
Adjusted Co-exposure Model[4]	12.9, [10.4, 15.4]	-25.3, [-27.3, -23.4]	-5.2, [-7.5, -2.8]	-11.1, [-13.6, -8.5]

[1] Adjusted and adjusted co-exposure models in Table 3.14 additionally adjusted for county-level percentage of poverty compared with those in the previous tables.

[2] Weighted by inverse variance of average birthweight.

[3] Adjusted for maternal age (<15, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (8th grade or less; 9th through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (Yes, No), parity (1st, 2nd, 3rd and over), and county-level percentage of poverty.

[4] Adjusted for the other PFAS, 1,4-dioxane, and all covariates in the adjusted model.

Table S3.15. Crude and Adjusted Associations between Population-weighted Average PFAS Water Concentration and Average Birthweight among Singleton Live Births in 87 Counties, 2013-2015 (Using $\frac{MRL}{\sqrt{2}}$ substitution)[1]

Model	The Change of Average Birthweight (g) for 1ng/L Increase in the Population-weighted Average PFAS Water Concentration: MLE, 95% CI[2]				
	PFOA	PFOS	PFHpA	PFHxS	Sum of four PFAS
Crude Model	-1.3, [-2.2, -0.4]	-3.2, [-3.4, -2.9]	-7.8, [-8.6, -7.1]	-4.2, [-4.5, -3.9]	-1.7, [-1.8, -1.6]
Adjusted Model[3]	-2.4, [-3.3, -1.5]	-1.7, [-2.0, -1.5]	-2.9, [-3.7, -2.2]	-4.3, [-4.6, -4.0]	-1.2, [-1.4, -1.1]
Adjusted Co-exposure Model[4]	6.0, [4.8, 7.3]	0.2, [-0.2, 0.5]	-1.9, [-2.8, -1.1]	-5.3, [-5.7, -4.9]	-1.2, [-1.4, -1.1]

[1] Adjusted and adjusted co-exposure models in Table 3.15 additionally adjusted for county-level percentage of poverty compared with those in the previous tables.

[2] Weighted by inverse variance of average birthweight.

[3] Adjusted for maternal age (<15, 15-19, 20-24, 25-29, 30-34, 35-39, 40-44, 45-49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (8th grade or less; 9th through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (Yes, No), parity (1st, 2nd, 3rd and over), and county-level percentage of poverty.

[4] Adjusted for the other PFAS, 1,4-dioxane, and all covariates in the adjusted model.

Table S3.16. Spearman Correlation between Percentage of Poverty and Percentage of Water Measurements with PFAS Detection for 551 Counties, 2013-2015

	Spearman correlation	p-value
PFOA	-0.07	0.09
PFOS	0.02	0.61
PFHpA	-0.08	0.08
PFHxS	-0.04	0.30

Table S3.17. Spearman Correlation between Percentage of Poverty and Population-weighted Average PFAS Water Concentrations for the 87 Counties with Detection of at least one of PFOA, PFOS, PFHpA, or PFHxS, 2013-2015

	Spearman correlation	p-value
PFOA	-0.18	0.10
PFOS	0.04	0.70
PFHpA	-0.13	0.22
PFHxS	-0.07	0.50

Table S3.18. Descriptive Statistics of Serum PFAS Concentrations (ng/ml) for the General US Population (Aged 12 Years and Older) from the National Health and Nutrition Examination Survey (NHANES).

	2015-2016 cycle of NHANES			
	Geometric Mean (95% CI)	Median (95% CI)	25th Percentile (95% CI)*	75th Percentile (95% CI)
PFOA	1.56 (1.47, 1.66)	1.57 (1.47, 1.77)	1.07 (0.97, 1.17)	2.47 (2.27, 2.57)
PFOS	4.72 (4.4, 5.07)	4.8 (4.4, 5.3)	2.8 (2.6, 3.0)	8.1 (7.3, 9.4)
PFHxS	1.18 (1.08, 1.30)	1.2 (1.1, 1.3)	0.7 (0.6, 0.8)	2.1 (1.8, 2.3)
PFHpA	-	-	-	-
PFNA	0.58 (0.54, 0.62)	0.6 (0.5, 0.6)	0.4 (0.3, 0.4)	0.9 (0.8, 1.0)
PFBS	-	-	-	-
	2013-2014 cycle of NHANES			
	Geometric Mean (95% CI)	Median (95% CI)	25th Percentile (95% CI)*	75th Percentile (95% CI)
PFOA	1.94 (1.76, 2.14)	2.07 (1.87, 2.20)	1.37 (1.17, 1.47)	3.07 (2.67, 3.37)
PFOS	4.99 (4.50, 5.52)	5.2 (4.8, 5.7)	3.1 (2.7, 3.4)	8.7 (8.0, 9.4)
PFHxS	1.35 (1.2, 1.52)	1.4 (1.2, 1.6)	0.8 (0.7, 0.9)	2.4 (2.2, 2.8)
PFHpA	< LOD	< LOD	< LOD	< LOD
PFNA	0.68 (0.61, 0.74)	0.7 (0.6, 0.7)	0.4 (0.4, 0.5)	1.0 (0.9, 1.2)
PFBS	< LOD	< LOD	< LOD	< LOD

Notes:

Limit of detection (LOD) for serum PFAS in survey cycle 2013-2014 and 2015-2016 are 0.10 ng/ml (= 100 ng/L = 100 ppt).

< LOD means less than the limit of detection.

- Not measured.

* The 25th percentile (95% CI) was not reported by CDC Fourth Report on Human Exposure to Environmental Chemicals (January 2019). We calculated the statistics accounting for complex survey design using NHANES data and the 'survey' package in R. The other percentiles and geometric means are identical to the statistics reported by CDC.

3.7 Supplemental Digital Content II

https://cdn-links.lww.com/permalink/ee/a/ee_2021_01_27_yzhu_ee-d-20-00015_sdc2.pdf

CHAPTER 4

PFAS in Drinking Water and Hypertensive Disorders of Pregnancy in the US during 2013-2015

Contents

4.1 Abstract

Background: PFAS widely exist in the environment and human bodies. Contaminated drinking water is one of the major exposure pathways for humans. Previous studies found weak or moderate associations between PFAS and hypertensive disorders of pregnancy (HDP).

Methods: We obtained the number of births and counts of HDP cases for singleton births multiply stratified by county, maternal age, race, education, smoking status, and parity from CDC WONDER, and PFAS water concentrations from EPA UCMR3 data in the US during 2013-2015. We used binomial regression on the multiply stratified HDP data to produce equal effect estimates and standard errors to those that would be derived from using individual-level data on binary HDP status and demographic covariates in logistic regression.

Results: After adjusting for demographic covariates, we found small but statistically significant positive associations between HDP and population-weighted average water concentrations (ng/L) of all four PFAS: Odds ratio (OR) = 1.009, 95% CI: [1.001, 1.016] per IQR increase in perfluorooctanoic acid (PFOA); 1.030, 95% CI: [1.021, 1.040] per IQR increase in perfluorooctane sulfonate (PFOS); 1.008, 95% CI: [1.005, 1.011] per IQR increase in perfluoroheptanoic acid (PFHpA); 1.007, 95% CI: [1.004, 1.010] per IQR increase in perfluorohexane sulfonic acid (PFHxS), and 1.032, 95% CI: [1.022, 1.042] per IQR increase in the sum of four PFAS. Further adjustment for co-exposures reversed the effect of PFOA from positive to inverse, and attenuated the effects of PFOS and PFHxS towards the null. After drinking water to serum concentration conversions, our effect estimates for PFOA, PFOS, and PFHxS are similar to previous studies.

Conclusions: We found a weak positive association between the PFAS mixture and HDP, although the generalizability is subject to inherent limitations of the public-available datasets.

Key Words: PFAS, public water supplies, HDP, PE, PIH

4.2 Introduction

PFAS are a large group of synthetic chemicals that exist widely in the environment. Contaminated drinking water, seafood, packaged fast food, daily consumer products (non-stick cookware, stain-resistant carpeting, and water repellent clothing), dust, and air are the major exposure pathways for humans (Jian et al. 2017; Sunderland et al. 2019; Zhu et al. 2021). Among nearly 5,000 types of PFAS, PFOA and PFOS are the two most extensively produced and studied chemicals, both of which have attracted extensive attention from the scientific and regulatory community (US EPA 2009). In laboratory-based animal studies, PFOA and PFOS have shown the potential for developmental toxicity and reproductive effects (Negri et al. 2017), but there are fewer studies of these health effects in humans.

Hypertensive disorders of pregnancy (HDP) complicate approximately 5-10% of pregnancies in the US. HDP includes both pregnancy-induced hypertension (PIH; or gestational hypertension) and preeclampsia (PE) (C8 Science Panel 2011; Borghese et al. 2020; Steenland et al. 2020; ATSDR 2021; Erinc et al. 2021), the latter of which is defined as new-onset hypertension combined with proteinuria (≥300 mg of protein excretion in a 24-hour urine collection) after 20 weeks of gestation (Milne et al. 2005). Modest state-level variation has been observed for HDP in the US (Butwick et al. 2020).

Most epidemiological studies on PFAS and HDP separated PE and PIH, and many focused on the effects of PFOA and PFOS only, while fewer studies investigated the potential effects of PFHxS and PFHpA. Among the previous studies, Savitz et al. (2012a) found a significant positive association with an adjusted odds ratio (AOR) of 1.16 (95% CI: [1.03, 1.30]) per interquartile range (IQR) increase in natural log PFOA between PE and PFOA based on historical exposure reconstruction with Bayesian time-dependent calibration;

Wikström et al. (2019) found a significant positive association between PE and PFOS with an AOR of 1.53 (95% CI: [1.07, 2.20]) per $\log_2$ unit increase in PFOS; Huang et al. (2019) found significant positive associations between perfluorobutane sulfonic acid (PFBS) and PE (AOR=1.81, 95% CI: [1.03, 3.17] per ln unit) and overall HDP (AOR=1.64, 95% CI: [1.09, 2.47] per ln unit). Rylander et al. (2020) found a significantly higher risk of PE comparing the 3rd quartile to the 1st quartile of serum PFHxS (AOR = 1.67, 95% CI: [1.02, 2.74]), Borghese et al. (2020) found a significant positive association between PE and PFHxS (AOR = 1.32, 95% CI: [1.03, 1.70] per $\log_2$ unit increase in plasma PFHxS concentration), and Darrow et al. (2013) found significant positive associations between PFOA, PFOS and PIH (AOR=1.27, 95% CI: [1.05, 1.55] per natural log unit increase in PFOA; AOR=1.47, 95% CI: [1.06, 2.04] per log unit increase in PFOS). The other studies only found weakly or moderately insignificant positive or inverse associations between PFAS and PE, PIH, or HDP (Stein et al. 2009; Savitz et al. 2012b; Starling et al. 2014; Huo et al. 2020; Birukov et al. 2021).

The inconsistent findings in previous studies may be due to the variation in study design, study population, case definition, exposure assessment, the timing of blood sampling, exposure level, restriction to nulliparous or not, covariates and co-exposure adjusted in the statistical models, statistical methods, etc. For example, some studies collected blood samples before or early in pregnancy (Darrow et al. 2013; Wikström et al. 2019; Huo et al. 2020; Rylander et al. 2020; Borghese et al. 2020; Birukov et al. 2021), while others collected blood samples in mid-pregnancy (Starling et al. 2014), at delivery or after pregnancy (Stein et al. 2009; Huang et al. 2019). Different from the studies that used measured serum PFAS concentrations, Savitz et al. (2012a; 2012b) analyzed the associations with PE and PIH based on environmentally modeled water and serum PFOA concentrations. HDP can adversely

affect kidney function during pregnancy, leading to decreased glomerular filtration rate (GFR) (August, 2013; Berry and Atta, 2016; Conti-Ramsden et al., 2019; Covella et al., 2019; Ishaku et al., 2021) and increased serum PFAS concentrations (Shankar et al. 2011; Watkins et al. 2013). Thus, the observed association between PFAS and HDP could be due to reverse causality in the studies with measured biomarkers, particularly those that sampled blood in mid-pregnancy or later.

During 2013-2015, the US Environmental Protection Agency (EPA) completed nationwide monitoring of six PFAS [PFOA, PFOS, PFHpA, PFHxS, perfluorononanoic acid (PFNA), and PFBS] at 4,908 public water systems (PWSs) under the third Unregulated Contaminant Monitoring Rule (UCMR3). The UCMR3 dataset is the most comprehensive data on PFAS in US public water supplies, covering all PWSs serving more than 10,000 people and a representative sample of 800 PWSs serving less than 10,000 people (US EPA, 2012). Overall, approximately 241 million people were served by the PWSs monitored under UCMR3 (Andrews and Naidenko, 2020). During 2013-2015, PFAS was detected in 1.6% of water samples and 4% of PWSs (Guelfo and Adamson 2018), which served 16.5 million US residents (Hu et al., 2016). With 90% of the US population being served by public water systems (US EPA, 2020), UCMR3 provides an important publicly available data source for researchers to investigate the health effects of PFAS. Based on UCMR3, Hurley et al. (2016) found significantly higher PFOA and PFOS concentrations in California women who resided in areas with detectable levels of PFOA and PFOS in public drinking water compared with those without detectable levels. Zhu and Bartell (2020) found a significant inverse association between the sum of PFAS and birthweight in the counties exposed to PFAS in drinking water in UCMR3.

Multiple US studies have investigated the associations between PFAS and HDP in communities with PFAS water contamination in West Virginia and Ohio, which are often referred to as "C8 Project" or "C8 Studies" (Stein et al. 2009; Nolan et al. 2010; Savitz et al. 2012a; Savitz et al. 2012b; Darrow et al. 2013). These are some of the largest available studies on this topic, but they also had some important limitations. First, some studies used self-reported PE without validation by medical records, which may be subject to recall bias (Stein et al. 2009; Savitz et al. 2012a). Second, Stein et al. (2009) is restricted to pregnancies occurring in five years before the mother's serum PFOA measurement, that is, their exposure assessment occurred after the outcome, which is a violation of temporality in epidemiology (Hill, 1965) and may introduce reverse causation. Because fetal transfer in pregnancy and breastfeeding after pregnancy are both important excretion pathways for PFOA in females (Beesoon et al. 2011; Fromme et al. 2010), the measured serum PFOA concentrations in a few years after pregnancy may not reflect the body burden of the women before pregnancy. Additionally, Savitz et al. (2012a; 2012b) assessed serum PFOA levels based on historical exposure reconstruction rather than actual measurements, and therefore may be subject to substantial exposure measurement error, though this approach largely avoided physiological confounding and reverse causation (Weisskopf and Webster 2017). Darrow et al. (2013) used a prospective study design with most pregnancies conceived after serum PFOA measurements, and was therefore not subject to the same concerns about temporality and historical exposure reconstruction as the other studies; these authors reported significant positive associations between PIH and PFOA and PFOS (AOR=1.27, 95% CI: [1.05, 1.55] per natural log unit increase in PFOA; AOR=1.47, 95% CI: [1.06, 2.04] per natural log unit increase in PFOS); and sub-analyses restricted to the births conceived after serum

measurements were consistent with the main results, yet with a stronger positive association between PIH and PFOS.

Although recent studies in other countries incorporated some understudied PFAS chemicals, such as PFNA, PFHpA, perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoA), perfluoroundecanoic acid (PFUA), PFHxS, and PFBS (Starling et al. 2014; Huang et al. 2019; Wikström et al. 2019; Huo et al. 2020; Rylander et al. 2020; Borghese et al. 2020; Birukov et al. 2021), the US C8 studies only investigated one or two PFAS chemicals: PFOA and/or PFOS (Stein et al. 2009; Savitz et al. 2012a; Savitz et al. 2012b; Darrow et al. 2013), and Nolan et al. (2010) used water service category (exclusively served by Little Hocking Water Association; partially served by Little Hocking Water Association; and not served by Little Hocking Water Association) as a crude exposure metric for PFOA. With PFOA and PFOS being gradually phased out in the US in the last two decades, and some similarities in reported health outcomes across the class of PFAS chemicals (ATSDR, 2021), other high use PFAS such as PFNA and PFHxS warrant investigation. Particularly, PFHxS was found to be ubiquitous in the serum of the US population during 2015-2016 (CDC, 2019). Using the UCMR3 data in this study, we were able to analyze the associations between PFAS and HDP more comprehensively by covering a larger population in the US and incorporating some understudied PFAS chemicals (i.e., PFHpA and PFHxS) compared to previous studies in the US. As an external exposure metric, PFAS water concentration is resistant to reverse causality or physiological confounding due to GFR, compared to PFAS serum concentration (Weisskopf and Webster 2017). In addition, the CDC WONDER data we used in this study provide HDP status as recorded on birth

certificates during the same years (2013-2015) covered by the UCMR3, facilitating the investigation of cross-sectional associations between PFAS in drinking water and HDP.

