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**FETAL EXPOSURE TO PERFLUORINATED COMPOUNDS:
DISTRIBUTION AND DETERMINANTS OF EXPOSURE AND
RELATIONSHIPS WITH WEIGHT AND SIZE AT BIRTH.**

by

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with the requirements for the degree of Doctor of Philosophy

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Abstract

BACKGROUND: Perfluorinated compounds (PFCs) such as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are surfactants used in a variety of commercial and industrial applications. Recent studies have documented widespread contamination of wildlife and human blood at low concentrations. Findings from animal studies have included developmental toxicity; however, limited data exist on the extent of *in utero* exposure in humans. The purpose of this dissertation is to describe the distribution and determinants of *in utero* exposure to PFCs and the relationship between cord serum concentrations of these compounds and size and weight at birth.

METHODS: A cross-sectional study of singleton newborn deliveries was conducted at the Johns Hopkins Hospital in Baltimore, MD. Cord blood samples were collected from the umbilical cord vein following delivery. We abstracted maternal and infant characteristics from clinical records maintained by the hospital. Cord serum samples were analyzed for 10 PFCs by on-line solid-phase extraction, coupled with reversed phase high-performance liquid chromatography-tandem mass spectrometry.

RESULTS: PFOS and PFOA were detected in 99 and 100 percent of samples, respectively, at concentrations lower than typically reported in adult serum collected from other regions in the United States. Other PFCs were detected less consistently. There were relatively few predictors of cord concentrations among the demographic characteristics available from the medical record, with the exception of race. On average, Asians and Blacks had higher PFOS concentrations than Whites. After adjusting for

potential confounders, both PFOS and PFOA were negatively associated with birth weight and ponderal index. A negative association was also observed with head circumference among vaginal but not Caesarian deliveries. In contrast, no consistent trend was observed between PFOS or PFOA and newborn length.

CONCLUSION: The findings of this research confirm that *in utero* exposure to PFCs is occurring among babies born in Baltimore City. Despite the relatively low serum concentrations compared to adults, we detected negative associations between PFOS and PFOA concentrations in cord serum and birth weight, head circumference, and ponderal index. Given the limited data on *in utero* exposure to PFOS and PFOA and effects in humans, future studies are needed to confirm these findings.

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Preface

This dissertation is organized in manuscript format. Chapter 1 presents an introduction to the dissertation. Chapters 2-4 make up the manuscript portion of the dissertation. Chapter 2 is a literature review of PFOS and PFOA production, exposure, and toxicity. Chapter 3 is a manuscript describing the distribution and determinants of cord serum PFC concentrations. Chapter 4 examines the relationships between cord serum PFOS and PFOA concentrations and measures of size and weight at birth. Chapter 5 summarizes the findings and implications of this research. The appendices included at the end of the dissertation detail the extensive analyses conducted in support of Chapter 4.

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CHAPTER 1

INTRODUCTION

Perfluorinated compounds (PFCs) comprise a class of man-made, fully fluorinated organic compounds that have been used in a variety of consumer and industrial applications for more than 50 years. These products include protective coatings for food-contact packaging, textile, carpet, and leather; non-stick cooking material; commercial and industrial surfactants (e.g., fire-fighting foams, electroplating baths); and insecticides (1;2). Although produced for many years, only recently have reports been published suggesting widespread exposure in wildlife and humans (3-5). The identification of pervasive exposure of the general U.S. population to one PFC, perfluorooctane sulfonate (PFOS), led its major manufacturer to announce in 2000 the phase-out of perfluorooctanyl-based products (6). The U.S. Environmental Protection Agency (EPA) has been evaluating a structurally-related compound, perfluorooctanoate (PFOA), on the basis of potential carcinogenic and developmental risks (7).

PFOS, PFOA, and related compounds have recently drawn regulatory and scientific attention due to their extreme persistence in the environment and biological systems, widespread contamination in the blood of wildlife and humans, and potential toxicity. Developmental toxicity is among the effects observed from PFOS and PFOA dosing in animal studies, including pregnancy loss, reduced birth weight, decreased gestational length, structural defects, developmental delays, and increased neonatal mortality (8-18). Although recent evidence suggests widespread human exposure to low levels of PFCs, there are limited data on fetal exposure to these chemicals and the potential effects of exposure on birth outcomes. Describing the extent of fetal exposure is important because

the fetus may be particularly susceptible to chemical exposures due to its rapid cellular growth and differentiation.

There are significant public health implications of disruptions to normal fetal growth and development. Babies born early or with low birth weight (<2,500 grams) are at increased risk of mortality in the first year of life (19;20). Babies born small or thin for gestational age due to fetal growth restriction are at greater risk for perinatal and childhood morbidity, including hypothermia, hypoglycemia, and/or asphyxia (21-27), neonatal intensive care unit admission (28), respiratory distress syndrome (29), reduced mental development in infancy (30), cerebral palsy (31), and reduced insulin sensitivity, a marker of type II diabetes risk (32). In addition to the neonatal and childhood impacts associated with fetal growth restriction, a growing body of research suggests that some metabolic diseases in adulthood may have their origins in fetal development. Over the last decade, a number of studies conducted in different countries have shown that small size at birth, modified by rapid childhood growth, is associated with coronary heart disease, type II diabetes, and their metabolic risk factors, including hypertension, hyperlipidemia, and reduced insulin sensitivity (33-38).

The goal of this research was to describe the magnitude and determinants of *in utero* exposure to PFCs and the association between cord serum concentrations of these compounds and measures of size and weight at birth. This research was conducted through the implementation of a hospital-based cross-sectional study of newborn deliveries at the Johns Hopkins Hospital in Baltimore, MD. From late 2004 through early

2005, cord blood specimens from a sample of deliveries occurring at the hospital were collected and information from medical records was abstracted for the mothers and infants.

In Chapter 2, I begin with a review of the literature on PFOS and PFOA, including production and use, human biomonitoring, exposure pathways, animal toxicity, and occupational epidemiology studies. In subsequent chapters, I describe the design of the cross-sectional study used to address the specific aims of this study. Chapter 3 focuses on the magnitude, distribution, and determinants of fetal exposure to PFCs. Chapter 4 presents the results of an epidemiological investigation into the association between fetal PFOS and PFOA exposure and measures of birth weight and birth size.

The results of this research provide data on the extent to which *in utero* exposure is occurring among pregnancies in Baltimore, MD. Such data are useful in the translation of developmental toxicity studies in animals to the estimation of potential human risk. Identification of determinants of cord concentrations may provide insights into exposure pathways and means for reducing human exposure. Finally, the epidemiologic investigation examines whether these data are consistent with an effect of PFOS or PFOA on birth weight and birth size parameters at environmentally-relevant concentrations.

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CHAPTER 2

PERFLUORINATED COMPOUNDS: PRODUCTION, USE, EXPOSURE, AND TOXICITY

Abstract

Perfluorinated compounds (PFCs) comprise a class of man-made, fully fluorinated organic compounds that have been used in a variety of consumer and industrial applications, including protective coatings for textile, carpets, and food-contact packaging; production of non-stick cooking material; and as industrial surfactants.

Despite evidence of widespread contamination at low levels in human blood, the specific pathways of human exposure to PFOS or PFOA are not well understood. Possible sources of exposure may include industrial releases, consumer product use, house dust ingestion, indoor air inhalation, or dietary pathways through environmental contamination; however, there are no clear data to suggest the relative contribution of these sources to human body burdens.

PFOS and PFOA are extremely persistent, both in the environment and in biological systems. Unlike traditional persistent pollutants, these compounds accumulate in the liver and serum, where they are bound to proteins. Both PFOS and PFOA are peroxisome proliferators and adverse effects observed in animal studies have included liver toxicity, disruption to lipid metabolism, alterations in endocrine function, and developmental toxicity. Limited occupational epidemiologic data are available in the form of retrospective cohort and medical surveillance studies. Key data gaps include a characterization of the pathways of human exposure and epidemiologic studies of exposure and human pregnancy outcomes.

Introduction

Perfluorinated compounds (PFCs) are man-made, fully fluorinated organic compounds found in a wide range of consumer and industrial products and processes. They are characterized by a carbon chain backbone in which carbon-hydrogen bonds have been replaced by carbon-fluorine bonds (1;2). The strength of the carbon-fluorine bond imparts resistance against degradation in the environment and makes these compounds extremely persistent. Many PFCs are oleophobic and hydrophobic, hence, providing utility as a repellent of soil, oil, and water. Further, the surface active properties of these compounds coupled with their thermal and chemical stability makes them particularly useful in harsh environments (e.g., semiconductor etching baths) (2). The chemical structures of two of the more widely studied PFCs, perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), are shown in Figure 2-1. The following sections describe the characteristics of these compounds, including the extent of production and use, data on human biomonitoring, possible exposure pathways, and a summary of the potential adverse effects identified in the toxicological and epidemiological literature.

Perfluorooctane sulfonate (PFOS)

Production, Use, and Characteristics

Perfluorooctane sulfonate (PFOS) and related precursor compounds (perfluorinated sulfonamides) are surfactants used in applications ranging from oil and water repellents for fabrics, apparel, carpets, and paper coatings to specialty chemical applications such as insecticides and fire fighting foams (3). These compounds are manufactured from a common starting material, perfluorooctanesulfonyl fluoride (POSF), which is produced

through a process known as electrochemical fluorination. This process results in a mixture of fluorochemical residual compounds, which remain in the final product and may ultimately degrade to PFOS (2).

POSF-based chemicals had been produced for over 40 years by the 3M Company, the dominant global producer of sulfonyl-based fluorochemicals (1-3). In 2000, global production of POSF by 3M was estimated to be 3,665 metric tons, 1,820 of which was either produced in the U.S. or entered the country through importation (4). As a result of reports suggesting widespread wildlife and human exposure to PFOS, 3M announced in 2000 that it would phase out the production of perfluorooctanyl-chemistries by 2002 (5).

PFOS is a completely fluorinated, eight-carbon chain compound with a sulfonyl group moiety (Figure 2-1). PFOS is relatively non-volatile and widespread exposure is thought to be due in part to degradation of volatile perfluorinated sulfonamide precursors (6). Once released, PFOS does not appear to be metabolized further in animals (1). The half-life in human serum has been estimated at 5.4 years from a study of 26 retired fluorochemical production workers (7). Although environmental monitoring suggests that PFOS can biomagnify in the food chain, PFCs are oleophobic and therefore do not accumulate in fats as do traditional persistent organic chemicals. Instead, PFOS has been shown to preferentially concentrate in the liver and blood serum of animals (8;9), where it is bound to proteins (10;11). Experimental data have shown an affinity for albumin, fatty acid binding protein, and steroid binding globulins in some species (10;11). PFOS also undergoes enterohepatic circulation in some species, which may contribute to the

long biological half-life (7). Due to this behavior, PFOS and other PFCs are not reported on a lipid basis, unlike many other polyhalogenated compounds.

Biomonitoring

Recent surveys of wildlife and humans have detected widespread contamination from PFOS. Measurable concentrations in serum have been observed in numerous species across many populated and remote regions of the world (8;12-14). Further, evidence suggests that wildlife (15;16) and human exposure among the general population have increased over the last several decades, at least through the 1980's (17;18).

Studies of workers exposed occupationally to PFOS have shown average serum levels on the order of one part per million and above (19;20). Among the general population, PFOS concentrations have been detected at parts per billion in the blood of most populations studied. Table 2-1 presents a summary of recent studies conducted across the world. These data show that significant variability in exposure may exist both between countries and within a country (Figure 2-2). In the U.S., serum PFOS concentrations in blood among adults (ages 20-69) was reported by Olsen et al. (2003) in a group of 645 blood donors in six different geographic regions. The median and the 90th percentile for all subjects were reported as 35.8 ng/mL and 70.7 ng/mL, respectively (21). Similar serum levels were observed in a study of 238 elderly subjects (ages 65+) in Seattle, Washington (22) and 598 children enrolled in a pediatric trial (23). These data suggest no significant age variation in human blood PFOS concentrations. These studies also found little evidence of gender differences in serum PFOS levels, consistent with many of

the other reports. Other studies conducted in the U.S. have found similar results (24;25), with the exception of a small set ($n = 30$ adults) of whole blood samples from Kentucky, in which median concentrations in blood (adjusted from whole blood to serum-equivalent using a factor of two) were reported as 81 ng/mL among females and 72 ng/mL among males (25).

More recently, Calafat et al. (2006) reported PFOS levels among 54 pooled serum samples from a subset of the 2001-2002 National Health and Nutrition Examination Survey (NHANES). The authors reported that White males and females had higher mean PFOS levels (40.2 and 24.0 ng/mL) than Black males (18.3 ng/mL) and females (17.9 ng/mL) or Mexican-American males (13.7 ng/mL) and females (10.4 ng/mL) (26). These data suggest that racial differences may exist in the proximity to sources or pathways of exposure in the U.S., although limited conclusions can be drawn from pooled data. The Centers for Disease Control and Prevention (CDC) will analyze individual serum samples from NHANES for a future National Human Exposure Report, which may shed light on the extent to which subgroups are differentially exposed to perfluorinated chemicals in the U.S. (26).

Significant variations in PFOS concentrations in human blood have been reported worldwide. Kannan et al. (2004) conducted a survey of PFOS levels in human blood from various countries around the world. After the U.S., the highest median levels were found in Poland, Korea, and Belgium. Median PFOS concentrations of around 10 ng/mL (serum-equivalent) were observed in samples from Malaysia, Brazil, and Colombia,

lower than what has been reported in the U.S. In contrast, relatively low serum concentrations were observed in specimens from Italy and India (25). Relatively high levels of PFOS were observed in another study in Poland (27), in which the median concentration in whole blood ranged from 9.7 to 34 ng/mL, depending on the subgroup. Studies conducted in Canada (28), Japan (12;18;29), and China (30) have also reported PFOS concentrations in human blood that approach or surpass those reported for the U.S. In fact, a Chinese study of 85 blood samples collected in 2004 reported a mean PFOS concentration of 52.7 ng/mL (serum-equivalent), with the highest concentration reported at 310 ng/mL (30). In contrast, among 44 human serum samples from Peru, only 20 percent of samples had detectable levels of PFOS (LOD: 0.4 ng/mL) and the 90th percentile concentration was just 0.7 ng/mL (31). When comparing these data it is important to note that many of these studies are small and not necessarily representative of the whole population of the country from which they were sampled. There may be significant variation in exposure within a country or geographic region, in addition to geographic variation between countries in exposure to fluorochemicals. Also it should be noted that the analyses were completed by different labs, using a variety of analytic methods, which may explain some of the observed variations.

The only previous reports of fetal exposure to PFCs come from two small studies of cord blood specimens. Inoue et al. (2004) measured PFOS in serum collected in Japan in 2003 from 15 maternal-fetal pairs. The authors reported the presence of PFOS in all 15 cord blood samples tested, at concentrations ranging from 1.6 to 5.3 ng/mL. PFOS concentrations in maternal and cord serum were highly correlated, with cord levels

approximately one-third that of the mother (32). In 13 pooled cord plasma samples in northern Canada, collected from 1994-2001, the mean PFOS level was 16.7 ng/mL (33).

Exposure Pathways

To date, there are limited data on the pathways of human exposure to PFOS. Due to the long-range transport necessary to carry PFOS to remote regions such as the Arctic, it has been hypothesized that volatile perfluoroalkyl sulfonamide precursors, which can degrade to PFOS (2), play a role in the widespread environmental contamination (6). Recent studies have identified these compounds in outdoor air (34-36) and it has been hypothesized that variations in atmospheric levels are due to the presence of point sources (34).

In the indoor environment, PFOS and fluorinated sulfonamides have been detected in house dust (37;38) and indoor air (36;39) at concentrations significantly greater than outdoor air (36;39). Kubwabo et al. (2005) report the presence of PFOS (among other PFCs) in the dust in a sample of homes in Canada. The levels of these compounds were positively correlated with the amount of carpet in the home and negatively correlated with the age of the home (37). Shoeib et al. (2005) measured perfluoroalkyl sulfonamides in dust and indoor air in Canadian homes and concluded that levels in air were not correlated with age of the home or percent of the home that was carpeted. The authors suggested, however, that dust ingestion could be a significant pathway for children (39). Further, a recent study reported the presence of residual sulfonamides in a commercial carpet protector, which could be a potential source of human exposure (40).

The presence of measurable levels of these contaminants in wildlife and game suggests that dietary exposure could play a role. Relatively high PFOS concentrations have been observed in Arctic polar bears (13;14;41), with evidence of biomagnification across trophic levels in this region (14). Several studies have shown the ability of PFOS to bioconcentrate substantially in fish tissue (12;42-45) and biomagnify in aquatic food chains (8;42-44;46), suggesting that fish consumption could be a plausible source of exposure. For example, mean bioconcentration factors (BCFs) reported for PFOS have ranged from approximately 1,000 to greater than 10,000, depending on species and location (12;42;44;45). Biomagnification factors (BMFs) have been reported to range from approximately 10 to 20 for PFOS (42;44). In a recent study in Poland, Falandysz et al. (2006) found that individuals with high fish consumption had elevated levels of PFOS in their blood relative to other groups (27). PFOS has also been detected in beef cattle, but at lower levels than fish (47). In a 3M-sponsored study of PFC concentration in food items, measurable concentrations of PFOS (LOD: 0.5 ng/g, wet weight) were found in only a small number of samples (48). PFOS has also been detected in surface waters (12;42;44;49) and tap water (49;50) at part per trillion levels in the U.S. and Japan, suggesting that exposure could occur through drinking water consumption.

Adverse Effects

General Toxicology

PFOS has been identified as a hepatic peroxisome proliferator that targets the liver and disrupts lipid metabolism in some animal species (51-53). Toxicity studies in animals

have shown marked reductions in serum cholesterol and/or triglycerides (54-57), which may be mediated through down-regulation of HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase, a key enzyme in cholesterol synthesis (56). Recent animal studies have shown that PFOS can affect thyroid hormone levels and other aspects of the neuroendocrine system (54;57-60). For example, Thibodeaux et al. (2003) found reductions in total and free thyroxine (T4) and serum triiodothyronine (T3) among pregnant rats dosed with PFOS, without a concomitant increase in serum thyroid stimulating hormone (TSH) (57). Luebker et al. (2005) also noted reductions in total T4 and T3 without elevated TSH during lactation, among rats dosed with PFOS during pregnancy (60). In evaluating other endocrine markers, Austin et al. (2003) found that high doses (10 mg/kg body weight intraperitoneally) of PFOS in rats affected estrus cycling, reduced serum leptin, elevated serum corticosterone, and elevated hypothalamic norepinephrine concentrations (58). A single study evaluated PFOS carcinogenicity in rats and identified increased risk of liver, thyroid, and mammary gland tumors (4).

Developmental Toxicology

Several studies have examined the potential for developmental toxicity of PFOS. PFOS has been shown to cross the placental barrier in rats, with fetal serum levels equivalent to or greater than maternal levels (60). Several effects on reproduction and development have been observed, including reduced maternal weight gain, reduced birth weight, decreased gestational length, birth defects, developmental delays, and increased neonatal mortality (57;59-63). Grasty et al. (2003) reported that PFOS dosing late in gestation was sufficient to induce neonatal mortality in rats and suggested that lung immaturity may be

responsible (61). However, in a more recent study, treating the animals with a rescue agent. (dexamethasone) did not result in improved survival. The authors did note evidence of lung immaturity by histopathology at birth, among PFOS-treated animals (64).

Occupational Epidemiology

Limited epidemiologic data are available in the form of a retrospective cohort study and medical surveillance in certain fluorochemical production plants. A retrospective cohort mortality study was conducted at a 3M POSF manufacturing plant in Decatur, Alabama, among all workers with at least one year of cumulative employment through the end of 1997. The authors reported elevated risks for bladder cancer among workers employed in a high exposure job, although based on only three observed cases. There were too few events to evaluate effects on liver cancer mortality, one of the a priori endpoints of interest (65). In addition to the small number of events for some of the endpoints, no data were available on potential confounders.

Medical surveillance of fluorochemical production employees has been conducted at 3M's plants in Decatur, Alabama and Antwerp, Belgium. In a series of studies using surveillance data from the last decade (1995, 1997, 2000), Olsen and colleagues examined the relationship between serum PFOS levels and hematology, clinical chemistry, and thyroid hormone assessments (19;66). In one study, PFOS exposure was significantly positively associated with total cholesterol (1997) and low-density lipoprotein (LDL) cholesterol (1997) and negatively associated with high-density

lipoprotein (HDL) cholesterol (1995). The authors suggest that these results could be confounded by body mass index (BMI) and age; however, the associations between serum PFOS and total cholesterol and HDL remained after adjusting for potential confounders (66). Although full details of the regression models were not provided, these results are noticeable because they differ in direction from the hypolipidemic effects observed in animal studies.

Among male fluorochemical production workers undergoing medical surveillance in 2000, those in the highest quartile of PFOS exposure had significantly higher serum triglycerides and serum triiodothyronine (T3). In multivariate analysis, significant positive associations were observed between PFOS exposure and total cholesterol and triglycerides. The positive association between PFOS and T3 levels remained in multivariate regression analysis, although no association was observed with TSH or T4 (19). Occupational surveillance investigations of this type may be prone to selection biases due to low participation rates (<40% in the 1995 and 1997 assessments) and the potential for workers adversely affected by exposure to be more likely to have left the job (i.e., healthy worker effect). However, selection bias would be less likely for sub-clinical effects, such as small changes in the clinical chemistry measurements reported here.

Perfluorooctanoate (PFOA)

Production, Use, and Characteristics

Perfluorooctanoic acid (PFOA) and its salts are used as chemical intermediates and processing aids in the production of fluoropolymers and fluoroelastomers (67). PFOA

can be manufactured through electrochemical fluorination or a process known as telomerization. It is estimated that fewer than 600 metric tons of PFOA are manufactured or imported into the U.S. per year (67). In 2002, 3M, the only U.S. manufacturer of PFOA, ceased production, at which point Dupont began producing PFOA for its fluoropolymer business needs (68).

PFOA is a fully fluorinated, eight carbon chain acid (Figure 2-1) which has the ability to form various salts. The salt most commonly used in industrial applications is ammonium perfluorooctanoate (AFPO), which dissociates to the free acid, PFOA, in water (69). Much like PFOS, PFOA is extremely stable in the environment and biological systems due to the strength of the carbon-fluorine bond (70). As a result, the half-life in humans is on the order of years (~3.8 years) (7). Similar to other PFCs, PFOA is oleophobic and therefore does not accumulate in fat tissue in the body. Instead, it is found in the liver and serum of animals (70), typically associated with proteins (11;71). In experimental systems among different species, PFOA has been shown to bind to albumin, fatty acid binding protein, and steroid binding globulins (10;11;71).

Human Biomonitoring

PFOA is the second most widely detected PFC in human blood, although it has generally been detected at levels lower than those of PFOS. A recent analysis of stored human sera showed an increase in PFOA concentrations among the general population from the mid-1970's to the late 1980's (17). Much like PFOS, general population serum concentrations are orders of magnitude lower than those of exposed production workers.

Table 2-2 and Figure 2-3 provide a summary of recent human biomonitoring studies of PFOA.

In the series of studies conducted by Olsen and colleagues, among children ($n = 598$), adults ($n = 645$), and the elderly ($n = 238$) in various regions of the U.S., the median serum level reported was between 4.2 and 5.1 ng/mL (21-23). Other smaller studies in the U.S. have reported higher levels. Calafat et al. (2006) reported a median level of 11.6 ng/mL among 23 pooled samples of serum collected from 1990-2002 (31). Kannan et al. (2004) reported a wide variation in median levels between samples collected from Michigan, Kentucky, and New York City. Among 75 adults in Michigan in 2000, the median serum PFOA level was below the limit of quantification (LOQ) of 3 ng/mL. Conversely, the median plasma PFOA level among 70 adults in New York City in 2002 was 25.2 ng/mL (25). In an analysis of 2001-2002 NHANES pooled samples, Calafat et al. (2006) report mean PFOA concentrations ranging from 2 to 7 ng/mL, depending on race and gender. Similar to PFOS, the highest levels were observed among White males and females in the study (26).

Varying levels of PFOA concentrations in serum or whole blood have been reported outside of the U.S. Some of the highest levels have been observed in Poland. Kannan et al. (2004) report median PFOA levels (adjusted from whole blood to serum-equivalent using a factor of two) close to 20 ng/mL among a sample of 25 adults collected in 2003 (25). However, another study in Poland reports median levels closer to what has been observed in the U.S. (27). Studies conducted on blood samples collected from Belgium

(25), Canada (28;33), Colombia (25), Japan (18), and Sri Lanka (72) all report mean or median PFOA plasma or serum concentrations in the range of 3 to 6 ng/mL, similar to levels reported in the U.S. Interestingly, the same study that reported some of the highest PFOS levels in China, showed a mean PFOA serum-equivalent concentration of 1.6 ng/mL (30). This suggests that the patterns of fluorochemical exposure may differ by region. Once again, it is important to note that these studies were not necessarily intended to provide a representative snapshot of the level of contamination in the population of a country, and cross-country comparisons should be made with this limitation in mind. Likewise some of the apparent geographic variability may be attributed to variability in laboratory methods.

Only two studies have reported the analysis of PFOA concentrations in umbilical cord blood. Inoue et al. (2004) did not detect PFOA in any of the 15 cord serum samples (LOD: 0.5 ng/mL) and in only 3 of 15 maternal samples collected from a population in Japan (32). By comparison, Tittlemier et al. (2004) reported detectable PFOA plasma levels with a mean of 3.4 ng/mL in 13 pooled cord samples collected from northern Canadian populations (33).

Exposure Pathways

Industrial releases during PFOA production may contribute to local or regional sources of pollution. However, PFOA contamination has been documented in many areas of the world, including the Arctic. Similar to PFOS, the physicochemical characteristics of PFOA make it unlikely to undergo long-range air transport and contaminate such remote

regions, the mechanism which dominates the global dispersion of other polyhalogenated organics. Recent studies have implicated fluorotelomer alcohols as possible precursors to PFOA contamination in these regions. These compounds have been measured widely in outdoor air (34;35), are estimated to have atmospheric lifetimes long enough for substantial transport (~20 days) (73), and have been shown to degrade to PFOA and other perfluorinated carboxylic acids in experimental chamber studies (74). Oceanic transport has also been hypothesized to contribute to the accumulation of these chemicals in remote regions, such as the Arctic (75).

Dinglasan-Panlilio et al. (2006) reported the presence of residual telomer alcohols in commercially available consumer and industrial products, suggesting these products could be a source of perfluorinated acids found in the environment and a potential source of human exposure (40). PFOA has also been detected in house dust (37;38), and like PFOS, in at least one study, levels correlated positively with the amount of carpet in the home and negatively with the age of the home (37).

Begley et al. (2005) studied the residual levels of PFOA present in several consumer products and the ability of PFOA to migrate into food during use. The authors detected only very low levels of PFOA residual in PTFE-coated pans and concluded that migration into food would not be a significant source of human exposure (76). Studies conducted by Dupont researchers failed to detect residual PFOA in PTFE-treated cookware (77). Some perfluorochemicals are approved for use as coatings on food-contact paper. These coatings are particularly useful for foods with a high fat content,

since the oleophobic properties of these compounds will prevent oil from leaking through the packaging (76). As a result, concern has arisen about the potential for exposure through fast food consumption (78). Begley et al. (2005) detected PFOA in microwave popcorn bags, though migration of PFOA into food was low (76). The authors did report substantial migration of fluorotelomers, which are found in the coatings applied to food-contact paper, and are believed to ultimately break down to PFOA.

To date, limited data are available on the extent to which diet may play a role in exposure. PFOA has been detected in surface water (42;44;49) and drinking water at part per trillion levels (49;50) in the U.S. and Japan. Drinking water contamination at higher levels has been reported in the U.S. as a result of direct PFOA releases (79). The potential for bioconcentration and biomagnification of PFOA appears to be less than that of PFOS (42-44). For example, BCFs for PFOA have been reported in the range of zero (i.e., undetectable concentrations in fish tissue) to approximately 200 (compared with 1,000-10,000 for PFOS) (42;44;45;80). Similarly, environmental surveys have suggested lower bioaccumulation potential for PFOA (42;44). In a study of PFC levels in food items by 3M, few samples were reported to be above the limits of detection for PFOA (0.5 ng/g, wet weight), similar to what was reported for PFOS (48).

Adverse Effects

General Toxicology

The primary target of PFOA toxicity is the liver (81-83). Like PFOS, PFOA has been identified as a peroxisome proliferator (84-86) and has been shown to have

hypolipidemic effects in some species (56). PFOA has been shown to cause liver tumors in rats, although it has been suggested that the proposed mechanism of peroxisome proliferation may not be relevant to humans (70;81). Increases in pancreatic and Leydig cell tumors in rats have also been observed, the latter of which may be due to increased estradiol levels in male rats (81;87). On the basis of this evidence, a recent draft report from EPA's Science Advisory Board concluded that PFOA should be considered a "likely human carcinogen" (88).

Developmental Toxicology

Recent studies have shown the potential for developmental toxicity, including reductions in body weight, delayed sexual maturation, and increased postnatal mortality in offspring (83;89-92). PFOA has been shown to cross the placenta in rats, with fetal levels at term approximately one-half of maternal levels (93). EPA recently conducted a draft risk assessment for PFOA developmental toxicity and concluded that the current margin of exposure may be cause for concern (91;94). However, the lack of measured serum levels in the offspring, uncertainty regarding whether the effects were due to prenatal, lactational, or postnatal exposure, and sex differences among rats in the elimination of PFOA have contributed to a large amount of uncertainty in the potential risk (91). In a recent study of PFOA in mice (among which sex differences in pharmacokinetics are not observed), a host of developmental effects were observed, including increased pregnancy loss, reduced fetal weight, reduced postnatal survival, and delays in postnatal growth and development (92).

Occupational Epidemiology

Several epidemiologic studies have been conducted among workers in PFOA production facilities. A retrospective cohort mortality study was conducted among 3,531 PFOA production workers employed for at least six months from 1947 to 1983 (95). Subjects were characterized as exposed (1+ month of work in the chemical division) or unexposed (non-chemical division employees) based on job histories. None of the cause-specific SMRs among chemical or non-chemical division employees was significantly elevated. However, in a proportional hazards model, length of employment in the chemical division was significantly associated with prostate cancer mortality, which could be considered plausible given the evidence from animal toxicity data suggesting reproductive hormone changes (87). An update of this study with longer follow-up (through 1997), modified eligibility requirements (including only workers employed for at least 1 year), and different exposure categories (defined as definite, probable, and no exposure to PFOA) reportedly showed no prostate cancer excess (91). These studies are likely to have some degree of exposure misclassification from the use of broad job categories as surrogates for exposure and are limited by small numbers of events for some of the endpoints.

Olsen et al. (1998) examined the association between serum PFOA levels and reproductive and other hormones among male PFOA manufacturing employees undergoing medical surveillance in 1993 and/or 1995. Significant associations between PFOA and hormone levels were not observed, with the exception of 17-alpha-hydroxyprogesterone in 1995. Subjects with PFOA levels above 30 ppm had serum

estradiol levels that were approximately 10% higher than those found in other workers, although the results may be somewhat confounded by bmi (96). The authors also report that prior research among this population found a similar increase in estradiol among the highly exposed, as well as a significant non-linear relationship.

As described above, Olsen et al. (2003) conducted a medical surveillance study in 2000 of 3M fluorochemical production workers, which included serum PFOS and PFOA measurements and hematological, clinical chemistry, and thyroid function tests (19). PFOA levels tended to track those of PFOS. Thus, PFOA was significantly positively associated with cholesterol, triglycerides, and serum T3, similar to associations observed for PFOS. Finally, the association between PFOA exposure and liver enzyme function and lipid levels was examined in a cross-sectional study of male 3M PFOA production workers in Cottage Grove, Minnesota. Total serum organic fluorine (TOF), which was used as a proxy for PFOA exposure, was not associated with cholesterol or hepatic enzyme activity, however, there was some evidence of interactions with other factors. One interaction observed was between PFOA and alcohol intake on HDL cholesterol, in which PFOA was found to diminish the protective effect of alcohol on HDL cholesterol level. Another significant interaction was observed between PFOA and bmi on hepatic enzyme concentrations in serum. Among obese workers only, PFOA exposure was associated with increased serum concentration of two enzymes associated with liver problems. Although gross changes in hepatic function were not observed, the authors conclude that PFOA may modify "the effects of endogenous and exogenous determinants of hepatic metabolism." (97)

Summary

Perfluorinated compounds (PFCs) represent a class of man-made, fully fluorinated organic compounds found in a wide range of consumer and industrial products and processes. Although produced for many years, only recently have reports documented widespread exposure of wildlife and humans. Variations in human blood concentrations have been reported between countries and within the U.S., but no clear variation in concentrations by age or gender has been observed. Future editions of CDC's "National Report on Human Exposure to Environmental Chemicals" will include PFCs and may provide insights into the extent of variation in serum levels between different subpopulations in the U.S.

Figure 2-4 summarizes the possible pathways of human exposure to these compounds in the context of the environmental public health paradigm. The specific pathways of human exposure to PFOS or PFOA are not well understood. Although possible sources of exposure may include industrial releases, consumer product use, dust/indoor air inhalation, or dietary pathways through environmental contamination, there are no clear data to suggest the relative contribution, if any, of these sources to human body burdens. Thus, key research is needed to elucidate the important pathways of human exposure. This should include studying the extent to which residual compounds are present and can escape household or consumer products; the levels of contamination in food and drinking water; and further defining the role of precursor compounds as sources of PFOS and PFOA in the environment.

PFOS and PFOA are extremely persistent, both in the environment and in biological systems. Unlike traditional persistent pollutants, these compounds accumulate in the liver and serum, where they are bound to proteins. Both PFOS and PFOA are peroxisome proliferators and adverse effects observed in animal studies have included liver toxicity, disruption to lipid metabolism, and changes in endocrine function. Some evidence of increased cancer risk has been reported in occupational epidemiology studies for both PFOS (bladder) and PFOA (prostate). Limited cancer bioassay data are available for PFOS. The EPA is currently evaluating the relevance of PFOA animal carcinogenicity data to humans and a recent draft report from EPA's Science Advisory Board concluded that PFOA should be considered a "likely human carcinogen" (88). Recent animal data has also suggested that developmental toxicity is a concern for both PFOS and PFOA. Despite the growing body of animal data, key gaps exist in the understanding of the potential toxicity of these compounds to humans. There are limited data on *in utero* exposure to these compounds, which are necessary to extrapolate developmental toxicity data from animals to humans. The mechanisms by which PFOS and PFOA cause adverse developmental effects in animals and the relevance to humans is not well understood. Finally, there are no studies of pregnancy outcomes in humans, in an occupational or general population setting.

The remaining chapters describe a cross-sectional study of newborns at the Johns Hopkins Hospital, conducted to evaluate PFC exposure *in utero* and relationship with fetal growth as measured by size and weight at birth. Given the evidence of widespread

contamination in humans and the evidence for placental transfer of these chemicals in animals, we hypothesize that this study will document fetal exposure among our study population of newborn deliveries. We will also test the hypothesis that *in utero* exposure to these compounds is associated with reductions in birth weight and/or birth size.

Table 2-1. Summary of studies measuring PFOS concentrations in human blood.

Country	N	Population	Matrix	Median (ng/mL)	Percent Detected	Year	Notes	Reference
USA - NYC	70	adults	plasma	42	100	2002		Kannan et al., 2004
USA - Michigan	75	adults (17-72)	serum	28.9 (F), 26.2 (M)	91 (F), 93 (M)	2000	Medians do not include values below LOQ.	Kannan et al., 2004
USA - Kentucky	30	adults (20-68)	whole blood	81 (F), 72 (M)	100	2002	Reported as serum-equivalent by multiplying by 2.	Kannan et al., 2004
USA	23 pooled samples	unk						
USA	54	unk	serum	31.1	100	1990-2002		Calafat et al., 2005
USA	pooled samples	ages 12 and above	serum	10.4-40.2, depending on race and gender	100	2001-2002	LSM estimates; NHANES, white males have highest levels	Calafat et al., 2006
USA	65	unk	serum	28.4	100	unk	Mean	Hansen et al., 2001
USA	598	children (2-12)	serum	36.7	100	1994-1995		Olsen et al., 2002 - 3M Report
USA	645	adults (20-69) elderly adults (65-96)	serum	35.8	~100	2000-2001		Olsen et al., 2003
USA	238	adults (ages 24-61)	serum	30.2	~100	unk	Non-detects set to 1/2 LLOQ	Olsen et al., 2004
Sri Lanka	30	adults (ages 24-61)	serum	3.3	100	2003	Non-detects set to 1/2 LLOQ Higher in urban area/conventional tea workers vs. organic tea workers	Guruge et al., 2005
Poland	60	adults (19-62)	whole blood	19.4 - 68, depending on subgroup	100	2003	Reported as serum-equivalent by multiplying by 2; Highest median among high fish consumers	Falandysz et al., 2006
Poland	25	adults (35-58) adults (men and pregnant women)	whole blood	33.8 (F), 40.9 (M)	100	2003	Reported as serum-equivalent by multiplying by 2.	Kannan et al., 2004
Peru	44		serum	0.7	20	2003	90th percentile; Median <LOD	Calafat et al., 2005

Country	N	Population	Matrix	Median (ng/mL)	Percent Detected	Year	Notes	Reference
Canada	10 pooled samples 13	female adults	plasma	36.9	100	1994-2001	Mean; northern Canada	Tittlemier et al., 2004
Canada	pooled samples	cord	plasma	16.7	100	1994-2001	Mean; northern Canada	Tittlemier et al., 2004
Malaysia	23	adults (21-26)	whole blood	12.7 (F), 13.1 (M)	100	2004	Reported as serum-equivalent by multiplying by 2.	Kannan et al., 2004
Korea	50	adults (15-95)	whole blood	11.3 (F), 21.7 (M)	100	2003	Reported as serum-equivalent by multiplying by 2.	Kannan et al., 2004
Japan	205	adults	serum	3.5 - 28.1, depending on region and gender	unk	2003	GM	Harada et al., 2004
Japan	15	pregnant women	serum	8.1	100	2003	Term births: 38-41 weeks	Inoue et al., 2004
Japan	15	cord	serum	2.5	100	2003	Term births: 38-41 weeks	Inoue et al., 2004
Japan	26	adults	whole blood	16.2	100	2002	Mean; reported as serum-equivalent by multiplying by 2.	Masanaga et al., 2002
Japan	10	adults (23-44)	whole blood	21	100	2002	Mean; the authors report as serum-equivalent using a factor of 2.5.	Taniyasu et al., 2003
Italy	50	adults (20-59)	serum	3.5 (F), 4.2 (M)	87.5 (F), 90.5 (M)	2001	Medians do not include values below LOQ.	Kannan et al., 2004
India	45	adults (17-48)	serum	2.5 (F), 1.3 (M)	55 (F), 50 (M)	2000	Medians do not include values below LOQ.	Kannan et al., 2004
Colombia	56	adults (20-29)	whole blood	7.3 (F), 8.1 (M)	100	2003	Reported as serum-equivalent by multiplying by 2.	Kannan et al., 2004
China	85	mostly adults	whole blood	52.7	unk	2004	Mean; reported as serum-equivalent by multiplying by 2.	Yeung et al., 2006
Canada	56	adults (20+)	serum	27.4	100	2002		Kubwabo et al., 2004
Brazil	29	adults (18-74)	whole blood	8.4 (F), 12.7 (M)	100	2003, 1998,	Reported as serum-equivalent by multiplying by 2.	Kannan et al., 2004
Belgium	20	adults (19-63)	plasma	10.4 (F), 17.6 (M)	100	2000		Kannan et al., 2004

Table 2-2. Summary of studies measuring PFOA concentrations in human blood.

Country	N	Population	Matrix	Median (ng/mL)	Percent Detected	Year	Notes	Reference
USA - NYC	70	adults	plasma	25.2	100	2002		Kannan et al., 2004
USA - Michigan	75	adults (17-72)	serum	<LOQ (3)	46 (F), 45 (M)	2000		Kannan et al., 2004
USA - Kentucky	30 23 pooled samples	adults (20-68)	whole blood	20 (F), 38.1 (M)	100	2002	Reported as serum-equivalent by multiplying by 2.	Kannan et al., 2004
USA	54	unk	serum	11.6	100	1990-2002		Calafat et al., 2005
USA	pooled samples	ages 12 and above	serum	2.1-7, depending on race and gender	100	2001-2002	LSM estimates; NHANES, white males have highest levels	Calafat et al., 2006
USA	65	unk	serum	6.4	100	unk	Mean; some samples were below LOQ, but above LOD.	Hansen et al., 2001
USA	598	children (2-12)	serum	5.1	96	1994-1995	Assuming values below LOQ equal 1/2 LOQ.	Olsen et al., 2002 - 3M Report
USA	645	adults (20-69) elderly adults (65-96)	serum	4.7	92	2000-2001	Non-detects set to 1/2 LLOQ	Olsen et al., 2003
USA	238	adults (ages 24-61)	serum	4.2	98	unk	Non-detects set to 1/2 LLOQ	Olsen et al., 2004
Sri Lanka	30	adults (ages 24-61)	serum	4	100	2003	Higher in urban area/conventional tea workers vs. organic tea workers	Guruge et al., 2005
Poland	60	adults (19-62)	whole blood	4.6 - 7.4, depending on subgroup	100	2003	Reported as serum-equivalent by multiplying by 2; Highest median among high fish consumers	Falandysz et al., 2006
Poland	25	adults (35-58) adults (men and pregnant women)	whole blood	23.2 (F), 18.4 (M)	100	2003	Reported as serum-equivalent by multiplying by 2.	Kannan et al., 2004
Peru	44		serum	0.1	25	2003	90th percentile; Median <LOD	Calafat et al., 2005

Country	N	Population	Matrix	Median (ng/mL)	Percent Detected	Year	Notes	Reference
Canada	10 pooled samples 13	female adults	plasma	2.2	100	1994-2001	Mean; northern Canada	Tittlemier et al., 2004
Canada	pooled samples	cord	plasma	3.4	100	1994-2001	Mean; northern Canada	Tittlemier et al., 2004
Malaysia	23	adults (21-26)	whole blood	<LOQ (10)	0	2004	Reported as serum-equivalent by multiplying by 2.	Kannan et al., 2004
Korea	50	adults (15-95)	whole blood	<LOQ (15)	19 (F), 25 (M)	2003	Reported as serum-equivalent by multiplying by 2.	Kannan et al., 2004
Japan	205	adults	serum	2.5 - 12.4, depending on region and gender	unk	2003	GM	Harada et al., 2004
Japan	15	pregnant women	serum	<LOD (0.5)	20	2003	Term births: 38-41 weeks	Inoue et al., 2004
Japan	15	cord	serum	<LOD (0.5)	0	2003	Term births: 38-41 weeks	Inoue et al., 2004
Japan	26	adults	whole blood	<LOQ (3.4)	0	2002		Masanuga et al., 2002
Italy	50	adults (20-59)	serum	<LOQ (3)	0	2001		Kannan et al., 2004
India	45	adults (17-48)	serum	<LOQ (3)	0 (F), 3 (M)	2000		Kannan et al., 2004
Colombia	56	adults (20-29)	blood	5.6 (F), 5.9 (M)	100	2003	Reported as serum-equivalent by multiplying by 2.	Kannan et al., 2004
China	85	mostly adults	whole blood	1.6	unk	2004	Mean; reported as serum-equivalent by multiplying by 2.	Yeung et al., 2006
Canada	56	adults (20+)	serum	3.4	73	2002	Mean; using only values above LOD.	Kubwabo et al., 2004
Brazil	29	adults (18-74)	whole blood	<LOQ (20)	0	2003	Reported as serum-equivalent by multiplying by 2.	Kannan et al., 2004
Belgium	20	adults (19-63)	plasma	2.4 (F), 4.3 (M)	75 (F), 100 (M)	1998, 2000	Medians do not include values below LOQ.	Kannan et al., 2004

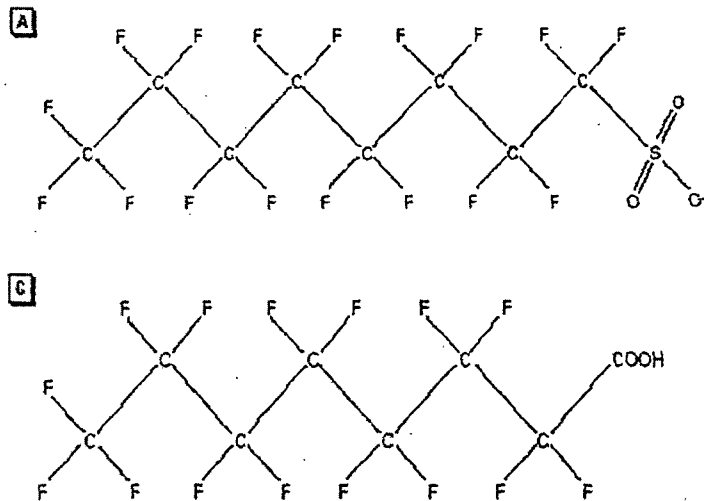


Figure 2-1. Chemical structure of PFOS (top) and PFOA (bottom).
Source: Austin et al. (2003)

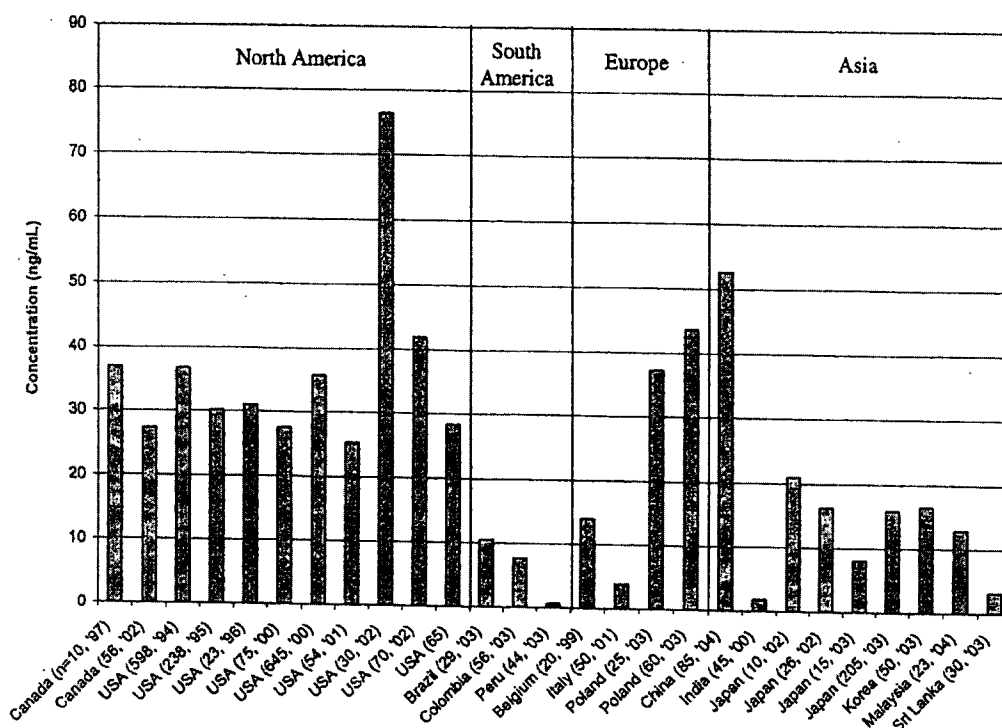


Figure 2-2. Summary of median or mean PFOS concentrations (ng/mL) in blood plasma or serum, from human biomonitoring studies.*

* Some studies measured PFOS in whole blood and reported concentrations as serum-equivalent by multiplying by a factor of two. Sample size and midpoint year of collection are listed in parentheses. If only subgroups were presented, the average concentration between the lowest and highest groups is used. See Table 2-1 for more detail. Missing bar indicates median level below LOD or LOQ.

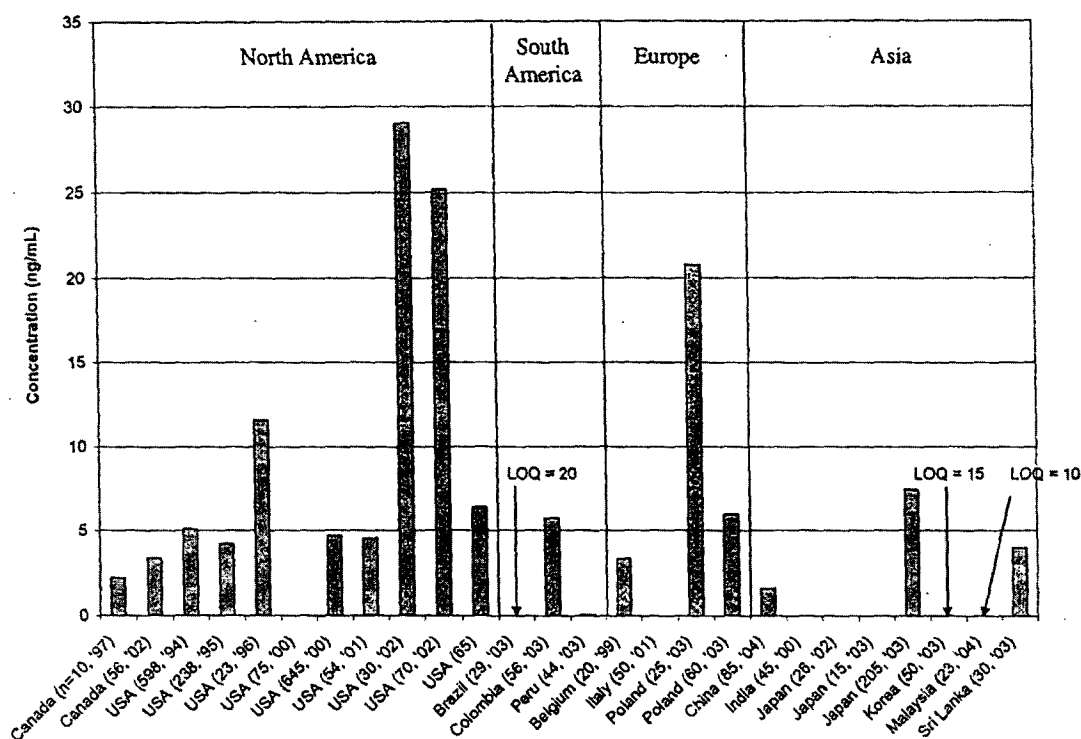


Figure 2-3. Summary of median or mean PFOA concentrations (ng/mL) in blood plasma or serum from human biomonitoring studies.*

* Some studies measured PFOS in whole blood and reported concentrations as serum-equivalent by multiplying by a factor of two. Sample size and midpoint year of collection are listed in parentheses. If only subgroups were presented, the average concentration between the lowest and highest groups is used. See Table 2-2 for more detail. Missing bar indicates median level below LOD or LOQ.

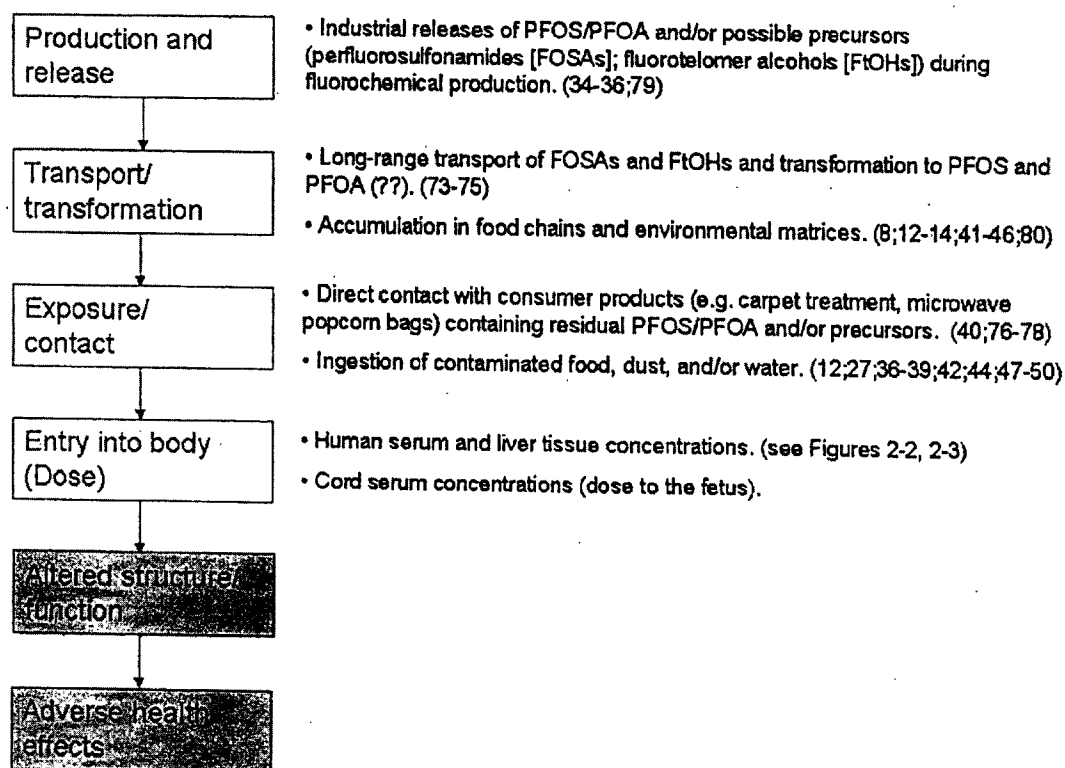


Figure 2-4. Environmental public health paradigm and possible pathways of human exposure to perfluorinated compounds.*

** Adapted from U.S. EPA, 2003 (98).*

Note: Relevant articles are denoted by reference numbers after each description.

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CHAPTER 3

**DISTRIBUTION AND DETERMINANTS OF
PERFLUORINATED COMPOUNDS IN CORD BLOOD**

Abstract

BACKGROUND: Perfluorinated compounds (PFCs) such as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are found globally in wildlife and in human blood samples at part per billion levels. Limited data are available on *in utero* exposure in the U.S.

METHODS: We conducted a hospital-based cross-sectional study in Baltimore, MD to examine levels and determinants of PFCs in newborns. Cord sera were analyzed for 10 PFCs by on-line solid-phase extraction, coupled with reversed phase high-performance liquid chromatography-tandem mass spectrometry (LODs: PFOS = 0.2 ng/mL; PFOA = 0.1-0.2 ng/mL). Demographic characteristics were abstracted from medical records.

RESULTS: PFOS and PFOA were detected in 99 and 100 percent of 299 umbilical cord blood samples, respectively, and concentrations were highly correlated (Pearson's $r = 0.64$ after natural log transformation, $p < 0.01$). The geometric mean concentrations of PFOS and PFOA were 5.0 ng/mL (range: ND-34.8 ng/mL) and 1.6 ng/mL (range: 0.3-7.1 ng/mL), respectively. Other PFCs were detected less frequently and at lower concentrations. Geometric mean concentrations of PFOS among Asians (6.0 ng/mL) and Blacks (5.1 ng/mL) were higher than among Whites (4.2 ng/mL), while PFOA levels were more evenly distributed by race. Slightly higher PFOA concentrations were found in babies born to obese mothers, primiparous mothers, and in female versus male babies. None of the other demographic characteristics, including maternal age, education, and living in the city limits, were associated with PFOS or PFOA cord concentrations.

CONCLUSION: The identification of PFOS and PFOA as the predominant compounds in cord blood is consistent with previous reports of PFC concentrations in humans.

Introduction

Perfluorinated compounds (PFCs) comprise a class of man-made, fully fluorinated organic compounds that have been used in a variety of consumer and industrial applications for more than 50 years. These applications include protective coatings for food-contact packaging, textile, carpets, and leather; production of non-stick cooking material; commercial and industrial surfactants (e.g., fire-fighting foams, electroplating baths); and insecticides (1;2). Although produced for many years, only recently have reports documented widespread exposure in wildlife and humans (3-5). The identification of pervasive exposure of the general U.S. population to one PFC, perfluorooctane sulfonate (PFOS), led its major manufacturer to announce in 2000 the phase-out of perfluorooctanyl-based products. The U.S. Environmental Protection Agency (EPA) has been evaluating a structurally-related compound, perfluorooctanoate (PFOA), on the basis of potential developmental risks (6).

PFCs are highly stable in the environment and biological systems. Based on serum analyses, the half-life in humans has been estimated at 5.4 years for PFOS and 3.8 years for PFOA (7). Many PFCs are surfactants, having both oleophobic and hydrophobic properties which provide utility as repellents of soil, oil, and water. Rather than accumulating in lipids like traditional persistent organic pollutants, these compounds partition in humans mainly between the liver and serum, where they are bound to proteins (4;8-12). Some of these compounds have also been shown to undergo enterohepatic circulation, which may contribute to their long half-life in the body (7).

PFOS has been identified as a hepatic peroxisome proliferator that targets the liver and disrupts lipid metabolism in some animal species (13-15). Toxicity studies in animals have shown marked reductions in serum cholesterol and/or triglycerides (16-19), which may be mediated through down-regulation of HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase, a key enzyme in cholesterol synthesis (18). Recent animal studies have shown that PFOS can affect thyroid hormone levels and other aspects of the neuroendocrine system (16;19-22). PFOS has been shown to induce developmental and reproductive effects in rats, such as reduced maternal weight gain, reduced birth weight, decreased gestational length, birth defects, developmental delays, and increased neonatal mortality (19;21-25). Among several occupational epidemiology studies, a notable finding was an elevated risk for bladder cancer among workers employed in a high exposure job, although based on only three observed cases (26).

The primary target of PFOA toxicity is also the liver (27-29). Like PFOS, PFOA has been identified as a peroxisome proliferator (30-32) and has been shown to have hypolipidemic effects in some species (18). PFOA has been shown to cause liver tumors in rats, although it has been suggested that the proposed mechanism of peroxisome proliferation may not be relevant to humans (9;27). Increases in pancreatic and Leydig cell tumors in rats have also been observed, the latter of which may be due to increased estradiol levels in male rats (27;33). On the basis of this evidence, a recent draft report from EPA's Science Advisory Board concluded that PFOA should be considered a "likely human carcinogen" (34). In a retrospective cohort mortality study conducted among PFOA production workers, length of employment in the chemical division was

significantly associated with prostate cancer mortality, which is plausible given the evidence from animal toxicity data suggesting reproductive hormone changes (33). An update of this study with longer follow-up reportedly showed no prostate cancer excess, although eligibility and exposure categories were changed (35). Animal toxicity studies have suggested the potential for developmental toxicity including pregnancy loss, reduced fetal weight, reduced postnatal survival, and delays in postnatal growth and development in rat and mouse pups exposed to high doses (29;35-38).

In human biomonitoring studies, PFOS and PFOA have been the predominant PFCs detected in human blood, although the concentrations observed vary geographically. In the U.S., mean serum concentrations of PFOS have been reported in the range of 30-40 ng/mL (5;39-41). Serum PFOA levels are considerably lower, with geometric mean concentrations estimated at around 5 ng/mL (5;39-41). Although no consistent association with age or gender has been reported, recent evidence from pooled samples from the 2001-2002 National Health and Nutrition Examination Survey (NHANES) suggests that White males and females have higher serum concentrations of PFCs than Blacks or Mexican-Americans (5). PFOS and PFOA have also been detected in the blood of individuals in the general population of many other countries, across several continents (3;42-50). In some developing countries (e.g., Peru), serum concentrations are considerably lower than in developed countries, suggesting that production and use of products containing these chemicals may contribute to the geographic variations observed (50). However, contamination has also been reported in biota from remote regions (e.g.

the Arctic) (13;14;41), which would require global transport of these chemicals or their precursors (51-55).

Despite the growing body of literature suggesting widespread human exposure, little is known about the presence of PFCs *in utero*. Such data are necessary to extrapolate developmental toxicity findings from animals to humans. The only previous reports of the presence of PFCs in the fetal circulation come from two small studies of cord blood specimens. A study of 15 maternal-fetal pairs in Japan confirmed that PFOS could cross the placental barrier in humans, albeit incompletely (56). Another small study in Northern Canada documented detectable levels of PFOS and PFOA in pooled cord blood samples (48). There is a lack of data on the extent of fetal exposure to PFCs in the United States. To fill in this gap, we measured concentrations of 10 PFCs in umbilical cord blood as part of a cross-sectional study of newborn deliveries at the Johns Hopkins Hospital in Baltimore, MD. We also examined the determinants of cord PFC levels in this population to identify whether demographic and/or socioeconomic factors were associated with *in utero* exposure to these chemicals.

Material and Methods

Subjects

We conducted a cross-sectional study of newborn deliveries at the Johns Hopkins Hospital in Baltimore, MD. The study received approval from the Johns Hopkins Medicine Institutional Review Board and was determined to be HIPAA exempt. The study was conducted anonymously and utilized only specimens that otherwise would

have been discarded and medical records that were available to hospital and study personnel. There was no requirement for informed consent due to the anonymization of all samples and data. Between November 26, 2004 and March 16, 2005 all singleton, live birth deliveries occurring in the Labor and Delivery Suite at the hospital were eligible for participation in the study. We excluded from the study population babies from multiple births.

Over the course of the study period, 609 live births occurred at the Johns Hopkins Hospital, of which 597 were singleton births. We obtained cord blood specimens from 341 of these, of which 42 had insufficient volume for laboratory analyses and were excluded from this study.

Cord blood samples were collected by hospital personnel immediately following delivery (57). Upon delivery, a section of the cord was cleaned with an alcohol wipe and blood was drawn from the umbilical cord vein using a sterile 60-mL Becton Dickinson (BD) syringe with an 18-gauge safety needle. A BD Vacutainer Blood Transfer Device was then attached to the syringe and 10 mL glass BD vacutainers were filled. After collection, cord blood specimens were stored in Labor and Delivery refrigerators. Within several hours, the specimens were picked up and taken across the street to a laboratory at the Johns Hopkins Bloomberg School of Public Health for processing. Tubes were centrifuged at 2400 rpm for 15 minutes for serum separation. Serum was aliquotted into 2 mL polypropylene prescreened cryovials and stored at -80°C . Frozen specimens were

transferred on dry ice to the Centers for Disease Control and Prevention (CDC) for laboratory analyses.

Medical Records

We abstracted maternal and infant characteristics from clinical databases maintained by the hospital. The data were abstracted by two study investigators concurrently, and a random 10 percent sample was verified by two others. The data collected included demographic information, anthropometric measures, medical history, and pregnancy-related characteristics. Age, race, education, marital status, and parity were based on self-report. Insurance type was recoded from the medical record as "Medicaid" or "Private," with "Medicaid" defined to include all forms of public assistance insurance. Body mass index was calculated from reported pre-pregnancy weight and height as the ratio of weight (in kilograms) to height (in meters) squared. Gestational age was based on the "best obstetric estimate" and categorized as term (≥ 37 full weeks) or preterm (< 37 full weeks). Infant gender was abstracted from the mother's medical record and confirmed with the infant record. Smoking status was defined using a combination of the maternal medical record and cord serum cotinine concentrations measured at birth. Using cutoffs from Jarvis et al. (1987), samples with cotinine concentrations below 1 ng/mL were included in the category of nonsmokers, cotinine concentrations between 1 and 14 ng/mL were categorized as passive smoking exposure, and samples with concentrations above 14 ng/mL were categorized as active smoking exposure (58). If the clinical record indicated that the mother reported smoking at any time during pregnancy, she was considered an active smoker regardless of the cotinine concentration in cord

blood at birth. The home address recorded in the maternal record was geocoded by a commercial service (Geolytics, Inc). We used the first five digits of the Census block group, referred to as the federal information processing standards (FIPS) code, to identify the boundaries of Baltimore City. Residence inside the city limits was defined using FIPS code: 24510.

Laboratory Analysis

Perfluorinated compounds.

Cord serum samples were analyzed for 10 PFCs by on-line solid-phase extraction (SPE), coupled with reversed phase high-performance liquid chromatography-tandem mass spectrometry. The method has been described in detail by Kuklenyik et al. (2005). Excellent recovery, precision, and reliability have been reported using this method for the detection of PFCs in human serum (59). Briefly, without protein precipitation, one aliquot of 100 μ L of serum was injected into a commercial column switching system allowing for concentration of the analytes on a SPE column. This column was placed automatically in front of an analytical column for chromatographic separation of the analytes. Detection and quantification were done using negative-ion TurboIonSpray ionization, a variant of electrospray ionization, tandem mass spectrometry. Detection limits were in the low nanogram per milliliter range for the following PFCs: perfluorooctane sulfonamide (PFOSA), 2-(*N*-ethyl-perfluorooctane sulfonamido) acetate (Et-PFOSA-AcOH), 2-(*N*-methyl-perfluorooctane sulfonamido) acetate (Me-PFOSA-AcOH), perfluorobutane sulfonate (PFBS), perfluorooctane sulfonate (PFOS), perfluoroheptanoate (PFHpA), perfluorooctanoate (PFOA), perfluorodecanoate (PFDeA),

perfluoroundecanoate (PFUA), and perfluorododecanoate (PFDoA). Although the analytical method allows for the quantification of perfluorohexane sulfonate (PFHxS) and perfluorononanoate (PFNA), these analytes could not be measured in the cord sera due to the presence of interferent compounds that eluted at the same retention times and shared precursor/product ion mass-to-charge ratios (m/z) with PFHxS and PFNA. Similarly, the precursor/product ion m/z transition used for the quantification of PFOS also had an interferent ion. Therefore, PFOS concentrations had to be calculated using another transition, normally used to confirm the presence of PFOS. The nature of these interferences is at present unknown. Quality control (QC) and reagent blank samples were included in each analytical batch along with the unknown samples. QC samples were evaluated according to modified Westgard rules.¹

Cotinine.

Serum cotinine was analyzed by CDC using the method described by Bernert et al. (1997). The analytic method uses high-performance liquid chromatography coupled with atmospheric pressure chemical ionization tandem mass spectrometry to measure serum cotinine concentrations with high accuracy and sensitivity (limit of detection [LOD] = 0.015 ng/mL). This method has been used to measure environmental tobacco smoke dose in large-scale surveys, including NHANES (60).

¹ Description of analytical method contributed by Antonia Calafat, Division of Laboratory Sciences, National Center for Environmental Health, CDC.