4.3 Methods

4.3.1 Study Population

We obtained the number of cases of HDP and the number of births (including those who had HDP and those who did not) for singleton births throughout the US during 2013-2015, multiply stratified by county, maternal age, bridged race, education, smoking status, and parity from CDC WONDER. In the CDC WONDER data, HDP is recorded as "pregnancy-associated hypertension" which includes diagnosis of either pregnancy-induced hypertension (PIH) or preeclampsia (PE). Following the practice in previous studies, we use the term "hypertensive disorders of pregnancy (HDP)" to represent pregnancy-associated hypertension (C8 Science Panel, 2011; Borghese et al., 2020; Steenland et al., 2020; ATSDR, 2021; Erinc et al., 2021). We merged the HDP cases and the number of births by combination of the stratification variables, excluding births with "unknown or not stated" HDP status from this study. We merged the CDC WONDER data on HDP and risk factors with UCMR3 data on PFAS in drinking water by county, which produced complete data for 551 large counties with more than 100,000 people. Table 4.1 presents the complete data (8,116,974 singleton births) we obtained for these 551 counties, accounting for 70.7% of all 11,484,590 singleton births in the US during 2013-2015. A flow chart (Figure S4.1) in the Supplemental Digital Content shows the details of exclusions.

4.3.2 UCMR3 Water Quality Data

We excluded PFNA and PFBS from this study because only 10 and 4 counties had detections for these two chemicals, respectively. The other four PFAS chemicals measured in UCMR3 (PFOA, PFOS, PFHpA, and PFHxS) were moderately to highly correlated with each other (Zhu and Bartell, 2020). We examined the other water quality indicators reported by the US EPA and found that 1,4-dioxane was the only other UCMR3 chemical moderately associated with the four PFAS ($r>0.3$) (Zhu and Bartell, 2020). A detailed description of the CDC WONDER and UCMR3 data, their limitations, and the merging process can be found in a previous paper (Zhu and Bartell, 2020). Among the 551 counties in the merged data, 87 counties had detection for at least one of PFOA, PFOS, PFHpA, and PFHxS. The number of singleton births (2,085,035 births) in these 87 counties accounted for 18.2% of the total number of singleton births in the US during 2013-2015.

Most counties have more than one public water supply (PWS), so we used two proxy indicators for PFAS exposure in the study: first, for the 551 counties in the complete merged data, we used the percentage of water measurements with PFAS detection by county (number of water samples with PFAS detections divided by the total number of water samples collected within the county) as the exposure indicator; second, for the 87 counties with PFAS detection in drinking water, we used the population-weighted average PFAS water concentrations by county (PWS-level average PFAS water concentrations weighted by population served by PWSs within a county) as the exposure indicator. We substituted values that were below the minimal reporting level (MRL) with $\mathrm{MRL}/\sqrt{2}$ in these 87 counties. Detailed descriptions of these two exposure indicators and summary statistics can be found in a previous paper (Zhu and Bartell, 2020).

4.3.3 Statistical Analyses

We assumed the outcome variable, the number of HDP cases within each stratum, follows a binomial distribution with the number of Bernoulli trials equal to the number of births within each stratum (n) and probability of HDP equal to p. We ran generalized linear models to examine the association between HDP and PFAS in drinking water using the *glm* function in R (version 4.1.0), where we specified the family as "binomial", the number of trials as the number of births (n), "success" as having HDP (Y), and "failure" as not having HDP (n-Y). Although the outcome variable HDP is reported at the group-level, because the binomial distribution is an aggregation of independent Bernoulli trials, using multiple-stratified data in binomial regression has the same likelihood function and produces the same results (effect estimates and standard errors) as we would get using individual-level data for the binary outcome variable in logistic regression, in which each individual birth is assumed to be a single Bernoulli trial with "success" representing having HDP and "failure" representing not having HDP. In both types of analyses, the logit of the probability of HDP (p) is modeled as a linear function of the explanatory variables. A simple example with simulated data in the Supplemental Digital Content shows the code to fit the equivalent *glm* models in R based on individual-level and multiple-stratified data, which can be extended to include interactions terms. However, in this study, we are limited to county-level exposure metrics, which may produce different results from using individual-level exposure information (e.g., PFAS measurements at each person's home and workplace).

We excluded the births with "Unknown or not stated" or "Excluded" education level, smoking status, and parity in the 551 counties (shown in Table 4.1) from statistical analyses. We ran three sets of binomial regression models, using the two types of exposure indicators

of PFAS separately. In the crude model, we examined the association between PFAS and HDP only. In the adjusted model, we examined the association while adjusting for maternal age, race, education, smoking status, and parity. In the adjusted co-exposure model, we adjusted for co-exposures including the other three PFAS and 1,4-dioxane in addition to the demographic covariates. We checked the linearity assumptions of binomial/logistic regressions by visually inspecting the scatter plots between continuous PFAS predictors and logit of HDP variable, which showed that PFAS variables are all quite linearly associated with the HDP outcome in logit scale (see Figure S4.2 and Figure S4.3 in Supplemental Digital Content). We evaluated the multi-collinearity by examining the generalized variance-inflation factors (GVIF) and GVIF^(1/(2*Df)), where Df is the degrees of freedom associated with the term (Fox and Monette, 1992). We found GVIF^(1/(2*Df)) < 3 for all terms in the adjusted co-exposure models using the two types of exposure indicators of PFAS, demonstrating no multi-collinearity in the adjusted co-exposure models (see Table S4.1 and Table S4.2 in the Supplemental Digital Content). We exponentiated the effect estimates from the binomial model output to obtain odds ratios measuring the associations between PFAS and HDP. The results derived from using the two exposure indicators are shown in Table 4.2 and Table 4.3, respectively.

Using the steady-state serum to drinking water conversion factors of 118:1 for PFOA, 129:1 for PFOS, and 202:1 for PFHxS derived from literature-based one-compartment pharmacokinetic models (Lu and Bartell, 2020), we converted the concentrations of PFOA, PFOS, and PFHxS in drinking water into the expected serum concentrations after long-term consumption of tap water. We explained the estimation of steady-state serum to drinking water ratios in the Supplemental Digital Content. The drinking water to serum conversions

of PFAS concentrations and odds ratios of HDP per μg/L increase in estimated steady-state serum PFAS concentrations in the 87 counties are reported in Table 4.4.

4.4 Results

Table 4.1. Predictors of HDP among Singleton Pregnancies in 551 Counties in the US, 2013-2015

Categories	No. of Births (%)	No. of HDP Cases
Total	8,116,974 (100%)	225,450 (100%)
Maternal Age (years)		
<15	2,436 (0.03%)	0 (0%)
15-19	472,950 (5.8%)	15,193 (6.7%)
20-24	1,712,821 (21.1%)	52,243 (23.2%)
25-29	2,343,447 (28.9%)	67,436 (29.9%)
30-34	2,315,191 (28.5%)	63,173 (28.0%)
35-39	1,074,932 (13.2%)	24,717 (11.0%)
40-44	191,832 (2.4%)	2,688 (1.2%)
≥45	3,365 (0.04%)	0 (0%)
Race		
American Indian or Alaska Native	30,764 (0.4%)	234 (0.1%)
Asian or Pacific Islander	612,144 (7.5%)	5,730 (2.5%)
Black or African American	1,348,057 (16.6%)	43,166 (19.1%)
White	6,126,009 (75.5%)	176,320 (78.2%)
Education		
8th grade or less	267,206 (3.3%)	3,553 (1.6%)

9th through 12th grade with no diploma	876,616 (10.8%)	19,322 (8.6%)
High school graduate or GED completed	1,905,846 (23.5%)	56,436 (25.0%)
Some college credit, but not a degree	1,663,504 (20.5%)	56,973 (25.3%)
Associate degree	571,084 (7.0%)	12,377 (5.5%)
Bachelor's degree	1,634,224 (20.1%)	50,619 (22.5%)
Master's degree	739,215 (9.1%)	16,366 (7.3%)
Doctorate or professional degree	198,732 (2.5%)	2,005 (0.9%)
Unknown or not stated	93,293 (1.2%)	1,346 (0.6%)
Excluded	167,254 (2.1%)	6,453 (2.9%)
Smoking Status		
No	7,428,369 (91.5%)	216,518 (96.0%)
Yes	365,603 (4.5%)	980 (0.4%)
Not reported	261,560 (3.2%)	7,651 (3.4%)
Unknown or not stated	61,442 (0.8%)	301 (0.1%)
Parity		
1st	3,272,130 (40.4%)	140,393 (62.3%)
2nd	2,570,880 (31.8%)	45,247 (20.1%)
3rd and over	2,251,921 (27.8%)	39,513 (17.5%)
Unknown or not stated	22,043 (0.3%)	297 (0.1%)

Table 4.2. Odds ratio of HDP per 10% increase in water measurements with PFAS detection in 551 counties in the US during 2013-2015 (No. of births = 7,692,730; No. of HDP cases = 215,957).

	PFOA	PFOS	PFHpA	PFHxS
Crude model[1]	1.004, 95% CI: [0.991, 1.018]	1.071, 95% CI: [1.057, 1.085]	1.036, 95% CI: [1.022, 1.050]	1.033, 95% CI: [1.019, 1.047]
Adjusted model[2]	1.002, 95% CI: [0.988, 1.015]	1.042, 95% CI: [1.029, 1.056]	1.011, 95% CI: [0.997, 1.025]	1.030, 95% CI: [1.016, 1.044]
Adjusted co-exposure model[3]	0.910, 95% CI: [0.882, 0.938]	1.015, 95% CI: [0.993, 1.038]	0.973, 95% CI: [0.945, 1.001]	1.116, 95% CI: [1.083, 1.151]

[1] Crude model: association between PFAS and HDP only.

2 Adjusted model: adjusted for maternal age (<15, 15–19, 20–24, 25–29, 30–34, 35–39, 40–44, 45–49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (eighth grade or less; ninth through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (yes, no), and parity (first, second, third and over).

[3] Adjusted co-exposure model: adjusted for the other three PFAS, 1,4-dioxane, and all covariates in the adjusted model.

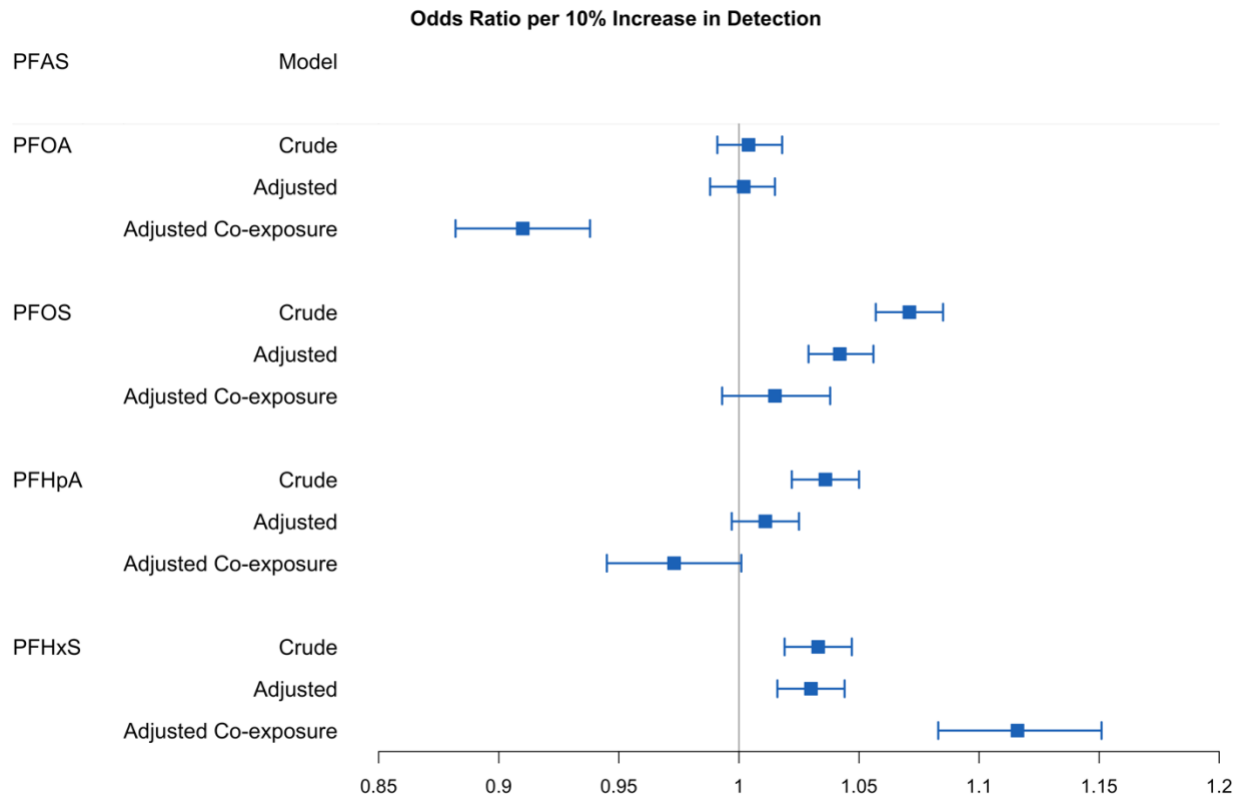

Figure 4.1. Odds ratio (95% CI) of HDP per 10% increase in water measurements with PFAS detection in 551 counties in the US during 2013-2015. Crude model: association between PFAS and HDP only. Adjusted model: adjusted for maternal age (<15, 15–19, 20–24, 25–29, 30–34, 35–39, 40–44, 45–49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (eighth grade or less; ninth through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (yes, no), and parity (first, second, third and over). Adjusted co-exposure model: adjusted for the other 3 PFAS, 1,4-dioxane, and all covariates in the adjusted model.

Table 4.3. Odds ratios with 95% confidence intervals (95% CI) of HDP per IQR (ng/L) increase in population-weighted average PFAS water concentration in 87 counties with detection of at least one of the four PFAS[1] (No. of births = 2,085,035; No. of HDP cases = 57,272).

	PFOA	PFOS	PFHpA	PFHxS	Sum of four PFAS
IQR IQR difference	[14.14, 14.90] 0.76	[28.28, 31.74] 3.46	[7.07, 7.42] 0.35	[21.21, 22.04] 0.83	[70.98, 78.26] 7.28
Crude model[2]	1.009, 95% CI: [1.001, 1.016]	1.033, 95% CI: [1.024, 1.042]	1.014, 95% CI: [1.011, 1.017]	1.007, 95% CI: [1.005, 1.010]	1.037, 95% CI: [1.027, 1.047]
Adjusted model[3]	1.009, 95% CI: [1.001, 1.016]	1.030, 95% CI: [1.021, 1.040]	1.008, 95% CI: [1.005, 1.011]	1.007, 95% CI: [1.004, 1.010]	1.032, 95% CI: [1.022, 1.042]
Adjusted co-exposure model[4]	0.976, 95% CI: [0.965, 0.986]	1.018, 95% CI: [1.003, 1.034]	1.014, 95% CI: [1.010, 1.018]	1.003, 95% CI: [0.999, 1.007]	1.031, 95% CI: [1.021, 1.041]

[1] Values that were below the minimal reporting levels (MRLs) were substituted with MRL/$\sqrt{2}$.

[2] Crude model: association between PFAS and HDP only.

3 Adjusted model: adjusted for maternal age (<15, 15–19, 20–24, 25–29, 30–34, 35–39, 40–44, 45–49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (eighth grade or less; ninth through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (yes, no), and parity (first, second, third and over).

[4] Adjusted co-exposure model: adjusted for the other three PFAS, 1,4-dioxane, and all covariates in the adjusted model.

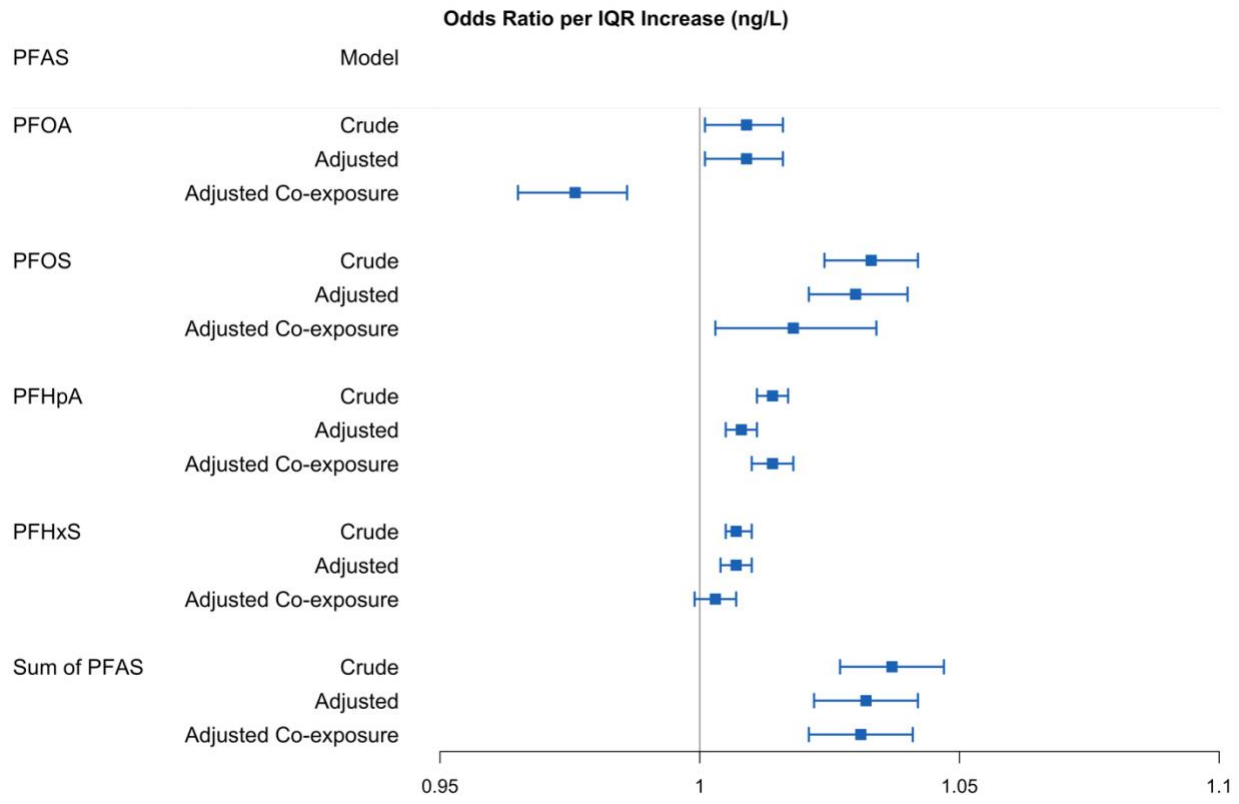

Figure 4.2. Odds ratio (95% CI) of HDP per IQR (ng/L) increase in the population-weighted average PFAS water concentration (ng/L = ppt = $10^{-3}\mu$g/L) in the 87 counties. Using MRL/$\sqrt{2}$ substitution for the non-detections. Crude model: association between PFAS and birthweight only. Adjusted model: adjusted for maternal age (<15, 15–19, 20–24, 25–29, 30–34, 35–39, 40–44, 45–49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (eighth grade or less; ninth through 12th grade with no diploma; high school graduate or GED completed; some college credit, but not a degree; associate degree; bachelor's degree; master's degree; doctorate or professional degree), smoking status (yes, no), and parity (first, second, third and over). Adjusted co-exposure model: adjusted for the co-expo- sures (the other 3 PFAS and 1,4-dioxane; or 1,4-dioxane only for the model includes the sum of PFAS), and all covariates in the adjusted model.