Statistical Analysis

We used descriptive statistics (geometric mean, median, interquartile range) to describe cord serum PFC concentrations. Because PFC concentrations were skewed to the right, all statistical tests were conducted on natural log-transformed concentrations. Pearson's correlation was used to test for linear relationships between PFCs. We used linear regression to describe univariate relationships between continuous predictors and PFC concentrations. The presence of non-linear relationships was explored using restricted cubic spline models.

Linear regression was also used to estimate the ratio of geometric mean concentrations (and 95 percent confidence intervals) among different categories of maternal characteristics. Under the linear regression model, the expectation (or average) of the natural log PFC concentration is described as follows:

$$E(\ln PFC) = \beta_0 + \beta_1 x_1 + \varepsilon$$

The regression coefficient, β_1 , is equal to:

$$\beta_1 = E(\ln PFC)_{x_1=1} - E(\ln PFC)_{x_1=0}$$

After exponentiating the coefficient, the equation reduces to:

$$e^{\beta_1} = \frac{e^{E(\ln PFC)_{x_1=1}}}{e^{E(\ln PFC)_{x_1=0}}} = \frac{GM(\ln PFC)_{x_1=1}}{GM(\ln PFC)_{x_1=0}}$$

The exponentiated coefficient can be interpreted as the ratio of geometric mean concentrations when $X_1=1$ versus when $X_1=0$. The 95 percent confidence interval on this ratio can be estimated similarly, by exponentiating the confidence intervals of the coefficient.

Multivariate linear regression was performed to compare geometric mean concentrations, after adjusting for other covariates. For all models, regression diagnostics were conducted and regression results were reported with and without robust standard errors in the presence of heteroskedasticity. Concentrations below the LOD ($<LOD$) were imputed a value equal to the LOD divided by the square root of two for all analyses (61). Statistical analyses were performed using STATA version 8.0 (StataCorp, College Station, TX).

Results

Table 3-1 summarizes the PFCs detected in umbilical cord serum. PFOA was detected in all samples and PFOS was detected in all but two samples, with corresponding geometric means of 1.6 ng/mL for PFOA (range: 0.3-7.1 ng/mL) and 4.9 ng/mL for PFOS (range: ND-34.8 ng/mL). The 95th percentile concentration was 3.4 ng/mL for PFOA and 12.4 ng/mL for PFOS. These two compounds made up most of the total concentration of the PFCs measured in these specimens (82%, on average). Four other compounds were detected in at least 20 percent of samples (PFOSA, Me-PFOSA-AcOH, PFDeA, PFUA). However, concentrations of these compounds were substantially lower than those of PFOS and PFOA. Because only PFOS and PFOA were detected in the majority of samples and the ranges of detections for the other compounds were small, further analyses were conducted only for PFOS and PFOA.

As expected, concentrations of both PFOS and PFOA were right skewed and became more Gaussian after natural log-transformation (Figure 3-1). However, both log-transformed distributions still deviated from normality based on the Shapiro-Wilk test ($p < 0.01$). Cord concentrations of PFOS and PFOA were highly correlated with one another (Figure 3-2; Pearson's $r = 0.64$, $p < 0.01$).

Figure 3-3 shows the distribution (median and interquartile range) of PFOS and PFOA concentrations by maternal and infant characteristics. In Tables 3-2 and 3-3, the ratios of geometric means comparing these subgroups are shown for PFOS and PFOA. The geometric mean concentrations of PFOS for Asians (6.0 ng/mL) and Blacks (5.1 ng/mL) were higher than for Whites (4.2 ng/mL), while PFOA levels were more evenly distributed by race. There was some evidence of heteroskedasticity in the comparison of geometric mean PFOS concentrations by race/ethnicity, so we generated linear regression estimates with robust standard errors as well. Using robust estimates, the difference in geometric mean concentrations between Asians and Whites was no longer statistically significant. Male babies had lower geometric mean concentrations than female babies for both compounds (PFOS: $p = 0.10$; PFOA: $p < 0.01$). There was a trend towards slightly higher average concentrations among obese ($\text{BMI} \geq 30 \text{ kg/m}^2$) and underweight ($< 18.5 \text{ kg/m}^2$) women, compared with normal weight ($18.5 - 24.9 \text{ kg/m}^2$) women, although only statistically significant for PFOA concentrations among obese women ($p < 0.01$). Evidence of a non-linear relationship with BMI was confirmed using restricted cubic spline models (Figures 3-4). Primiparous and term births were associated with slightly higher average cord concentrations of PFOS and PFOA, but the differences were not

statistically significant in univariate models. There were no other significant predictors of cord concentrations among the remaining covariates, which included age, education, insurance type, marital status, smoking status, and living inside the city limits. Similar relationships were observed when all the variables were included in a multivariate model (Tables 3-2 and 3-3). When examining covariates as continuous measures, no significant linear trends were observed between PFOS or PFOA and maternal age (Figure 3-5), gestational age (Figure 3-6) or cord cotinine concentration (Figures 3-7, 3-8).

Discussion

This study confirms earlier findings indicating that the developing fetus is exposed to persistent PFCs *in utero*. We detected PFOS and PFOA in 99 and 100 percent of 299 umbilical cord blood samples, respectively, at a hospital in Baltimore City. The geometric mean concentrations of PFOS and PFOA were 4.9 and 1.6 ng/mL, respectively. Other PFCs were detected less frequently and at lower concentrations. The identification of PFOS and PFOA as the predominant compounds detected in cord blood is consistent with previous reports of PFC concentrations in the blood of individuals in the United States.

Cord concentrations of PFOS and PFOA were found to be strongly correlated with one another. The correlation between these compounds is of interest because they arise from different industrial sources and their presence likely differs in consumer products. Further, there is no evidence that PFOS or PFOA can degrade or metabolize into one another. To date, the pathways of human exposure to PFOS and PFOA are not well

understood. It has been hypothesized that volatile precursor compounds (perfluorinated sulfonamides and fluorotelomer alcohols) may contribute to the widespread contamination observed in remote regions (51;53). Recently, residual amounts of these compounds have been identified in commercial and consumer products, including carpet protectors (62) and microwave popcorn bags (63). PFOS and PFOA have also been identified in house dust and/or indoor air (64-67). If exposure through consumer products were occurring and the use of these products were correlated, this could explain the correlation observed in human serum samples.

Alternatively, environmental contamination of these compounds has been well-documented in regions as far away as the Arctic (68;69). PFOS (and PFOA to a lesser extent) has been shown to bioconcentrate in fish (44;70-72) and biomagnify in aquatic food chains (4;70-73), suggesting that fish consumption could be a plausible source of exposure. In a recent study in Poland, Falandysz et al. (2006) found that individuals with high fish consumption had elevated concentrations of PFOS (and PFOA to a lesser extent) in their blood relative to other groups (42). The correlation between PFOS and PFOA in blood may reflect the co-occurrence and uptake of these compounds through secondary pathways, such as dietary or drinking water consumption. Although the specific pathways of exposure are still uncertain, these data would indicate that PFOS and PFOA share common pathways to the population of women of childbearing age in this region.

The analytic method used to measure human serum concentrations of PFCs has been described in detail (59). Excellent precision has been reported using this on-line method. At concentrations in the range of what we observed in the current study, CVs of 10 percent were reported for both PFOS and PFOA (59). In this study, measurement error for PFOS may be greater, because the precursor/product ion transition normally used for quantification could not be used due to coelution with another interferent analyte transition ion. In general, measurement error would be expected to bias bivariate associations to the null if the error is completely random (74). Thus, it may contribute to the lack of differences observed in PFC concentrations between many of the subgroups under study.

Cord PFOS and PFOA concentrations in this study were generally within the range of previous reports from Japan and Canada (Figure 3-9). Inoue et al. (2004) measured PFOS and PFOA in serum collected in Japan in 2003 from 15 maternal-fetal pairs. The authors reported the presence of PFOS in all 15 cord blood samples tested, at concentrations ranging from 1.6 to 5.3 ng/mL (56). In the same study, PFOA was detected in only 3 maternal samples and no fetal samples (LOQ: 0.5 ng/mL). In a study of 13 pooled cord plasma samples in northern Canada, collected from 1994-2001, arithmetic mean concentrations of PFOS and PFOA were 16.7 and 3.4 ng/mL, respectively, higher than the average concentrations reported here (48).

Inoue et al. (2004) reported that concentrations of PFOS in cord blood were approximately one-third those of maternal concentrations (56). If we assume this

relationship would be observed in the present study, then this population may have lower exposure than reported for other parts of the United States. The geometric mean PFOS serum concentration reported here, 5 ng/mL, would correspond to 15 ng/mL in the mothers. This represents less than half of the average concentrations reported in the United States, which are in the range of 30-40 ng/mL (5;39-41). However, in a recent analysis of pooled 2001-2002 NHANES samples, mean concentrations of PFOS among women of childbearing age were 24 and 18 ng/mL, for Whites and Blacks, respectively (5). Differences in reported concentrations may result, in part, from varied use and exposure to fluorochemically-treated consumer products, different dietary habits, and temporal trends in human exposure. Alternatively, the relationship between maternal and fetal concentrations may be different in this population than what has been reported by Inoue et al. (2004). Further, this study was not intended to be representative of the full U.S. population and regional differences in exposure may exist. The hospital in which this study was conducted is located in an urban area on the East Coast of the United States. The patients delivering at this hospital are likely to represent a diverse mixture of subjects, including individuals from the surrounding community, faculty and staff from the adjacent medical institutions, as well as transfers from outside of the Baltimore area.

In this study, very few maternal factors were observed to be predictors of cord PFC concentrations. PFOS and PFOA concentrations were relatively constant across maternal age. This lack of association with age is consistent with reports among other populations. Olsen and colleagues conducted three separate studies of PFC concentrations in the blood of children, adults, and the elderly, and found similar geometric mean PFOS and PFOA

concentrations (39-41). If exposure to these compounds were a function of the type of consumer products and materials in the home, one might expect to observe a difference in serum concentrations between individuals of different socioeconomic status. However, none of the socioeconomic measures in our study (e.g., education, insurance, marital status, living within the city limits) were associated with fetal concentrations. It should be noted that a significant amount of data was missing on insurance status and self-reported level of education may not be a precise measure of attained education for the study participants younger than age 18 ($n = 25$). Thus, there may be better metrics (e.g., income, wealth) with which to examine the presence of socioeconomic disparities in the distribution of fetal levels of these compounds. Overall, cord concentrations were relatively low, specifically for PFOA, such that even statistically significant differences estimated by the ratio of geometric means may reflect minor absolute differences in dose. For example, we did find a difference in cord PFOA concentrations by maternal bmi (obese versus normal weight) and infant gender (male versus female), however the absolute difference in geometric means between the groups in each comparison was only 0.3 ng/mL.

In our study population, babies of Asians and Blacks had somewhat higher PFOS concentrations than those of Whites. This is in contrast to an analysis of pooled serum samples from 2001-2002 NHANES, in which White females of child-bearing age had higher levels than Black females (5). There are several possible reasons for differences in this relationship, including the use of pooled versus individual serum samples, differences in exposure patterns between the populations under study, or variations in

placental transfer by race. Interestingly, the three highest PFOS concentrations observed in this study were all among Asians (two from China). Several studies conducted in Asian countries have reported concentrations of PFOS in the general population at or above what has been observed in the United States (44-47). In fact, a Chinese study of 85 blood samples collected in 2004 reported a mean PFOS concentration of 52.7 ng/mL (47), substantially higher than what has been typically reported in the United States (40). These data are consistent with regional variability in exposure, for which race/ethnicity may represent a surrogate marker.

In summary, our findings confirm the presence of *in utero* exposure to PFOS and PFOA, and less so, to other PFCs under study. These data suggest that exposure is occurring among a population of women of childbearing age in the Baltimore area, at levels that are similar to or slightly below such women across the United States. Concentrations of PFOS and PFOA were highly correlated, possibly due to common pathways for exposure. Further, *in utero* serum concentrations of PFOS appear to be higher in Asian and Black babies, when compared to White babies. Future studies should measure maternal serum concentrations in addition to cord serum concentrations, to describe the extent of placental transfer of these compounds. The next chapter examines the relationship between cord serum concentrations, birth weight, and birth size in this population.

Table 3-1. Perfluorinated chemicals (PFCs) measured in cord blood serum and reported in units of ng/mL.

Compound*	Limit of Detection (LOD)	% Above LOD	Geometric Mean** (Range)	Mean % of Total PFC
PFOSA	0.05	26%	0.05 (ND - 0.8)	0.8%
Et-PFOSA-AcOH	0.2	1%	0.14 (ND - 0.5)	2.0%
Me-PFOSA-AcOH	0.2	40%	0.20 (ND - 1.8)	3.0%
PFBuS	0.1	3%	0.07 (ND - 0.2)	1.0%
PFOS	0.2	99%	4.95 (ND - 34.8)	62%
PFHpA	0.4	2%	0.29 (ND - 2.6)	4.0%
PFOA	0.1-0.2	100%	1.57 (0.3 - 7.1)	20%
PFDeA	0.2	24%	0.17 (ND - 1.1)	2.4%
PFUA	0.2	34%	0.20 (ND - 1.9)	2.9%
PFDoA	0.2	5%	0.15 (ND - 1.7)	2.0%

* PFOSA = perfluorooctane sulfonamide; Et-PFOSA-AcOH = 2-(*N*-ethyl-perfluorooctane sulfonamido) acetate; Me-PFOSA-AcOH = 2-(*N*-methyl-perfluorooctane sulfonamido) acetate; PFBuS = perfluorobutane sulfonate; PFOS = perfluorooctane sulfonate; PFHpA = perfluoroheptanoate; PFOA = perfluorooctanoate ; PFDeA = perfluorodecanoate; PFUA = perfluoroundecanoate; PFDoA = perfluorododecanoate.

** Assuming non-detects (ND) equal LOD/ $\sqrt{2}$.

Table 3-2. Ratios (and 95% confidence intervals) of geometric mean PFOS concentrations in cord blood serum by maternal and infant characteristics.

Characteristic	N	PFOS	
		Univariate Model	Adjusted Model*
Age group			
<18 years	25	1.00 (0.76-1.31)	1.02 (0.73-1.43)
18-35 years	250	---	---
>35 years	24	1.00 (0.76-1.32)	1.13 (0.84-1.53)
Race			
White	64	---	---
Asian	25	1.43 (1.06-1.95)	1.43 (1.02-2.01)
Black	210	1.23 (1.02-1.48)	1.28 (0.98-1.68)
Education			
<HS diploma	87	---	---
HS diploma	97	1.07 (0.88-1.30)	1.05 (0.84-1.32)
1-4 years college	69	1.01 (0.82-1.25)	1.06 (0.79-1.42)
5+ years college	42	1.07 (0.84-1.37)	1.05 (0.70-1.58)
Insurance			
Public assistance	98	---	---
Private	116	1.03 (0.86-1.24)	1.10 (0.86-1.41)
Marital status			
Unmarried	198	---	---
Married	101	0.95 (0.81-1.11)	0.90 (0.67-1.19)
Body mass index			
Underweight (<18.5)	16	1.22 (0.87-1.73)	1.22 (0.85-1.75)
Normal (18.5-24.9)	135	---	---
Overweight (25-29.9)	65	0.98 (0.80-1.20)	0.97 (0.78-1.19)
Obese (30+)	72	1.13 (0.93-1.37)	1.11 (0.90-1.37)
Parity			
Primiparous	125	---	---
Multiparous	174	0.92 (0.79-1.08)	0.91 (0.76-1.08)
Smoking status			
Non-smoker	222	---	---
Passive	21	1.07 (0.79-1.44)	1.05 (0.76-1.46)
Active	56	0.88 (0.73-1.08)	0.92 (0.73-1.15)
Infant gender			
Female	133	---	---
Male	166	0.88 (0.76-1.02)	0.87 (0.74-1.02)
Inside city limits?			
No	92	---	---
Yes	207	1.04 (0.88-1.22)	0.94 (0.75-1.17)
Preterm birth			
No	260	---	---
Yes	39	0.86 (0.69-1.08)	0.90 (0.70-1.14)

* Adjusted model includes all variables listed in the table.

The following data were missing: 4 observations for education, 85 for insurance, and 11 for BMI. Missing data were treated as an indicator term in regression models.

Table 3-3. Ratios (and 95% confidence intervals) of geometric mean PFOA concentrations in cord blood serum by maternal and infant characteristics.

Characteristic	N	PFOA	
		Univariate Model	Adjusted Model*
Age group			
<18 years	25	0.94 (0.77-1.15)	0.98 (0.77-1.25)
18-35 years	250	---	---
>35 years	24	0.95 (0.77-1.17)	1.07 (0.86-1.33)
Race			
White	64	---	---
Asian	25	1.06 (0.84-1.34)	1.06 (0.83-1.35)
Black	210	1.11 (0.97-1.27)	1.12 (0.92-1.36)
Education			
<HS diploma	87	---	---
HS diploma	97	1.10 (0.95-1.27)	1.14 (0.96-1.34)
1-4 years college	69	1.02 (0.87-1.19)	1.16 (0.94-1.44)
5+ years college	42	1.06 (0.88-1.27)	1.21 (0.90-1.62)
Insurance			
Public assistance	98	---	---
Private	116	0.94 (0.82-1.07)	0.95 (0.80-1.14)
Marital status			
Unmarried	198	---	---
Married	101	0.95 (0.84-1.07)	0.99 (0.81-1.22)
Body mass index			
Underweight (<18.5)	16	1.16 (0.90-1.50)	1.13 (0.87-1.47)
Normal (18.5-24.9)	135	---	---
Overweight (25-29.9)	65	1.08 (0.94-1.25)	1.04 (0.90-1.21)
Obese (30+)	72	1.21 (1.06-1.40)	1.19 (1.02-1.39)
Parity			
Primiparous	125	---	---
Multiparous	174	0.90 (0.81-1.01)	0.86 (0.76-0.98)
Smoking status			
Non-smoker	222	---	---
Passive	21	1.06 (0.85-1.33)	1.07 (0.84-1.35)
Active	56	1.01 (0.88-1.17)	1.10 (0.93-1.29)
Infant gender			
Female	133	---	---
Male	166	0.81 (0.73-0.91)	0.82 (0.73-0.92)
Inside city limits?			
No	92	---	---
Yes	207	1.05 (0.93-1.19)	1.01 (0.86-1.19)
Preterm birth			
No	260	---	---
Yes	39	0.88 (0.74-1.04)	0.88 (0.74-1.05)

* Adjusted model includes all variables listed in the table.

The following data were missing: 4 observations for education, 85 for insurance, and 11 for BMI. Missing data were treated as an indicator term in regression models.

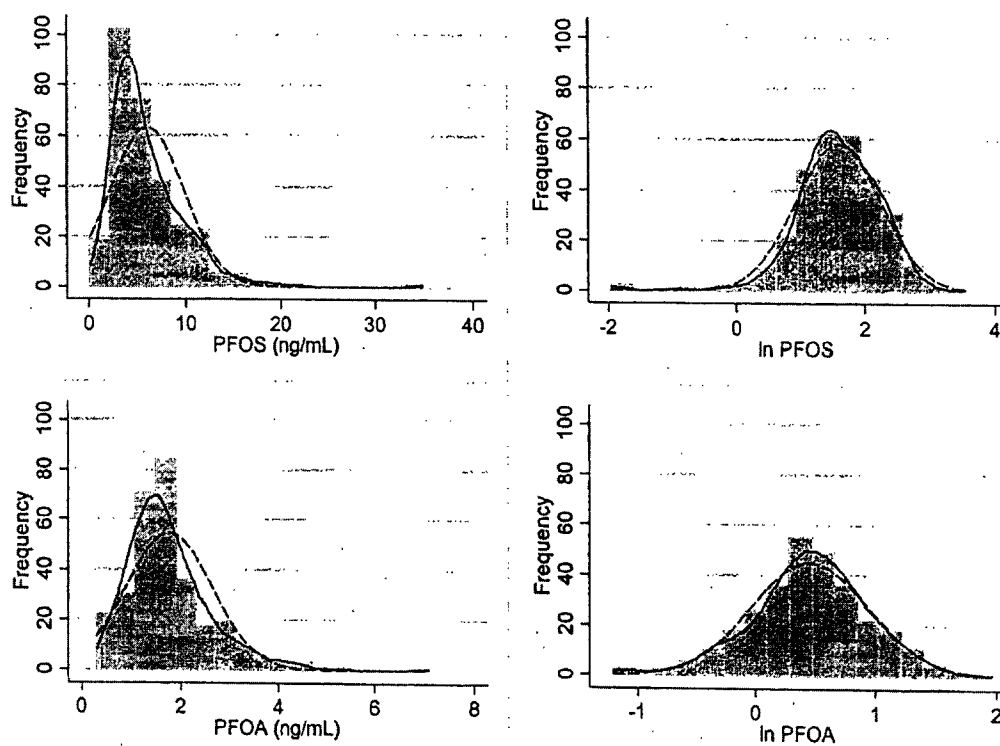


Figure 3-1. Distributions of PFOS and PFOA concentrations in cord blood serum.

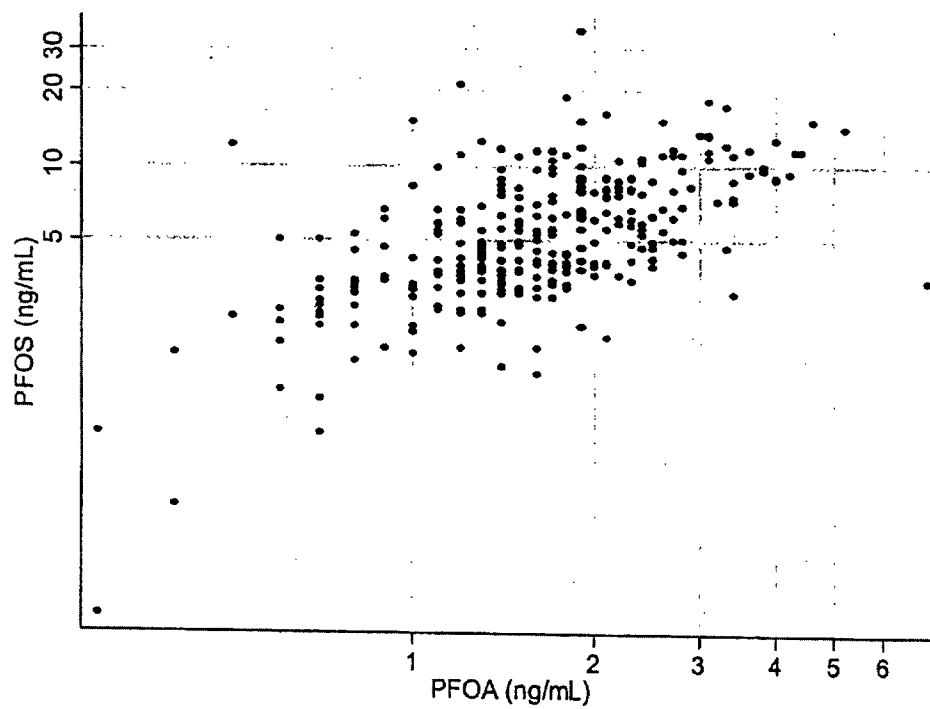


Figure 3-2. Correlation between PFOS and PFOA concentrations in cord blood serum (n = 299).
* $r = 0.64$

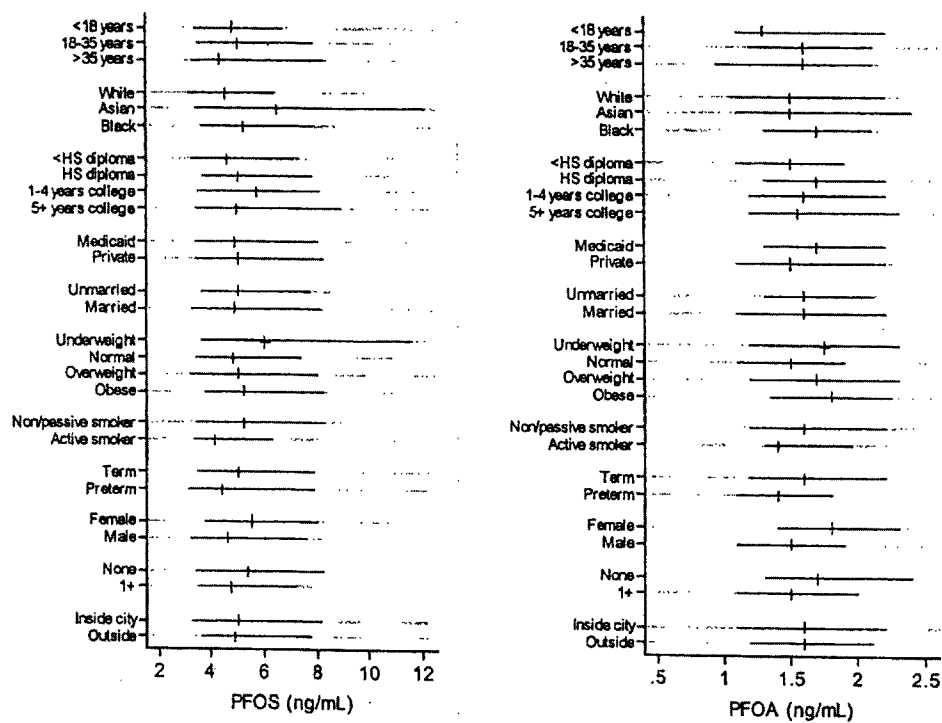


Figure 3-3. Distribution of PFOS and PFOA concentrations in cord blood serum (median and interquartile range) by select characteristics.

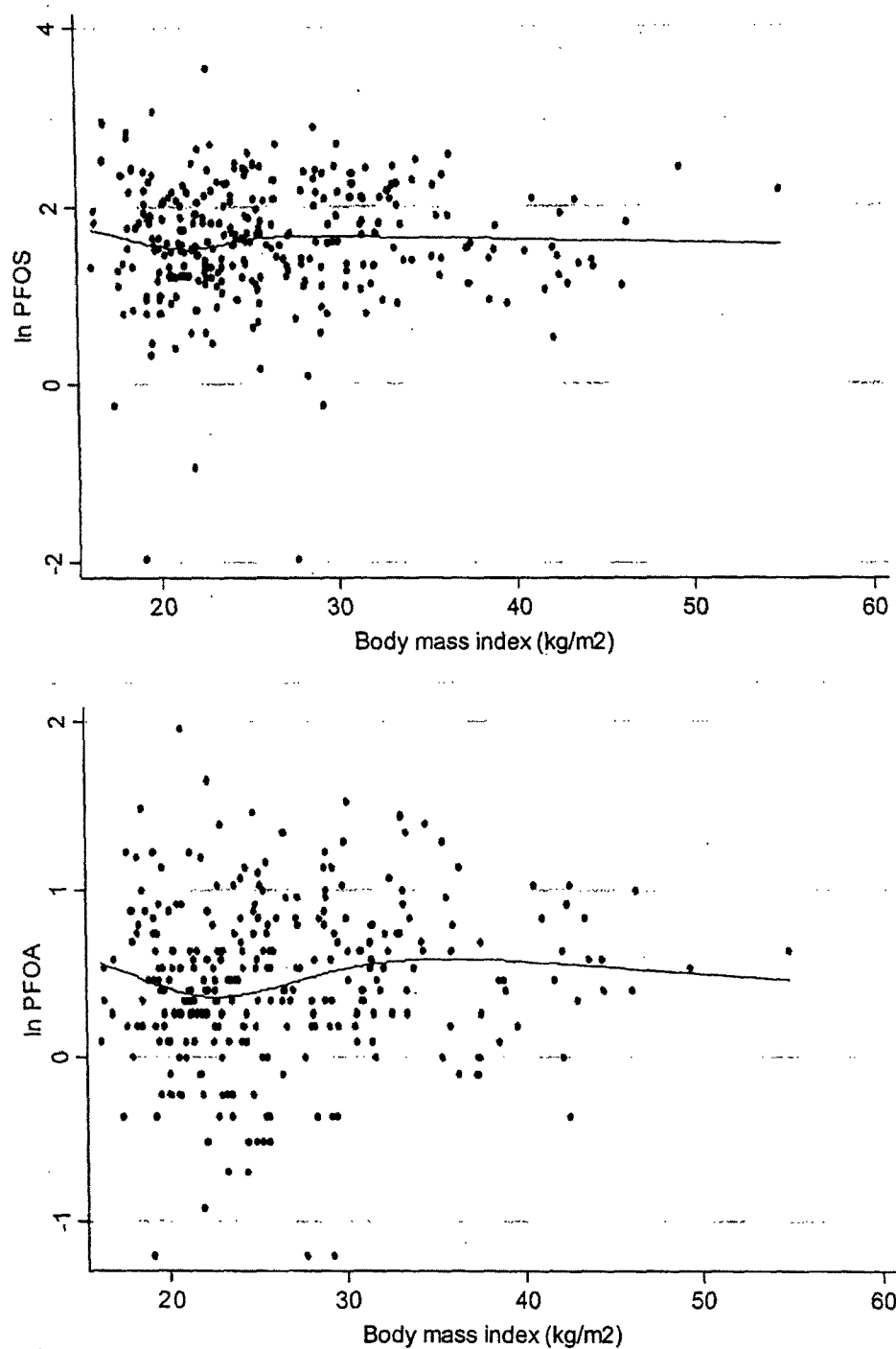


Figure 3-4. PFOS (top) and PFOA (bottom) concentrations in cord blood serum versus maternal pre-pregnancy body mass index (n=288).

Note: Line reflects predicted values from a restricted cubic spline model.

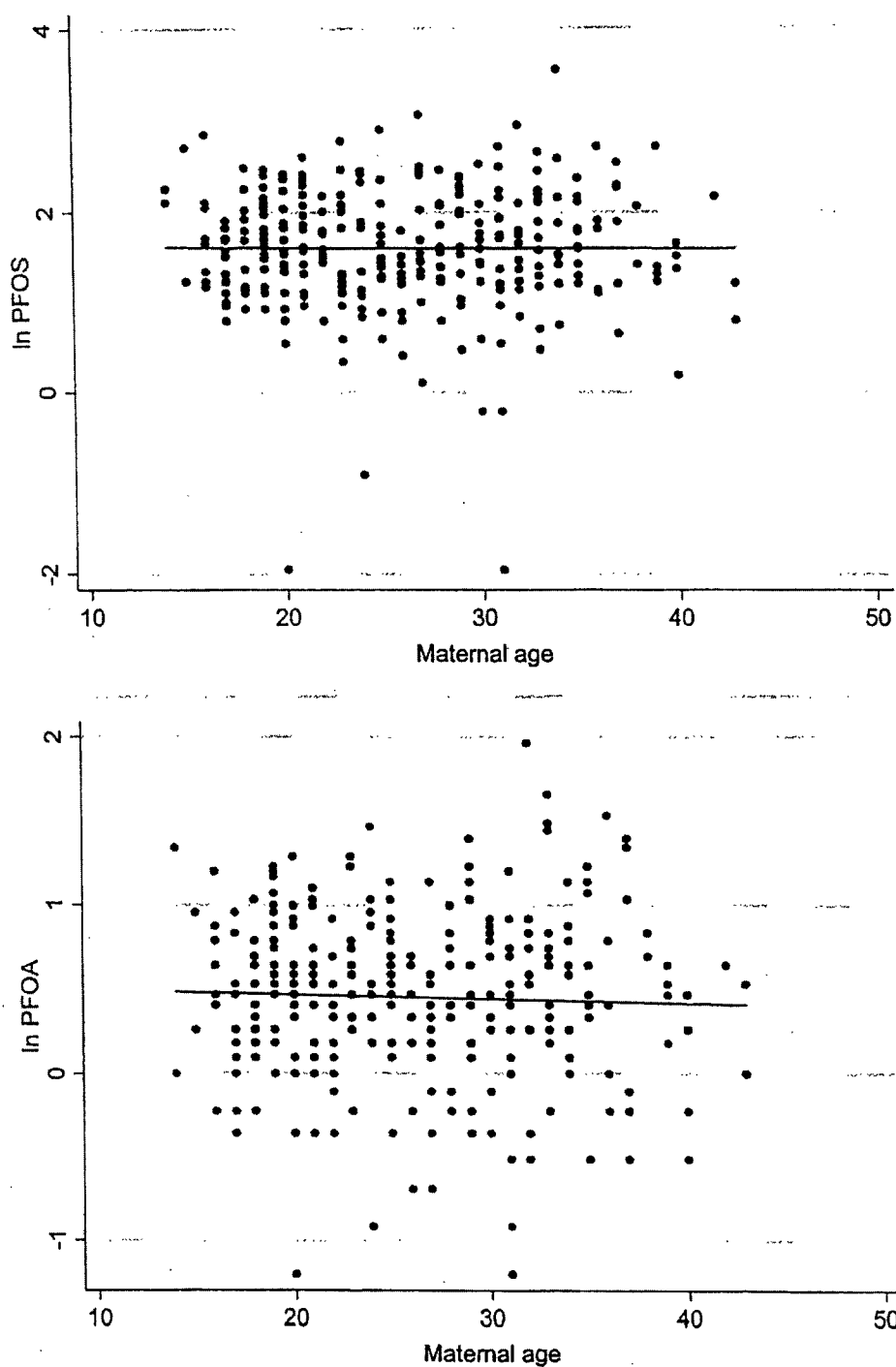


Figure 3-5. PFOS (top) and PFOA (bottom) concentrations in cord blood serum versus maternal age (n=299).