Table 4.4. Drinking water to serum conversions of PFAS concentrations and odds ratios with 95% CI of HDP per μg/L increase in estimated steady-state serum PFAS concentrations after long-term consumption of tap water in 87 counties with detection of at least one of the four PFAS[1] (No. of births = 2,085,035; No. of HDP cases = 57,272).

	PFOA	PFOS	PFHpA	PFHxS
Mean of population-weighted average UCMR3 water concentration (ng/L)	14.84	30.58	7.74	22.60
Steady-state serum to drinking water ratio	118:1	129:1	-	202:1
Mean predicted serum concentration after drinking water to serum conversion (μg/L)	1.7	3.8	-	4.4
Crude model[2]	1.10, 95% CI: [1.01, 1.20]	1.07, 95% CI: [1.05, 1.10]	-	1.05, 95% CI: [1.03, 1.06]
Adjusted model[3]	1.09, 95% CI: [1.01, 1.20]	1.07, 95% CI: [1.05, 1.09]	-	1.04, 95% CI: [1.03, 1.06]
Adjusted co-exposure model[4]	0.76, 95% CI: [0.67, 0.86]	1.04, 95% CI: [1.01, 1.08]	-	1.02, 95% CI: [0.995, 1.04]

[1] Values that were below the minimal reporting levels (MRLs) were substituted with MRL/$\sqrt{2}$.

[2] Crude model: association between PFAS and HDP only.

[3] Adjusted model: adjusted for maternal age (<15, 15–19, 20–24, 25–29, 30–34, 35–39, 40–44, 45–49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (eighth grade or less; ninth through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (yes, no), and parity (first, second, third and over).

[4] Adjusted co-exposure model: adjusted for the other three PFAS, 1,4-dioxane, and all covariates in the adjusted model.

Based on the first proxy indicator, water measurements with PFAS detection in 551 counties, we found a null association between HDP and PFOA, significant positive associations between HDP and PFOS, PFHpA, and PFHxS. Adjusting for demographic covariates partly explained the associations between HDP and PFOS, and PFHpA, thus attenuating the effect estimates towards the null. Additional adjustments for co-exposures further attenuated the positive association between PFOS and HDP towards the null, changed the effects for PFOA and PFHpA to the inverse, and increased the effect size in the positive direction for PFHxS (see Table 4.2 and Figure 4.1).

The IQRs for the second proxy indicator, population-weighted average PFAS water concentration in 87 counties, are shown in Table 4.3. Based on the second proxy indicator, we found small but statistically significant positive associations between each of the four PFAS and HDP in the crude and adjusted models. Adjusting for demographic covariates partly explained the associations between HDP and PFOS, and PFHpA, slightly attenuating the effect sizes towards the null. Additional adjustments for co-exposures changed the effect of PFOA from null to inverse. We also observed a significant positive association between the sum of four PFAS and HDP, which is robust to adjustments for demographic covariates and the co-exposure 1,4-dioxane (see Table 4.3 and Figure 4.2).

Using steady-state serum to drinking water ratios of 118:1 for PFOA, 129:1 for PFOS, and 202:1 for PFHxS derived from literature-based pharmacokinetic models (Lu and Bartell, 2020), we converted the population-weighted average water concentrations (ng/L) into the expected serum concentrations (μg/L) after long-term consumption of tap water. The estimation of steady-state serum to drinking water ratios were explained in the Supplemental Digital Content. The average serum concentrations after the drinking water to

serum conversion are 1.7 μg/L for PFOA, 3.8 μg/L for PFOS, and 4.4 μg/L for PFHxS. The effect estimates for PFOA, PFOS, and PFHxS after adjusting for demographic covariates are shown in Table 4.4, which are reversed from positive to inverse for PFOA, and attenuated towards the null for PFOS and PFHxS after further adjustments for co-exposures (see Table 4.4).

4.5 Discussion

To our knowledge, our analyses of 8,116,974 US singleton births during 2013-2015 uses the most comprehensive database for estimating of the associations between HDP and four common PFAS (i.e., PFOA, PFOS, PFHpA, and PFHxS) in public water supplies. We found a null association between HDP and the detection of PFOA, and small positive associations between HDP and detections of PFOS, PFHpA, and PFHxS after adjusting for demographic covariates. Further adjusting for co-exposures (the other three PFAS and 1,4-dioxane) changed the effect estimates differently for different PFAS chemicals.

Among the 2,085,035 singleton births in the 87 counties with exposure to PFAS in drinking water, we observed significant positive associations between HDP and population-weighted average water concentrations of all four PFAS and the sum of four PFAS, after adjusting for demographic covariates. Although further adjustment for co-exposures (PFOS, PFHpA, PFHxS, and 1,4-dioxane) reversed the effect of PFOA from positive to inverse, exposure amplification bias due to residual confounding could occur when investigating correlated exposure mixtures with common sources in the same model (Weisskopf et al., 2018).

To facilitate the comparisons with other studies that used serum PFAS concentrations as the exposure metric, we also used steady-state serum to drinking water ratio to convert the population-weighted average PFAS water concentrations to the predicted serum concentrations after long-term consumption of tap water. After the conversion, our effect estimates for PFOA (AOR = 1.09, 95% CI: [1.01, 1.20] per μg/L), PFOS (1.07, 95% CI: [1.05, 1.09] per μg/L), and PFHxS (1.04, 95% CI: [1.03, 1.06] per μg/L) after adjusting for demographic covariates are similar to the weakly/moderately positive effect estimates found in most previous studies (Stein et al. 2009; Savitz et al. 2012a, 2012b; Huang et al. 2019; Huo et al. 2020; Rylander et al. 2020; Borghese et al. 2020; Birukov et al. 2021).

Strengths of our study include a large sample size of 8,116,974 US singleton births that represents 71% of all singleton births in the US during 2013-2015, information on the counties and states of the deliveries, HDP status based on birth records, and availability of multiply stratified data on HDP and key demographic covariates, equivalent to individual-level data on those variables. Particularly, the multiply stratified data structure allows the use of binomial regression to derive equivalent effect estimates and standard errors as using individual-level data on HDP and demographic covariates in logistic regression; and the large sample size provides sufficient statistical power and allows for precise quantifications of the effect estimates of PFAS exposure using two different exposure metrics. We also used two approaches to measure PFAS exposure, which produced similar results: null associations for PFOA in the crude and adjusted models, yet inverse associations for PFOA in the adjusted co-exposure model; positive associations for PFOS in the crude and adjusted models, and null association for PFOS in the adjusted co-exposure model; and small positive associations for

PFHxS in the crude and adjusted models. The similarities in these two approaches further add credibility in our findings.

Our study also has some limitations. First, data suppression, de-identification, and some missingness in CDC WONDER restricted our analyses to 551 and 87 large counties in the US with populations ≥100,000, respectively, for the two sets of analyses using different exposure metrics, which limits our ability to generalize the results to the entire US (Zhu and Bartell 2020). Also, birth certificates may capture HDP information imperfectly in the CDC database, although a previous validation study concluded that HDP is "reported with a reasonable level of accuracy" in birth and hospital discharge data (Robert et al., 2008). Second, the reported detections of PFAS in drinking water were dependent on the minimal reporting limits used in UCMR3, which likely underestimated the presence of PFAS in U.S water systems (Andrews and Naidenko 2020) and may have introduced some measurement error in our averaged water concentrations. Out of the 551 large counties covered in this study, only 87 counties had detections for at least one of the four PFAS, including 58 counties with detections for PFOA, 49 counties with detections for PFOS, 48 counties with detections for PFHpA, and 38 counties with detections for PFHxS. We also did not have individual-level information on type of water consumed to account for the use of bottled water, private well water, or public drinking water. Third, although we fit three sets of models (crude model, adjusted model, and adjusted co-exposure model) to better explore the associations between HDP and each PFAS chemical, we cannot rule out potential uncontrolled or incompletely controlled confounding, which may bias our results. Adjusting for education, in particular, shifted the parameter estimates more than other sociodemographic variables for our analyses using the first proxy indicator for PFAS exposure (see Table S4.3 in the

Supplemental Digital Content), suggesting possible residual confounding if education alone may not adequately capture the effects of socioeconomic status on HDP. Another limitation is our use of county-level PFAS water data, instead of individual-level measurements of PFAS in personal water or serum, which may have contributed to exposure measurement error. Additionally, Borghese et al. (2020) found that infant sex may be an effect modifier for PFAS and HDP. We tried but did not incorporate the stratified analysis by infant sex in this study due to additional data suppression after further stratification, which led to smaller strata and the inability to obtain standard error estimates.

4.6 Conclusion

We linked two publicly available databases (CDC WONDER and EPA UCMR3) to conduct a nationwide study on PFAS water concentrations and HDP. Our results show a weak positive association between the PFAS mixture and HDP, although the generalizability is subject to inherent limitations of the two datasets. Future studies using serum measurements of PFAS in early pregnancies would be a valuable addition to the body of research on this topic.

4.7 Acronyms

HDP: hypertensive disorders of pregnancy

PE: preeclampsia

PIH: pregnancy-induced hypertension

PFAS: per- and polyfluoroalkyl substances

PFOA: perfluorooctanoic acid

PFOS: perfluorooctane sulfonate

PFHxS: perfluorohexane sulfonic acid

PFHpA: perfluoroheptanoic acid

PFBS: perfluorobutane sulfonic acid

PFNA: perfluorononanoic acid

PFDA: perfluorodecanoic acid

PFUnDA: perfluoroundecanoic acid

PFDoA: perfluorododecanoic acid

PFUA: perfluoroundecanoic acid

4.8 Supplemental Digital Content

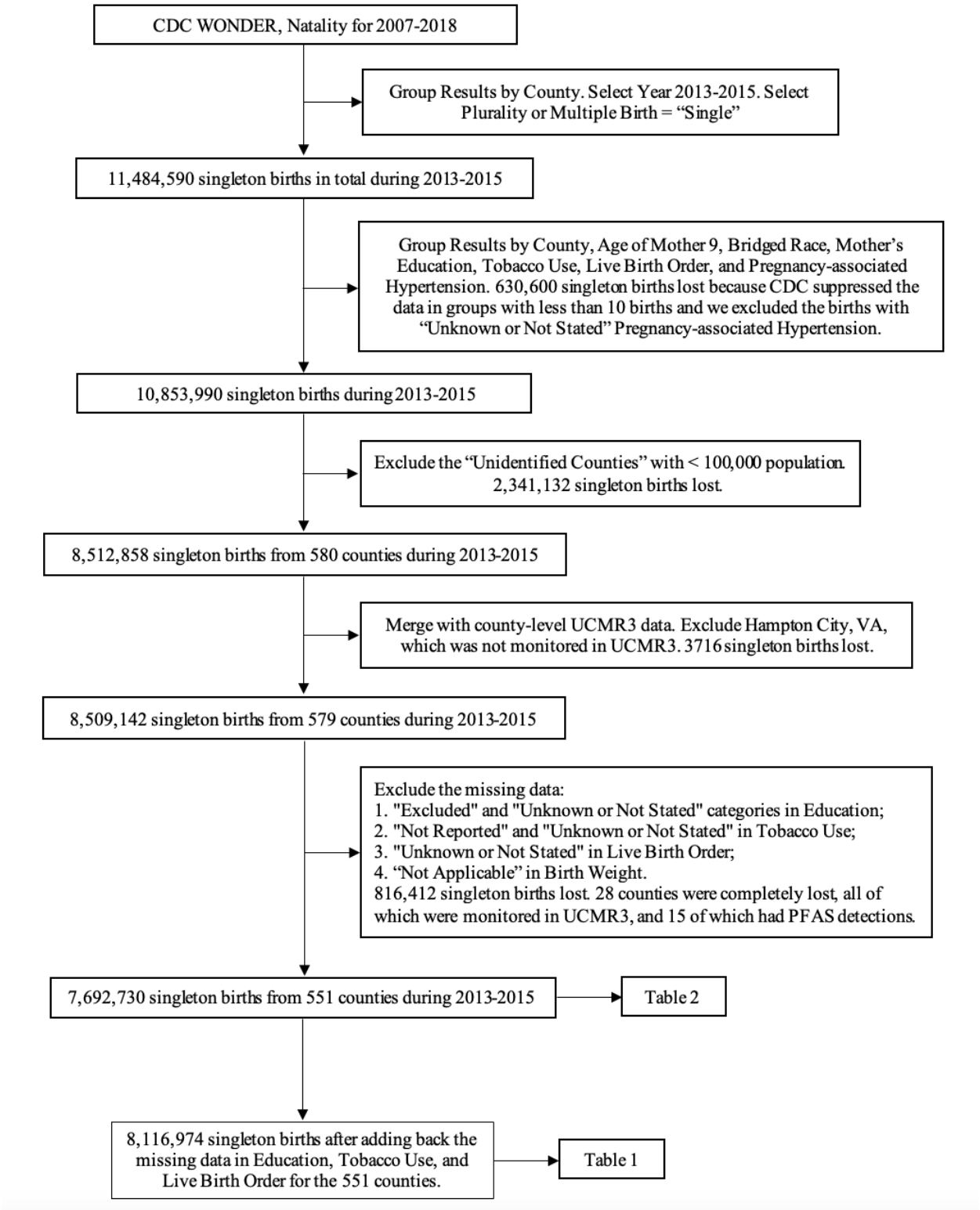

Figure S4.1. Study Profile

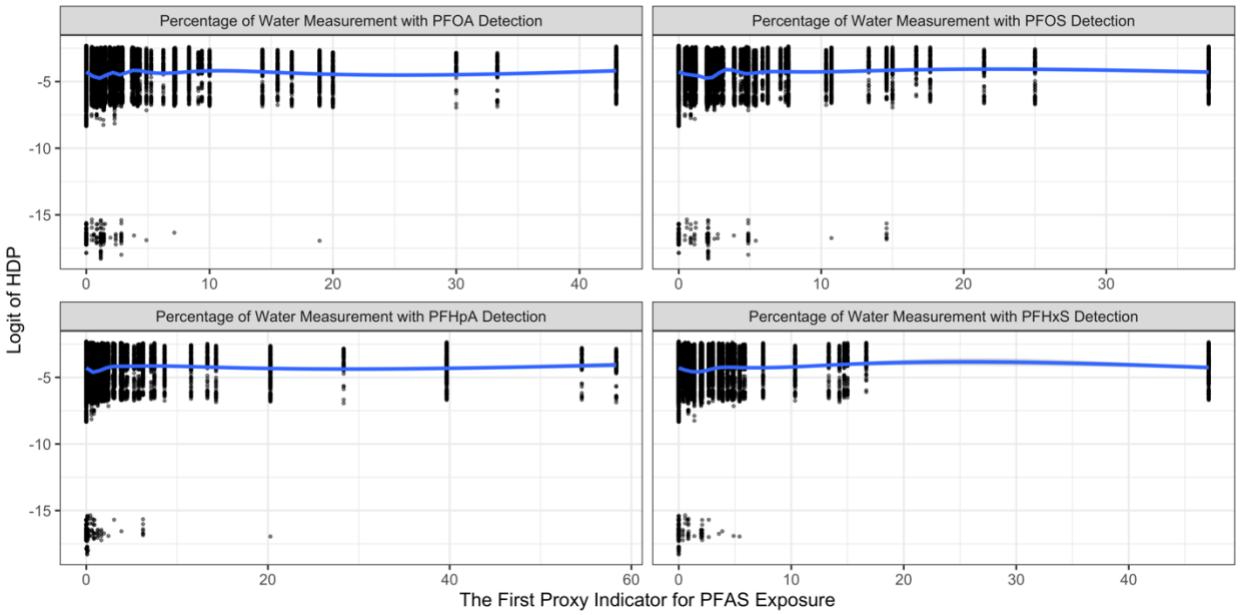

Figure S4.2. Scatter Plot between the First Proxy Indicator for PFAS Exposure and the Logit of HDP.

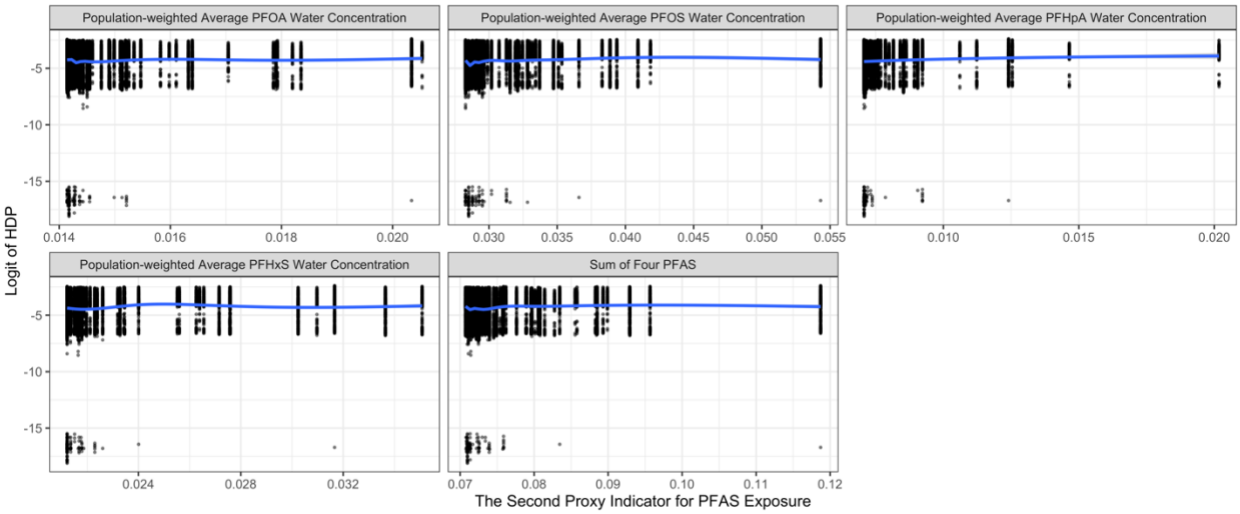

Figure S4.3. Scatter Plot between the Second Proxy Indicator for PFAS Exposure and the Logit of HDP.

Table S4.1. Generalized Variance-Inflation Factors (GVIF) for the Adjusted Co-exposure Models using the First Exposure Indicator - Percentage of water measurement with PFAS detection.

	GVIF	Df	GVIF^(1/(2*Df))
PFOA	2.82	1	1.68
PFOS	5.16	1	2.27
PFHpA	4.50	1	2.12
PFHxS	4.92	1	2.22
1,4-dioxane	1.11	1	1.05
Maternal age	1.69	7	1.04
Maternal race	1.06	3	1.01
Education	1.62	7	1.03
Smoking status	1.00	1	1.00
Parity	1.25	2	1.06

Table S4.2. Generalized Variance-Inflation Factors (GVIF) for the Adjusted Co-exposure Models using the Second Exposure Indicator - Population-weighted average PFAS concentration.

	GVIF	Df	GVIF^(1/(2*Df))
PFOA	2.08	1	1.44
PFOS	2.81	1	1.68
PFHpA	1.92	1	1.39
PFHxS	1.89	1	1.37
1,4-dioxane	1.31	1	1.15
Maternal age	1.64	7	1.04
Maternal race	1.07	3	1.01
Education	1.57	7	1.03
Smoking status	1.00	1	1.00
Parity	1.26	2	1.06

We fitted four additional models adding the sociodemographic confounders one at a time in additional to those shown in Table 4.2 in the manuscript to examine the effects of different sociodemographic variables. Model 1 adjusted for maternal age. Model 2 additionally adjusted for race based on Model 1. Model 3 additionally adjusted for education based on Model 2. Model 4 additionally adjusted for smoking status (yes, no) based on Model 3.

Table S4.3. Odds ratio with 95% confidence intervals (95% CI) of HDP per 10% increase in water measurements with PFAS detection in 551 counties in the US during 2013-2015 (No. of births = 7,692,730; No. of HDP cases = 215,957).

	PFOA	PFOS	PFHpA	PFHxS
Crude model[1]	1.004, [0.991, 1.018]	1.071, [1.057, 1.085]	1.036, [1.022, 1.050]	1.033, [1.019, 1.047]
Model 1	1.003, [0.990, 1.017]	1.074, [1.060, 1.088]	1.033, [1.019, 1.046]	1.029, [1.015, 1.044]
Model 2	1.007, [0.993, 1.020]	1.070, [1.056, 1.084]	1.022, [1.009, 1.036]	1.030, [1.016, 1.045]
Model 3	0.997, [0.983, 1.010]	1.065, [1.052, 1.079]	1.013, [1.00, 1.027]	1.020, [1.006, 1.034]
Model 4	1.001, [0.987, 1.014]	1.056, [1.042, 1.070]	1.013, [0.999, 1.026]	1.022, [1.008, 1.036]
Adjusted model[2]	1.002, [0.988, 1.015]	1.042, [1.029, 1.056]	1.011, [0.997, 1.025]	1.030, [1.016, 1.044]
Adjusted co-exposure model[3]	0.910, [0.882, 0.938]	1.015, [0.993, 1.038]	0.973, [0.945, 1.001]	1.116, [1.083, 1.151]

[1] Crude model: association between PFAS and HDP only.

[2] Adjusted model: adjusted for maternal age (<15, 15–19, 20–24, 25–29, 30–34, 35–39, 40–44, 45–49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (eighth grade or less; ninth through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (yes, no), and parity (first, second, third and over).

[3] Adjusted co-exposure model: adjusted for the other three PFAS, 1,4-dioxane, and all covariates in the adjusted model.

We also fitted four additional models adding the sociodemographic confounders one at a time in additional to those shown in Table 4.3 in the manuscript to examine the effects of different sociodemographic variables.

Table S4.4. Odds ratios (95% CI) of HDP per IQR (ng/L) increase in population-weighted average PFAS water concentration in 87 counties with detection of at least one of the four PFAS[1] (No. of births = 2,085,035; No. of HDP cases = 57,272).

	PFOA	PFOS	PFHpA	PFHxS	Sum of four PFAS
IQR	[14.14, 14.90]	[28.28, 31.74]	[7.07, 7.42]	[21.21, 22.04]	[70.98, 78.26]
IQR difference	0.76	3.46	0.35	0.83	7.28
Crude model[2]	1.009, 95% CI: [1.001, 1.016]	1.033, 95% CI: [1.024, 1.042]	1.014, 95% CI: [1.011, 1.017]	1.007, 95% CI: [1.005, 1.010]	1.037, 95% CI: [1.027, 1.047]
Model 1	1.005, 95% CI: [0.997, 1.013]	1.029, 95% CI: [1.020, 1.038]	1.013, 95% CI: [1.011, 1.016]	1.005, 95% CI: [1.002, 1.008]	1.031, 95% CI: [1.022, 1.041]
Model 2	0.996, 95% CI: [0.988, 1.004]	1.016, 95% CI: [1.007, 1.026]	1.009, 95% CI: [1.006, 1.012]	1.002, 95% CI: [0.999, 1.005]	1.016, 95% CI: [1.006, 1.026]
Model 3	0.993, 95% CI: [0.985, 1.001]	1.016, 95% CI: [1.007, 1.025]	1.008, 95% CI: [1.005, 1.011]	1.008, 95% CI: [1.005, 1.011]	1.013, 95% CI: [1.003, 1.022]
Model 4	1.003, 95% CI: [0.995, 1.011]	1.029, 95% CI: [1.020, 1.038]	1.009, 95% CI: [1.006, 1.012]	1.003, 95% CI: [1.0004, 1.006]	1.026, 95% CI: [1.016, 1.036]
Adjusted model[3]	1.009, 95% CI: [1.001, 1.016]	1.030, 95% CI: [1.021, 1.040]	1.008, 95% CI: [1.005, 1.011]	1.007, 95% CI: [1.004, 1.010]	1.032, 95% CI: [1.022, 1.042]
Adjusted co-exposure model[4]	0.976, 95% CI: [0.965, 0.986]	1.018, 95% CI: [1.003, 1.034]	1.014, 95% CI: [1.010, 1.018]	1.003, 95% CI: [0.999, 1.007]	1.031, 95% CI: [1.021, 1.041]

[1] Values that were below the minimal reporting levels (MRLs) were substituted with MRL/$\sqrt{2}$.

[2] Crude model: association between PFAS and HDP only.

[3] Adjusted model: adjusted for maternal age (<15, 15–19, 20–24, 25–29, 30–34, 35–39, 40–44, 45–49, ≥50), race (American Indian or Alaska Native, Asian or Pacific Islander, Black or African American, White), education (eighth grade or less; ninth through 12th grade with no diploma; High school graduate or GED completed; Some college credit, but not a degree; Associate degree; Bachelor's degree; Master's degree; Doctorate or professional degree), smoking status (yes, no), and parity (first, second, third and over).

[4] Adjusted co-exposure model: adjusted for the other three PFAS, 1,4-dioxane, and all covariates in the adjusted model.

Methods to estimate steady-state serum to drinking water ratios

The conversion factors were estimated from literature-based one-compartment pharmacokinetic models

$$CF = \frac{I}{k \cdot V} = \frac{I}{\frac{ln2}{t_{1/2}} \cdot V}$$

where I is the water ingestion rate, $t_{1/2}$ is the half-life of PFAS, and V is the volume of distribution.

For example, assuming the water ingestion rate is I=16.6 ml/kg/day (Lu and Bartell, 2020), PFOS serum half-life is 3.4 years (Li et al., 2018), and volume of distribution is V_{PFOS}=0.23 L/kg (Thompson et al., 2010), then the steady-state ratio for serum:water concentration of PFOS is

$$CF_{PFOS} = \frac{16.6\ ml/\ kg/day * 0.001\ L/ml * 365.25\ day/year}{\frac{ln2}{3.4\ year} * 0.23 L/kg} = 129.31$$

Assuming the PFOA serum half-life is 2.3 years (Bartell et al., 2010) and volume of distribution is $V_{PFOA} = 0.17$ L/kg (Thompson et al., 2010), then the steady-state ratio for serum:water concentration of PFOA is

$$CF_{PFOA} = \frac{16.6\ ml/\ kg/day * 0.001\ L/ml * 365.25\ day/year}{\frac{ln2}{2.3\ year} * 0.17 L/kg} = 118.35$$

Assuming the PFHxS serum half-life is 5.3 years (Li et al., 2018) and volume of distribution is $V_{PFHxS} = 0.23$ L/kg (Zhang et al., 2013), then the steady-state ratio for serum:water concentration of PFOA is

$$CF_{PFHxS} = \frac{16.6\ ml/\ kg/day * 0.001\ L/ml * 365.25\ day/year}{\frac{ln2}{5.3\ year} * 0.23 L/kg} = 201.57$$

Example R code for fitting binomial regression models with multiple-stratified data

```
set.seed(1)
n <- 100
library(dplyr)
library(extraDistr)

Smoke <- rcat(n, prob = c(0.1, 0.90), labels = c("Yes", "No"))
Age <- rcat(n, prob = c(0.5, 0.5), labels = c("<40", ">=40"))
p <- 0.25 * (Smoke == "Yes" & Age == "<40") + 0.1 * (Smoke == "No" & Age == "<40") +
  0.2 * (Smoke == "Yes" & Age == ">=40") + 0.25 * (Smoke == "No" & Age == ">=40") + 0.05
y <- rbinom(n, 1, p)


# logistic regression based on individual-level data
data.0 <- data.frame(Smoke, Age, p, y)

mod.0 <- glm(y ~ Smoke + Age, family = “binomial”, data = data.0)
summary(mod.0)


# binomial regression based on group-level multiple-stratified data
data.1 <- data.0 %>% group_by(Smoke, Age) %>% summarize(n = n(), n.y = sum(y)) %>%
mutate(p = 0.25 * (Smoke == "Yes" & Age == "<40") + 0.1 * (Smoke == "No" & Age == "<40")
+ 0.2 * (Smoke == "Yes" & Age == ">=40") + 0.25 * (Smoke == "No" & Age == ">=40") + 0.05)

mod.1 <- glm(cbind(n.y, n-n.y) ~ Smoke + Age, family = "binomial", data = data.1)
summary(mod.1)

# mod.0 and mod.1 produce the same results
```

CHAPTER 5

Retrospective Exposure Reconstruction using Approximate Bayesian Computation: A Case Study on Perfluorooctanoic Acid and Preeclampsia

Contents

5.1 Abstract

Background: In environmental epidemiology, measurements of toxicants in biological samples are often used as individual exposure assignments. It is common to obtain only one or a few exposure biomarkers per person and use those measurements to represent each person's relevant toxicant exposure for a given health outcome, even though most exposure biomarkers can fluctuate over time. When the timing of the exposure reflected by the biomarker measurement is misaligned with disease development especially if it occurs after the disease outcome, results could be subject to reverse causality or exposure measurement error.

Objective: This study aimed to use an approximate Bayesian computation (ABC) method to improve PFOA exposure estimates and characterize the effects of PFOA on preeclampsia in the C8 Studies.

Methods: Serum PFOA concentrations were measured in blood samples collected during 2005-2006 in West Virginia and Ohio (the C8 Studies), and residential and water use histories and pregnancy outcomes were obtained from self-reports. Our previous results may have been influenced by the choice of methods for characterizing PFOA exposures. Here we use an ABC method to combine measured PFOA serum concentrations and environmentally modeled PFOA concentrations to reconstruct historical PFOA exposures. We also expanded our previous work by assuming more realistic lognormal distributions for key input parameters in the exposure and pharmacokinetic models.

Results: Compared to using fixed values of model parameters and Monte Carlo simulations, ABC produced similar Spearman correlations between estimated and measured serum PFOA concentrations, yet substantially reduced the mean squared error by over 50%. Based on

ABC, compared to previous studies, we found a similar adjusted odds ratio (AOR) for the association between PFOA and preeclampsia.

Conclusions: Bayesian combination of modeled exposure and measured biomarker concentrations can reduce exposure measurement error compared to modeled exposure.

Key Words: Preeclampsia, Historical Exposure Reconstruction, Approximate Bayesian Computation, One-compartment Pharmacokinetic Model

5.2 Introduction

In environmental epidemiology, researchers typically use one of two approaches for exposure assessment: first, "contact-based" exposure models that combine individual time-activity patterns and measurements of the pollutants in the air, soil, food, water, and consumer products (Fjeld et al., 2007); second, "validated" biomarker measurements that assess the exposure concentrations in blood, hair, or other tissues obtained from the participants (Paustenbach and Galbraith, 2006). Many researchers prefer using "validated" biomarkers rather than "contact-based" exposure models (Paustenbach and Galbraith, 2006; Schisterman and Albert, 2012; Rappaport et al., 2015) due to greater objectivity and less reliance on self-reports of diet, water consumption, and other complex time-activity patterns. However, biomarker measurements are often difficult and expensive to obtain, therefore, many epidemiologists have used only one exposure biomarker per pollutant and study participant (Borghese et al., 2020; Johnson et al., 2014, Hertz-Picciotto et al., 2010; McKean et al., 2015; Starling et al., 2014; Steenland et al., 2018; Huang et al., 2019; Huo et al., 2020; Rylander et al., 2020; Wikström et al., 2019), which may not align with the disease induction period. For example, the C8 Studies in the U.S. only collected one serum sample for most participants at enrollment during 2005-2006 (Savitz et al., 2012a, 2012b; Stein et al., 2009). However, the pregnancy outcomes occurred from 1990 to 2005 or 2006, and most of them were before the corresponding biomarker exposure measurement, which is a violation of temporality in epidemiology in establishing causality (Hill, 1965). Exposure assessment methods that combine contact-based models with biomarker measurements, in ways that respect the temporal and pharmacokinetic relationships among the different types of information, may be beneficial in these settings (Georgopoulos et al., 2009).

To avoid potential reverse causality due to the violation of temporality and to ensure that exposure estimates were temporally aligned with the health outcomes under investigation, Shin et al. (2011a) developed a suite of environmental fate and transport models to estimate the yearly air and groundwater concentrations of PFOA from 1951 to 2008. The model outputs (air and groundwater PFOA concentrations) were then linked to each study participant based on their detailed self-reported residential and work histories to estimate the yearly inhalation and ingestion doses, i.e., the "contact-based" exposure model developed by Shin et al., 2011b. The individual yearly total doses (the sum of inhalation and ingestion doses) were input into a one-compartment pharmacokinetic model under the assumption of yearly piecewise constant intake rate to estimate each participant's yearly serum PFOA concentrations, which were then used in a series of epidemiological studies to investigate the associations between PFOA and health outcomes (Savitz et al., 2012a, 2012b; Barry et al., 2013; Vieira et al., 2013; Watkins et al., 2013; Winquist and Steenland, 2014; Darrow et al., 2016). The conceptual framework of Shin et al. (2011a, 2011b) is summarized in Figure 5.1. For example, based on the historical exposure construction (Shin et al., 2011a, 2011b), Savitz et al. (2012a) found a weak association between PFOA and self-reported preeclampsia [adjusted odds ratio (AOR) = 1.13, 95% CI: 1.00, 1.28, for an interquartile range (IQR) increase in natural log-transformed PFOA].