(PFOS: $\beta = -0.0007$, $p = 0.90$; PFOA: $\beta = -0.0026$, $p = 0.54$)

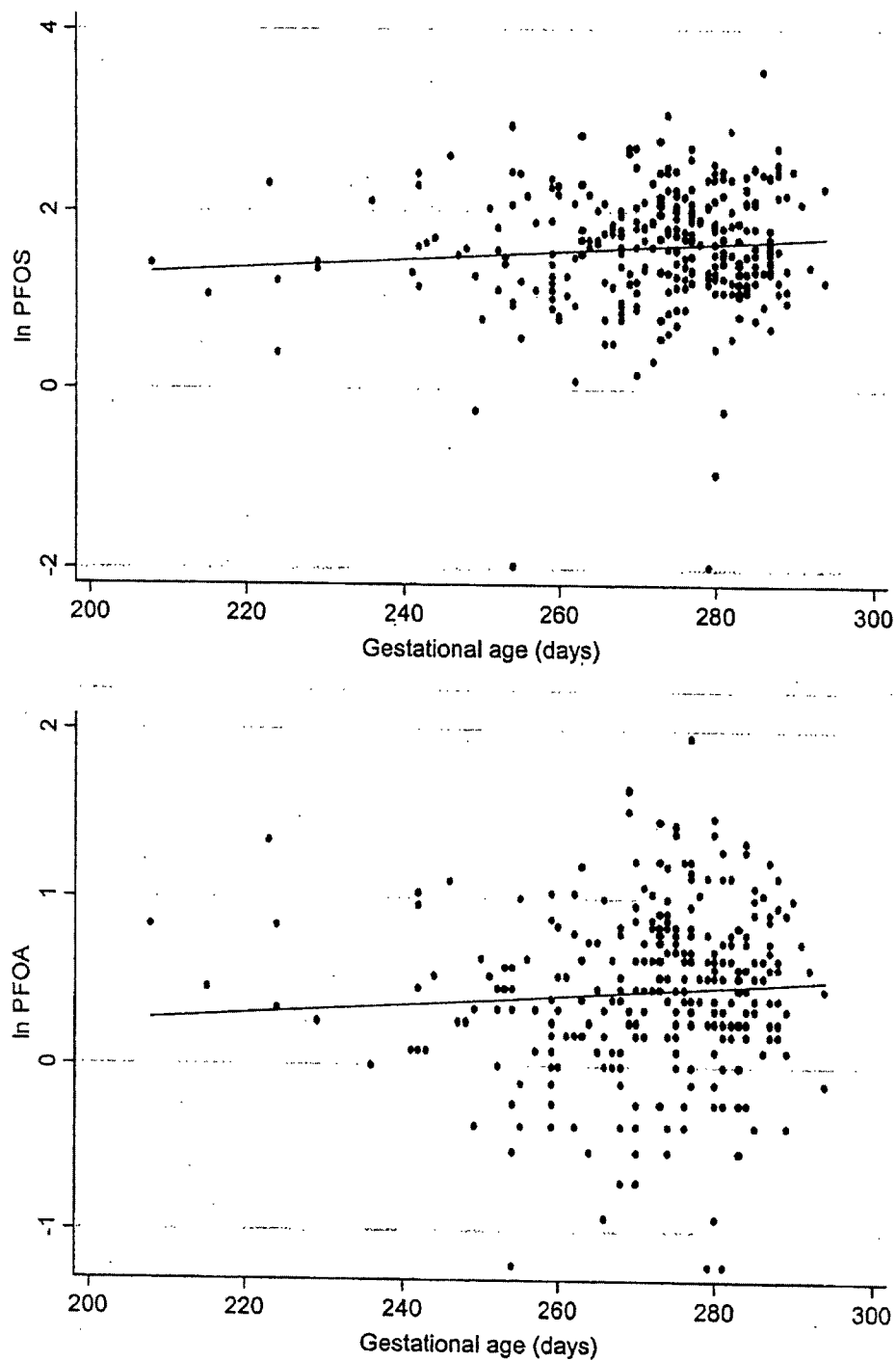


Figure 3-6. PFOS (top) and PFOA (bottom) concentrations in cord blood serum versus gestational age (n = 299).

(PFOS: $\beta = 0.0046$, $p = 0.10$; PFOA: $\beta = 0.0028$, $p = 0.17$)

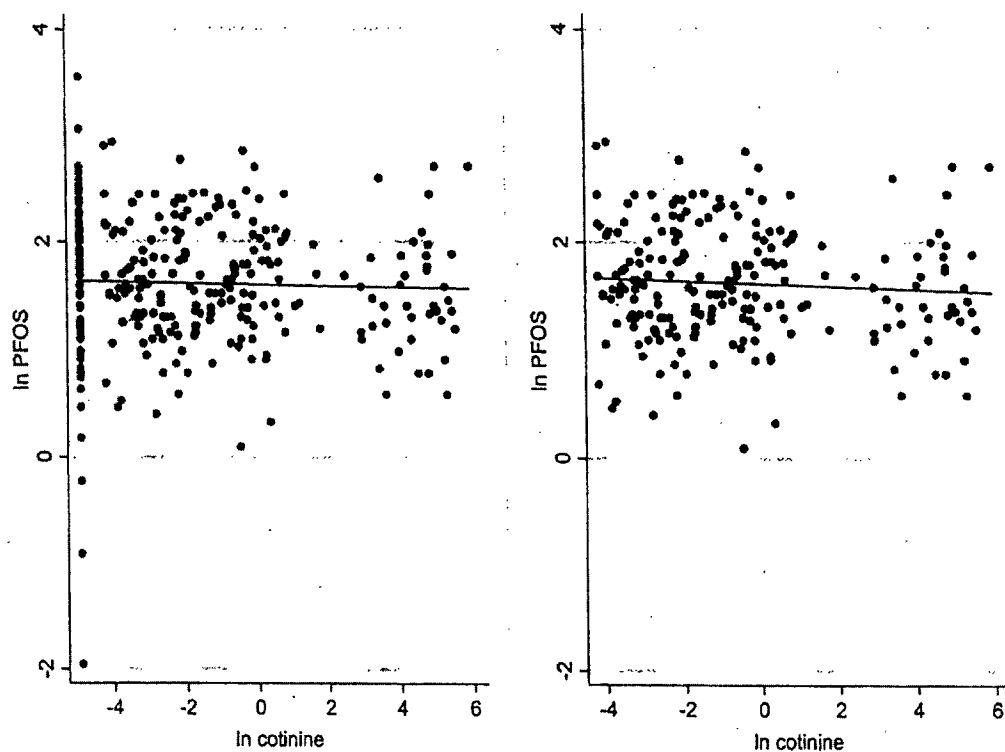


Figure 3-7. PFOS (ng/mL) versus cotinine (ng/mL) concentrations in cord blood serum, with ($n = 286$) and without ($n = 211$) the inclusion of non-detectable cotinine values.

(With NDs: $\beta = -0.0061$, $p = 0.62$; Without NDs: $\beta = -0.0131$, $p = 0.32$)

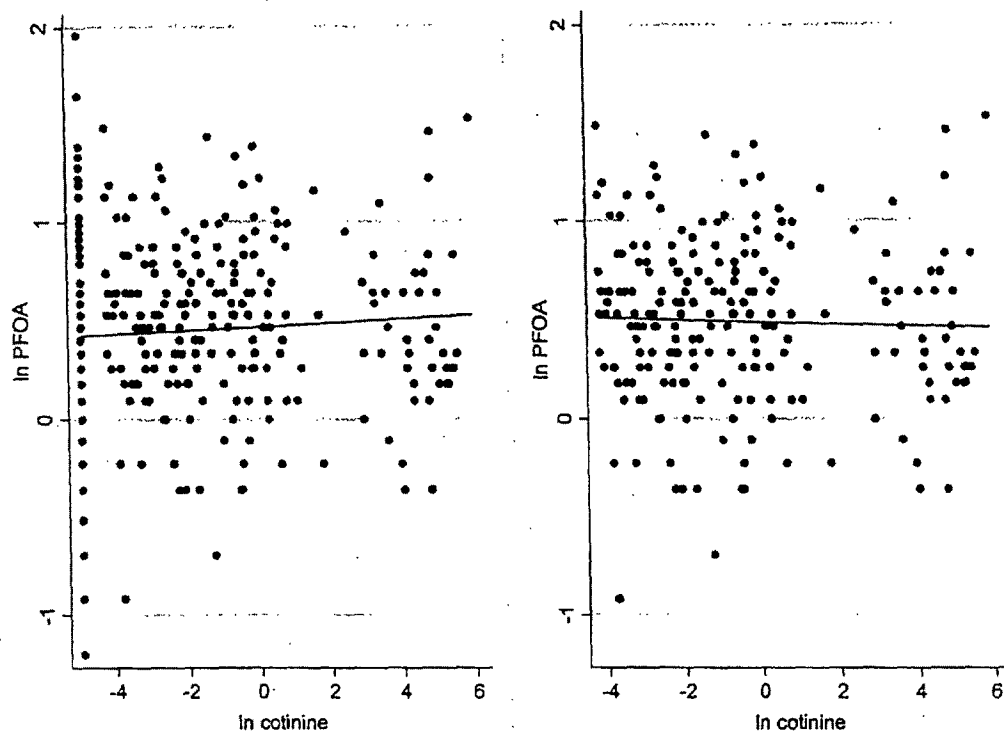


Figure 3-8. PFOA (ng/mL) versus cotinine (ng/mL) concentrations in cord blood serum, with (n = 286) and without (n = 211) the inclusion of non-detectable cotinine values.

(With NDs: $\beta = 0.0095$, $p = 0.31$; Without NDs: $\beta = -0.0049$, $p = 0.63$)

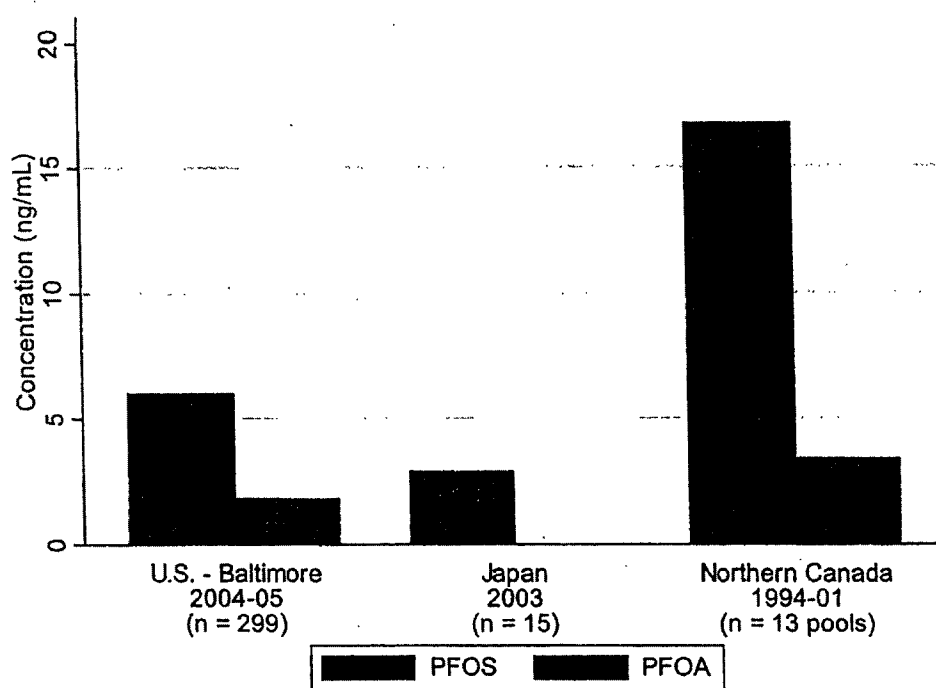


Figure 3-9. Comparison of mean PFOS and PFOA concentrations (ng/mL) measured in cord blood serum or plasma.

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CHAPTER 4

AN EPIDEMIOLOGIC INVESTIGATION OF FETAL EXPOSURE TO PFOS AND PFOA AND RELATIONSHIPS WITH WEIGHT AND SIZE AT BIRTH

Abstract

BACKGROUND: Perfluorinated compounds (PFCs) such as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA) are used in a variety of consumer and industrial applications. Recent studies have reported developmental toxicity among rodents dosed with PFOS and PFOA, two widely used surfactants. We previously documented *in utero* exposure to PFCs among newborn deliveries at a hospital in Baltimore, MD.

METHODS: In this hospital-based cross-sectional study, we examine the relationship between cord concentrations of PFOS or PFOA and birth weight (grams), newborn head circumference (cm), length (cm), and ponderal index ($\text{g}/\text{cm}^3 \times 100$). Multiple births and babies with major congenital anomalies were excluded from the study population ($n = 293$). Cord serum samples were analyzed for PFOS and PFOA by on-line solid-phase extraction, coupled with reversed phase high-performance liquid chromatography-tandem mass spectrometry. Medical record data were obtained from maternal and infant charts.

RESULTS: After adjusting for potential confounders, both PFOS and PFOA were negatively associated with birth weight (per increase from 25th to 75th percentile: $\beta = -58$ grams, 95% CI: -125, 9 for PFOS; $\beta = -58$ grams, 95% CI -119, 3 for PFOA) and ponderal index (per increase from 25th to 75th percentile: $\beta = -0.062$, 95% CI -0.104, -0.021 for PFOS; $\beta = -0.039$, 95% CI: -0.077, -0.001 for PFOA). A negative association was also observed with head circumference, but only among vaginal deliveries. These associations were independent of cord serum lipid concentrations.

CONCLUSION: Despite relatively low serum concentrations, we detected negative associations between PFOS and PFOA concentrations in cord serum and birth weight and size. Future studies should attempt to replicate these findings in other populations.

Introduction

Perfluorinated compounds (PFCs) comprise a class of man-made, fully fluorinated organic compounds that have been used in a variety of consumer and industrial applications for more than 50 years (1;2). Although produced for many years, only recently have reports surfaced suggesting widespread exposure in wildlife and humans (3-5). Two of the most widely detected and studied compounds in this class are perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA). PFOS and related compounds (perfluorinated sulfonamides) are surfactants used in applications ranging from oil and water repellents for fabrics, apparel, carpets, and paper coatings to specialty chemical applications such as insecticides and fire fighting foams (2). Because of the evidence of human exposure to PFOS, its major U.S. manufacturer announced in 2000 the phase-out of all perfluorooctanyl-based products. PFOA and its salts are used as chemical intermediates and processing aids in the production of fluoropolymers and fluoroelastomers (6) and are still being produced in the United States.

Both PFOS and PFOA have shown the potential for developmental toxicity in animal studies. PFOS has been shown to induce developmental and reproductive effects in rats and mice, such as reduced birth weight, decreased gestational length, structural defects, developmental delays, and increased neonatal mortality (7-12). Recent studies have reported developmental toxicity from PFOA in rodents as well, including pregnancy loss, reduced fetal weight, reduced postnatal survival, and delays in postnatal growth and development in offspring (13-17). The U.S. Environmental Protection Agency is currently evaluating PFOA for potential developmental risks to humans (18). PFOS and

PFOA have also been identified as peroxisome proliferators (19-24) and have been shown to have hypolipidemic effects in several animal species. Toxicity studies in animals have shown marked reductions in serum cholesterol and/or triglycerides (7;25-27), which may be mediated through down-regulation of HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase, a key enzyme in cholesterol synthesis (27). Conversely, a few cross-sectional occupational studies conducted among fluorochemical production employees have reported positive relationships between serum lipids and PFOS and/or PFOA concentrations (28;29). No epidemiologic studies have evaluated the impacts of PFC exposure on fetal growth and development.

The fetus is likely to be sensitive to the availability of cholesterol and triglycerides, due to its rapid cellular growth, differentiation, and body weight accumulation during development (30). Disruptions to normal fetal growth and development have been associated with effects across the lifespan, including adverse neonatal and childhood outcomes (31-36) and metabolic diseases in adulthood (37;38). The most common approaches for identifying potentially growth-restricted newborns are classifying birth weight, length, and head circumference by gestational age. Newborns below the 10th percentile of weight or size are typically classified as small-for-gestational age, even though it is recognized that such a classification will miss some growth-restricted babies and identify others who are simply constitutionally small (39;40). Body proportionality has been used to define asymmetric growth, which has been associated with increased severity of growth restriction (41) and adverse neonatal outcomes (42-47). One measure

of disproportionate growth restriction is the ponderal index ($\text{weight}/\text{length}^3 \times 100$), a measure of body mass at birth.

In a previous report, we documented cord blood concentrations of PFOS and PFOA in a population of newborn deliveries occurring in a Baltimore city hospital. In this study, we examine the relationship between these concentrations and measures of birth weight and birth size parameters, including head circumference, length, and ponderal index.

Material and Methods

Subjects

We conducted a cross-sectional study of newborn deliveries at the Johns Hopkins Hospital in Baltimore, MD. The study received approval from the Johns Hopkins Medicine Institutional Review Board and was determined to be HIPAA exempt. The study was conducted anonymously and utilized only specimens that otherwise would have been discarded and medical records that were available to study personnel. There was no requirement for informed consent due to the anonymization of all samples and data. Between November 26, 2004 and March 16, 2005 all singleton, live birth deliveries occurring in the Labor and Delivery Suite at the hospital were eligible for participation in the study. We excluded from the study population women who gave birth to more than one child or women who delivered a single child but had an initial twin gestation with fetal loss at 20 weeks or greater. Newborns with major congenital anomalies likely to impact fetal growth were excluded as well.

Over the course of the study period, 609 live births occurred at the Johns Hopkins Hospital, of which 597 were singleton births. We obtained cord blood specimens from 341 of these, of which 42 had insufficient volume for laboratory analyses and were excluded from this study. An additional five singleton births had major congenital anomalies indicated on the birth record and one birth was identified as an initial twin gestation with demise of the fetal twin at 20 weeks. These subjects were excluded, leaving a total of 293 study participants (Figure 4-1).

Cord blood samples were collected from the umbilical cord vein by hospital personnel immediately following delivery (48). Upon delivery, a section of the cord was cleaned with an alcohol wipe and blood was drawn using a sterile 60-mL Becton Dickinson (BD) syringe with an 18-gauge safety needle. A BD Vacutainer Blood Transfer Device was then attached to the syringe and 10 mL glass BD vacutainers were filled. After collection, cord blood specimens were stored in Labor and Delivery refrigerators. Within several hours, the specimens were picked up and taken across the street to a laboratory at the Johns Hopkins Bloomberg School of Public Health for processing. Tubes were centrifuged at 2400 rpm for 15 minutes for serum separation. Serum was aliquotted into 2 mL polypropylene prescreened cryovials and stored at -80°C . Frozen specimens were transferred on dry ice to the Centers for Disease Control and Prevention (CDC) for laboratory analyses.

Medical Records

We abstracted maternal and infant characteristics from clinical databases maintained by the hospital. Maternal information was abstracted by two study investigators concurrently, and a random 10 percent sample was verified by two others. The data collected included demographic information, anthropometric measures, medical history, and pregnancy-related characteristics. Age, race, education, marital status, and parity were based on self-report. Body mass index was calculated from reported pre-pregnancy weight and height as the ratio of weight (in kilograms) to height (in meters) squared. Gestational age was based on the "best obstetric estimate." Infant gender was abstracted from the mother's medical record and confirmed with the infant record. Information on maternal health conditions were abstracted from the medical record. Smoking status was defined using a combination of the maternal medical record and cord serum cotinine concentrations measured at birth. Using cutoffs from Jarvis et al. (1987), samples with a cotinine concentration of 1 to 14 ng/mL were categorized as passive smoking exposure and samples with a concentration above 14 ng/mL were categorized as active smoking exposure (49). Serum samples with cotinine concentrations below 1 ng/mL were included in the category of nonsmokers. If the clinical record indicated that the mother reported smoking at any time during pregnancy, she was considered an active smoker regardless of the cotinine concentration in cord blood at birth. Because it was not possible to measure passive smoking exposure earlier in pregnancy and misclassification is likely to exist between passive and non-smokers, smoking status was dichotomized to active and passive/non-smoker in statistical analyses.

Infant anthropometric measures were abstracted from the infant medical record. Birth weight in grams was obtained from the infant record and confirmed with the maternal record. Head circumference and length in centimeters were abstracted from the infant record. Ponderal index was calculated as the ratio of birth weight in grams to length in centimeters cubed, multiplied by 100 ($birthweight/length^3 \times 100$). We examined the relationships between gestational age, birth weight, length, and head circumference to identify outlying values. Outliers were identified and weight and size measurements were verified using the infant and maternal record.

Laboratory Analysis

Pefluorinated compounds

Cord serum samples were analyzed for PFOS and PFOA by on-line solid-phase extraction (SPE), coupled with reversed phase high-performance liquid chromatography-tandem mass spectrometry. The method has been described in detail by Kuklenyik et al. (2005). Excellent precision has been reported using this method for the detection of PFCs in human serum (50). Briefly, without protein precipitation, one aliquot of 100 μ L of serum was injected into a commercial column switching system allowing for concentration of the analytes on a SPE column. This column was placed automatically in front of an analytical column for chromatographic separation of the analytes. Detection and quantification were done using negative-ion TurboIonSpray ionization, a variant of electrospray ionization, tandem mass spectrometry. Detection limits were in the low nanogram per milliliter range for PFOS and PFOA. In these cord serum samples, the precursor/product ion m/z transition used for the quantification of PFOS had an

interferent ion. Therefore, PFOS concentrations had to be calculated using another transition, normally used to confirm the presence of PFOS. The nature of this interference is at present unknown. Quality control (QC) and reagent blank samples were included in each analytical batch along with the unknown samples. QC samples were evaluated according to modified Westgard rules.² All laboratory analyses were conducted by investigators blinded to the characteristics of study subjects.

Cotinine.

Serum cotinine was analyzed by CDC using the method described by Bernert et al. (1997). The analytic method uses high-performance liquid chromatography coupled with atmospheric pressure chemical ionization tandem mass spectrometry to measure serum cotinine concentrations with high accuracy and sensitivity (limit of detection [LOD] = 0.015 ng/mL). This method has been used to measure environmental tobacco smoke dose in large-scale surveys, including NHANES (51).

Statistical Analysis

We used descriptive statistics to characterize the study population. We used Fisher's Exact test and Wilcoxon's Rank Sum test to compare subjects with missing data on key covariates to those with complete data. We used descriptive statistics appropriate for right-skewed data to characterize cord PFOS and PFOA concentrations. Spearman rank correlation was used to estimate the correlation between cord levels of the two compounds. Because PFOS and PFOA concentrations were skewed to the right, all

² Description of analytical method contributed by Antonia Calafat, Division of Laboratory Sciences, National Center for Environmental Health, CDC.

statistical tests were conducted on natural log-transformed concentrations. Natural log-transformed concentrations were also used as independent variables in regression analysis to minimize the potential influence of outliers on the regression coefficients. Samples below the detection limit for PFOS, PFOA, and cotinine were assumed to be equal to the detection limit divided by the square root of two for all analyses (52).

We conducted univariate and multivariate linear regression analyses to examine the associations between PFOS or PFOA and birth weight, newborn head circumference, newborn length, and ponderal index. The inclusion of covariates in the regression model was based on *a priori* knowledge of the key determinants of birth weight and birth size. The covariates included in the primary adjusted models were: smoking status, maternal age, gestational age, race, maternal pre-pregnancy body mass index (BMI), weight gain during pregnancy, maternal height, parity, gender, diabetes, and hypertension. Diabetes was defined to include subjects with pre-existing or gestational diabetes, assuming both conditions would result in increased fetal weight for age. Similarly, hypertension was defined to include subjects with pre-eclampsia, pregnancy-induced hypertension, and chronic hypertension, which were expected to result in decreased weight for age. For consistency, the same set of covariates was included in the primary regression model for each endpoint, with the exception of delivery mode (Vaginal versus Caesarian section), which was included as a predictor of head circumference. The specification of these variables in the model was based on the shape of the empirical relationships between the covariates and the different endpoints (Appendix A). Based on the empirical evidence, a quadratic term for maternal age was included in regressions of birth weight, head

circumference, and ponderal index, but not for length. A quadratic term for gestational age was included in regressions of head circumference only. All other terms were included in the models as linear or categorical (indicator) variables. We explored the possible inclusion of other variables (e.g. education level, marital status, insurance status), but were dropped because they had no material effect on the coefficient estimates.

A small minority (<4 percent) of the study population was missing data on pre-pregnancy weight, height, and/or weight gain during pregnancy, which are important predictors of birth weight and size. Those subjects missing data on at least one of these variables were more likely to be smokers and deliver preterm. However, they were similar with respect to other characteristics, including demographics, birth weight, anthropometric measures at birth, and cord levels of PFOS and PFOA (Table B-1). We examined two different approaches for handling those missing data: (1) complete case analysis (i.e. dropping observations for which one of the above measures is missing, also called "listwise" deletion) and (2) imputing the missing data with the median value of height, weight, and/or weight gain. Both approaches produced similar results (Table B-3), therefore, only the latter approach is presented here. As a sensitivity analysis, we examined the impact of imputing BMI or weight gain with extreme values and found no material effect on the regression coefficients of PFOS or PFOA.

We also conducted a series of sensitivity analyses to examine the impact of modeling assumptions on the regression estimates. We examined the impact of covariate specification in regression models, including the use of cotinine concentration instead of

smoking status to control for the effect of smoking, and we explored the presence of non-linearities among the potential confounders using restricted cubic spline models. We also conducted regressions on untransformed PFOS and PFOA concentrations to evaluate whether inferences were consistent with the primary log-linear models.

Regression diagnostics were conducted for all models, including examination of fit, heteroskedasticity, and influence. Statistical analyses were performed using STATA version 8.0 (StataCorp, College Station, TX).

Results

Table 4-1 shows the characteristics of the study population. The mean maternal age at delivery was approximately 26 years of age, with a range of 14 to 43. Eight percent of births occurred to mother's less than 18 years of age at delivery. The majority of study participants were Black (71%; n=208), followed by White (21%; n=60) and Asian (9%; n=25). Over 60 percent of the mothers in our study had a high school diploma or less, while about 14 percent had some amount of post-graduate education. Approximately two-thirds of the study mothers were unmarried. While only 6 percent of the mothers were underweight prior to pregnancy ($\text{BMI} < 18.5 \text{ kg/m}^2$), close to 50 percent were classified as overweight or obese ($\text{BMI} > 25 \text{ kg/m}^2$). The average net weight gain during pregnancy (weight gain minus birth weight) was 22.7 pounds, with a wide distribution observed ($\text{SD} = 17.8 \text{ lbs}$). More than 40 percent of babies were first-born. Approximately 19 percent of mothers were categorized as smokers on the basis of cord cotinine levels at birth and self-report. Among study subjects, the rates of low birth

weight (<2500 grams) and preterm birth (<37 completed weeks) were 11 and 13 percent, respectively. The distribution of birth weight, newborn length, head circumference, and ponderal index is shown in Table 4-2.

PFOS was detected in greater than 99 percent of infants' cord blood samples and PFOA was detected in 100 percent of samples. The distribution of PFOS and PFOA concentrations in cord blood is shown in Table 4-2. The median cord serum concentration of PFOS was 5 ng/mL, with a range from below the limit of detection (<LOD) to 34.8 ng/mL. The median PFOA concentration was 1.6 ng/mL, with a range from 0.3 ng/mL to 7.1 ng/mL. Concentrations of PFOS and PFOA in cord blood were highly correlated (Spearman rank correlation coefficient: 0.58; $p < .01$).

Table 4-3 shows the regression model results of birth weight, length, head circumference, and ponderal index on natural log transformed PFOS and PFOA. After adjusting for potential confounders, both PFOS and PFOA were negatively associated with birth weight. For PFOS, an average reduction in birth weight of 58 grams (95% CI -125, 9) was associated with a change in cord concentration from the 25th to 75th percentile. For PFOA, an increase from the 25th to 75th percentile was associated with an average birth weight decline of 58 grams (95% CI -119, 3). In both models, significant predictors of birth weight included gestational age, smoking status, maternal BMI, parity, gender, maternal height, net weight gain during pregnancy, and diabetes (Tables C-1, C-2).

For head circumference, a negative association was observed with $\ln(\text{PFOS})$ in a univariate analysis ($p=0.14$). Under the multivariate model, an increase in PFOS concentration from the 25th to 75th percentile was associated with an average decrease in head circumference of 0.27 centimeters (95% CI: -0.48, -0.06). For PFOA, a negative association was observed in both univariate ($p=0.03$) and multivariate analyses ($p=0.02$). In the multivariate model, an increase from the 25th to 75th percentile of PFOA was associated a decrease in mean head circumference of 0.23 centimeters (95% CI: -0.42, -0.04). The predicted relationships between PFOS or PFOA and head circumference based on the unadjusted and adjusted regression models are displayed in Figure 4-2. Based on the results of multivariate models, significant predictors of head circumference were gestational age, smoking status, maternal BMI, parity, race, maternal height, net weight gain during pregnancy, diabetes, hypertension, and delivery mode (Tables C-3, C-4).

Delivery mode was highly significant predictor of head circumference, with babies born by Caesarean section (C-section) having larger head circumferences, on average, compared with vaginal deliveries, after adjusting for potential confounders. Because babies born by C-section do not have head molding during birth, we included an interaction term between PFOS or PFOA and delivery mode, which was statistically significant ($p<0.05$). Among vaginal births, a large negative association was observed between PFOS or PFOA and head circumference. Among C-sections, there was a non-significant positive association between PFOS or PFOA and head circumference. Table

4-4 shows estimated regression coefficients for PFOS and PFOA under the interaction model.

Contrary to the associations with birth weight and head circumference, neither PFOS nor PFOA was significantly associated with newborn length in univariate or multivariate models (Table 4-3). The following covariates were significant predictors of newborn length in multivariate models: gestational age, smoking status, maternal BMI and gender. Maternal height, net weight gain during pregnancy, and diabetes were marginally significant (Tables C-5, C-6).

The association between PFOS and PFOA and ponderal index is shown in Table 4-3. Negative associations were observed for both PFOS and PFOA with ponderal index in univariate and multivariate models. After adjustment, an increase in PFOS concentration from the 25th to 75th percentile was associated with an average decline in ponderal index of 0.062 (95% CI: -0.104, -0.021). An increase in PFOA from the 25th to 75th percentile was associated with a mean decrease in ponderal index of 0.039 (95% CI: -0.077, -0.001). Figure 4-3 displays the associations between PFOS or PFOA and ponderal index, based on results from the univariate and multivariate regression models. Based on the results of multivariate models, the only other significant predictors of ponderal index were gestational age, maternal age parity, and net weight gain during pregnancy (Tables C-7, C-8).

We conducted a series of sensitivity analysis to assess the impact of model and variable specification on the results. The associations observed were predominantly insensitive to different specifications of potential confounders (e.g. use of cotinine as a continuous measure, non-linear smooth functions of height, weight, and weight gain) (Appendices D-G). We also examined the consistency of inferences when the multivariate regressions were run on untransformed PFOS and PFOA. Figure 4-4 shows the univariate relationship between PFOS or PFOA and head circumference, comparing the predicted fit from log-linear and linear models. A similar comparison is made for ponderal index in Figure 4-5. As shown in the figures, the choice of a log-linear or linear model had little impact on the estimates obtained. Similar inferences were obtained in multivariate models as well when comparing log-linear with linear functions (Figures D-7, E-7, F-7, G-7).

Discussion

In a previous study, we documented the extent and determinants of fetal cord concentrations of PFOS and PFOA in a hospital in Baltimore. In this study, we examined the relationship between these cord concentrations with birth weight and birth size. Our results showed negative associations between PFOS and PFOA with birth weight, ponderal index, and head circumference, after adjusting for potential confounders. Gestational age was the strongest confounder in this study and adjustment tended to strengthen the associations observed. No significant associations were observed between either PFOS or PFOA and newborn length. These associations were relatively consistent,

after conducting a series of sensitivity analyses examining the impacts of different covariate adjustments, model specifications, and treatment of missing data.

Ponderal index has been used by clinicians and epidemiologists as a measure of thinness at birth and an indicator of disproportionate or asymmetric growth restriction. Because fetal weight gain and soft tissue mass increases dramatically in the third trimester and the body becomes more proportional, it has been hypothesized that early insults during gestation result in symmetric growth restriction and later insults, asymmetric growth restriction. However, some empirical evidence conflicts with this notion about the timing of fetal growth disruption (39;53;54). Kramer et al. (1989) showed that disproportionality increased with increasing severity of growth restriction, suggesting the presence of a continuum of fetal growth restriction, rather than two distinct patterns (41). Regardless, several studies have shown associations between low ponderal index and risk of adverse neonatal outcomes (42-47). It has been reported that other metrics may better predict infant body fat mass, including birth weight alone and birth weight:length ratio (55-58). We conducted regression analyses of birth weight:length ratio and birth weight adjusted for length and found similar results to models of ponderal index (Figures G-7 through G-10). These results taken together suggest that at a given length and gestational age, infant body weight is lower among babies with higher cord levels of PFOS and PFOA.

The toxicology literature provides evidence of developmental effects among animals dosed with PFOS and PFOA, albeit at substantially higher levels than observed here (7-

17). Additionally, hypolipidemic effects have been observed in animals in response to PFOS and PFOA exposure (7;25-27). In occupational studies, associations between exposure and cholesterol and triglyceride levels have been observed as well, although in the opposite direction (28;29;59). As a result of their potential effects on lipid metabolism, our observations of reduced weight for length would seem plausible. The availability of triglycerides and cholesterol are essential to the developing fetus for the accumulation of body fat and development of cell membranes and steroid hormones (30). In this study, we had cord serum cholesterol and triglyceride measurements available on the study subjects. However, controlling for total lipids, total cholesterol, or triglycerides had no impact on the associations between PFOS or PFOA and ponderal index, suggesting that the observed associations were independent of serum lipid levels (Appendix H).

We also observed a negative association between PFOS and PFOA concentrations with head circumference. Head circumference is correlated with brain weight and volume (60-62) and reduced head circumference has been associated with poor cognitive development in childhood, particularly among growth restricted babies (63-68). However, our findings were somewhat puzzling given that the associations with PFOS and PFOA appeared to be completely restricted to vaginal deliveries. It could be expected that if an association existed, it would be more apparent among elective C-sections, due to the avoidance of head molding that accompanies labor. In our study, there was no evidence of a negative association when restricting the analysis further to elective C-sections. However, it should be noted that there were only 24 elective C-

sections among the full study population. Future studies should examine this association further.

There are some potential limitations to this cross-sectional study, described below, which should lead to cautious interpretation of the results. In this analysis we used an on-line SPE method for the detection of low-level PFC concentrations in human serum. The precision of the method has described in detail by Kuklennyik et al (2005), with low coefficients of variation (CV) reported for PFOS and PFOA (50). In this study, however, measurement error for PFOS may be greater, because the precursor/product ion transition normally used for quantification could not be used due to coelution with another interferent analyte transition ion. However, measurement error would be expected to bias bivariate associations to the null if the error is completely random (69).

As described above, there was missing data on possible confounders among a small minority (<4 percent) of study subjects. However, when examining different approaches to handling missing data (i.e. complete case analysis versus median imputation), similar results were found. Similarly, the associations observed were relatively consistent with the inclusion of different sets of covariates in the model and the specification of those variables (including the use of non-linear smoothing functions for potential confounders). The use of medical records as the principle source for data on potential confounders is likely to result in some degree of misclassification. However, we observed the expected relationships between key predictors and birth outcomes, suggesting that the degree of residual confounding is likely to be small.

When comparing the log-linear and linear models, relatively consistent findings were observed. In some cases, the use of a linear model resulted in PFOA coefficients that did not achieve statistical significance, although the magnitude of the associations was qualitatively similar to the log-linear model. The associations between PFOS and the endpoints under study were insensitive to the functional form of the model. The log-linear and linear models provide a similar fit to the data, thus, it is not clear which model is the most appropriate for characterizing the shape of the relationships between cord concentrations and birth weight and birth size.

In this analysis, we adjusted for the major known determinants of birth size and weight. It remains possible that other unmeasured factors, such as diet, may be confounding the relationships observed in this study. The maternal diet would be expected to be related to weight and size of the fetus, and ponderal index has often been considered a measure of nutritional status (46). Further, it is possible that the consumption of contaminated food or water, including the use of fast-food containers, is a pathway of exposure to PFOS and/or PFOA. However, to confound the relationships observed, undernutrition would have to be positively associated with PFOS or PFOA exposure.

Our study population represented a group of individuals with more risk factors for adverse birth outcomes than the United States as a whole. Compared to national estimates, the subjects in our population were more likely to be Black, teenagers, unmarried, and cigarette smokers (Table B-2) (70). This is perhaps not surprising given

the location of the hospital in an urban and disadvantaged community. Although not quantified, these subjects may also have higher rates of other risk factors for poor outcomes, such as substance abuse and infections. It is not clear what, if any, impact the presence of concomitant risk factors would have on the results reported here, but future studies should be conducted in other settings to confirm these findings. It should be noted, however, that we observed relationships between other key determinants and birth weight consistent with the scientific literature, such as mother's age, smoking status, mother's weight and height, diabetes, and hypertension (Appendix B), which improves our confidence in the generalizability of these results to other settings.

In summary, we observed a negative association between PFOS and PFOA concentrations in cord blood and birth weight, ponderal index, and head circumference. Although effects on lipid metabolism have been among the more sensitive effects observed in animal and human data, the associations observed here were independent of cord lipid levels. We suggest cautious interpretation of this study until findings can be replicated in other populations.

Table 4-1. Study population characteristics.

Characteristic	Mean(SD) or N (%)
Maternal age	25.9 (6.6)
<18 years	24 (8.2)
18-35 years	246 (84.0)
>35 years	23 (7.8)
Race	
White	60 (20.5)
Asian	25 (8.5)
Black	208 (71.0)
Education	
<HS diploma	86 (29.8)
HS diploma	96 (33.2)
1-4 years college	65 (22.5)
5+ years college	42 (14.5)
Marital status	
Unmarried	196 (66.9)
Married	97 (33.1)
Body mass index	26.7 (7.0)
Underweight (<18.5)	16 (5.6)
Normal (18.5-24.9)	132 (46.6)
Overweight (25-29.9)	64 (22.6)
Obese (30+)	71 (25.1)
Net weight gain (lbs)	22.7 (17.8)
Maternal height (inches)	64.4 (2.7)
Primiparous	
No	171 (58.4)
Yes	122 (41.6)
Smoking status	
Non/Passive smoker	238 (81.2)
Active	55 (18.8)
Infant gender	
Female	131 (44.7)
Male	162 (55.3)
Preterm birth	
No (≥ 37 weeks)	255 (87.0)
Yes (<37 weeks)	38 (13.0)
Low birth weight	
No ($\geq 2,500$ grams)	262 (89.4)
Yes (<2,500 grams)	31 (10.6)

Missing data excluded from the calculation of means and percentages.
The following data were missing: 4 observations for education, 10 for BMI, 8 for weight gain, and 4 for maternal height.

Table 4-2. Distribution of cord concentrations of PFOS and PFOA and study endpoints.

	Percentiles								
	N	Mean	Min	10th	25th	50th	75th	90th	Max
<u>Concentration</u>									
PFOS (ng/mL)	293	6.0	<LOD*	2.5	3.4	5	7.9	11.1	34.8
PFOA (ng/mL)	293	1.8	0.3	0.8	1.2	1.6	2.1	2.8	7.1
<u>Endpoints</u>									
Birth weight (grams)	293	3,200	1,145	2,495	2,777	3,209	3,562	4,190	5,166
Head circumference (cm)	289	33.5	26.0	31	32.5	33.5	34.5	35.5	39.0
Length (cm)	288	50.0	39.0	47	48.5	50.0	52.0	53.5	56.5
Ponderal index	288	2.54	1.79	2.15	2.37	2.55	2.72	2.89	3.41

* LOD = 0.2 ng/mL

Table 4-3. Estimated change in birth weight and birth size parameters with a change in PFOS or PFOA concentrations equal to one ln-unit or from the 25th to 75th percentile.

Model	PFOS		PFOA		p-value
	Per ln-unit	Coefficient (95% CI)	Per ln-unit	Coefficient (95% CI)	
Birth weight					
Univariate	-37 (-139, 64)	-31 (-117, 54)	-97 (-234, 40)	-54 (-131, 23)	p = 0.47
Adjusted*	-69 (-149, 10)	-58 (-125, 9)	-104 (-213, 5)	-58 (-119, 3)	p = 0.09
Head circumference					
Univariate	-0.22 (-0.52, 0.07)	-0.19 (-0.44, 0.06)	-0.46 (-0.87, -0.06)	-0.26 (-0.48, -0.03)	p = 0.14
Adjusted*	-0.32 (-0.56, -0.07)	-0.27 (-0.48, -0.06)	-0.41 (-0.76, -0.07)	-0.23 (-0.42, -0.04)	p = 0.01
Length					
Univariate	0.25 (-0.21, 0.72)	0.21 (-0.18, 0.6)	-0.06 (-0.69, 0.57)	-0.03 (-0.39, 0.32)	p = 0.29
Adjusted*	0.13 (-0.26, 0.52)	0.11 (-0.22, 0.44)	-0.10 (-0.64, 0.44)	-0.06 (-0.36, 0.24)	p = 0.52
Ponderal index					
Univariate	-0.070 (-0.119, -0.021)	-0.059 (-0.101, -0.018)	-0.074 (-0.140, -0.007)	-0.041 (-0.078, -0.004)	p = 0.005
Adjusted*	-0.074 (-0.123, -0.025)	-0.062 (-0.104, -0.021)	-0.070 (-0.138, -0.001)	-0.039 (-0.077, -0.001)	p = 0.003

* Multivariate models adjusted for gestational age, maternal age, body mass index, race, parity, smoking, baby gender, height, net weight gain, diabetes, and hypertension. For head circumference, adjusted model includes delivery mode (C-section/Vaginal).

Table 4-4. Change in head circumference with a unit change in ln(PFOS) or ln(PFOA) concentration, among Caesarean section and vaginal deliveries.

Delivery mode	PFOS		PFOA	
	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Vaginal	-0.46 (-0.73, -0.19)	p<0.01	-0.62 (-1.0, -0.23)	p<0.01
C-section	0.31 (-0.23, 0.86)	p=0.26	0.23 (-0.44, 0.91)	p=0.50

* Regression coefficients are estimated from a multivariate model with an interaction term between PFOS or PFOA and delivery mode. Coefficients are adjusted for gestational age, maternal age, body mass index, race, parity, smoking, baby gender, height, net weight gain, diabetes, and hypertension

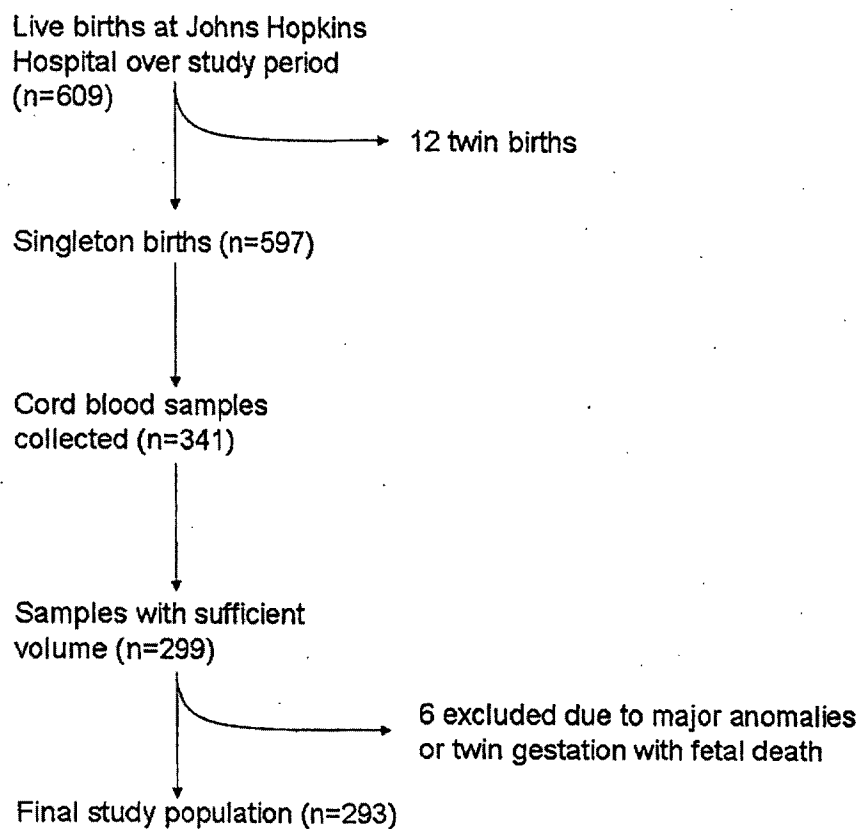


Figure 4-1. Flow chart of study population.

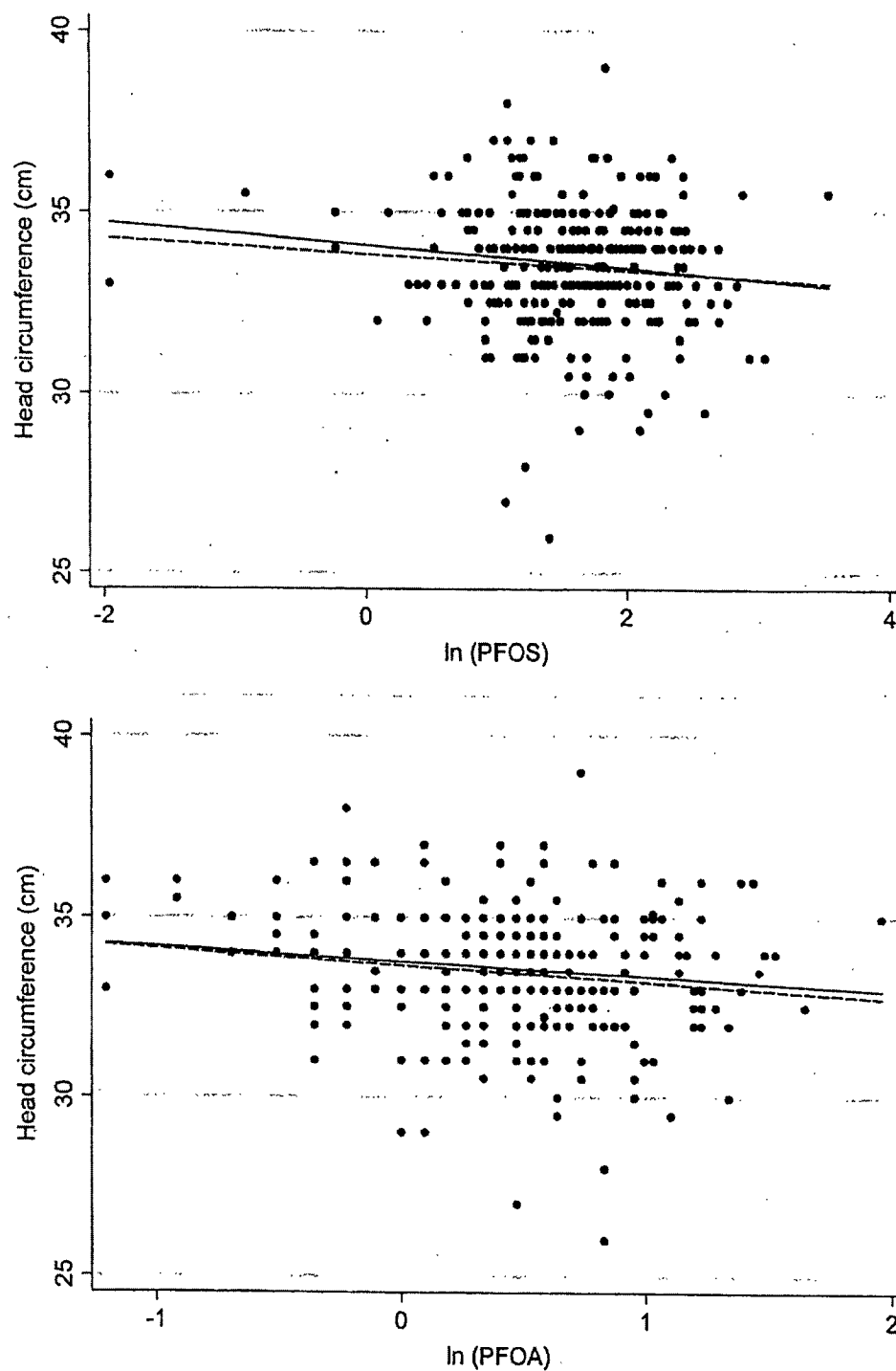


Figure 4-2. Head circumference versus $\ln(\text{PFOS})$ and $\ln(\text{PFOA})$, before and after adjustment for potential confounders.

Note: The dotted line denotes the predicted fit from linear regression. The solid line denotes the predicted fit from a multivariate regression. See Table 4-3 for regression coefficients.

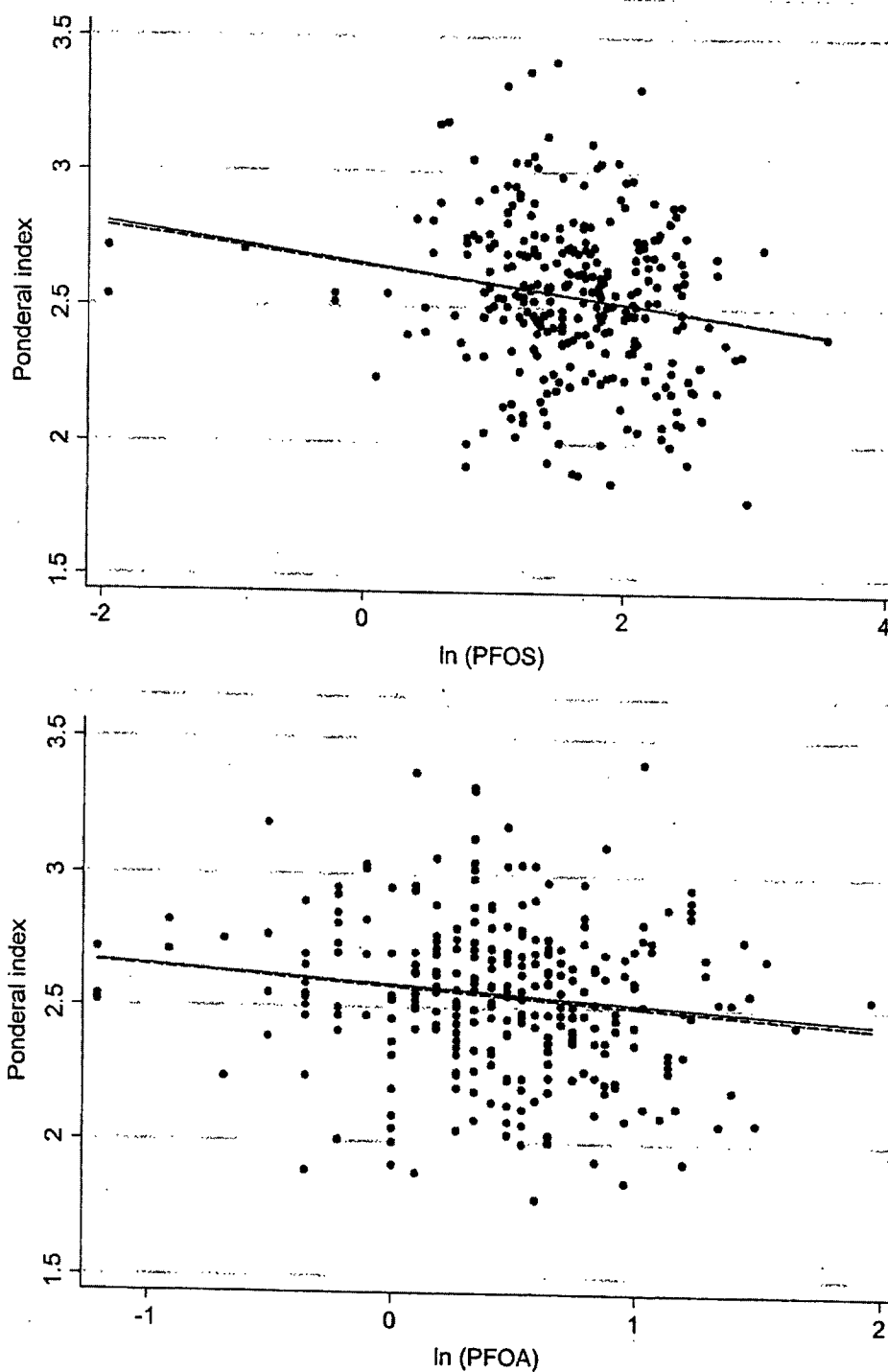


Figure 4-3. Ponderal index versus $\ln(\text{PFOS})$ and $\ln(\text{PFOA})$, before and after adjustment for potential confounders.

Note: The dotted line denotes the predicted fit from linear regression. The solid line denotes the predicted fit from a multivariate regression. See Table 4-3 for regression coefficients.

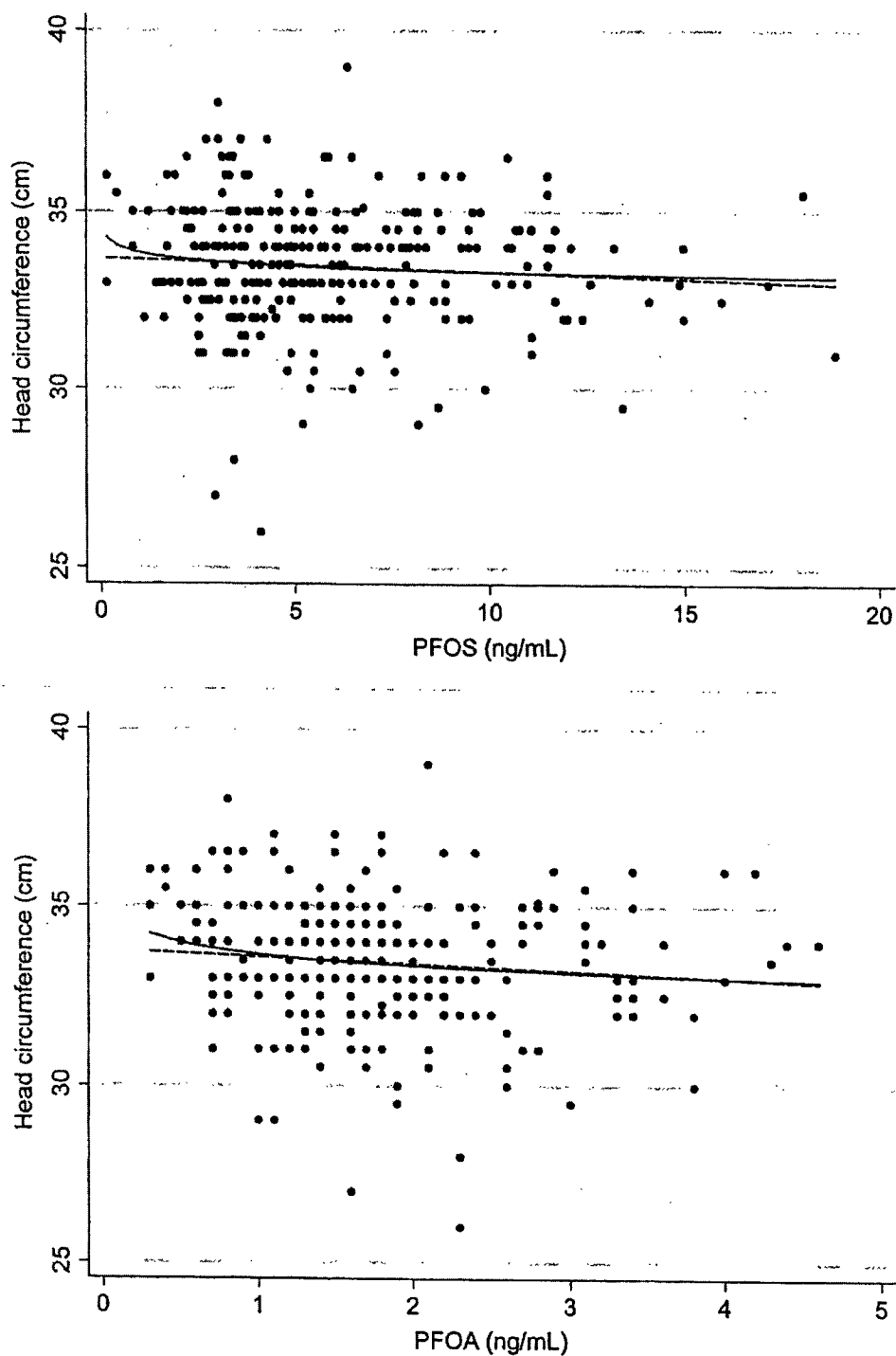


Figure 4-4. Relationship between head circumference and PFOS, using log-linear and linear models.

Note: PFOS concentrations above 20 ng/mL and PFOA concentrations above 5 ng/mL are excluded from figure for display purposes. See Table 4-3 for log-linear model regression coefficients.

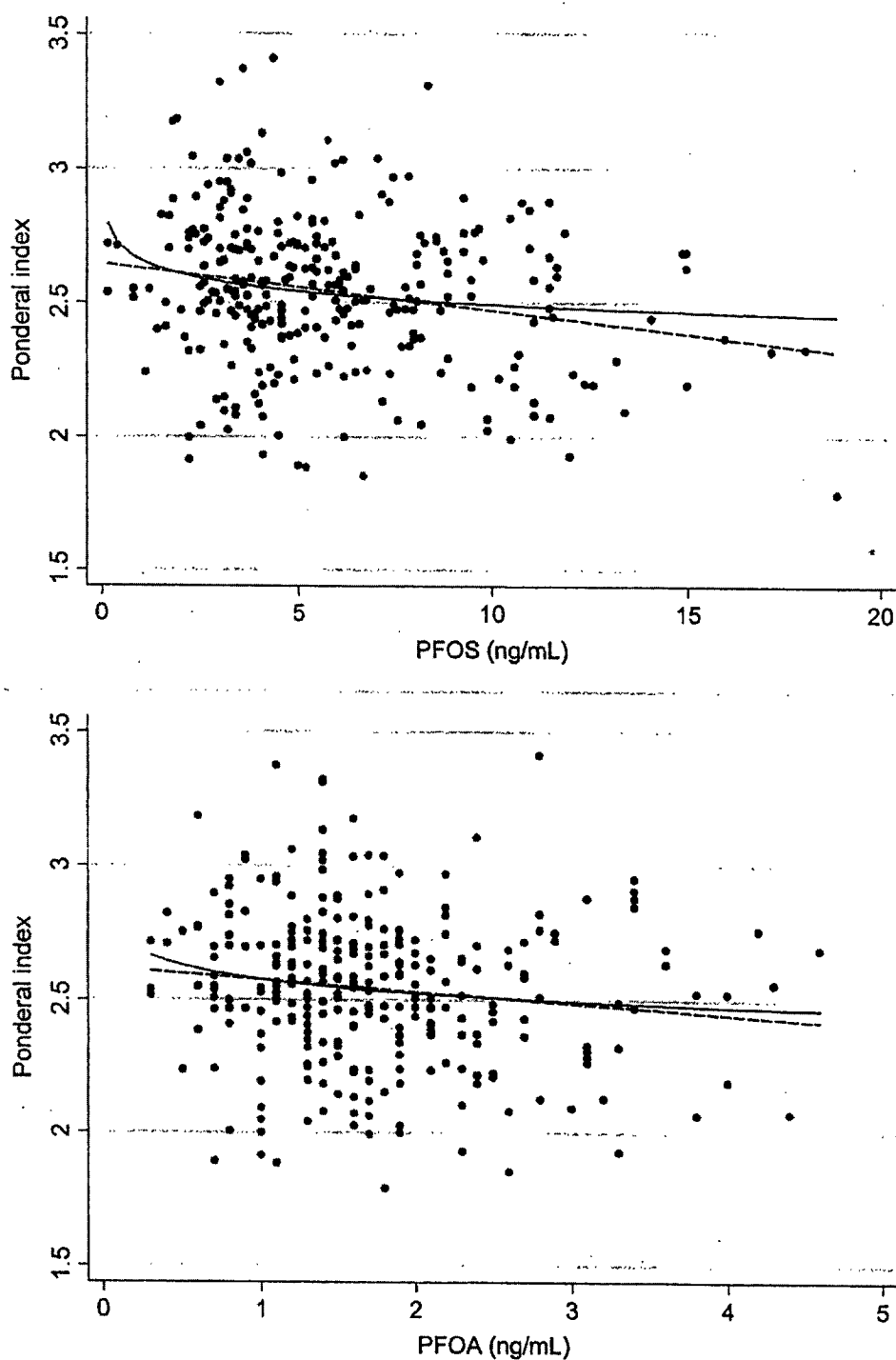


Figure 4-5. Relationship between ponderal index and PFOS, using log-linear and linear models.

Note: PFOS concentrations above 20 ng/mL and PFOA concentrations above 5 ng/mL are excluded from figure for display purposes. See Table 4-3 for log-linear model regression coefficients.

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CHAPTER 5

CONCLUSIONS

This chapter serves as a conclusion to the dissertation titled "Fetal Exposure to Perfluorinated Compounds (PFCs): Distribution and Determinants of Exposure and Relationships with Weight and Size at Birth." Below I reiterate the specific aims of my dissertation and summarize my findings, along with their implications for public health, environmental policy, and future research.

- Specific Aim 1: Review the literature on perfluorinated compounds, including production and use, human biomonitoring and exposure pathways, and toxicity and epidemiology (Chapter 2).
- Specific Aim 2: Conduct a study to describe the distribution and determinants of PFC cord concentrations among a sample of newborn deliveries occurring at the Johns Hopkins Hospital in Baltimore, MD (Chapter 3).
- Specific Aim 3: Conduct an epidemiologic investigation into the relationship between PFC cord concentrations and birth weight, newborn head circumference, crown-heel length, and ponderal index (Chapter 4).

Summary of Findings

In Chapter 2, I summarized the literature on perfluorinated compounds, including human biomonitoring, exposure pathways, animal toxicity, and occupational epidemiology studies. From this literature review, it is apparent that human exposure to two perfluorinated compounds, PFOS and PFOA, is widespread, as documented by biomonitoring studies conducted in a wide range of countries. It is also clear that a wide

variation in exposure is occurring, as human blood concentrations in some countries were considerably lower than others (Figures 2-2 and 2-3). On the basis of environmental monitoring, it has been established that contamination with PFOS and PFOA is observed even in remote regions, such as the Arctic (1;2). This provides some insight to possible exposure pathways and suggests that direct contact with consumer products is not the only possible source of exposure. Potential sources of exposure may include industrial releases, consumer product use, dust/indoor air inhalation, drinking water, or dietary pathways through environmental contamination; however, there are no clear data to suggest the relative contribution, if any, of these sources to human exposure.

Developmental toxicity of PFOS and PFOA has been noted among the effects observed in animal studies. PFOS has been shown to induce developmental and reproductive effects in rats and mice, including reduced birth weight, decreased gestational length, structural defects, developmental delays, and increased neonatal mortality (3-8). PFOA has been shown to cause pregnancy loss, reduced fetal weight, reduced postnatal survival, and delays in postnatal growth and development in offspring of rodents (9-13). PFOS and PFOA have also been shown to have hypolipidemic effects in several animal species (3;14-16). Characterization of *in utero* exposure among humans is necessary for extrapolating developmental risk from animal data. Further, the relevance of animal studies conducted at high doses to humans exposed at much lower levels is uncertain. The direct evaluation of reproductive and developmental endpoints in humans would shed light on the issue of health effects from low dose exposures.

In Chapter 3, I examined the distribution and determinants of PFC concentrations in cord blood from a sample of newborn infants delivered at the Johns Hopkins Hospital in Baltimore, MD. The results of this analysis confirmed the presence of *in utero* exposure to PFOS and PFOA, and less so, to other PFCs under study. Lower concentrations were observed in cord serum when compared with typically reported concentrations in adult serum in the United States. This is consistent with incomplete incomplete transfer across the placenta by simple diffusion because of the relatively large size of these compounds, their affinity for binding to proteins in serum, and other physical characteristics such as their surfactant properties. Differences in protein concentration between maternal and fetal blood may also play a role.

As expected, the predominant PFCs detected in the cord samples were PFOS and PFOA, with detection rates of 99 and 100 percent, respectively. Other PFCs were detected less frequently and, when detected, occurred at much lower levels. Among the demographic characteristics available from the medical record, there were relatively few predictors of cord concentrations. Many persistent pollutants tend to accumulate with age as the opportunity for exposure and accumulation of the chemical increase with age. However, consistent with previous findings, we observed no such association with these compounds and age. In fact, a predictive model with age, race, education, insurance status, marital status, body mass index, parity, smoking status, infant gender, preterm birth, and an indicator for whether the mother lived inside Baltimore city limits explained only 6 percent of the variation in cord PFOS concentrations and 11 percent of the variation in cord PFOA concentrations. Whether examined singly or in multivariate models,

relatively few of the demographic factors measured in this study were predictive of *in utero* exposure.

One notable exception to the above was that Asian babies had the highest average cord serum PFOS concentrations. Most of the mothers were not Asian-American, but instead were born in Asian countries. The three highest PFOS concentrations were among babies born to individuals from Asia, two from China. This is of interest because some of the highest levels of PFOS contamination in humans have been reported in China (Figure 2-2). Although we did not find evidence for significant variability within our region (i.e., within Baltimore vs. outside of Baltimore city), these data are consistent with variability in exposure over larger geographic regions, for which race/ethnicity may represent a surrogate marker. However, this must be interpreted with caution, given that data on levels of PFCs in human populations are limited. More extensive monitoring is needed to understand these exposure patterns.

This study provided an opportunity to examine socioeconomic status as a predictor of cord concentrations. The study population of mothers delivering at Johns Hopkins Hospital represents a diverse mixture of individuals, including those from the surrounding community, Johns Hopkins faculty and staff, and individuals transported from outside the area. We found no evidence of a socioeconomic or urban environment gradient in cord concentrations of PFOS or PFOA. Because socioeconomic status is likely associated with the types of consumer products and materials in the home, lack of a gradient in serum concentrations by socioeconomic status may imply that consumer

products are not major sources of exposure. Rather, exposure may be occurring from sources that are more ubiquitous and less associated with socioeconomic level, such as widely consumed foods, drinking water, and/or air. Future studies could include a survey component to characterize possible sources of exposure in the home environment as predictors of serum concentrations of PFOS and PFOA.