The sophisticated "contact-based" exposure model developed by Shin et al. (2011b) is potentially prone to systematic biases since it depends on numerous variables in the fate and transport models (Shin et al., 2011a), subjective self-reported water ingestion rates, and complicated time-activity patterns, many of which have large uncertainties. Avanasi et al. (2016a, 2016b, 2016c) subsequently used Monte Carlo simulations to account for the

uncertainties in PFOA water concentrations, water ingestion rates, and geocoding in the "contact-based" exposure model and pharmacokinetic parameters. The authors found similar associations between PFOA and preeclampsia to Savitz et al. (2012a), suggesting that effect estimates of PFOA and preeclampsia in the C8 Studies were largely driven by the rank order of exposure estimates of study participants, rather than the exact exposure amount (Avanasi et al., 2016a, 2016b, 2016c).

Although having accounted for uncertainties, studies by Avanasi et al. (2016a, 2016b, 2016c) were still solely based on historical exposure construction (Shin et al., 2011a, 2011b), without any adjustment or calibration for measured serum PFOA concentrations in 2005 or 2006. Biomarker measurements contain useful information regarding exposure, and when used in conjunction with modeled exposure, can improve the retrospective prediction of exposure (Georgopoulos et al., 2009).

Considering the strengths and limitations of the previous studies (Shin et al., 2011b, 2014; Avanasi et al., 2016a, 2016b, 2016c) and the complexity of the "contact-based" exposure model (Shin et al., 2011b), here we apply an approximate Bayesian computation (ABC) method to calibrate the yearly serum PFOA concentrations for the participants in the C8 Studies (i.e., approximate Bayesian calibration). The ABC method combines the single biomarker measurement and the environmentally modeled exposure, which is likely to reduce exposure measurement error. Incorporating environmental modeled exposure should also diminish any impact of reverse causality, compared to only using a single biomarker measurement which may have been influenced by the outcome.

ABC is a method that relaxes the need for a likelihood function in favor of summary statistics to facilitate inferences for complex Bayesian models; it has been extensively used

in biological and environmental sciences (Beaumont et al., 2002; Blum, 2010; Vrugt and Sadegh, 2013; Sadegh and Vrugt, 2014). In ABC, we simulate data based on candidate parameter values drawn from probability distributions, similar to Monte Carlo "uncertainty analysis" methods long practiced in exposure and risk assessment (Cox and Baybutt, 1981), and then compare the simulated data with observed data (measured values such as biomarker concentrations) using a defined "distance" function. We discard the candidate parameter values when the simulated data is not close enough to the observed data (Turner and Zandt, 2012). Specifically, we expand the previous approaches by incorporating uncertainties associated with key input parameters in both the previously developed exposure and pharmacokinetic models (Figure 5.1). We then investigate the association between PFOA and preeclampsia based on the reconstructed historical exposure estimate using the ABC method.

5.2.1 Previous Bayesian analysis of these data

In earlier work with these data, Shin et al. (2014) calibrated the retrospective exposure estimates by incorporating the one-time serum PFOA concentration measured in 2005 or 2006 for each participant in a simplified Bayesian framework. The authors derived a closed-form posterior distribution of the PFOA dose vector under the assumption of multivariate normal distribution for the prior dose vector during the 58 years (Shin et al., 2014); the posterior mean of the yearly total dose is a weighted average of the prior yearly dose (estimated from the environmental fate and transport models and "contact-based" exposure model) and the steady-state dose implied by the measured serum PFOA concentration for each participant. When the posterior mean was used in place of the original

exposure estimate for each participant estimated by Shin et al. (2011b), the association between PFOA and self-reported preeclampsia was slightly higher and statistically significant (AOR = 1.16, 95% CI: 1.03, 1.30 per IQR increase in natural log PFOA) (Savitz et al., 2012a).

Although the closed-form posterior in Shin et al. (2014) provides a straightforward way to update the PFOA dose vector to incorporate serum PFOA measurements within a complex "contact-based" exposure model (Shin et al., 2011b), the doses have an unrestricted range from negative infinity to positive infinity, requiring post-hoc adjustments to exclude negative doses, and the approach can suffer from computational difficulties due to high-dimensional singular matrices. In addition, the posterior mean of the yearly total dose was previously input directly into the one-compartment pharmacokinetic model under the assumption of yearly piecewise constant intake rate to estimate the yearly serum PFOA concentration, without incorporating posterior uncertainties in the dose estimates, while holding the PFOA half-life and the volume of distribution constant in the pharmacokinetic model (Shin et al., 2014). Our new ABC approach addresses those limitations by using lognormal water concentration distributions that disallow negative doses, specifying probability distributions for pharmacokinetic parameters, and using the full range of posterior exposure assignments in the epidemiologic analysis, rather than just the posterior mean.

5.3 Methods

5.3.1 Study population

The C8 Studies are a set of large, inter-related population-based health studies in the mid-Ohio River Valley, where high levels of PFOA were measured in drinking water due to contamination from the DuPont Washington Works Plant in West Virginia (Frisbee et al., 2009). The current analysis is restricted to 10,149 pregnancies that occurred from 1990 to the study enrollment in 2005 or 2006 in 6,134 women participants in the C8 Studies, excluding those who had occupational exposure to PFOA (Avanasi et al., 2016a). The average number of pregnancies is 1.65 (range: 1–6) among the recruited women in this study. A serum measurement of PFOA was taken for each participant at enrollment in 2005 or 2006. A detailed description of the study population and their demographic information can be found elsewhere (Savitz et al., 2012a).

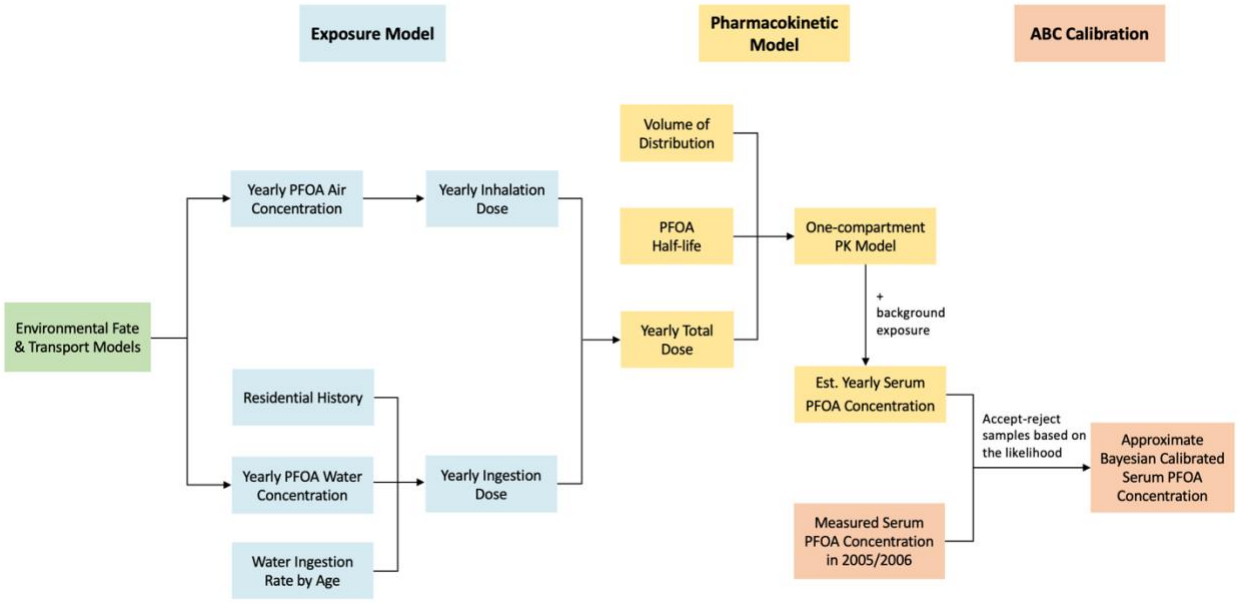

Figure 5.1. A Visualization of Shin et al. (2011a, 2011b)'s Conceptual Framework of Models.

5.3.2 Environmental fate and transport model

We obtained the annual estimated PFOA water concentrations (μg/L) in six water districts from the environmental fate and transport models developed by Shin et al. (2011a) (shown in Figure 5.2). To account for the hierarchical nature of the data, for each of the water districts, we assumed a uniform distributed hyperprior U (1, 10) for the ratio of standard deviation (σ_j) to the mean (μ_j) of the PFOA water concentration in year *j* (*j*=1, …, 58), due to vague prior information on the standard deviation of the estimated PFOA water concentrations.

$$\tau = \frac{\sigma_j}{\mu_j} \sim U(1, 10), \tag{1}$$

We assumed a multivariate lognormal distribution MVLN ($\mu_{log}, \Sigma {\sigma_{log}}^2$) for the vector of PFOA water concentrations in 58 years in each water district based on the predicted PFOA water concentrations shown in Figure 5.2. μ_{log} is a vector with the length of 58, and each element equal to

$$\mu_{log,j} = log\left(\frac{\mu_j^2}{\sqrt{\mu_j^2 + \sigma_j^2}}\right) = log\left(\frac{\mu_j}{\sqrt{1+\tau^2}}\right), \tag{2}$$

where μ_j is the PFOA water concentration in year *j*;

$$\sigma_{log} = \sqrt{log\,(1 + \frac{\sigma_j^2}{\mu_j^2})} = \sqrt{log\,(1+\tau^2)}, \tag{3}$$

where τ is sampled from the uniform distribution $U(1, 10)$ in each Monte Carlo iteration. Σ is a 58×58 first-order autocorrelation matrix AR(1) with autocorrelation factor equal to 0.75 (Shin et al., 2014), that is, the correlation between PFOA water concentration in year *i* and

year j is $0.75^{|i-j|}$. In sensitivity analyses, we also tried different values (0.5 and 0.9) for the autocorrelation factor.

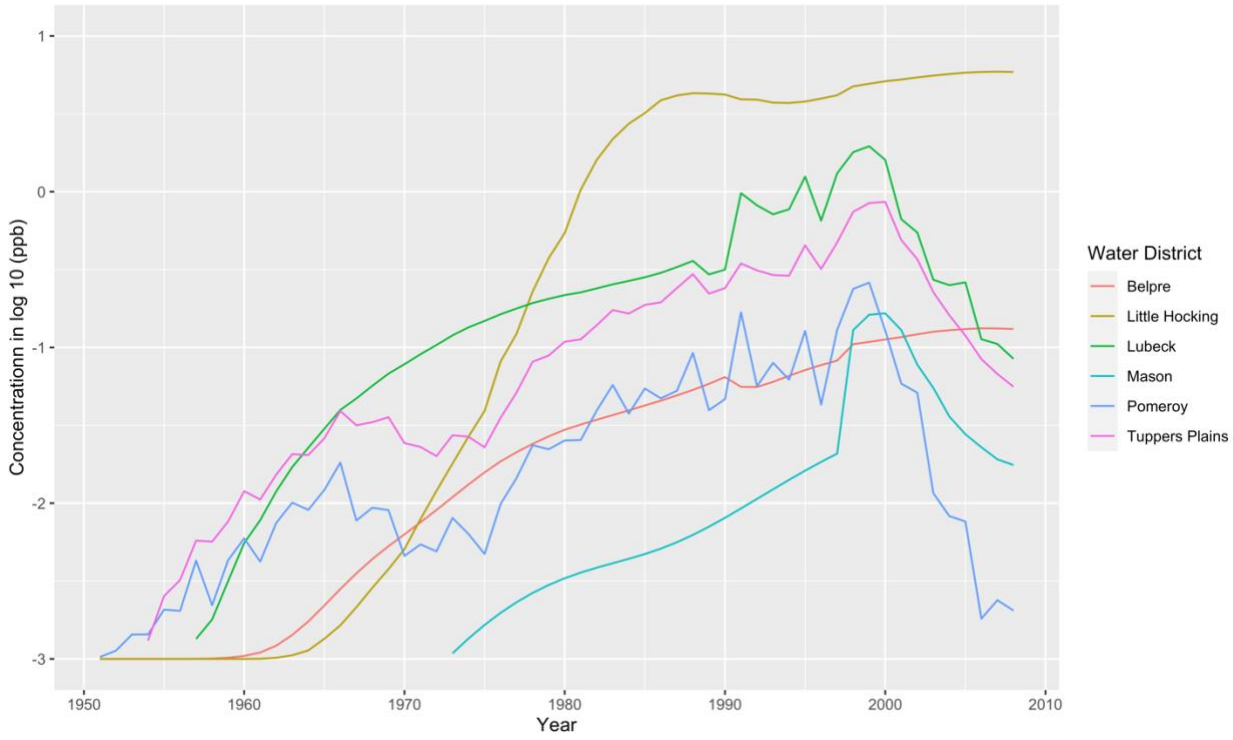

Figure 5.2. Annual average calibrated predicted PFOA concentrations (ppb) in log 10 scale in six water districts.

5.3.3 "Contact-based" exposure model

The "contact-based" exposure model provides exposure estimation based on water ingestion and air inhalation rates, and residential and work histories of the study participants (Shin et al., 2011b). The yearly total dose was computed as a sum of yearly ingestion dose and inhalation dose. Because water ingestion is the dominant exposure pathway for PFOA in the C8 Studies (Shin et al., 2011a, 2011b) during the time period of interest, we held the yearly inhalation dose constant for each participant and only accounted for uncertainty in the ingestion dose. The yearly ingestion dose is a product of PFOA water

concentration (μg/L) and yearly water intake (L). We assumed for each of the participants who had work histories, their water ingestion was constructed by 70% of residential and 30% of workplace water ingestions, which resulted in the highest correlation between predicted and observed serum PFOA concentration in 2005 or 2006 (Shin et al., 2011b). For the participants who did not report a water ingestion rate, we sampled age-specific water ingestion rates from the lognormal distributions shown in Table 5.1 throughout their lifetime during 1951-2008. We obtained the means (μ) and 95th percentiles ($p_{0.95}$) of age-specific shrinkage estimation of long-term water ingestion rates from Cuvelier and Bartell (2021). We used the formulas below to calculate the standard deviation (SD), log mean (μ_{log}), and log SD (σ_{log}) of the long-term water ingestion rate.

$$SD = (p_{0.95} - \mu)/1.645 \quad (4)$$

$$\mu_{log} = log\ (\mu^2/\sqrt{\mu^2 + \sigma^2}) \quad (5)$$

$$\sigma_{log} = \sqrt{log\ (1 + \frac{\sigma^2}{\mu^2})} \quad (6)$$

In each Monte Carlo iteration, we randomly sampled a percentile of the water ingestion rate for each participant from a uniform distribution $U(0,1)$ and used the same percentile throughout the individual's lifetime to compute the age-specific water ingestion rate from the quantile function of lognormal distribution (Avanasi et al., 2016b). In sensitivity analysis, we also used standard short-term water ingestion rates from the latest version of the U.S. Environmental Protection Agency (EPA) Exposure Factors Handbook (U.S. EPA, 2019) for the Monte Carlo simulation (Table S5.2).

Table 5.1. The parameters of the log-normal distribution for age-specific shrinkage estimation of long-term water ingestion rate (L/day) (Cuvelier and Bartell, 2021).

Age group	Mean (μ)	95th Percentile ($p_{0.95}$)	SD	Log mean (μ_{log})	Log SD (σ_{log})
0 to <2	0.438	0.913	0.289	-1.006	0.601
2 to <16	0.576	1.092	0.314	-0.682	0.510
16 to <21	0.995	1.809	0.495	-0.116	0.470
21 to <50	1.552	2.862	0.796	-0.323	0.483
50+	1.490	2.615	0.684	-0.303	0.437

5.3.4 One-compartment pharmacokinetic model

Assuming that the PFOA concentration is zero at birth (i.e., $\hat{C}_0 = 0$) and subsequent piecewise constant intake, that is, the intake rate I_j is a constant within each year j (j = 1, 2, …, m), where m is the number of years of life for each woman from their birth year (1947-1990) to their pregnancy year or year of serum measurement t (1990-2005). The estimated cumulative serum PFOA concentration at time t is

$$\hat{C}_t = \sum_{j=1}^{m} \frac{I_j}{k \cdot V}(1 - e^{-k}) \cdot e^{-k(m-j)}$$

$$= \sum_{j=1}^{m} \left(\frac{1 - e^{-k}}{k \cdot V}\right) e^{-k(m-j)} I_j \qquad (7)$$

based on the constant-source first-order removal model during the period of each piece of dose and first-order removal model after each piece of dose (Bartell, 2003; Fjeld et al., 2007; Shin et al., 2014).