In Chapter 4, I presented the results of an epidemiologic investigation of fetal exposure to PFOS and PFOA and associations with birth weight, newborn head circumference, length, and ponderal index. These birth size parameters have been used as indicators of growth restriction *in utero*, through the use of classifications such as "small-for-gestational age," based on cutoffs at the lower tail of the distributions. In this study, I examined the associations between cord concentrations and these endpoints across the full range of the distribution. We found negative associations between both PFOS and PFOA with birth weight, head circumference, and ponderal index. In contrast, no consistent trend was observed for PFOS or PFOA and newborn length. The associations observed were fairly robust to the inclusion of additional covariates in the model, specification of continuous covariates in the model, and functional form of the model. Additionally, these associations were independent of cord lipid levels at birth. These findings were consistent with toxicology data that has found decreased weight of newborn animals when mothers were dosed with PFOS and PFOA.

One finding that was not completely intuitive was the interaction between PFOS or PFOA and delivery mode on head circumference. As expected, babies born through

Caesarian sections had larger head circumferences, on average, than those born through vaginal delivery, after accounting for other predictors of head circumference. Because of this phenomenon which is likely due to head molding, one might have expected that any effect of PFOS or PFOA on head circumference would be stronger among Caesarian sections than among vaginal deliveries. However, we observed a strong negative association among vaginal deliveries and a small positive association among Caesarian sections. The reason for this interaction is unclear at present and future research should examine this further.

A major strength of this study was the state-of-the-art analytical method conducted by the Centers for Disease Control and Prevention for quantifying cord serum PFC concentrations (17). There is some uncertainty as to the extent of measurement error for PFOS because the precursor/product ion transition normally used for quantification could not be used due to an interferent ion. However, an increase in random measurement error should make a statistical association more difficult to detect (18). Maternal nutritional status was not measured in this study and could be a potential confounder in the relationship between PFOS or PFOA concentrations and birth weight or birth size. However, this would only be the case if poor nutrition was correlated with higher levels of PFOS and PFOA, or vice-versa. More research is needed to elucidate the pathways of human exposure to determine whether these concerns are founded.

Adding further to our confidence in the validity of these findings was the replication of previously reported associations between other predictors and birth outcomes: Negative

associations were observed between smoking status and birth weight, head circumference and length; maternal age was associated with birth weight in an inverse U-shape, where both young and older mothers tended to have lighter babies; diabetes was associated with heavier and larger babies and hypertension with lighter and smaller ones; primiparous women had lighter and smaller babies compared with multiparous; maternal anthropometry was a strong predictor of birth weight and size; males tended to be bigger than female babies; and larger head circumferences were observed among Caesarian sections versus vaginal deliveries, after adjusting for the key predictors of birth weight and size.

Public Health Implications

There are significant public health implications of disruptions to normal fetal growth and development. Babies born early or with low birth weight (<2,500 grams) are at increased risk of mortality in the first year of life (19;20). In 2001, the infant mortality rate among low birth weight births in the U.S. was more than 25-fold greater than among babies with birth weights above 2,500 grams (19). In 2002, the combination of preterm birth and unspecified LBW was the second leading cause of infant death (20). Babies born small for gestational age due to fetal growth restriction are at greater risk for perinatal and childhood morbidity, including hypothermia, hypoglycemia, and/or asphyxia (21-27), neonatal intensive care unit admission (28), respiratory distress syndrome (29), reduced mental development in infancy (30), cerebral palsy (31), and reduced insulin sensitivity, a marker of type II diabetes risk (32).

In addition to the neonatal and childhood impacts associated with fetal growth restriction, a growing body of research suggests that some metabolic diseases in adulthood may have their origins in fetal development. Over the last 15 years, a number of studies conducted in different countries have shown that small size at birth, modified by rapid childhood growth, is associated with risk of coronary heart disease, type II diabetes, and their metabolic risk factors, including hypertension, hyperlipidemia, and reduced insulin sensitivity (33-38). A number of hypotheses have been suggested to explain this phenomenon, including the 'Barker hypothesis', which presumes that birth weight represents a marker of fetal nutrition and that lack of optimal nutrition coupled with catch-up growth predisposes individuals to developing metabolic diseases in adulthood (33;39;40). Barker and colleagues have suggested that the processes of "developmental plasticity" and "compensatory growth" are responsible for the increased disease risk later in life (34;40). The basis for this theory is the presence of "a critical period when a system is plastic and sensitive to the environment, followed by loss of plasticity and a fixed functional capacity" (39). It is suggested that the fetus can adapt *in utero* to undernourishment through metabolic changes in order to improve short-term survival. These metabolic changes may put the individual at greater risk for heart disease and diabetes later in life, specifically, when entering an environment of adequate nutrition after birth and excess compensatory growth (29;35;36). Interestingly, the increased risk of adult disease is observed across a range of what could be considered "normal" birth weights. For example, among 10,636 men in a community in England, hazard ratios for death from coronary heart disease increased monotonically with decreasing birth weight. Babies born at weights between 5.5-6.5 lbs (approximately 2,500-3,000 grams) were 29

percent more likely to die from CHD than babies born at ≥ 10 lbs (33). These findings suggest that even small decrements in fetal growth can affect long-term disease risk. The major hypotheses regarding the fetal origins of adult disease have been focused on nutrition as the key mechanism underlying this process. However, if exposure to persistent pollutants were causally related to reduced fetal growth, this could be an alternate mechanism.

Our study was not intended to examine the risk of fetal growth restriction associated with increased cord blood concentrations of PFOS or PFOA. Instead, we examined the association between exposure and birth size parameters across the full distribution of birth weight, head circumference, length, and ponderal index. We identified an association between increased exposure and reduced birth weight, head circumference, and ponderal index across the range of these endpoints in our study population. A linear model suggests that a shift in the distribution would occur evenly across the population. Babies with birth size parameters in the middle of this range would not necessarily be classified as growth restricted if head circumference or ponderal index were reduced by a small amount. However, one could expect that a similar reduction in birth size among those babies at the lower end of the distribution could result in more of these babies classified as growth restricted and lead to a greater risk of adverse outcomes among those infants. When comparing the 5th to 95th percentile of PFOS cord concentrations, the changes in birth weight, head circumference, and ponderal index were, on average, 134 grams, 0.61 centimeters, and $0.14 \text{ g/cm}^3 \times 100$ respectively, after adjusting for potential confounders. For a baby born at 37 completed weeks, this represents a shift from the 16th

to 10th percentile of birth weight in the U.S. (41). At term, a change in ponderal index of 0.14 represents a shift from the 25th percentile to the 10th percentile of ponderal index distribution (42). Thus, changes of this magnitude would be associated with more babies being classified as small-for-gestational age. Additionally, if the observations made by Barker and others hold, shifts throughout the range of the birth weight distribution may result in increased future morbidity.

It is interesting to note that a significant percentage of the mother's in our study were overweight or obese and, subsequently, there were a larger proportion of large-for-gestational age babies than small-for-gestational age babies. For example, based on the birth weight for gestational age standards used by the hospital, almost 15 percent of babies were classified as large-for-gestational age (LGA), while only 4 percent were classified as SGA. Thus, one could argue that macrosomia may be as important a health problem to the well-being of our study population as growth restriction.

Policy Implications

Comparisons with Animal Toxicity Data

In the present study, we observed negative associations between both PFOS and PFOA and measures of weight and size at birth. The doses at which developmental effects were observed in the animal toxicity literature are considerably greater than what was observed in our study. Table 5-1 gives an overview of results from the animal developmental toxicity data, by showing the lower confidence limits of the benchmark dose associated with a 5 percent increase in response (BMDL₅). Although these estimates are, in part,

dependent on the model used to fit the observed data, they provide a useful comparison between human exposure levels and animal toxicity studies. Among the most sensitive effects observed in these developmental studies were sternal defects in mice for PFOS and reduced ossification in mice for PFOA. The mouse has been suggested as a better model for PFOA human developmental risk because the female rat has the ability to rapidly eliminate the chemical. These estimates are mainly reported in mg/kg/day of external dose. For comparison with human data it is necessary to convert these units to serum concentrations. The corresponding serum levels associated with these BMDL's are not readily available, however, they can be bounded using data from the studies. For PFOS and sternal defects in rats, the BMDL₅ of 0.122 mg/kg/day would result in fetal serum concentrations between 0.188 and 36 ppm (3;9). For PFOA and reduced ossification in mice, a BMDL₅ of 0.6 mg/kg/day is equal to less than 20 ppm in maternal serum (13), corresponding to <10 ppm in the fetus assuming one-half is transferred through the placenta (43). Luebker et al. (2005) report a BMDL₅ of 0.39 mg/kg for PFOS and reduced birth weight, which corresponded to a fetal serum level of about 34 ppm in the rat (6). These serum concentrations are orders of magnitude greater than what has been observed in our study and are higher than the serum concentrations typically reported among occupationally-exposed individuals. It is interesting to note that in several of the studies, serum concentrations among the control group are higher than we report here. For example, Thibodeaux et al. (2003) reports mean fetal serum PFOS concentrations among the control group of 188 ppb at birth (3;9), compared with approximately 5 ppb in our study. Additionally, animal studies are typically conducted with small numbers of animals and have low statistical power. Thus, as designed, these

animal studies may not be capable of examining the adverse effects of low-dose exposure.

Current Regulatory Actions

As of 2000, global production of perfluorooctanesulfonyl fluoride-based chemicals was estimated to be 3,665 metric tons, 1,820 of which was either produced in the U.S. or entered the country through importation (44). In 2000, the major manufacturer of PFOS-based materials announced they would end production by 2002 (45). U.S. EPA subsequently promulgated a Significant New Use Rule to limit the production and use of a group of related perfluoroalkyl sulfonated compounds (46). Because of the long half-life in humans and the environment, it may take years before concentrations decline noticeably. It is estimated that fewer than 600 metric tons of PFOA are manufactured or imported into the U.S. per year (47). In response to concern about the widespread PFOA contamination and potential carcinogenicity and developmental toxicity risk, the U.S. EPA issued an enforceable consent agreement, which requires manufacturers to conduct testing on the fate and transport of fluoropolymers made with PFOA (48). The U.S. EPA has also entered into voluntary agreements with the major manufacturers of PFOA to reduce emissions and residual content in finished products and work towards eliminating them completely (49). Despite this progress, the policy options for reducing human exposure to these compounds are tied to improving our understanding of the major sources and pathways of exposure, along with the extent to which precursor compounds contribute to exposure. These and additional future research needs are described below (Table 5-2).

Future Research Needs

Subsequent studies of fetal exposure and birth outcomes should be conducted to confirm the findings reported here, ideally among a population with a wider range of exposure. These studies should be conducted in settings where subjects have low rates of pregnancy-related complications, including co-morbidities such as diabetes and pre-eclampsia, intrapartum infection, and substance abuse. Such studies would reduce the chance that findings could result from confounding due to these strong determinants of adverse pregnancy outcomes. Additionally, studies of birth outcomes among women occupationally exposed or among communities exposed at higher levels than the general population would provide insight to the potential for developmental effects.

Hospital-based cross-sectional studies are relatively straightforward to conduct and useful for studying the effects of persistent pollutants on birth outcomes. Because these compounds are persistent and maternal levels will not fluctuate over a 9-month time period, cord blood concentrations at birth can be seen as a measure of exposure across the duration of pregnancy. Future studies could improve on this design by measuring maternal concentrations of these compounds as well. This would allow a more direct analysis of the extent to which these chemicals cross the placenta and an examination of patterns of variation in placental transfer. Additionally, oversampling babies with low birth weight and/or small size for gestational age would improve the statistical power to detect an association between exposure and birth outcomes.

We were able to conduct this study in a relatively short period of time because it was cross-sectional and used pre-existing medical record data. However, there would be added advantages to conducting a longitudinal study across the duration of pregnancy. First, serial ultrasounds could be used to examine fetal growth trajectory to distinguish growth restricted from constitutionally small babies. Second, surveys could be administered to the mothers to identify dietary, lifestyle, and household determinants that may be predictors of exposure and/or birth outcomes. A biomarker of nutritional status could also be used to supplement dietary data obtained from surveys.

Animal studies are necessary to further elucidate the mechanisms which have led to observations of developmental toxicity in rats and mice. Possible mechanisms for these effects include increased membrane fluidity and permeability (50), disrupted cell communication through gap junctions (51), displacement of endogenous ligands from proteins (such as liver fatty acid binding protein) (52), or disrupted thyroid hormone production (3;5;6;14). In addition to the possible human health effects, key data gaps exist in understanding the pathways of human exposure. Future studies are needed to elucidate these pathways further, so that steps can be taken to minimize human exposure to these compounds.

In summary, the findings of this research confirm that *in utero* exposure to PFOS and PFOA is occurring among babies born in Baltimore City. Despite the relatively low serum concentrations, we detected negative associations between PFOS and PFOA concentrations in cord serum and birth weight, head circumference, and ponderal index.

Future studies are needed to replicate these findings in other settings. Given the widespread human exposure to these compounds documented by biomonitoring studies, impacts on fetal growth could have significant public health implications.

Table 5-1. Benchmark Dose Estimates from Selected Developmental Toxicity Studies of PFOS and PFOA.

Chemical	Reference	Species	Endpoint	BMDL5 (mg/kg/day)	Serum concentration conversion
PFOS	Luebker et al., 2005	Rat	Gestational age	0.31	Fetal serum levels at birth: 0.1 mg/kg/d - 9.1 ppm 0.4 mg/kg/d - 34.3 ppm 1.6 mg/kg/d - 101 ppm 3.2 mg/kg/d - 164 ppm
			Birth weight	0.39	
			Postnatal survival	0.89	
			Postnatal weight	0.27	
			Postnatal weight gain	0.28	
	Thibodeaux et al., 2003	Rat	Sternal defects	0.122	Fetal serum levels at birth: (see ref 9) 0 mg/kg/d = 0.188 ppm 1 mg/kg/d = 36 ppm 2 mg/kg/d = 72 ppm 3 mg/kg/d = 87 ppm 10 mg/kg/d = 108 ppm
			Cleft palate	3.33	
PFOA	Lau et al., 2003	Mouse	Sternal defects	0.016	At term, 10 mg/kg/d = 179 ppm in maternal serum.
			Cleft palate	3.53	
		Rat	Postnatal survival	0.58	Fetal serum levels at birth: (see ref 9) 0 mg/kg/d = 0.188 ppm 1 mg/kg/d = 36 ppm 2 mg/kg/d = 72 ppm 3 mg/kg/d = 87 ppm 10 mg/kg/d = 108 ppm
	Lau et al., 2006	Mouse	Postnatal survival	3.88	
		Mouse	Ossification	0.6	Maternal serum levels: 1 mg/kg/d ~ 20 ppm 3 mg/kg/d ~ 40 ppm 5 mg/kg/d ~ 75 ppm
			Postnatal body weight	0.86	
			Birth weight	4.3	
	Butenoff et al., 2005	Rats	Days to preputial separation	22 = 29 ppm	Concentrations (ppm) estimated from dose (mg/kg/d) using pharmacokinetic data.
			Post-lactational mortality (F)	22 = 29 ppm	
			Post-lactational mortality (M)	24 = 32 ppm	
			Days to vaginal patency	30 = 40 ppm	
			Pre-weaning mortality	34 = 45 ppm	
			Postnatal body weight	44 = 59 ppm	

Table 5-2. Data gaps and future research needs.

• Human exposure pathways ○ Role of precursor compounds ○ Use of consumer products versus environmental contamination
• Mechanisms of developmental toxicity in animals ○ Cell signaling ○ Membrane fluidity ○ Protein binding ○ Thyroid hormones
• Replication of current epidemiologic study in different settings ○ Occupational or highly-exposed populations ○ Survey component ○ Measurement of maternal and fetal serum concentrations

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APPENDIX A

KEY DETERMINANTS OF BIRTH WEIGHT, HEAD CIRCUMFERENCE, LENGTH AND PONDERAL INDEX

This appendix presents the univariate relationships between key determinants of low birth weight and birth size from the epidemiologic literature with these endpoints in our study. Key determinants of birth weight include baby gender, parity, multiple gestation, maternal age, body composition (height and weight), lifestyle (e.g. smoking, drug abuse, nutrition), and health complications (e.g. hypertension, diabetes) (1). We examined the associations between the determinants available in this study and birth weight, along with head circumference, length, and ponderal index. This analysis serves two purposes. First, the confirmation of established relationships between these determinants and endpoints serves to increase our confidence in the validity of the data abstracted for the present study. Second, the observed dose-response relationships between the continuous predictors and these endpoints were used to inform the specification of these variables in the regression modeling conducted and presented in the manuscript. The scatterplots below include a non-parametric smoothing function as well as the predicted fit based on regression with a linear or quadratic term. The need for a quadratic term was based on a significant coefficient ($p < .05$) when added to a model with only the linear term. Analysis of variance or Student's t-test was used to compare mean values of birth weight, head circumference, length, and ponderal index between levels of categorical variables.

Reference

- (1) United Nations Children's Fund and World Health Organization. Low birthweight: country, regional and global estimates. Geneva: WHO, 2004.

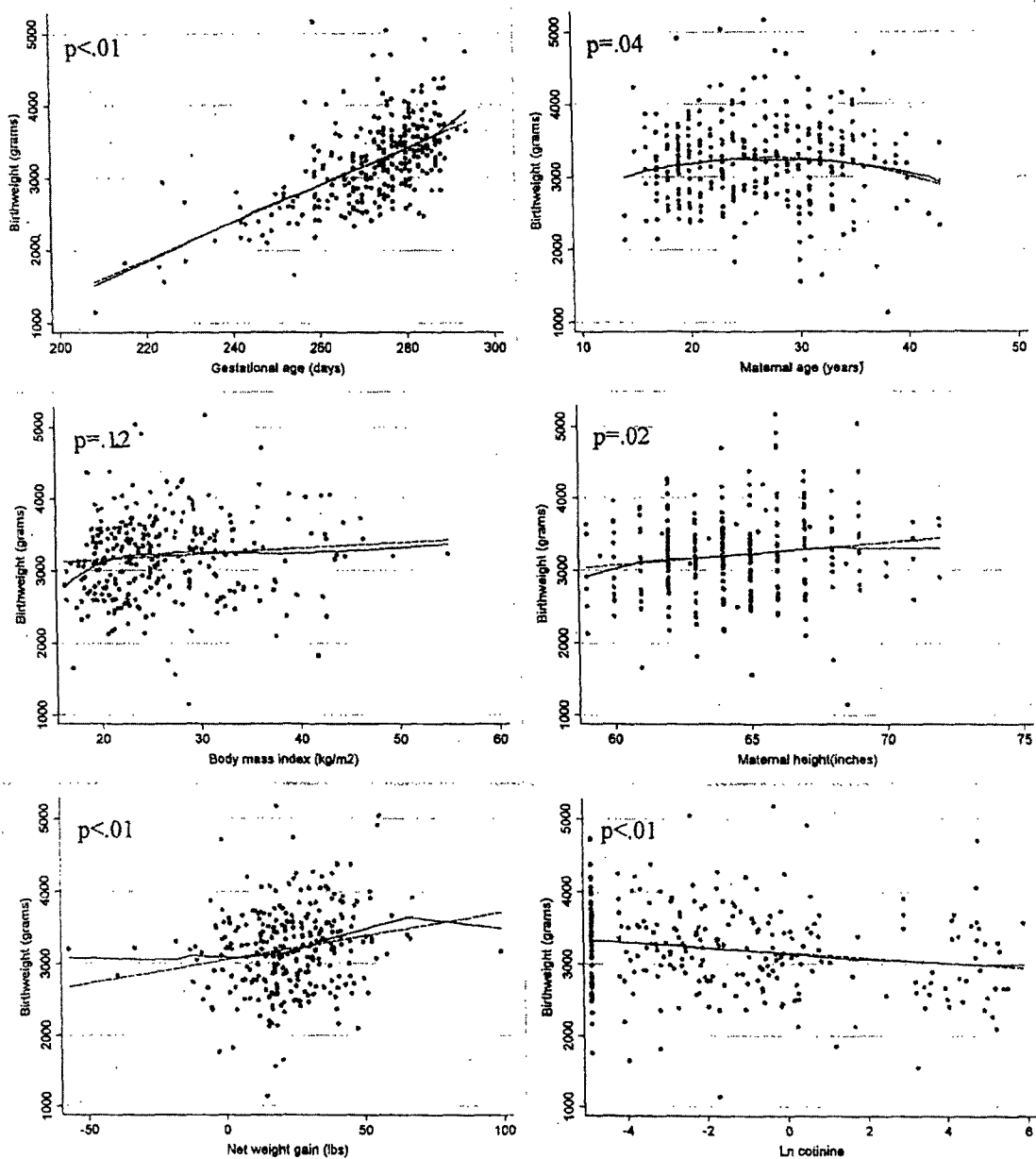


Figure A-1. Key determinants of birth weight, Baltimore THREE Study.

* Solid line based on non-parametric smoothing function. Dashed line denotes predicted linear regression fit. P-values are from linear or quadratic term in regression model.

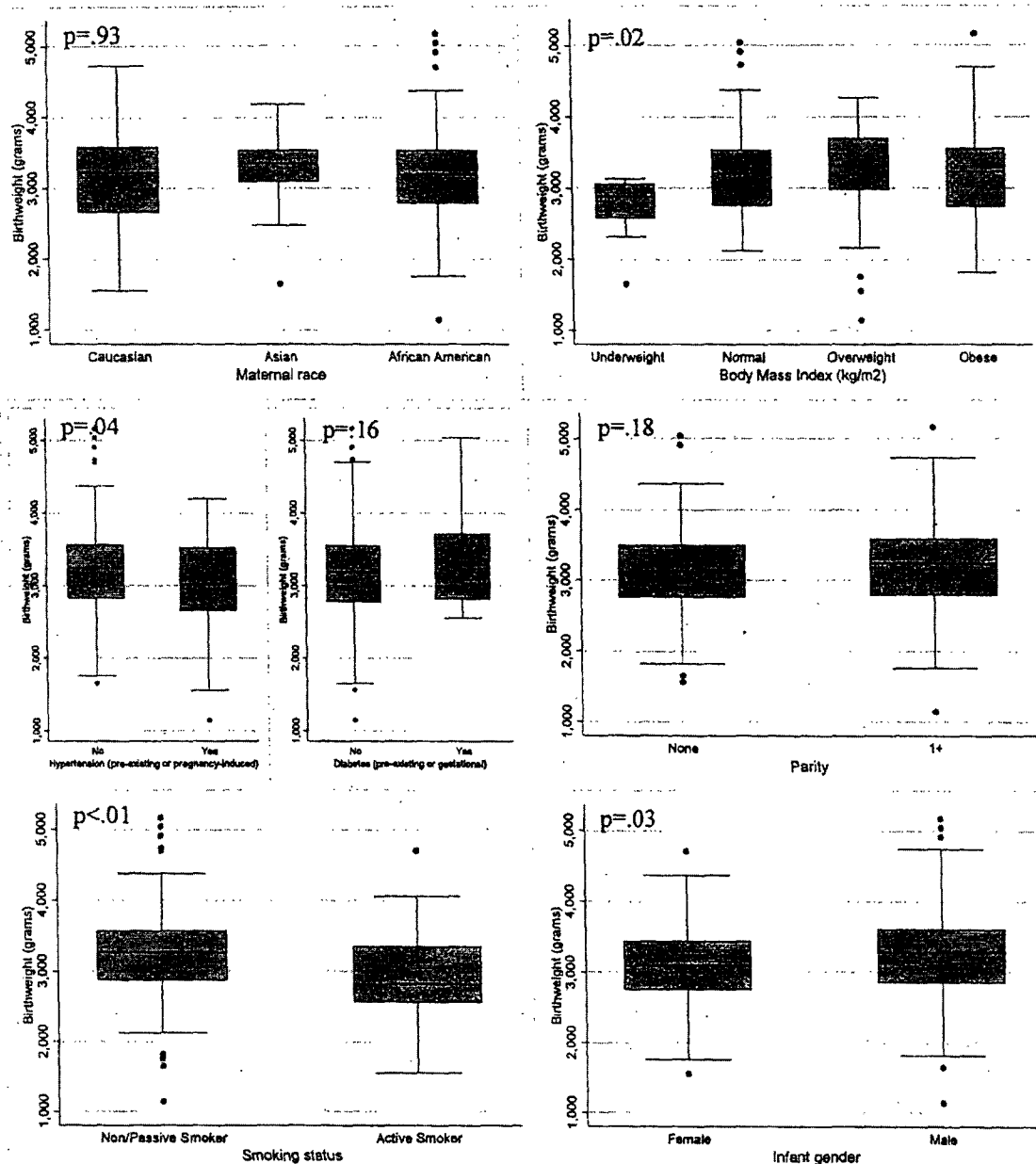


Figure A-2. Distribution of birth weight (grams) by subject characteristics, Baltimore THREE Study.

* P-values from ANOVA comparing means between groups.

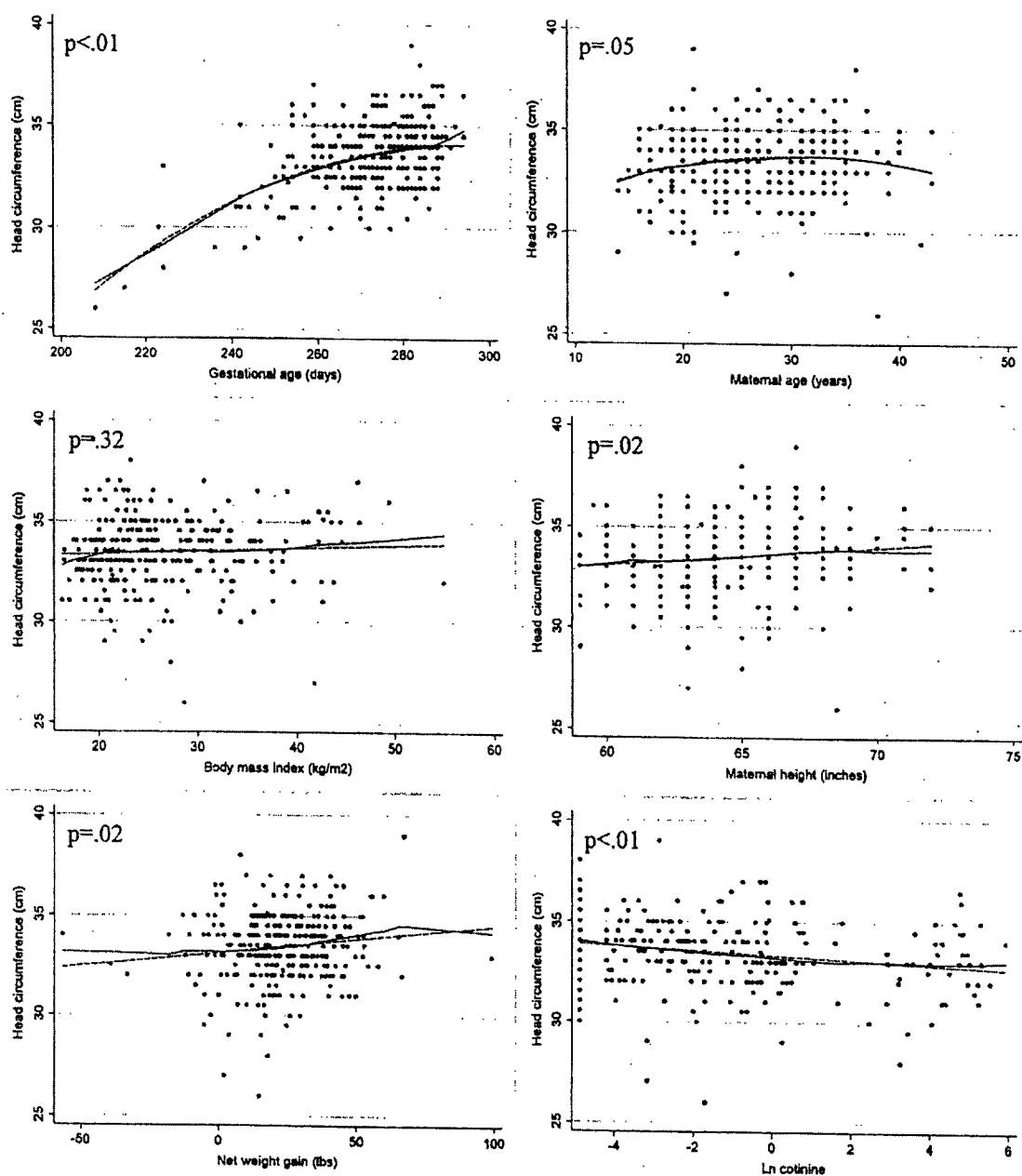


Figure A-3. Key determinants of newborn head circumference, Baltimore THREE Study.

* Solid line based on non-parametric smoothing function. Dashed line denotes predicted linear regression fit. P-values are from linear or quadratic term in regression model.

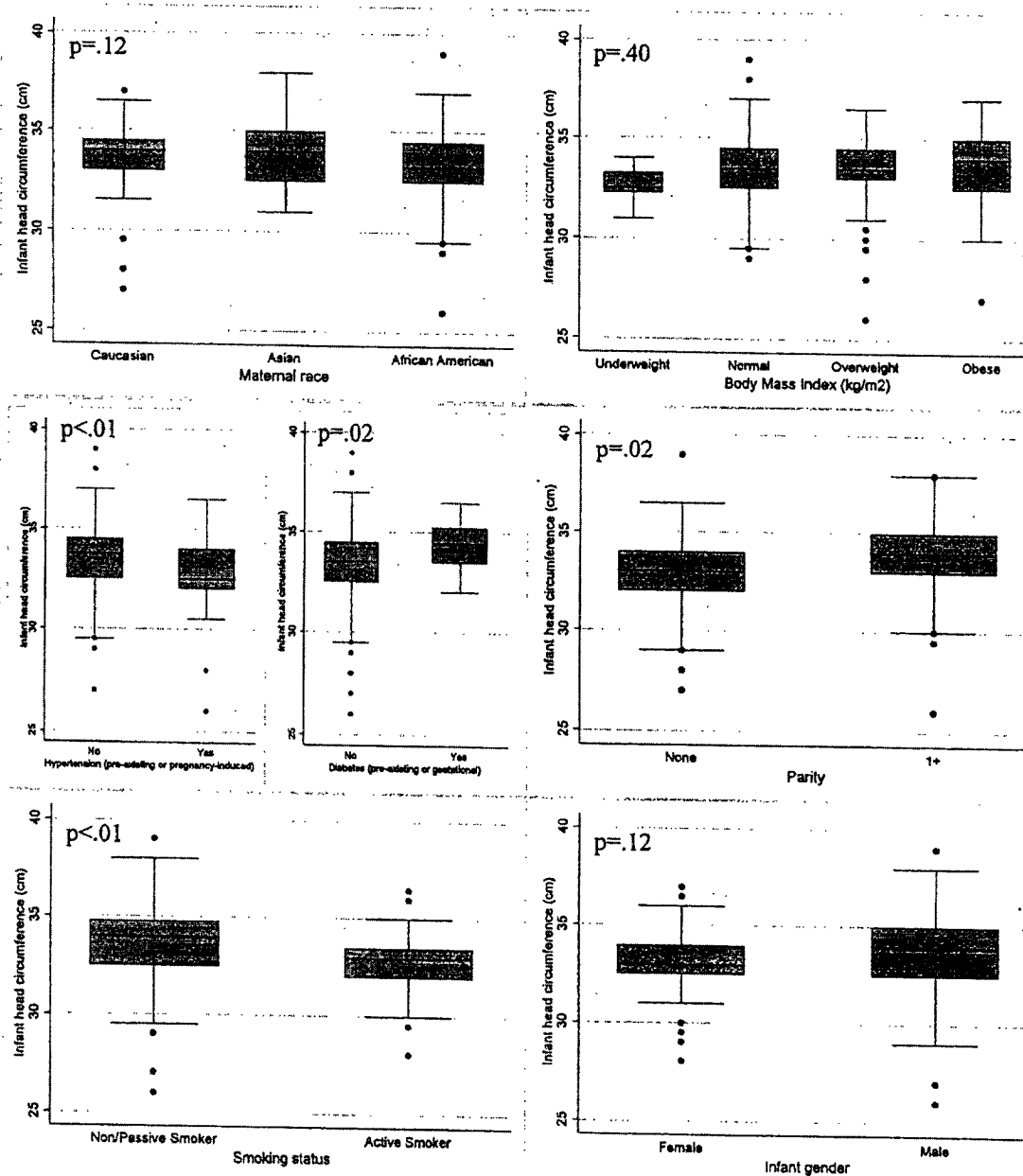


Figure A-4. Distribution of head circumference (cm) by subject characteristics, Baltimore THREE Study.

* P-values from ANOVA comparing means between groups.

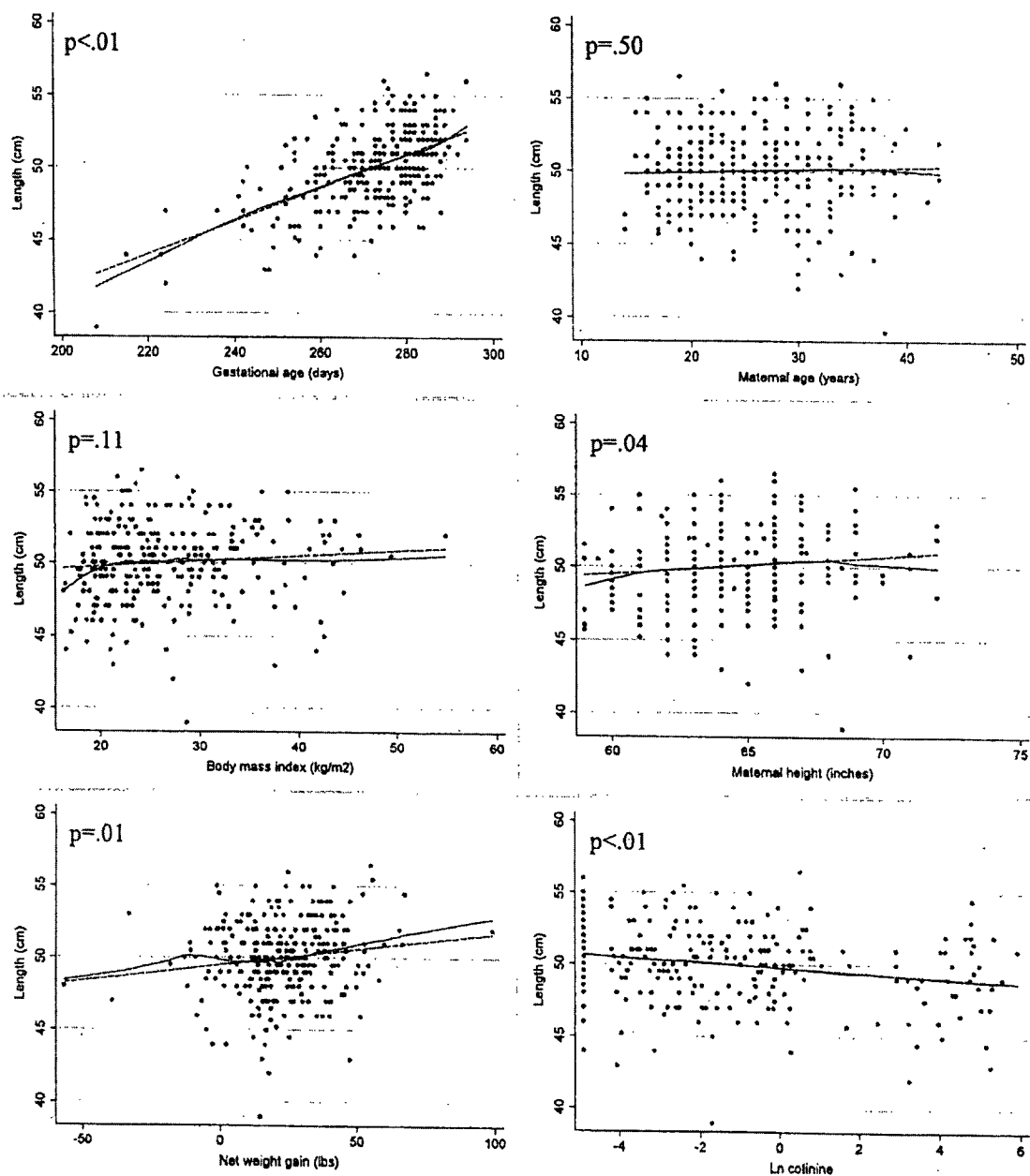


Figure A-5. Key determinants of newborn length, Baltimore THREE Study.

* Solid line based on non-parametric smoothing function. Dashed line denotes predicted linear regression fit. P-values are from linear or quadratic term in regression model.

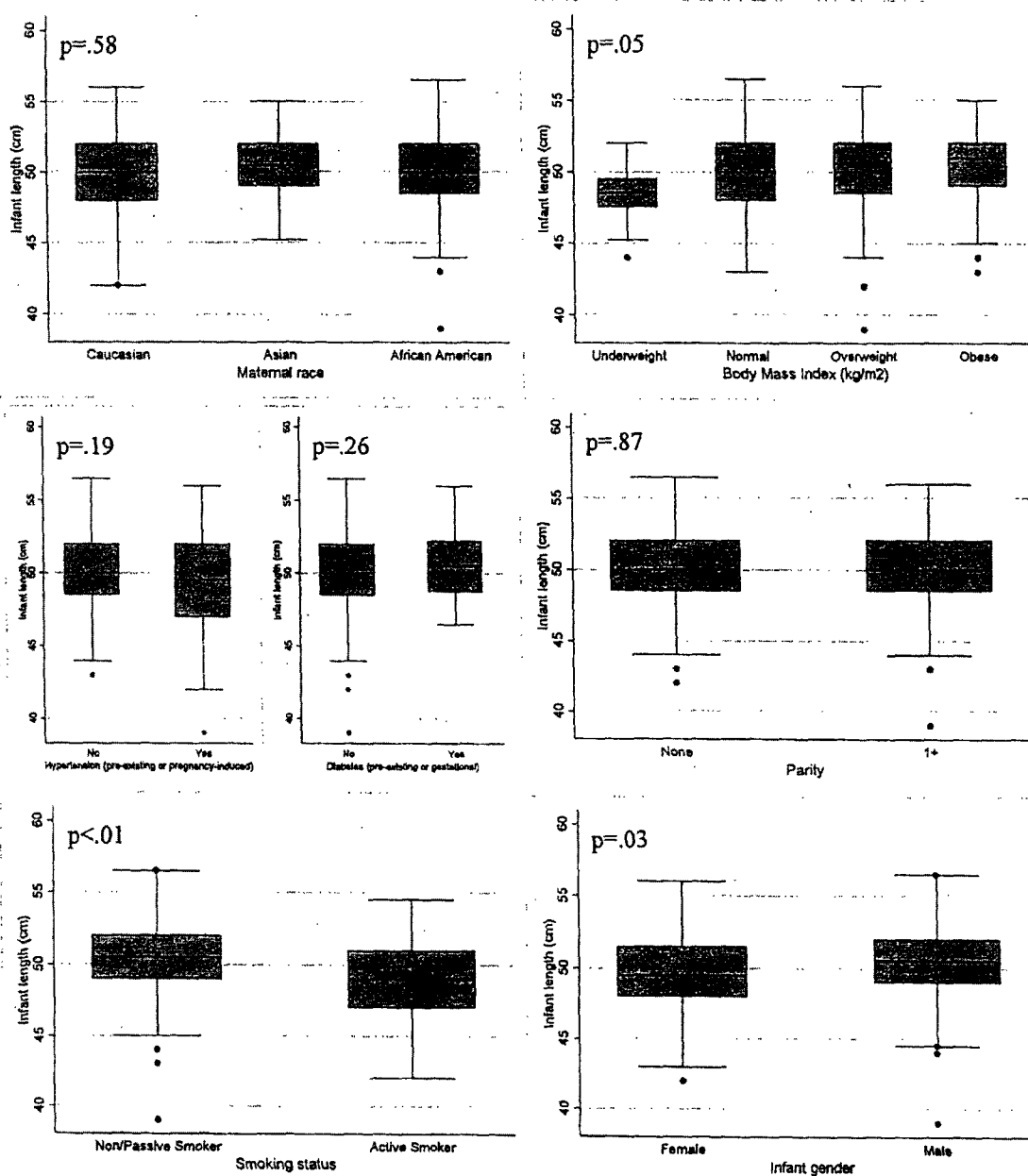


Figure A-6. Distribution of length (cm) by subject characteristics, Baltimore THREE Study.

* P-values from ANOVA comparing means between groups.

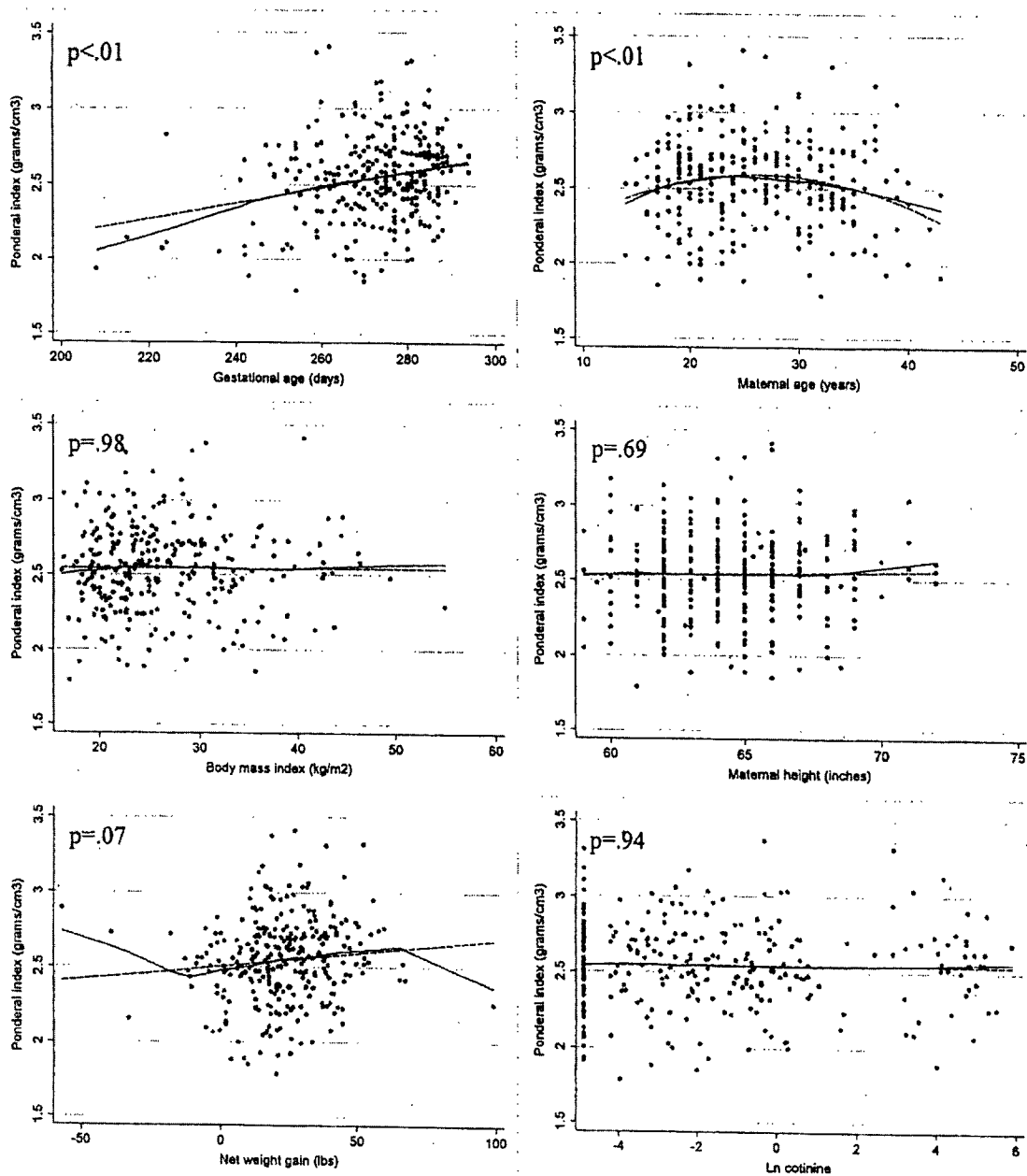


Figure A-7. Key determinants of newborn ponderal index, Baltimore THREE Study.

* Solid line based on non-parametric smoothing function. Dashed line denotes predicted linear regression fit. P-values are from linear or quadratic term in regression model.

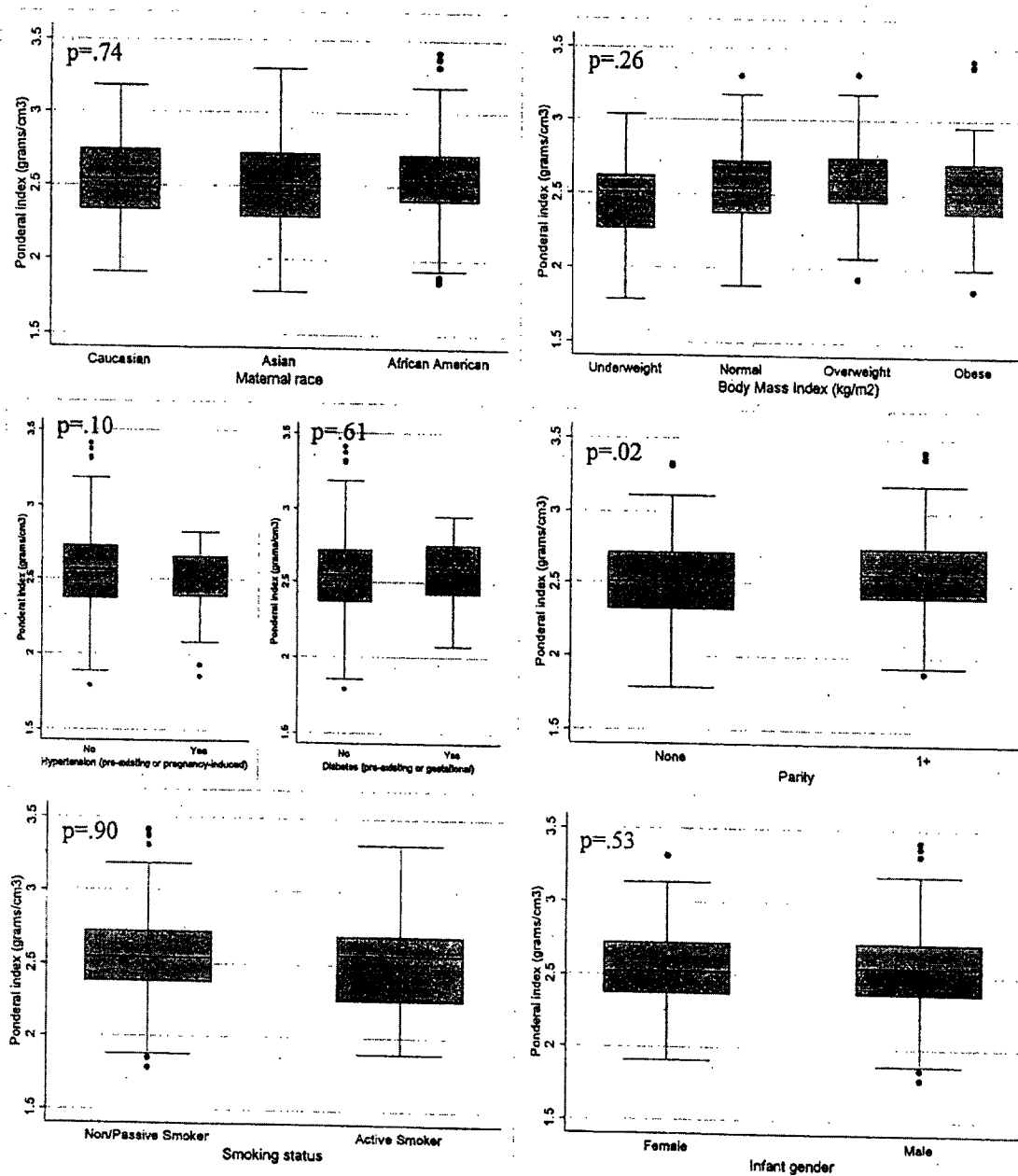


Figure A-8. Distribution of ponderal index by subject characteristics, Baltimore THREE Study.

* P-values from ANOVA comparing means between groups.

APPENDIX B

**REPRESENTATIVENESS OF STUDY POPULATION AND
SUBJECTS WITH MISSING DATA**

Table B-1. Characteristics of study subjects missing information on pre-pregnancy weight, height, and/or weight gain.

Characteristic	N (%), Mean (SD), or Median (IQR)		p-value
	Non-missing (n=282)	Missing (n=11)	
<u>Mother</u>			
Maternal age			
<18	23 (8)	1 (9)	p=.89
18-35	238 (84)	8 (73)	
35+	21 (7)	2 (18)	
Race			
White	59 (21)	1 (9)	p=.36
Asian	25 (9)	0 (0)	
Black	198 (70)	10 (91)	
Education			
<HS degree	79 (28)	7 (64)	p=.59
HS degree	94 (34)	2 (18)	
1-4 yrs college	63 (23)	2 (18)	
5+ yrs college	42 (15)	0 (0)	
Marital status			
Married	186 (66)	10 (91)	p=.24
Unmarried	96 (34)	1 (9)	
Primiparous			
No	121 (43)	1 (9)	p=.11
Yes	161 (57)	10 (91)	
Smoking status			
Non/passive	232 (82)	6 (55)	p=.02
Active	50 (18)	5 (45)	
<u>Infant</u>			
Baby gender			
Female	123 (44)	8 (73)	p=.70
Male	159 (56)	3 (27)	
Preterm			
No	247 (88)	8 (73)	p<.01
Yes	35 (12)	3 (27)	
Low birth weight			
No	253 (90)	9 (82)	p=.13
Yes	29 (10)	2 (18)	
Gestational age (days)	272 (13)	261 (20)	p=.03
Birth weight (grams)	3201 (584)	3168 (784)	p=.62
Length (cm)	50.0 (2.7)	51.1 (2.0)	p=.28
Head circumference (cm)	33.4 (1.7)	33.8 (1.9)	p=.62
Ponderal index (g/cm ³)	2.54 (0.29)	2.51 (0.32)	p=.97
<u>Cord concentrations (ng/mL)</u>			
PFOS	5.0 (3.4-8.0)	4.2 (3.2-6.0)	p=.47
PFOA	1.6 (1.2-2.1)	1.5 (1.1-2.0)	p=.65
Cotinine	0.10 (<LOD-0.84)	2.9 (0.07-121)	p<.01

Note: p-values based on Wilcoxon Rank Sum test or Fisher's Exact test. Missing data on other variables excluded from comparisons. Sample sizes differ for length (n=279 vs 9), head circumference (n=280 vs 9), ponderal index (n=279 vs 9), education (n=278 vs 11) and cotinine (269 vs. 11).

Table B-2. Comparing the study population with all births occurring at Johns Hopkins Hospital over the study period and with U.S. Vital Statistics from 2003-2004.

Characteristic	Study Population	Percentages All Live Births*	Full U.S. Population**
Maternal age			
<20 yrs of age	19.8	12.6 ^a	10.3
Maternal race			
White	20.5	n/a	78.9
Black	71.0	n/a	14.8
Asian	8.5	n/a	5.1
Marital status			
Unmarried	66.9	n/a	35.7
Married			
Smoking status			
Non-smoker	15.4 ^b	n/a	10.7
Smoker			
Birth weight (grams)			
≥ 2,500	89.4	84.9	91.9
< 2,500 (LBW)	10.6	15.1	8.1
Gestational age (weeks)			
≥ 37 (term)	87.0	82.0	87.5
< 37 (preterm)	13.0	18.0	12.5

* Assuming all multiple births were preterm and low birth weight.

** From Hoyert et al, 2006, except for race data, which is from CDC Wonder for 2002 (See <http://wonder.cdc.gov/wonder/help/Natality.html>). Includes multiple births.

a. Below 19 years of age.

b. Based on self-report. Smoking rate at the end of pregnancy as determined by cotinine level was 18.8%.

Table B-3. Comparing regression results using different methods for handling missing data.

Model	PFOS		PFOA	
	Coefficient (95% CI)	p-value	Coefficient (95% CI)	p-value
Birth weight				
<i>Complete Case (n=282)</i>				
Univariate	-43 (-144, 59)	p = 0.41	-106 (-245, 33)	p = 0.13
Adjusted	-64 (-143, 14)	p = 0.11	-87 (-196, 23)	p = 0.12
<i>Median Imputation (n=293)</i>				
Univariate	-37 (-139, 64)	p = 0.47	-97 (-234, 40)	p = 0.17
Adjusted	-69 (-149, 10)	p = 0.09	-104 (-213, 5)	p = 0.06
Head circumference				
<i>Complete Case (n=280)</i>				
Univariate	-0.20 (-0.51, 0.10)	p = 0.19	-0.45 (-0.87, -0.04)	p = 0.03
Adjusted	-0.28 (-0.52, -0.03)	p = 0.03	-0.37 (-0.72, -0.02)	p = 0.04
<i>Median Imputation (n=289)</i>				
Univariate	-0.22 (-0.52, 0.07)	p = 0.14	-0.46 (-0.87, -0.06)	p = 0.03
Adjusted	-0.32 (-0.56, -0.07)	p = 0.01	-0.41 (-0.76, -0.07)	p = 0.02
Length				
<i>Complete Case (n=279)</i>				
Univariate	0.29 (-0.19, 0.76)	p = 0.23	-0.03 (-0.67, 0.62)	p = 0.94
Adjusted	0.20 (-0.19, 0.59)	p = 0.32	0.01 (-0.53, 0.55)	p = 0.97
<i>Median Imputation (n=288)</i>				
Univariate	0.25 (-0.21, 0.72)	p = 0.29	-0.06 (-0.69, 0.57)	p = 0.85
Adjusted	0.13 (-0.26, 0.52)	p = 0.52	-0.10 (-0.64, 0.44)	p = 0.71
Ponderal index				
<i>Complete Case (n=279)</i>				
Univariate	-0.077 (-0.127, -0.028)	p < 0.01	-0.081 (-0.149, -0.013)	p = 0.02
Adjusted	-0.082 (-0.132, -0.032)	p < 0.01	-0.073 (-0.143, -0.003)	p = 0.04
<i>Median Imputation (n=288)</i>				
Univariate	-0.070 (-0.119, -0.021)	p < 0.01	-0.074 (-0.140, -0.007)	p = 0.03
Adjusted	-0.074 (-0.123, -0.025)	p < 0.01	-0.070 (-0.138, -0.001)	p = 0.05

* Regression coefficients represent the change in birth weight and birth size parameters with a unit change in ln(PFOS) or ln(PFOA) concentration. Multivariate models adjusted for gestational age, maternal age, body mass index, race, parity, smoking, baby gender, height, net weight gain, diabetes, and hypertension. For head circumference, adjusted model includes delivery mode (C-section/Vaginal). "Complete case" model excludes observations with missing bmi, height, and/or weight gain.

APPENDIX C

**FULL MODEL RESULTS FOR MULTIVARIATE
REGRESSION ANALYSES**

Table C-1. Coefficients from multivariate regression of birth weight on ln(PFOS).*

Independent variable	Coef.	Std. Err.	P>t	95% CI	
Ln(PFOS)	-64.3	40.0	0.109	-143.2	14.5
Gestational age (days)	24.4	2.1	0	20.3	28.4
Smoker (Yes/No)	-174.5	72.0	0.016	-316.4	-32.7
Maternal age (years)	35.3	32.7	0.282	-29.1	99.7
Maternal age squared	-0.7	0.6	0.252	-1.8	0.5
Normal weight (vs Underweight)	203.1	115.8	0.081	-24.9	431.1
Overweight (vs Underweight)	311.7	125.6	0.014	64.3	559.0
Obese (vs Underweight)	298.6	127.4	0.02	47.7	549.5
Asian (vs White)	-81.1	108.1	0.454	-294.0	131.8
Black (vs White)	-104.2	76.6	0.175	-255.0	46.6
Parity (1+ vs Zero)	151.4	60.3	0.013	32.6	270.2
Baby gender (Male vs Female)	139.7	52.8	0.009	35.7	243.7
Maternal height (inches)	22.5	10.0	0.026	2.8	42.2
Net weight gain (lbs)	5.6	1.6	0	2.5	8.7
Diabetes (Yes/No)	245.5	103.5	0.018	41.8	449.2
Hypertension (Yes/No)	-138.4	87.3	0.114	-310.3	33.4
constant	-5633.2	902.0	0	-7409.2	-3857.1

* Results from "complete case" analysis.

Table C-2. Coefficients from multivariate regression of birth weight on ln(PFOA).

Independent variable	Coef.	Std. Err.	P>t	95% CI	
Ln(PFOA)	-86.7	55.7	0.121	-196.3	22.9
Gestational age (days)	24.4	2.1	0	20.4	28.5
Smoker (Yes/No)	-163.5	71.8	0.024	-304.9	-22.2
Maternal age (years)	36.1	32.7	0.271	-28.3	100.5
Maternal age squared	-0.7	0.6	0.245	-1.8	0.5
Normal weight (vs Underweight)	204.0	115.8	0.079	-24.1	432.0
Overweight (vs Underweight)	317.9	125.4	0.012	70.9	564.9
Obese (vs Underweight)	306.6	127.6	0.017	55.4	557.7
Asian (vs White)	-95.9	107.3	0.372	-307.2	115.4
Black (vs White)	-105.8	76.5	0.168	-256.4	44.9
Parity (1+ vs Zero)	143.4	61.0	0.019	23.3	263.5
Baby gender (Male vs Female)	133.1	53.6	0.014	27.7	238.6
Maternal height (inches)	22.4	10.0	0.026	2.7	42.2
Net weight gain (lbs)	5.4	1.5	0.001	2.4	8.5
Diabetes (Yes/No)	242.7	103.7	0.02	38.5	447.0
Hypertension (Yes/No)	-135.8	87.2	0.121	-307.4	35.9
constant	-5716.6	897.4	0	-7483.6	-3949.6

* Results from "complete case" analysis.

Table C-3. Coefficients from multivariate regression of head circumference on ln(PFOS).

Independent variable	Coef.	Std. Err.	P>t	95% CI	
Ln(PFOS)	-0.276	0.126	0.03	-0.524	-0.027
Gestational age (days)	0.600	0.140	0	0.325	0.875
Gestational age squared	-0.001	0.000	0	-0.002	-0.001
Smoker (Yes/No)	-0.458	0.227	0.045	-0.906	-0.011
Maternal age (years)	0.114	0.103	0.271	-0.089	0.317
Maternal age squared	-0.002	0.002	0.264	-0.006	0.002
Normal weight (vs Underweight)	0.585	0.367	0.113	-0.138	1.307
Overweight (vs Underweight)	0.631	0.404	0.12	-0.165	1.427
Obese (vs Underweight)	0.889	0.406	0.029	0.090	1.688
Asian (vs White)	0.230	0.342	0.501	-0.443	0.904
Black (vs White)	-0.534	0.242	0.028	-1.010	-0.057
Parity (1+ vs Zero)	0.569	0.193	0.003	0.190	0.949
Baby gender (Male vs Female)	0.232	0.167	0.164	-0.096	0.561
Maternal height (inches)	0.103	0.032	0.002	0.039	0.167
Net weight gain (lbs)	0.012	0.005	0.013	0.003	0.022
Diabetes (Yes/No)	0.781	0.330	0.019	0.132	1.430
Hypertension (Yes/No)	-0.860	0.275	0.002	-1.401	-0.319
Delivery type (C-section vs Vaginal)	0.772	0.206	0	0.367	1.177
constant	-61.973	18.795	0.001	-98.981	-24.965

* Results from "complete case" analysis.

Table C-4. Coefficients from multivariate regression of head circumference on ln(PFOA).

Independent variable	Coef.	Std. Err.	P>t	95% CI	
Ln(PFOA)	-0.368	0.177	0.039	-0.716	-0.019
Gestational age (days)	0.576	0.140	0	0.300	0.852
Gestational age squared	-0.001	0.000	0	-0.002	0.000
Smoker (Yes/No)	-0.410	0.227	0.072	-0.857	0.036
Maternal age (years)	0.117	0.103	0.257	-0.086	0.320
Maternal age squared	-0.002	0.002	0.254	-0.006	0.002
Normal weight (vs Underweight)	0.580	0.368	0.116	-0.144	1.304
Overweight (vs Underweight)	0.641	0.404	0.114	-0.155	1.438
Obese (vs Underweight)	0.911	0.406	0.026	0.111	1.711
Asian (vs White)	0.169	0.339	0.62	-0.500	0.837
Black (vs White)	-0.540	0.242	0.027	-1.016	-0.063
Parity (1+ vs Zero)	0.542	0.194	0.006	0.160	0.925
Baby gender (Male vs Female)	0.205	0.169	0.226	-0.128	0.538
Maternal height (inches)	0.103	0.033	0.002	0.039	0.167
Net weight gain (lbs)	0.011	0.005	0.019	0.002	0.021
Diabetes (Yes/No)	0.778	0.330	0.019	0.128	1.429
Hypertension (Yes/No)	-0.847	0.275	0.002	-1.388	-0.307
Delivery type (C-section vs Vaginal)	0.783	0.206	0	0.377	1.189
constant	-59.154	18.900	0.002	-96.369	-21.938

* Results from "complete case" analysis.

Table C-5. Coefficients from multivariate regression of length on ln(PFOS).

Independent variable	Coef.	Std. Err.	P>t	95% CI	
Ln(PFOS)	0.198	0.197	0.317	-0.191	0.586
Gestational age (days)	0.112	0.010	0	0.092	0.132
Smoker (Yes/No)	-0.796	0.355	0.026	-1.496	-0.096
Maternal age (years)	0.019	0.025	0.44	-0.030	0.069
Normal weight (vs Underweight)	0.809	0.568	0.156	-0.310	1.927
Overweight (vs Underweight)	0.887	0.616	0.151	-0.326	2.101
Obese (vs Underweight)	1.325	0.625	0.035	0.095	2.555
Asian (vs White)	-0.292	0.527	0.58	-1.329	0.745
Black (vs White)	-0.368	0.378	0.332	-1.112	0.377
Parity (1+ vs Zero)	0.216	0.290	0.457	-0.355	0.786
Baby gender (Male vs Female)	0.713	0.260	0.007	0.201	1.226
Maternal height (inches)	0.089	0.050	0.077	-0.010	0.187
Net weight gain (lbs)	0.014	0.008	0.059	-0.001	0.029
Diabetes (Yes/No)	0.897	0.507	0.078	-0.102	1.896
Hypertension (Yes/No)	-0.234	0.428	0.584	-1.078	0.609
constant	11.553	4.081	0.005	3.518	19.587

* Results from "complete case" analysis.

Table C-6. Coefficients from multivariate regression of length on ln(PFOA).

Independent variable	Coef.	Std. Err.	P>t	95% CI	
Ln(PFOS)	0.009	0.274	0.973	-0.531	0.549
Gestational age (days)	0.112	0.010	0	0.092	0.132
Smoker (Yes/No)	-0.823	0.355	0.021	-1.522	-0.124
Maternal age (years)	0.021	0.025	0.403	-0.029	0.071
Normal weight (vs Underweight)	0.776	0.569	0.174	-0.345	1.896
Overweight (vs Underweight)	0.844	0.616	0.172	-0.370	2.058
Obese (vs Underweight)	1.321	0.626	0.036	0.088	2.554
Asian (vs White)	-0.223	0.524	0.67	-1.255	0.808
Black (vs White)	-0.308	0.378	0.416	-1.052	0.437
Parity (1+ vs Zero)	0.192	0.294	0.513	-0.386	0.770
Baby gender (Male vs Female)	0.685	0.264	0.01	0.165	1.205
Maternal height (inches)	0.084	0.050	0.095	-0.015	0.182
Net weight gain (lbs)	0.015	0.008	0.045	0.000	0.030
Diabetes (Yes/No)	0.848	0.509	0.097	-0.155	1.851
Hypertension (Yes/No)	-0.280	0.428	0.514	-1.123	0.563
constant	12.107	4.061	0.003	4.111	20.104

* Results from "complete case" analysis.

Table C-7. Coefficients from multivariate regression of ponderal index on ln(PFOS).

Independent variable	Coef.	Std. Err.	P>t	95% CI	
Ln(PFOS)	-0.0817	0.0253	0.001	-0.1316	-0.0318
Gestational age (days)	0.0046	0.0013	0	0.0020	0.0071
Smoker (Yes/No)	-0.0109	0.0457	0.812	-0.1008	0.0790
Maternal age (years)	0.0371	0.0207	0.074	-0.0036	0.0779
Maternal age squared	-0.0008	0.0004	0.039	-0.0015	0.0000
Normal weight (vs Underweight)	0.0311	0.0730	0.671	-0.1127	0.1748
Overweight (vs Underweight)	0.0783	0.0792	0.324	-0.0777	0.2342
Obese (vs Underweight)	0.0129	0.0803	0.872	-0.1452	0.1711
Asian (vs White)	-0.0263	0.0681	0.7	-0.1604	0.1079
Black (vs White)	-0.0288	0.0486	0.553	-0.1245	0.0668
Parity (1+ vs Zero)	0.0905	0.0381	0.018	0.0156	0.1655
Baby gender (Male vs Female)	-0.0066	0.0335	0.844	-0.0724	0.0593
Maternal height (inches)	0.0017	0.0064	0.791	-0.0109	0.0143
Net weight gain (lbs)	0.0020	0.0010	0.039	0.0001	0.0040
Diabetes (Yes/No)	0.0668	0.0652	0.306	-0.0615	0.1951
Hypertension (Yes/No)	-0.0890	0.0551	0.107	-0.1974	0.0194
constant	0.8081	0.5745	0.161	-0.3230	1.9393

* Results from "complete case" analysis.

Table C-8. Coefficients from multivariate regression of ponderal index on ln(PFOA).