Let

$$w_j = \left(\frac{1 - e^{-k}}{k \cdot V}\right) e^{-k(m-j)} \tag{8}$$

be the time-varying weight, k is the elimination rate of PFOA in serum and V is the volume of distribution (L). The elimination rate k is inversely proportional to the half-life of PFOA ($t_{1/2}$).

$$k = \frac{ln2}{t_{1/2}} \tag{9}$$

Then we can compute the estimated yearly PFOA serum concentration for each participant as a weighted sum of annual exposure contributions from previous years.

$$\hat{C}_t = \sum_{j=1}^{m} w_j I_j \tag{10}$$

Based on the above estimated serum PFOA concentration, we used a normal likelihood for the measured serum PFOA concentration in year 2005 or 2006 with mean equal to the estimated serum PFOA concentration in year 2005 or 2006 and variance equal to σ_ε^2 (Shin et al., 2014).

$$C_t \sim N(\sum_{j=1}^{m} w_j I_j \, , \sigma_\varepsilon^2) \tag{11}$$

To account for inter-individual differences in pharmacokinetic parameters, we assumed the half-life of PFOA ($t_{1/2}$) follows the lognormal distribution as below. The parameters were calculated based on the arithmetic mean and confidence interval (2.3 years, 95% CI: [2.1, 2.4]) reported by Bartell et al. (2011).

$$t_{1/2} \sim \text{LN}(0.83, 0.03) \tag{12}$$

We assumed the volume of distribution per body weight (V/kg) follows the lognormal distribution below. The parameters were calculated from the arithmetic mean (0.198 L/kg) and standard deviation (0.069 L/kg) reported by Butenhoff et al. (2004).

$$V \sim \mathrm{LN}(-1.68, 0.34) \quad (13)$$

For participants who reported body weight at the time of the survey in 2005 or 2006, we assumed that their body weight stayed constant since the age of 18 (Shin et al., 2011b), and we multiplied the sampled volume of distribution per weight (L/kg) by the self-reported body weight (kg) to obtain the volume of distribution V (L) in those years. We obtained the recommended age-specific body weight for women from the 2011 version of U.S. EPA Exposure Factors Handbook Chapter 8 (U.S. EPA, 2011) to calculate the volume of distribution for the participants before the age of 18. For participants who did not report body weight at the time of the survey, we used recommended age-specific body weight for women from the U.S. EPA Exposure Factors Handbook (U.S. EPA, 2011) to calculate the volume of distribution throughout their lifetime during 1951-2008.

5.3.5 Historical exposure estimation methods

To facilitate comparisons between different exposure estimate methods, we conducted three sets of analyses using: (a) fixed values of model parameters, similar to Shin et al., (2011b) and the analysis of PFOA and preeclampsia in Savitz et al. (2012a), (b) Monte Carlo simulations of model parameters, similar to Avanasi et al., (2016b), and (c) approximate Bayesian calibration of the exposure estimates, a novel approach proposed in the current study. However, our analyses (a) and (b) are not direct replicates of Shin et al., (2011b), Savitz et al. (2011a), and Avanasi et al., (2016b) because the prior information on

some model parameters (i.e., age-specific water ingestion rate, PFOA half-life) have been updated over the last 10 years.

(a) Exposure estimates using fixed values of model parameters

First, we ran the analyses using the exposure estimates based on the fixed values of PFOA water concentration shown in Figure 5.2, PFOA half-life of 2.3 years, volume of distribution of 0.198 L/kg, and mean of age-specific shrinkage estimation of long-term water ingestion rate shown in Table 5.1. That is, we only applied the mean component of each distribution, without accounting for uncertainty characterized by the variance component. Our analysis is similar to but slightly different from Savitz et al. (2012a) in that we excluded the participants with occupational exposure to PFOA (Avanasi et al., 2016a), and we also applied more recent estimate of PFOA half-life of 2.3 years (Bartell et al., 2010) rather than 3.5 years (Olsen et al., 2007) used by Savitz et al. (2012a); additionally, we applied recently published age-specific shrinkage estimates of long-term water ingestion rates (Cuvelier and Bartell, 2021) rather than the standard short-term water ingestion rates from the 2009 version of the U.S. EPA Exposure Factors Handbook used by Shin et al. (2011b) and Savitz et al. (2012a).

(b) Exposure estimates based on Monte Carlo simulations of model parameters

Second, we ran Monte Carlo simulations (with 1,000 iterations) of exposure estimates. That is, we computed 1,000 exposure estimates for each participant by taking 1,000 random samples from the assumed distributions of PFOA water concentrations, PFOA half-life, volume of distribution, and age-specific water ingestion rates. We kept all the 1,000 samples and did not adjust for the serum measurement of PFOA in 2005 or 2006. Our analysis is

similar to yet slightly different from Avanasi et al., (2016b) in that we applied more recent estimate of PFOA half-life of 2.3 years reported by Bartell et al. (2010) rather than 3.5 years reported by Olsen et al. (2007), and we applied age-specific shrinkage estimation of long-term water ingestion rate (Cuvelier and Bartell, 2021) rather than the standard short-term water ingestion rate based on the 2011 version of the U.S. EPA Exposure Factors Handbook, we also used a larger number of iterations than Avanasi et al. (2016b).

(c) Approximate Bayesian calibration of the exposure estimates

Third, we calibrated the estimated serum PFOA concentrations in the years of pregnancies (10,149 pregnancies in total) based on the one-time serum measurement of PFOA obtained in 2005 or 2006 for each of the 6,134 women. We ran 1000 iterations of the Monte Carlo simulations and then accepted or rejected samples of estimated year-by-year PFOA serum concentrations for all participants based on the normal likelihood calculation below

$$L \propto \exp\left(-\sum_{i=1}^{n=6,134} \frac{1}{2\sigma_\varepsilon^2}\left(C_t - \sum_{j=1}^{m} w_j I_j\right)^2\right) \quad (14)$$

where n = 6,134 is the sample size (number of pregnant women) in this study. We tried different values for the standard deviation ($\sigma_\varepsilon = 3C_t, 4C_t, 5C_t, 6C_t, 10C_t$, and $100C_t$) and rejected the samples when the likelihood function was less than $\varepsilon = 10^{-300}$ in statistical software R (version 4.0.3), indicating a large difference between the estimated ($\sum_{j=1}^{m} w_j I_j$) and measured serum concentrations (C_t) in 2005 or 2006.

For the above three different methods, we calculated the Pearson and Spearman correlation coefficients between estimated serum PFOA concentrations and measured

serum PFOA concentrations in 2005 or 2006. We also computed the mean squared error (MSE) and mean absolute error (MAE) to measure the differences between estimated serum PFOA concentrations and measured serum PFOA concentrations in 2005 or 2006.

5.3.6 Generalized estimating equation

As in previous analyses of these data, we used generalized estimating equations (GEE) with a logit link function to analyze the association between reconstructed historical serum PFOA concentration and preeclampsia, while accounting for exposure year, maternal age, education, smoking status, and parity (Savitz et al., 2012a). We used an exchangeable correlation structure in GEE to account for the correlation among multiple pregnancies from the same women (Savitz et al., 2012a). We estimated AOR for preeclampsia for an IQR increase in natural log-transformed PFOA after adjustment for the covariates mentioned above (Savitz et al., 2012a). We applied two strategies to compute AOR. First, we ran the epidemiological analysis using the average PFOA concentrations across all accepted samples. Second, we ran the analyses based on all accepted samples and then took an average of the effect estimates and computed the variance using the law of total variance (Avanasi et al., 2016b). We assumed approximate normality to compute 95% probability intervals (PI, i.e., the 2.5^{th} and 97.5^{th} percentiles) for the AOR, reflecting the contribution of both exposure uncertainty and traditional sampling error to the precision of the health effect estimate (Avanasi et al., 2016a). When the 95% PI for the AOR excluded 1, we considered the effect estimate to be statistically significant. We used statistical software R 4.1.0 for all data analysis. The R code can be found in the Supplemental material.

5.4 Results

Based on the fixed values of model parameters, we found a Pearson correlation of 0.62 and a Spearman correlation of 0.67 between the estimated and measured serum PFOA concentrations in 2005 or 2006 for the 6,134 women participants in this study (Table 5.2), similar to the previous finding for all 45,276 participants in the C8 studies (Spearman correlation = 0.67 in Shin et al., 2011b). Based on the fixed values of model parameters, we found an AOR of 1.15 (95% PI: 0.96, 1.37) for preeclampsia for an IQR increase in natural log-transformed PFOA. Monte Carlo simulations with 1,000 iterations produced similar results in epidemiological analyses, despite a 23.7% increase in MSE and a 14.2% increase in MAE, compared to those calculated based on the fixed values of model parameters (Table 5.2).

Approximate Bayesian calibration of exposure estimates produced similar Pearson and Spearman correlations, as well as similar epidemiological associations (Table 5.2), although MSE and MAE are 24.6%-72.1% lower than those based on exposure estimate using fixed values of model parameters and 33.9%-77.5% lower than those based on Monte Carlo simulations shown in Table 5.2, respectively; and the MSE and MAE further decreased as we increased the standard deviation of the measured serum PFOA concentration (σ_ε) in the likelihood function from $3C_t$ to $10C_t$ (Table 5.2). Increasing the standard deviation of the measured serum PFOA concentration (σ_ε) in the likelihood function to $100C_t$ produced an acceptance rate of 99% and much higher MSE and MAE.

We also found a slightly larger effect estimate, as shown by AOR, based on the posterior mean of exposure estimate, using ABC compared with using fixed values of model parameters and Monte Carlo simulations (Table 5.2), which might be due to the

improvement in exposure estimate because random error in exposure measurement can attenuate the effect estimate towards the null (Hutcheon et al., 2010). Using different values of autocorrelation (0.5, 0.9) in ABC produced similar results in sensitivity analyses (Table S5.1). We also found similar results using standard water ingestion rates reported by the U.S. EPA (Table S5.3) to those using the shrinkage estimation of long-term water ingestion rates in ABC (Table 5.2).

Table 5.2. Exposure estimates using three different methods and epidemiological analysis results.

Fixed values of model parameters[1]			Pearson correlation[5]	Spearman correlation[5]	MSE[6]	MAE[6]	AOR and 95% CI	
			0.62	0.67	22384.00	48.86	1.15 [0.96, 1.38]	
Monte Carlo Simulations[2]			Pearson correlation[5]	Spearman correlation[5]	MSE[6]	MAE[6]	AOR and 95% PI based on the average of simulated PFOA serum concentrations	AOR and 95% PI based on all simulated samples and rule of total variance
			0.65	0.67	27692.30	55.78	1.15 [0.96, 1.37]	1.14 [0.88, 1.46]
Approximate Bayesian calibration of exposure estimates combining contact-based exposure estimates with the biological measurement[3]	Assumption on σ_ε	Acceptance rate[4]	Pearson correlation[5]	Spearman correlation[5]	MSE[6]	MAE[6]	AOR and 95% PI based on the posterior mean of exposure estimate	AOR and 95% PI based on all the accepted samples and rule of total variance
	$\sigma_\varepsilon = 3C_t$	12.5%	0.64	0.67	8911.11	30.58	1.17 [0.88, 1.56]	1.14 [0.83, 1.58]
	$\sigma_\varepsilon = 4C_t$	24.0%	0.66	0.67	8192.02	29.13	1.17 [0.90, 1.53]	1.14 [0.84, 1.55]
	$\sigma_\varepsilon = 5C_t$	36.0%	0.66	0.67	7178.79	27.48	1.17 [0.91, 1.49]	1.14 [0.86, 1.53]
	$\sigma_\varepsilon = 6C_t$	42.9%	0.66	0.67	7057.48	27.22	1.17 [0.92, 1.49]	1.14 [0.86, 1.52]
	$\sigma_\varepsilon = 10C_t$	68.4%	0.66	0.67	6234.05	27.22	1.16 [0.94, 1.44]	1.14 [0.87, 1.49]
	$\sigma_\varepsilon = 100C_t$	99.0%	0.65	0.67	10640.83	36.86	1.15 [0.95, 1.39]	1.13 [0.88, 1.47]

[1] All the values were calculated based on fixed values of PFOA water concentrations in the six water districts shown in Figure 5.2, PFOA half-life of 2.3 years, volume of distribution of 0.198 L/kg, and mean of age-specific shrinkage estimation of long-term water ingestion rate shown in Table 5.1.

[2] Monte Carlo simulations of model parameters (PFOA water concentrations in the six water districts shown in Figure 5.2, PFOA half-life, volume of distribution, and age-specific shrinkage estimation of long-term water ingestion rate) with 1,000 iterations.
[3] Assuming autocorrelation factor of 0.75 and age-specific shrinkage long-term water ingestion rate reported by Cuvelier and Bartell (2021).
[4] Acceptance rate of ABC based on the likelihood function. We rejected the simulated samples when the likelihood is less than $\varepsilon = 10^{-300}$ in statistical software R.
[5] Correlation coefficients between estimated serum PFOA concentrations and measured serum PFOA concentrations in 2005 or 2006.
[6] Summary of errors between estimated serum PFOA concentrations and measured serum PFOA concentrations in 2005 or 2006.

5.5 Discussion

In this study, we used an ABC method to reconstruct/calibrate the historical PFOA exposure estimates. Based on the reconstructed exposure estimates, our effect estimate of PFOA on preeclampsia is similar to previous findings based on the same population (Savitz et al., 2012a; Avanasi et al., 2016a, 2016b, 2016c). Although the ABC method has the best performance in terms of exposure estimates with lower MSE and MAE, the general epidemiological results are insensitive to the methods of exposure reconstruction applied in this study, i.e., using fixed values of model parameters, Monte Carlo simulations of model parameters, or approximate Bayesian calibration of exposure estimates based on the biological measurement (Table 5.2).

Strengths of this study include the Bayesian combination of modeled PFOA concentration with measured PFOA concentrations in serum samples using the ABC method to improve the exposure estimate compared with previous C8 studies (Savitz et al., 2012a; Avanasi et al., 2016a, 2016b, 2016c), and the use of more realistic lognormal prior distributions for the year-by-year PFOA water concentrations in the six water districts than the multivariate normal distribution assumption in Shin et al. (2014). We also accounted for inter-individual variability in model parameters including water ingestion rate, PFOA half-life, and volume of distribution.

Due to the complexity of the "contact-based" exposure model, we considered but did not implement the Bayesian method using standard packages such as STAN or JAGS, which use Markov Chain Monte Carlo methods that substantially add to the computational burden. Future work should be done to increase the speed of this program, with the possibility of turning it into a R package. Although some previous studies have applied a fully Bayesian

method that fits a joint model for the exposure and health outcome data (Gryparis et al., 2009), doing so uses the disease outcome to update the exposure status, which is more likely to introduce reverse causality effects. Therefore, we modeled the exposure data independently of the outcome and did not include the exposure-disease model or the outcome data in our accept-reject sampling criterion.

Despite reconstruction/calibration of modeled serum PFOA concentrations based on the biomarker measurements, our results are still heavily driven by the estimated PFOA serum concentration based on the environmental fate and transport models, exposure model, and pharmacokinetic models, rather than measured serum PFOA. This is because only a single serum sample for each participant was collected, and the half-life driven time-varying weights in the piecewise constant intake model are relatively small for PFOA, so the measured biomarkers only have a substantial influence on the exposure calibration for the years just before the 2005-2006 biomarker measurements, and less impact on exposure estimation for the 1990s. This is a limitation of the present analysis but also a strength of the Bayesian calibration method, as the time-varying weights are appropriately dependent on the timing and physiology of the measured biomarkers. For studies incorporating multiple biomarker measurements over time, or biomarkers with longer half-lives, the Bayesian calibration method would place greater weight on the biomarker measurements, improving the calibration.

In addition, our sophisticated environmental, exposure, and pharmacokinetic models (i.e., fate and transport models, "contact-based" exposure model, one-compartment pharmacokinetic model under the assumption of yearly piecewise constant intake rate) included a large number of parameters, which are prone to systematic bias; and the

estimation of serum PFOA concentration ($\sum_{j=1}^{m} w_j I_j$) might be mis-specified. This could explain why the MSE and MAE further decreased as we increased the standard deviation of the measured serum PFOA concentration (σ_ε) in the likelihood function from $3C_t$ to $10C_t$ in Table 5.2. Although we applied an accept-reject sampling criterion in the ABC method, we only rejected the samples when the likelihood function (14) was less than $\varepsilon = 10^{-300}$ in R, i.e., the group of samples with a large discrepancy between estimated ($\sum_{j=1}^{m} w_j I_j$) and measured serum concentrations (C_t) in 2005 or 2006. Therefore, our accept-reject sampling criterion may not be strict enough to only keep "close enough" observed data and simulated data as typical ABC procedures would do (Turner and Zandt, 2012), and only extremely unrealistic values of simulated data were excluded. Thus, the decreasing MSE and MAE in Table 5.2 might be explained by systematic bias or model misspecification, in which case, assuming a large standard error for the likelihood (14) may work better in quantifying uncertainties and simulating data that are more realistic and closer to the observed data. Increasing the standard deviation of the measured serum PFOA concentration (σ_ε) in the likelihood function to $100C_t$ produced an acceptance rate of 99.0% and much higher MSE and MAE, indicating that the optimal standard deviation in this setting is somewhere between $10C_t$ and $100C_t$.

Although we expect that using ABC (other Bayesian approaches) to combine environmental exposure models and biomarker measurements will improve epidemiologic studies by reducing exposure measurement error and limiting the potential for reverse causation, compared to using either exposure assessment approach in isolation, epidemiologic effect estimates based on the ABC method may still be susceptible to some degree of reverse causation in certain settings due to incorporating biomarker

measurements. For that reason, it may be valuable to compare the epidemiologic effect estimate using biomarkers alone to the effect estimate using environmental models alone, prior to employing the ABC method, as large differences in the two effect estimates may be indicative of reverse causation (Watkins et al., 2013; Weisskopf and Webster, 2017).

5.6 Conclusion

In this study, we used an ABC method to combine measured PFOA biomarker concentrations with environmentally modeled PFOA concentrations to improve PFOA exposure estimates and characterize the health effects of PFOA on preeclampsia in the C8 Studies. We found smaller measurement errors and slightly larger effect sizes when using approximate Bayesian calibration, compared to when setting fixed values of key parameters in the exposure and pharmacokinetic models or using Monte Carlo simulations without adjustment to the biomarker measurements. Our results demonstrate the effectiveness of using the ABC method to reduce exposure measurement error in environmental exposure estimates. We believe these modifications may improve the effect estimate of PFOA on preeclampsia in the C8 studies. It is an important scientific question, as PFOA is a very active topic of policy discussion regarding regulation and medical monitoring, which depend heavily on the interpretation of epidemiologic evidence regarding its potential health effects.

5.7 Acronyms

PFOA: perfluorooctanoic acid

ABC: Approximate Bayesian Computation

IQR: interquartile range

AOR: adjusted odds ratio

PI: probability interval

MSE: mean squared error

MAE: mean absolute error

GEE: generalized estimating equations

5.8 Supplemental Material

Table S5.1. Sensitivity analysis with different assumptions of autocorrelation factor and $\sigma_\varepsilon = 4C_t$.

Autocorrelation factor	Acceptance rate[1]	Pearson correlation[2]	Spearman correlation[2]	MSE[3]	MAE[3]	AOR[4] based on the posterior mean of exposure estimate	AOR[4] based on all the accepted samples and rule of total variance
0.5	18.8%	0.66	0.67	7385.55	27.70	1.17, 95% PI: [0.91, 1.49]	1.14, 95% PI: [0.87, 1.51]
0.75	24.0%	0.66	0.67	8192.02	29.13	1.17, 95% PI: [0.90, 1.53]	1.14, 95% PI: [0.84, 1.55]
0.9	33.3%	0.65	0.67	8375.14	29.55	1.16, 95% PI: [0.88, 1.54]	1.13, 95% PI: [0.82, 1.56]

[1] Acceptance rate of ABC based on the likelihood function. We rejected the simulated samples when the likelihood is less than $\varepsilon = 10^{-300}$ in statistical software R.

[2] Correlation coefficients between estimated serum PFOA concentrations and measured serum PFOA concentrations in 2005 or 2006.

[3] Summary of errors between estimated serum PFOA concentrations and measured serum PFOA concentrations in 2005 or 2006.

[4] Adjusted odds ratios for an interquartile range (IQR) increase in natural log-transformed estimated serum PFOA concentrations.

Table S5.2. The parameters of the log-normal distribution for all age groups for standard short-term water ingestion rate (L/day) based on the latest version of the U.S. EPA Exposure Factors Handbook Chapter 3 (U.S. EPA, 2019).

Age group	Mean	95th Percentile	Standard Deviation (SD)	Log mean	Log SD
1 to <2	0.245	0.658	0.251	-1.765	0.847
2 to <3	0.332	0.901	0.346	-1.470	0.857
3 to <6	0.338	0.836	0.303	-1.379	0.768
6 to <11	0.455	1.258	0.488	-1.170	0.875
11 to <16	0.562	1.761	0.729	-1.070	0.993
16 to <21	0.722	2.214	0.907	-0.799	0.973
21 to <50	1.277	3.353	1.262	-0.096	0.826
50 to <60	1.419	3.388	1.197	0.081	0.733
60 to <70	1.394	3.187	1.090	0.094	0.691
70 to <80	1.214	2.641	0.868	-0.012	0.642

Table S5.3. Approximate Bayesian calibration of exposure estimates, and results of epidemiological analyses (assuming autocorrelation factor of 0.75 and age-specific short-term water ingestion rate reported by U.S. EPA, 2019).

Standard deviation	Acceptance rate[1]	Pearson correlation[2]	Spearman correlation[2]	MSE[3]	MAE[3]	AOR[4] based on the posterior mean of exposure estimate	AOR[4] based on all the accepted samples and rule of total variance
$\sigma_\varepsilon = 3C_t$	14.1%	0.64	0.68	8975.16	30.81	1.14, 95% PI: [0.85, 1.53]	1.12, 95% PI: [0.81, 1.56]
$\sigma_\varepsilon = 4C_t$	27.1%	0.65	0.68	8048.46	28.89	1.17, 95% PI: [0.89, 1.53]	1.14, 95% PI: [0.84, 1.56]
$\sigma_\varepsilon = 5C_t$	35.7%	0.65	0.67	7493.59	27.95	1.18, 95% PI: [0.91, 1.52]	1.14, 95% PI: [0.84, 1.55]
$\sigma_\varepsilon = 6C_t$	43.5%	0.65	0.68	7037.42	27.22	1.16, 95% PI: [0.91, 1.48]	1.14, 95% PI: [0.85, 1.52]

[1] Acceptance rate of ABC based on the likelihood function. We rejected the simulated samples when the likelihood is less than $\varepsilon = 10^{-300}$ in statistical software R.

[2] Correlation coefficients between estimated serum PFOA concentrations and measured serum PFOA concentrations in 2005 or 2006.

[3] Summary of errors between estimated serum PFOA concentrations and measured serum PFOA concentrations in 2005 or 2006.

[4] Adjusted odds ratios for an interquartile range (IQR) increase in natural log-transformed estimated serum PFOA concentrations.

CHAPTER 6

Potential Effects of Long-term Exposure to Air Pollution on Dementia: A Longitudinal Analysis in American Indians Aged 55 Years and Older

Contents

6.1 Abstract

Background: Air pollution has been identified as an important risk factor for dementia in previous studies. As a unique population with a history of colonization, American Indians have long been disproportionately affected by air pollution. However, few studies have investigated the effects of air pollution on the risk of developing dementia in American Indians. This study aims to address this important knowledge gap.

Method: We downloaded county-level average air pollution data in 2003-2007 from the land-use regression models developed by the Center for Air, Climate, & Energy Solutions (CACES) and merged with data from the Indian Health Service (IHS) National Data Warehouse and related electronic health record databases between fiscal year (FY) 2007-2013. We assigned air pollution exposure to IHS users based on their county of residence and calculated 5-year averages of $PM_{2.5}$, O_3, and NO_2 in each county. A total of 32,564 American Indians who were 55+ years old in FY2007, used IHS services at least once each year, and were dementia free between FY2007-2009 were included in the main analysis. We employed Cox proportional hazard models with age as the time scale to examine the association of air pollution with dementia incidence, adjusting for potential confounders.

Results: The $PM_{2.5}$ levels in the IHS counties were lower than those in all US counties during 2003-2013, while the O_3 levels in the IHS counties were higher than the US in most of the years, and the NO_2 levels in the IHS counties were similar to that of the US, especially during those years before the baseline. We observed higher rate of incident dementia cases per 1,000 participants in IHS counties with higher O_3 levels. In multivariable Cox regression models, we found a significant positive association between dementia and county-level O_3 with a hazard ratio (HR) of 1.29 (95% CI: 1.05-1.59) per 1 ppb standardized O_3, while the

associations between dementia and $PM_{2.5}$, and NO_2 were not statistically significant. We found similar results in sensitivity analyses using longer lags between air pollution and dementia.

Conclusion: Our study suggests that exposure to O_3 is associated with the higher risk of dementia in American Indians. Future efforts to reduce exposure to air pollution might help lower dementia risk in American Indian communities.

6.2 Introduction

Air pollution, an important source of premature mortality and morbidity, is unevenly distributed across the US (Dockery et al., 1993). In the United States, people of color experience greater exposure to air pollution than White (Tessum et al., 2021). The disparity was found in people at all income levels across states, urban, and rural areas (Tessum et al., 2021). As a distinct population with "historical trauma" caused by forced colonization (Grayshield et al., 2015), Native Americans who live in tribes face disproportionate health impacts from air pollution due to the oil and gas industry that emits volatile organic compounds (VOCs) and nitrogen oxides (NO_x), which react in sunlight to form ground-level ozone (O_3) pollution that is harmful to human health (CATF, 2018; US EPA, 2021).

Long-term/chronic exposure to $PM_{2.5}$, O_3, NO_x or nitrogen dioxide (NO_2), the major toxic substances in the air, can trigger local inflammation and oxidative stress in the brain (Block and Calderon-Garciduenas, 2009; Béjot et al., 2018), which may play a role in neurodegeneration processes and lead to dementia (Peters et al., 2019; Power et al., 2016). Calderón-Garcidueñas et al. (2002) established the first linkage between air pollution and neurodegenerative disease in 32 healthy dogs in a highly polluted urban region in Southwest Metropolitan Mexico City. Subsequent epidemiological studies in humans further investigated the association in different populations. However, the results are inconsistent, ranging from significant to weak, null, or negative effects, possibly due to the variations in study designs, populations, air pollutants, sources of exposure, exposure levels, windows of exposure assessment, follow-up periods, outcome assessment, disease subtypes, statistical methods, uncontrolled confounding factors, etc.

Although most of the studies found positive associations between incident dementia/dementia hospitalization/cognitive decline and exposure to $PM_{2.5}$ (Cacciottolo et al., 2017; Chen et al., 2017; Carey et al., 2018; Grande et al., 2020; Jung et al., 2015; Shaffer et al., 2021; Shi et al., 2021; Tonne et al., 2014; Weuve et al., 2012), NO_x/NO_2 (Carey et al., 2018; Chang et al., 2014; Chen et al., 2017; Grande et al., 2020; Oudin et al., 2016; Shi et al., 2021), or O_3 (Cerza et al., 2019; Cleary et al., 2018; Jung et al., 2015; Wu et al., 2015), some did not find significant associations or even found negative associations between the outcome and $PM_{2.5}$ (Cleary et al., 2018; Loop et al., 2013), NO_x/NO_2 (Oudin et al., 2017), or O_3 (Chen et al., 2017; Carey et al., 2018). For example, Oudin et al. (2018) found the association differed by the source of $PM_{2.5}$, i.e., $PM_{2.5}$ from traffic exhaust was associated with incident dementia, while $PM_{2.5}$ from residential wood burning was not. In contrast, Tonne et al. (2014) found that $PM_{2.5}$ from traffic was not associated with cognitive change. Also, some studies reported different results for different air pollutants (Carey et al., 2018; Chen et al., 2017; Cleary et al., 2018; Cerza et al., 2019; Jung et al., 2015; Shi et al., 2021). For example, Cerza et al. (2019) found positive associations between O_3 and dementia hospitalization, yet a negative association between NO_2 and dementia hospitalization in Rome. However, Carey et al. (2018) reported positive associations between $PM_{2.5}$, NO_2 and dementia, yet a negative association between O_3 and dementia in London. In addition to the above studies, Wang et al. (2022) recently reported reduced dementia risk associated with air quality improvement with regards to $PM_{2.5}$ and NO_2 in older women in the US.

Although multiple previous studies examined the association between air pollution and dementia, no study that we are aware of has investigated the problem in Native Americans specifically. As a unique population in the US, Native American communities have

less insurance coverage, and worse access and utilization of health services than Whites (Zuckerman et al., 2004), and thus have long been disproportionately affected by the high burden of dementia and closely related cardiovascular diseases (CVD) (Browne et al., 2016; Galloway, 2005). With detailed individual-level information on dementia diagnosis and associated risk factors and county-level geographic information, the Indian Health Service National Data Warehouse and related electronic health records can be linked to public-use air pollution data, providing an invaluable opportunity for us to investigate the effects of air pollution on dementia risk in American Indians. To our knowledge, this is the first longitudinal study that specifically investigates the long-term effects of air pollution on dementia incidence in American Indians.

6.3 Methods

6.3.1 Indian Health Service (IHS) Data Project

The IHS provided healthcare services (hospitals, clinics, and health center/station) to approximately 2.56 million American Indians/Alaska Natives (AI/ANs) in 574 federally recognized Tribes in 37 states, accounting for more than one-third of AI/AN population in the US (US DHHS IHS, 2020). We obtained data from the IHS Improving Health Care Delivery Data Project (IHS Data Project) data infrastructure that includes health status, service use information for over 640,000 AI/ANs during fiscal years (FY) 2007-2013, representing about 30% of AI/ANs who use IHS services (O'Connell et al., 2014).

6.3.2 Air pollution Data

We obtained yearly county-level air pollution data for three common air pollutants ($PM_{2.5}$, O_3, and NO_2) for contiguous US counties from land-use regression models developed by the Center for Air, Climate, & Energy Solutions (CACES) (Kim et al., 2018). The models were built based on publicly available air pollution concentration measurements from US EPA regulatory monitors, information on land use (e.g., locations of major/minor roads, elevation, urban/rural area information), and satellite-derived estimates of air pollution to predict concentrations at locations without measurements (Kim et al., 2018). Model estimates are annual average values for $PM_{2.5}$, NO_2, and annual warm-season average during May through September of the daily maximum 8-hour moving average ground-level O_3. Ground-level O_3 is more easily formed during the warm season (Jacob, 1999) and this metric has long been used in environmental epidemiology to study the long-term health effects of ground-level O_3 (Jerrett et al., 2009; Shi et al., 2021). The CACES group provided air pollution

prediction at multiple geographic units including county, census tract, and census block group. In this study, we linked the IHS users to the air pollution data based on their earliest county information on record during the 7 years of follow-up from FY2007 to FY2013 and conducted the analyses at the county level, because county is the smallest standard geographic unit recorded on file for the IHS users, and is recorded for 99.8% of the IHS users. We excluded Alaska Natives from this study because the CACES group did not include Alaska in their air pollution modeling. We averaged the estimated air pollution levels obtained from CACES over the 5-year period before baseline (i.e., from 2003 to 2007), the most used length of period for averaging (Grande et al., 2020; Shi et al., 2021), to represent the long-term exposure to air pollution and linked those exposures to the IHS users based on their county of residence.

6.3.3 Study Population

For the FY2007-2013 IHS cohort, we restricted our study population to those who were at least 55 years old at enrollment in FY2007, used IHS services at least once each year, and were dementia-free at baseline between FY2007-2009 in this study to better capture dementia incidence. We restricted our study participants to those who live in the contiguous US, where the air pollution data is readily available from CACES. We also excluded the counties with ≤ 5 participants in the data. Our final data included a total of 32,564 American Indians from 95 counties in the contiguous US.

6.3.4 Survival Analyses

We used Cox proportional hazard regression models to examine the association of air pollution with dementia incidence. Because aging is considered as the greatest risk factor for the development of dementia, we used age rather than time-on-study as the time scale and accounted for left truncation with truncation time specified as age in FY2007, which allows for the non-parametric specification of the age effect and can automatically adjust for the confounding effect of age in the elderly population (Cancholа et al., 2003; Lamarca et al., 1998; Shaffer et al., 2021).

We adjusted for gender, 14-category study region, and modifiable lifestyle-related cardiometabolic risk factors including depression, diabetes, hypertension, and CVD at baseline (Zaninotto et al., 2018; Goins et al., 2021). Neighborhood disadvantage is another important risk factor for personal health as suggested by previous studies (Kind et al., 2014; Kind et al., 2018). Therefore, in addition to the above individual-level confounders, we further adjusted for county-level socioeconomic status (SES), i.e., percentage of poverty in all races and percentage of education below high school completion in people aged 25 years and older in all races, from the American Community Survey (ACS) 2010-2014.

We computed robust standard errors to account for the fact that between-county variations may be larger than within-county variations in air pollution levels and sociodemographic characteristics (Freeman, 2006). We standardized air pollution levels (i.e., subtract by mean and then divide by the standard deviation) before running statistical models to make the effect estimates comparable across different pollutants. We checked the proportional hazard (PH) assumption by evaluating the independence between Schoenfeld residuals and time (Grambsch and Therneau, 1994), and did not find significant relationship

between residuals and time, i.e., the PH assumption is supported. A two-sided p value <0.05 was considered statistically significant. All statistical analyses were performed on R 3.6.2.

6.4 Results

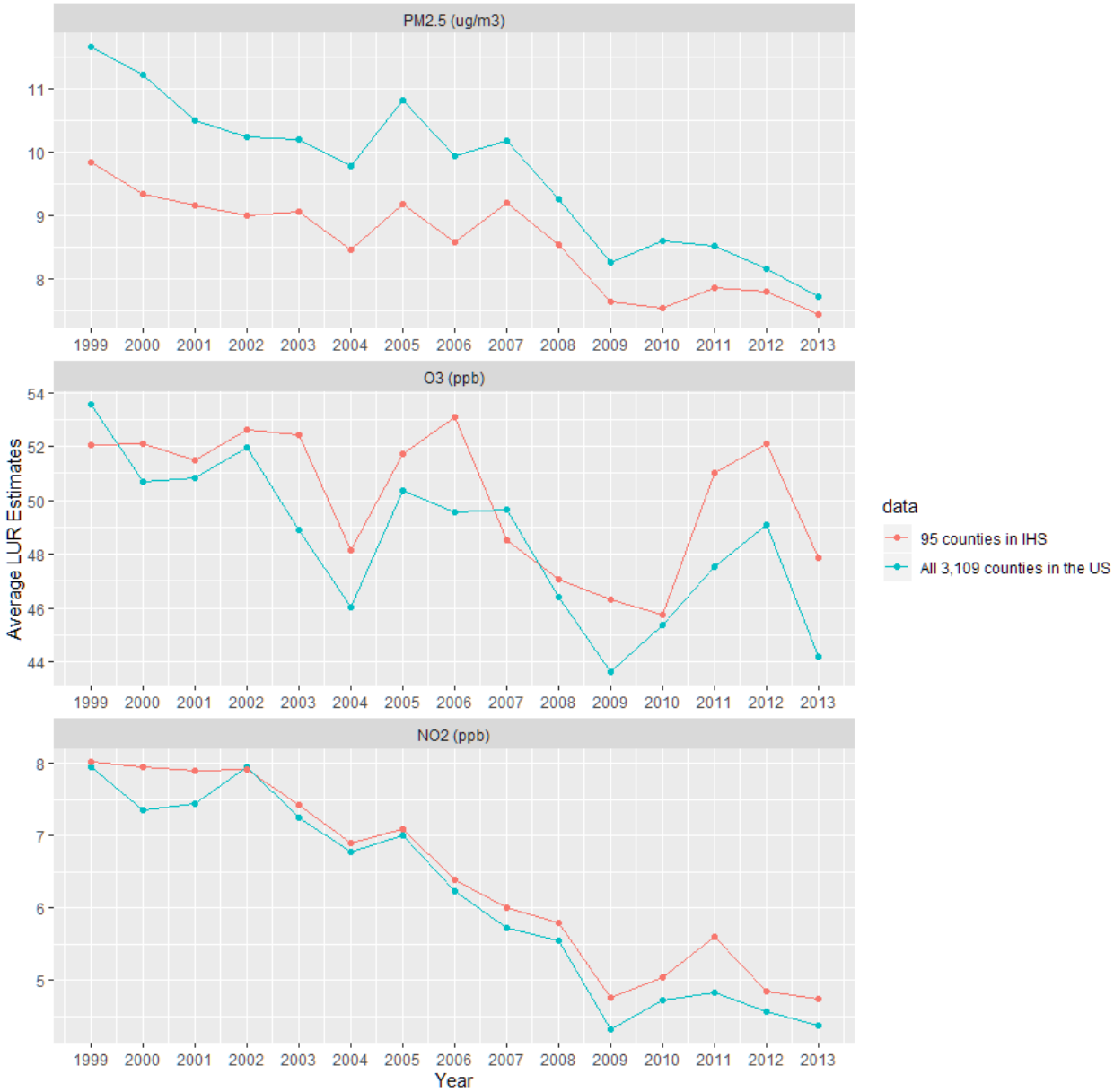

Figure 6.1. Yearly average air pollution levels of $PM_{2.5}$, O_3, and NO_2 in all 3,109 US counties vs. 95 IHS counties in this study.

Table 6.1. Descriptive Statistics of Individual-level Characteristics of the Study Participants (n=32,564)

	All (n=32,564)		**With Dementia** (n=1,121)		**Without Dementia** (n=31,443)	
	n	%	n	%	n	%
Age group (based on age in FY2007)						
55-65	17,794	54.64%	163	14.54%	17,631	56.07%
65-75	10,208	31.35%	405	36.13%	9,803	31.18%
75+	4,562	14.01%	553	49.33%	4,009	12.75%
Gender						
Male	13,287	40.80%	414	36.93%	12,873	40.94%
Female	19,277	59.20%	707	63.07%	18,570	59.06%
Region						
East	1,196	3.63%	74	6.55%	1,122	3.53%
Northern Plains	3,140	9.54%	130	11.51%	3,010	9.47%
Pacific Coast	1,256	3.82%	33	2.92%	1,223	3.85%
Southern Plains	12,093	36.74%	307	27.19%	11,786	37.08%
Southwest	9,305	28.27%	464	41.10%	8,841	27.81%
Other	5,925	18.00%	121	10.72%	5,804	18.26%
Baseline depression						
Yes	6,582	20.21%	280	24.98%	6,302	20.04%
No	25,982	79.79%	841	75.02%	25,141	79.96%
Baseline diabetes						
Yes	15,144	46.51%	546	48.71%	14,598	46.43%
No	17,420	53.49%	575	51.29%	16,845	53.57%
Baseline hypertension						
Yes	25,713	78.96%	949	84.66%	24,764	78.76%
No	6,851	21.04%	172	15.34%	6,679	21.24%
Baseline CVD						
Yes	12,942	39.74%	630	56.20%	12,312	39.16%
No	19,622	60.26%	491	43.80%	19,131	60.84%
Medicaid						
Yes	4,908	15.07%	295	26.32%	4,613	14.67%
No	27,656	84.93%	826	73.68%	26,830	85.33%
Private insurance						
Yes	8,458	25.97%	223	19.89%	8,235	26.19%
No	24,106	74.03%	898	80.11%	23,208	73.81%

Table 6.2. Descriptive Statistics of County-level Variables of the Study Participants.

	Min	1st Quartile	Median	Mean	3rd Quartile	Max	SD
$PM_{2.5}$ ($\mu g/m^3$)[1]	3.83	5.78	10.65	8.90	11.33	15.85	2.82
O_3 (ppb)[1]	31.44	51.89	52.84	52.35	53.72	57.72	3.59
NO_2 (ppb)[1]	1.71	5.25	6.17	7.18	8.36	21.28	3.22
% Below poverty[2]	7.5	18.4	22.5	23.75	27.3	52.6	8.28
% Education below high school completion[3]	5.3	12.5	15.5	16.5	19.4	28.4	4.84

[1] Average air pollution level in 2003-2007.

[2] Percentage below poverty in all races, ACS 2010-2014.

[3] Percentage of education level below high school completion in people aged 25 years and older in all races, ACS 2010-2014.

Table 6.3. Adjusted Associations between Air Pollution Levels and Dementia Risk (HR per 1-unit Increase in Standardized Air Pollution Levels, 95% CI).

	$PM_{2.5}$	NO_2	O_3
Model 1	0.78, 0.71-0.85***	0.99, 0.88-1.10	1.11, 0.97-1.26
Model 2	0.69, 0.57-0.84***	0.93, 0.84-1.03	1.41, 1.18-1.68***
Model 3	0.71, 0.58-0.86***	0.94, 0.86-1.03	1.42, 1.19-1.68***
Model 4	0.74, 0.61-0.88**	0.94, 0.86-1.02	1.40, 1.19-1.65***
Model 5	0.76, 0.62-0.93**	0.94, 0.87-1.01	1.46, 1.24-1.72***
Model 6	0.88, 0.68-1.14	0.98, 0.90-1.06	1.37, 1.10-1.70**

Notes:

Model 1 adjusted for gender.

Model 2 further adjusted for the 14-category study region.

Model 3 further adjusted for baseline depression, diabetes, hypertension, and CVD.

Model 4 further adjusted for insurance (Medicaid and private insurance).

Model 5 further adjusted for county-level % poverty and % education below high school completion in all races.

Model 6 further adjusted for the other two air pollutants.

* p-value < 0.05

** p-value < 0.01

*** p-value < 0.001

Overall, our study included 32,564 American Indians who were at least 55 years old in FY2007, used IHS services at least once each year, and were dementia-free between the baseline years of FY2007-2009. Descriptive statistics of individual-level and county-level characteristics of the study participants were shown in Table 6.1 and Table 6.2, respectively. We simplified the study regions into six categories in Table 6.1 due to confidentiality concerns. The air pollution levels in IHS counties covered in this study are lower than the EPA safety standard of 12 $\mu g/m^3$ for $PM_{2.5}$, 53 ppb for NO_2, and 70 ppb for a daily maximum of 8-hour average O_3 (US EPA, 2018; US EPA, 2020a; US EPA, 2020b).

We examined the correlation between each pair of air pollutants before analysis in case of potential multicollinearity, $PM_{2.5}$, NO_2, and O_3 were only weakly correlated with each other with Spearman correlation coefficients below 0.3 in the 95 IHS counties covered in this study (r=0.25 between $PM_{2.5}$ and O_3, r=0.19 between $PM_{2.5}$ and NO_2, and r=0.29 between O_3 and NO_2 in the 95 IHS counties).

Figure 6.1 shows the yearly average air pollution levels in all 3,109 counties in the contiguous US and in the 95 IHS counties where our study participants resided. The $PM_{2.5}$ levels in the 95 IHS counties were consistently lower than that in all 3,109 counties in the contiguous US during the period from 1999 to 2013, while O_3 levels in the 95 counties were higher than that in all 3,109 counties in the US in most of the years, and the NO_2 levels in the 95 counties were similar to that of the US, especially during those years before the FY2007 (Figure 6.1). We also observed a higher rate of incident dementia cases per 1,000 participants in IHS counties with higher O_3 levels.

In Table 6.3, we presented the adjusted associations between dementia and air pollution from a series of Cox regression models. After adjusting for socio-demographic

confounders and co-exposures, we found a strong positive association between dementia and O_3 with a hazard ratio (HR) of 1.29 (95% CI: 1.05-1.59) per 1 ppb standardized O_3, while the associations between dementia and $PM_{2.5}$, and NO_2 were not statistically significant. We found similar results in sensitivity analyses using longer lags between air pollution (i.e., using 5-year average air pollution in 2000-2004) and dementia.

6.5 Discussion

In this study, we investigated the associations between three common air pollutants ($PM_{2.5}$, O_3, and NO_2) and all-cause dementia in a large elderly American Indian population aged 55 years and older in the contiguous US using a longitudinal study design. The effects of air pollution on dementia have been previously explored in numerous studies in the US (Kioumourtzoglous et al., 2016; Shaffer et al., 2021; Shi et al., 2021; Wang et al., 2022) and other countries around the world (Grande et al., 2020; Carey et al., 2018; Cerza et al., 2019; Chen et al., 2017; Oudin et al., 2016; Jung et al., 2015; Wu et al., 2015), with most studies conducted in urban areas. However, no study has investigated the problem in American Indian tribes specifically, where the oil and gas industry emit VOCs and NO_x, which combine to form ground-level O_3 pollution (CATF, 2018; US EPA, 2021). To our knowledge, this is the first longitudinal study that uses the IHS data to investigate the long-term effects of air pollution on dementia incidence in American Indians.

Overall, we observed consistent statistically significant strong positive associations between O_3 and incident dementia in all models, negative associations between $PM_{2.5}$ and incident dementia yet the associations became insignificant after further adjusting for county-level SES and co-exposures, and nearly null associations between NO_2 and incident

dementia in all models. Our results are supported by the findings from Cleary et al. (2018), who observed an increased rate of cognitive decline associated with O_3 yet not with $PM_{2.5}$ in a heterogeneous and broadly distributed cohort of Alzheimer's disease (AD) (the leading subtype of dementia) participants in the US, and Chen and Schwartz (2009) who observed consistent associations between O_3 and reduced cognitive performance in US adults. Our results are also similar to that of Cerza et al. (2019) who found a significant positive association between O_3 and dementia hospitalization, a null association between NO_x and dementia hospitalization, yet mixed findings between $PM_{2.5}$ and different dementia subtypes (i.e., a positive association with vascular dementia yet a negative association with AD) in Rome.

Although multiple epidemiological studies have found associations between $PM_{2.5}$ and elevated dementia risk (Kioumourtzoglous et al., 2016; Carey et al., 2018; Chen et al., 2017; Grande et al., 2020; Jung et al., 2015; Shaffer et al., 2021; Shi et al., 2021), our study is different from those studies in that the American Indian tribes are mostly rural areas where the $PM_{2.5}$ levels are much lower than the overall US averages during 1999-2013 (Figure 6.1), which might explain the inverse associations between $PM_{2.5}$ and dementia in the crude and some adjusted models in Table 6.3.

Strengths of this study include using electronic health records with detailed individual-level information on dementia diagnosis, cardiovascular risk factors including depression, diabetes, hypertension, and CVD, and demographic information to investigate the association between air pollution and dementia risk, and our longitudinal study design with the requirement of a three-year "clean period" without dementia diagnosis for our

study participants, which may provide stronger evidence for causal link than cross-sectional studies.

This study also has several limitations. First, our air pollution exposure was only assessed at the county level because county code is the smallest standard geographic information on record for the study participants, covering nearly all the study participants, although the county-level exposure estimate may not be an accurate proxy for long-term environmental exposure (Greenland, 2001). The exposure aggregation at the county level can reduce statistical power (i.e., increase standard errors of effect estimates) compared to fully individual-level studies. However, it is relatively free from classical error bias that tends to attenuate the effect estimate towards the null compared to other studies that assessed air pollution exposure based solely on individual-level residential addresses while ignoring exposure at the workplace, during the regular commute or other social/physical activities (Navidi et al., 1994; Paul et al., 2019). Collecting more detailed residential and work addresses for the IHS users and evaluating the air pollution exposure based on this individual-level information would be a valuable addition to the current study. Second, we were only able to adjust for major risk factors for dementia at the individual level including age, gender, depression, diabetes, hypertension, and CVD at baseline using the IHS database, and there was no information on record for apolipoprotein (APOE) genotype, smoking status, alcohol consumption, and physical activities for the study participants, which could also be confounders or effect modifiers (Livingston et al., 2020; Shaffer et al., 2021). However, Wang et al. (2022) reported that the associations between air pollution and dementia did not substantially differ by APOE genotype in US elderly women. Future studies could further investigate how APOE genotype might impact the association between air pollution and

dementia in American Indians. Third, identifying dementia patients via clinical diagnostic codes likely underestimates the prevalence and incidence of dementia (both diagnosed and undiagnosed) in the population under consideration. Furthermore, a 3-year of "washout" period may not be long enough to capture all the baseline prevalent dementia patients, which means some of the prevalent dementia patients might have been misclassified as incident cases.

6.6 Disclaimer

Based on the feedback and advise I receive, I will update the results. The updated results will be disseminated more broadly in

1) an upcoming presentation at Alzheimer's Association International Conference,

2) a manuscript that will be submitted for publication, and

3) community summaries written at the 8th grade and 11 grade reading levels for dissemination within non-research communities where our data came from.

These documents will be submitted for review by Indian Health Service and Tribes before being disseminated beyond our Program in Public Health.

CHAPTER 7: Conclusions

In this dissertation, I discussed exposure to two groups of modifiable environmental risk factors: PFAS and air pollution, and associated effects on susceptible populations including infants, pregnant women, and elderly people in the US.

PFAS is a group of emerging pollutants in the environment that are less known than air pollution, despite the fact that they have been found ubiquitous in the general US population. In Chapter 2, we examined the associations between serum PFAS concentrations and the type of residential flooring among the general US population aged 12 years and older using the 2005-2006 NHANES survey data. We concluded that low pile carpeting was associated with increased serum concentrations of PFHxS and MeFOSAA in the general US population, while no clear association has been observed between the other types of carpeting and serum PFAS concentrations. Further studies would be needed to fully understand PFAS formulations in different types of carpeting.

In Chapters 3 and 4, we conducted two nationwide studies investigating the impacts of PFAS detected in drinking water on birthweight and HDP status, respectively. We used county-level aggregated PFAS data from EPA UCMR3 and county-level multiple-stratified birth/pregnancy data from CDC WONDER. We found an average change in birthweight of -1.0 g (95% CI: -1.1, -0.9) per ng/L increase in the sum of PFOA, PFOS, PFHpA, and PFHxS concentrations in public water supplies, and we observed negative associations between all PFAS chemicals and birthweight adjusting for demographic confounders; we also found a weak positive association between the PFAS mixture and HDP, although the generalizability is subject to inherent limitations of the two public-available datasets.

As it is a generally held belief in public health that interventions or preventive strategies should be targeted at population subgroups where most cases could potentially be prevented, in Chapters 5 and 6 we further conducted two subgroup analyses using data from the C8 project and IHS project, respectively, focusing on two susceptible population subgroups with significantly higher levels of environmental exposure to PFOA and O_3 in the US, respectively. Chapter 5 focuses on a group of pregnant women who resided in the mid-Ohio River Valley during 1990-2006, where high levels of PFOA were measured in drinking water due to contamination from the DuPont Washington Works Plant in West Virginia; and Chapter 6 focuses on a group of elderly American Indians who resided in tribes during 2007-2013, where the O_3 level is above the national average due to the oil and gas industry there.

In Chapter 5, we used an ABC method to combine modeled PFOA exposure and measured biomarker concentrations to reduce exposure measurement error compared to previous studies (Savitz et al., 2012a; Avanasi et al., 2016a, 2016b, 2016c) that used the same data source in the C8 studies, and then investigate the association between PFOA and preeclampsia based on the new PFOA estimates. Compared to using fixed values of model parameters (Savitz et al., 2012a) and Monte Carlo simulations (Avanasi et al., 2016a, 2016b, 2016c), ABC produced similar Spearman correlations between estimated and measured serum PFOA concentrations, yet substantially reduced the mean squared error by over 50%. Based on ABC, compared to previous studies (Savitz et al., 2012a; Avanasi et al., 2016a, 2016b, 2016c), we found a similar adjusted odds ratio (AOR) for the association between PFOA and preeclampsia, suggesting a moderately effect of PFOA on preeclampsia in the C8 studies.

In addition to infants and pregnant women, elderly people are also susceptible to environmental pollutants. In Chapter 6, we investigated the impacts of air pollution on dementia in American Indians aged 55 years and older by linking the public-use county-level air pollution levels of three major air pollutants $PM_{2.5}$, NO_2, and O_3 to the IHS electronic health records. Our study suggests that exposure to O_3 is associated with a higher risk of dementia in American Indians. Future efforts to reduce exposure to air pollution might help lower dementia risk in American Indian communities.

PFAS and air pollution are similar in that they can both cause oxidative stress and inflammation in various parts of the human body, contributing to disease progressions. However, few studies have estimated the joint/combined health effects of PFAS and air pollution on susceptible populations, which could be a future research direction. Bayesian kernel machine regression (BKMR) models (Bobb et al., 2015) and weighted quantile sum (WQS) regression models (Tanner et al., 2019) may be appropriate in this case.

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