Independent variable	Coef.	Std. Err.	P>t	95% CI	
Ln(PFOA)	-0.0733	0.0356	0.04	-0.1434	-0.0032
Gestational age (days)	0.0046	0.0013	0	0.0020	0.0072
Smoker (Yes/No)	0.0021	0.0461	0.964	-0.0886	0.0928
Maternal age (years)	0.0381	0.0210	0.07	-0.0032	0.0793
Maternal age squared	-0.0008	0.0004	0.037	-0.0015	0.0000
Normal weight (vs Underweight)	0.0364	0.0738	0.623	-0.1090	0.1818
Overweight (vs Underweight)	0.0895	0.0800	0.264	-0.0680	0.2469
Obese (vs Underweight)	0.0204	0.0813	0.802	-0.1397	0.1804
Asian (vs White)	-0.0484	0.0684	0.48	-0.1831	0.0862
Black (vs White)	-0.0391	0.0490	0.426	-0.1356	0.0575
Parity (1+ vs Zero)	0.0872	0.0389	0.026	0.0106	0.1637
Baby gender (Male vs Female)	-0.0078	0.0343	0.821	-0.0753	0.0597
Maternal height (inches)	0.0024	0.0065	0.708	-0.0103	0.0152
Net weight gain (lbs)	0.0018	0.0010	0.074	-0.0002	0.0037
Diabetes (Yes/No)	0.0715	0.0661	0.28	-0.0586	0.2016
Hypertension (Yes/No)	-0.0803	0.0556	0.15	-0.1897	0.0292
constant	0.6473	0.5774	0.263	-0.4896	1.7841

* Results from "complete case" analysis.

APPENDIX D

BIRTH WEIGHT REGRESSION SENSITIVITY ANALYSES

The following sensitivity analyses are provided:

- Covariate adjustment (see models below)
- Covariate specification (non-linearities)
- Untransformed PFC independent variable

Models

A: Univariate

B: Adjusted for gestational age.

C: Adjusted for gestational age, maternal age, maternal age squared, body mass index, race, parity, smoking, baby gender, height, and net weight gain.

D: Model C + diabetes and hypertension.

E: Model C + maternal education, insurance status, and marital status.

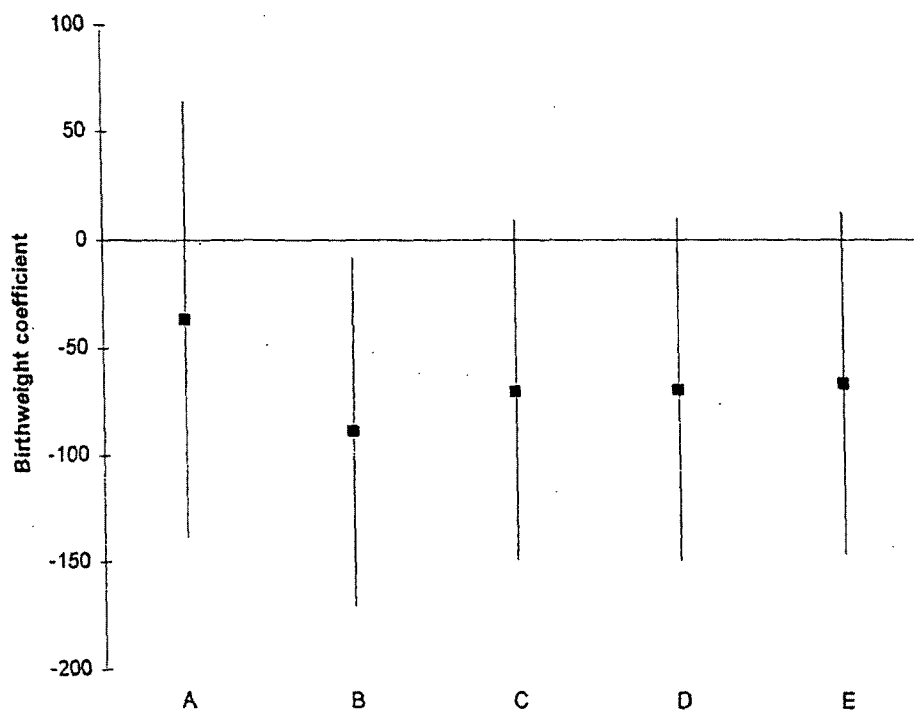


Figure D-1. Coefficients from linear regression of birth weight on ln(PFOS), adjusted by different sets of covariates.

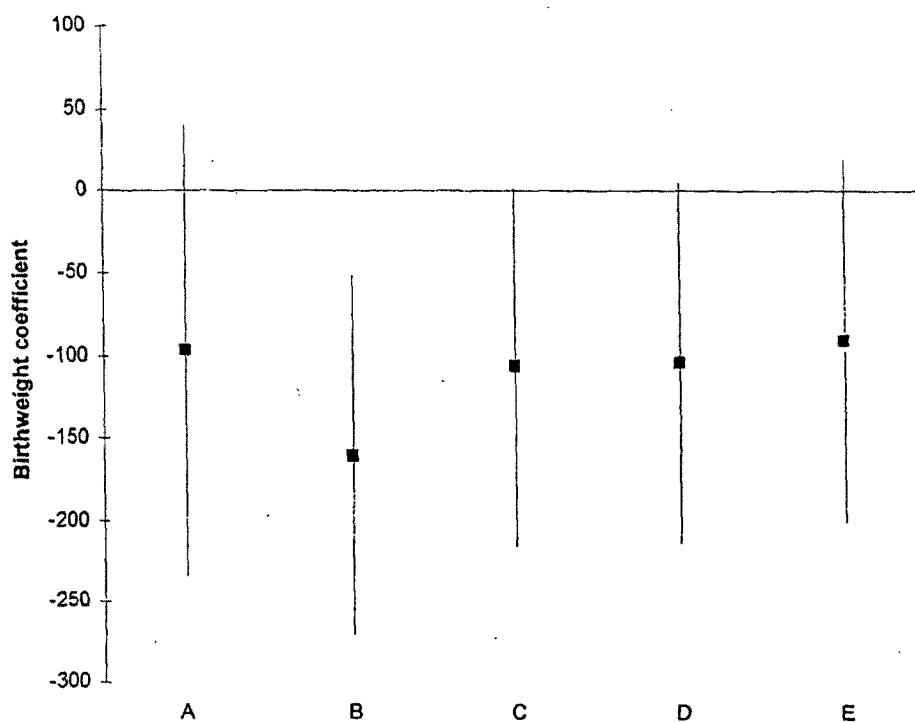


Figure D-2. Coefficients from linear regression of birth weight on ln(PFOA), adjusted by different sets of covariates.

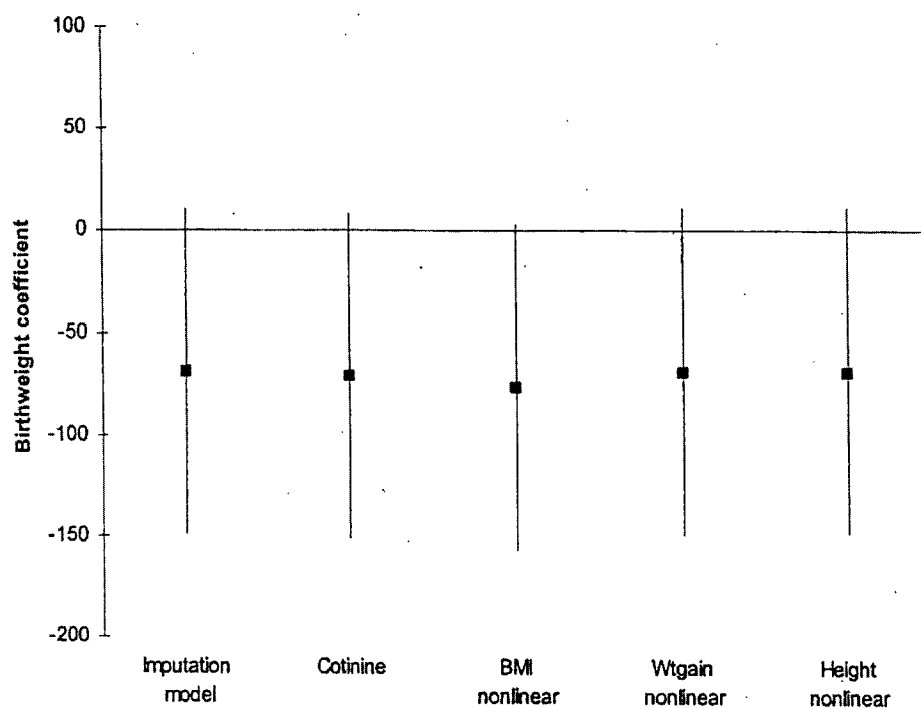


Figure D-3. Coefficients from linear regression of birth weight on $\ln(\text{PFOS})$, with different specifications of potential confounders.

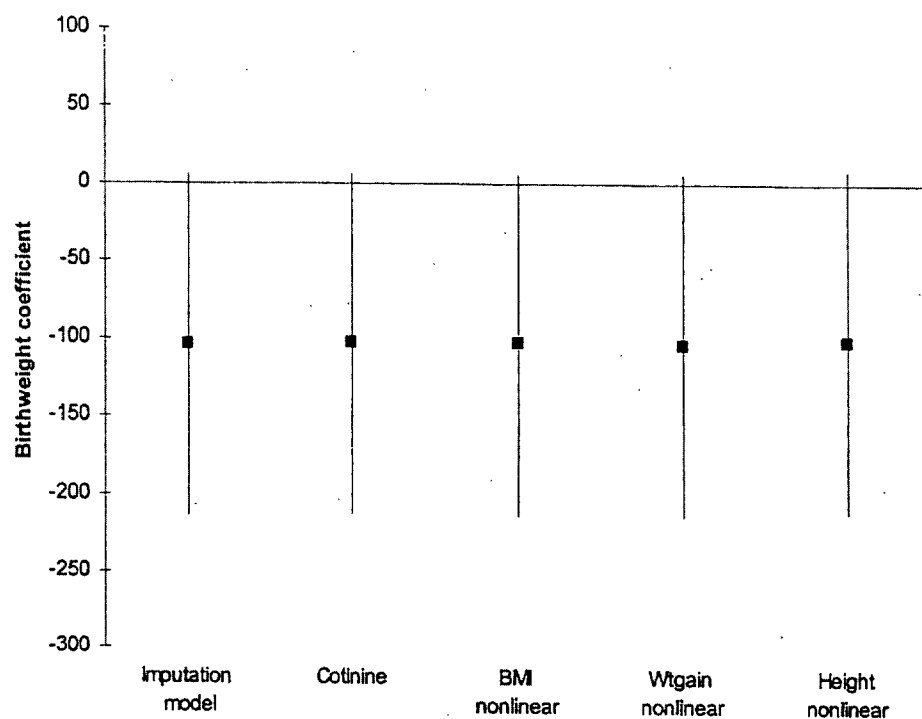


Figure D-4. Coefficients from linear regression of birth weight on $\ln(\text{PFOA})$, with different specifications of potential confounders.

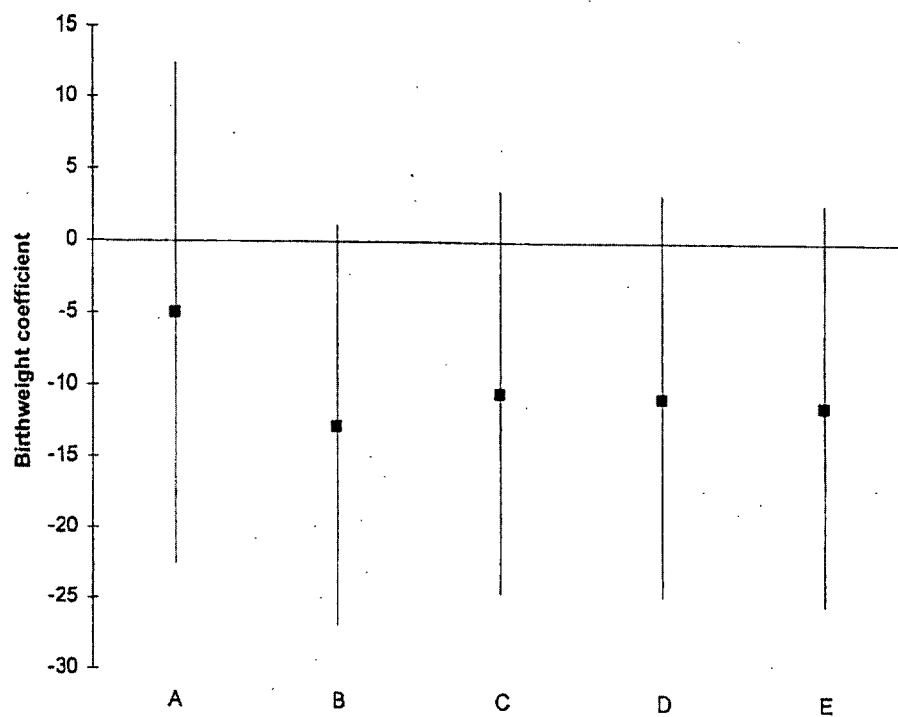


Figure D-5. Coefficients from linear regression of birth weight on PFOS, with adjustment by different sets of covariates.

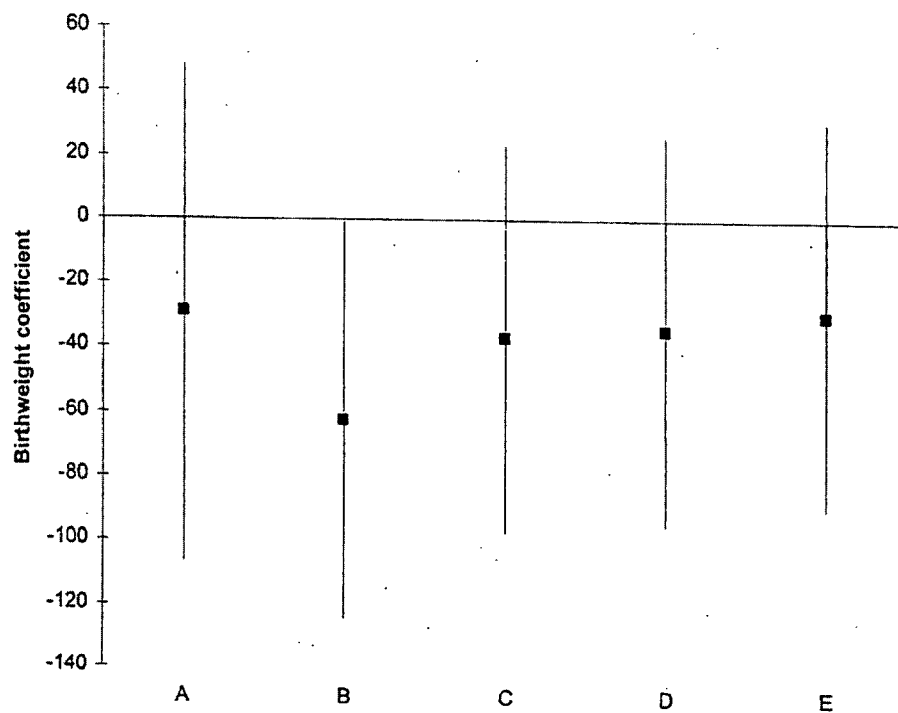


Figure D-6. Coefficients from linear regression of birth weight on PFOA, with adjustment by different sets of covariates.

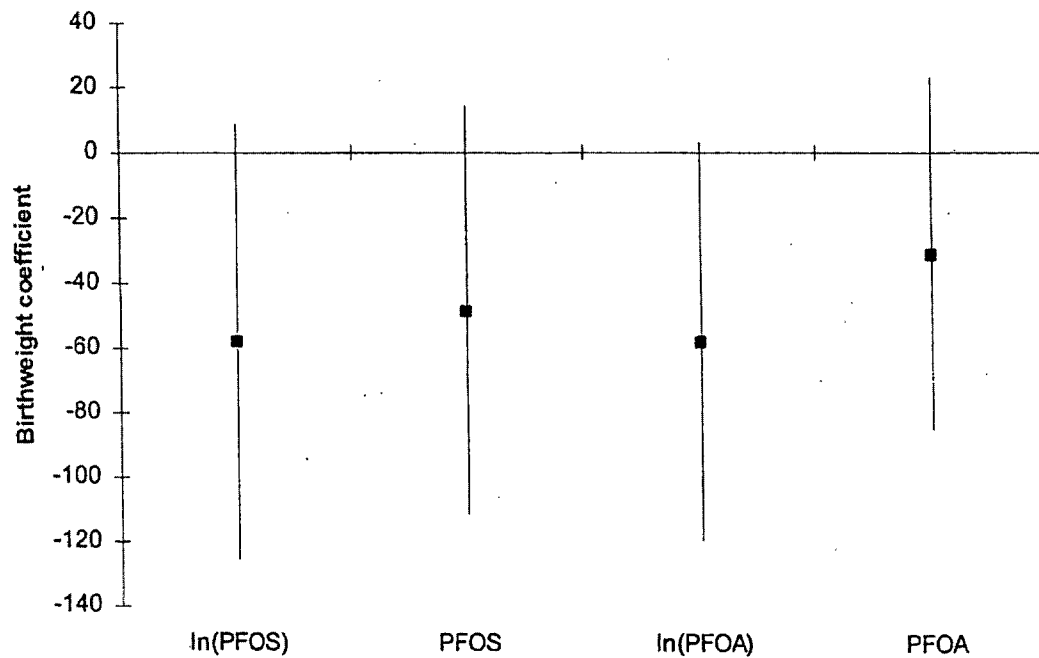


Figure D-7. Adjusted birth weight coefficients associated with a change equal to the IQR, comparing log-linear and linear models.

APPENDIX E

**NEWBORN HEAD CIRCUMFERENCE REGRESSION
SENSITIVITY ANALYSES**

The following sensitivity analyses are provided:

- Covariate adjustment (see models below)
- Covariate specification (non-linearities)
- Untransformed PFC independent variable

Models

A: Univariate

B: Adjusted for gestational age, gestational age squared.

C: Adjusted for gestational age, gestational age squared, maternal age, maternal age squared, body mass index, race, parity, smoking, baby gender, height, net weight gain, and delivery mode.

D: Model C + diabetes and hypertension.

E: Model C + maternal education, insurance status, and marital status.

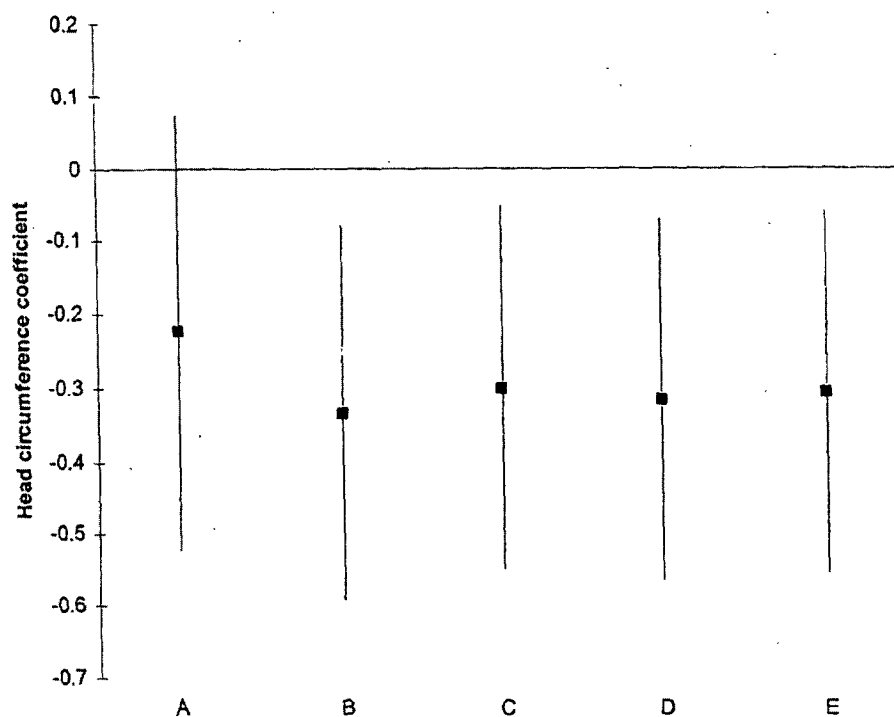


Figure E-1. Coefficients from linear regression of head circumference on $\ln(\text{PFOS})$, with adjustment by different sets of covariates.

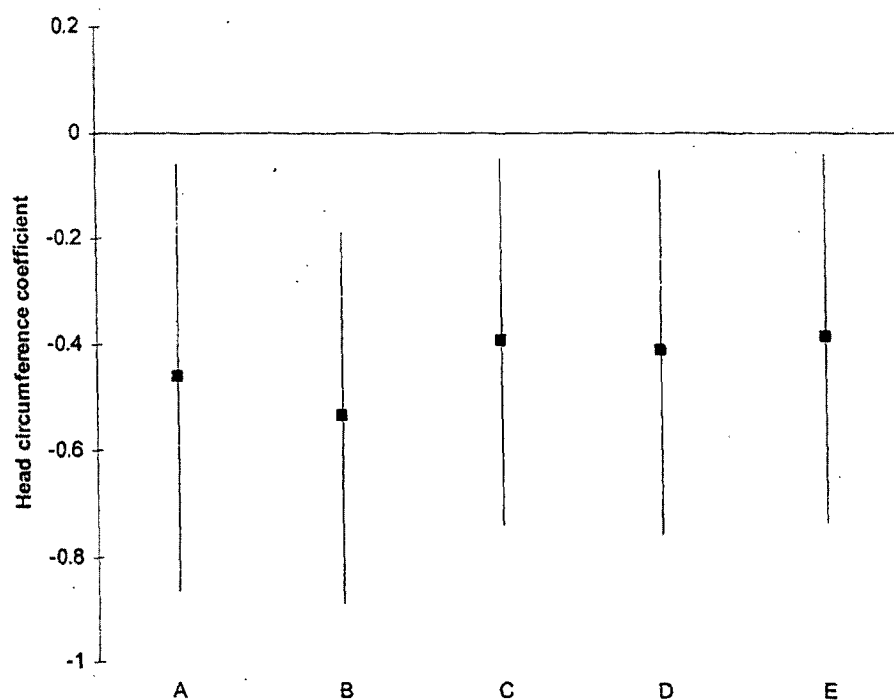


Figure E-2. Coefficients from linear regression of head circumference on $\ln(\text{PFOA})$, with adjustment by different sets of covariates.

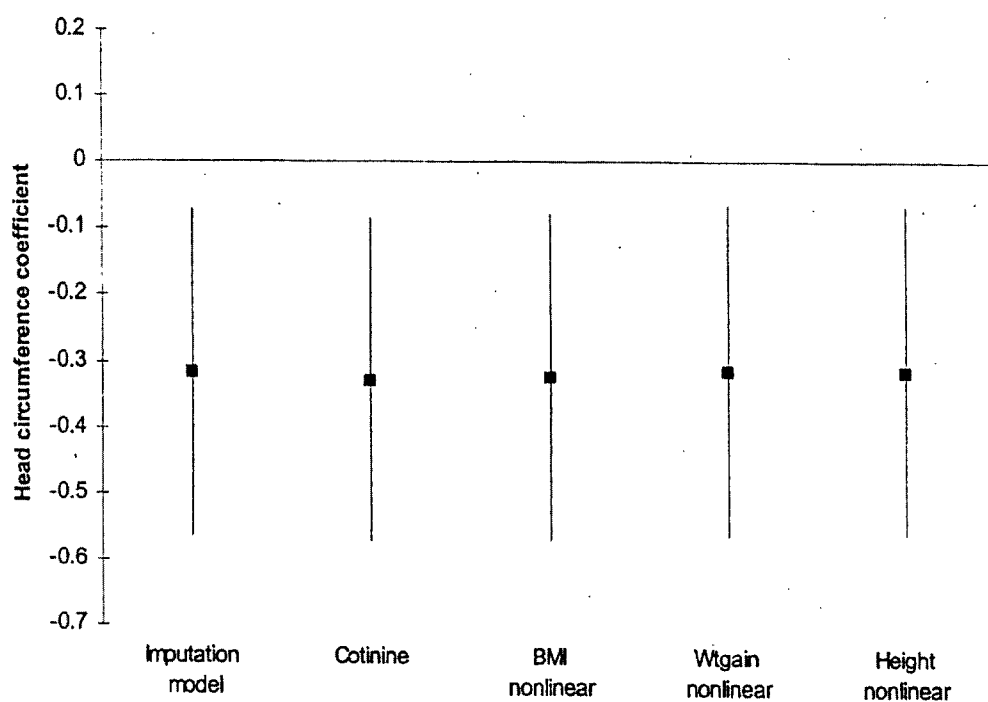


Figure E-3. Coefficients from linear regression of head circumference on $\ln(\text{PFOS})$, with different specifications of potential confounders.

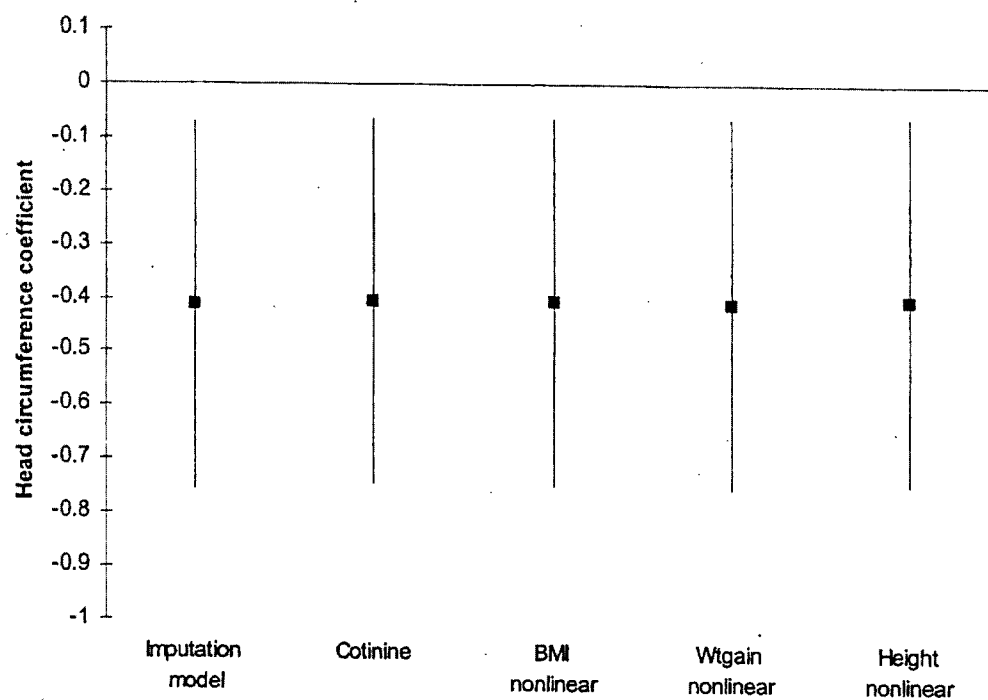


Figure E-4. Coefficients from linear regression of head circumference on $\ln(\text{PFOA})$, with different specifications of potential confounders.

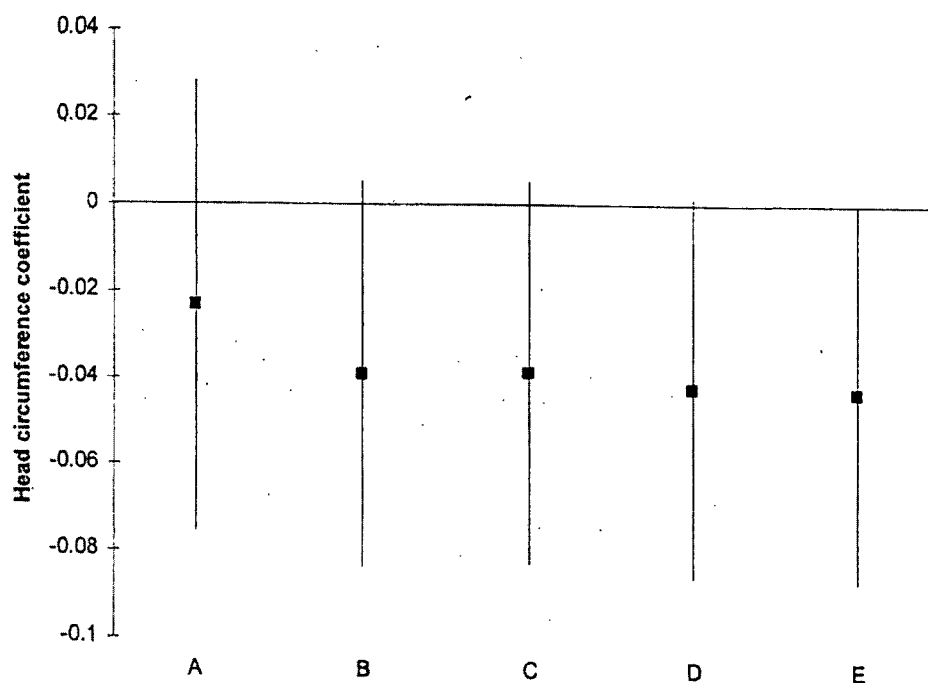


Figure E-5. Coefficients from linear regression of head circumference on PFOS, with adjustment by different sets of covariates.

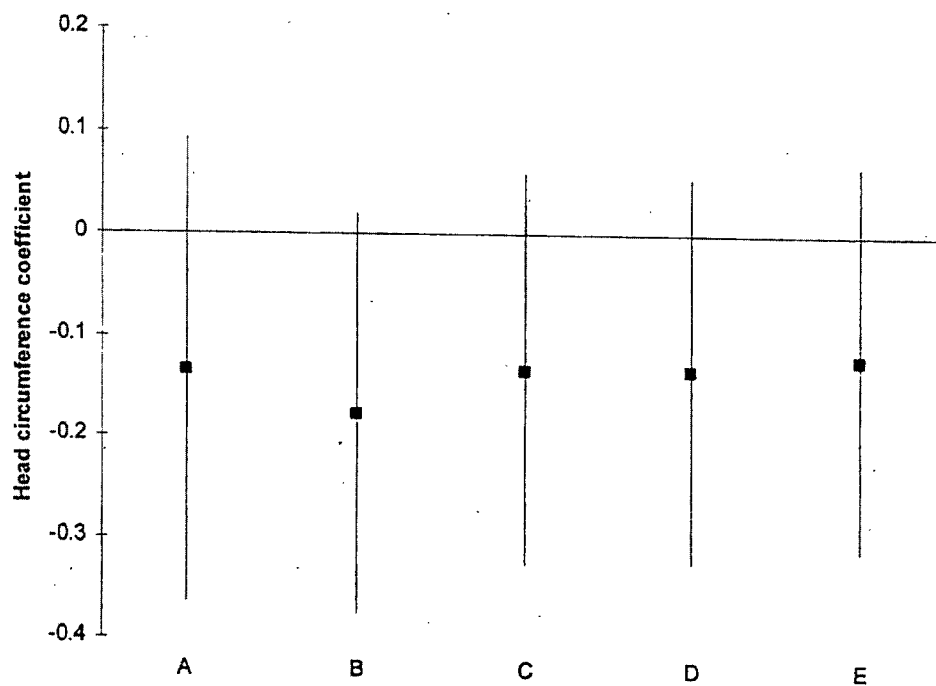


Figure E-6. Coefficients from linear regression of head circumference on PFOA, with adjustment by different sets of covariates.

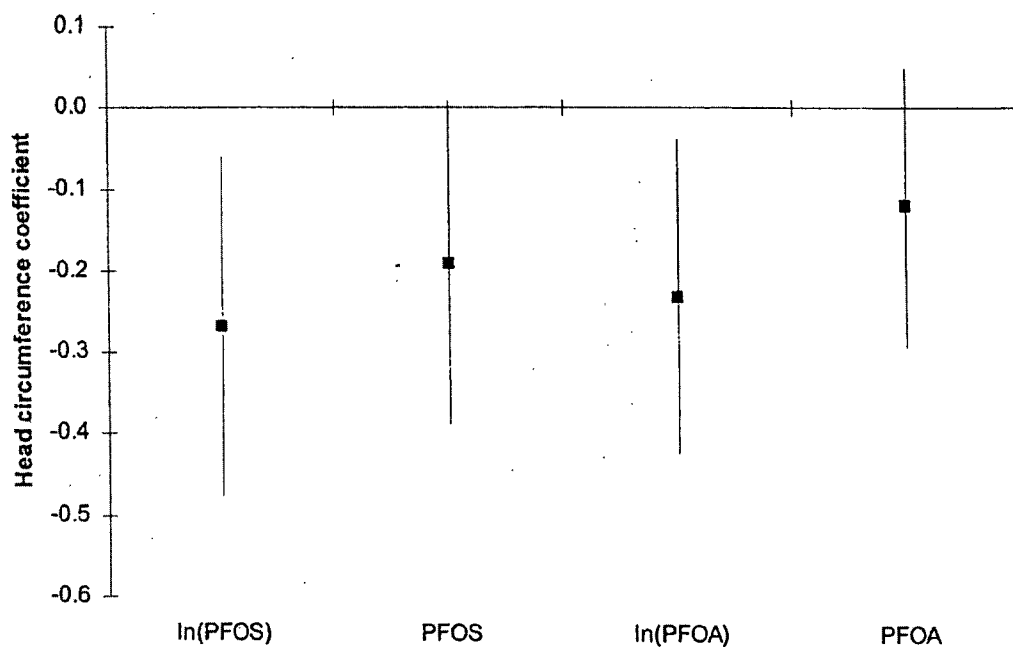


Figure E-7. Adjusted head circumference coefficients associated with a change equal to the IQR, comparing log-linear and linear models.

APPENDIX F
NEWBORN LENGTH REGRESSION SENSITIVITY
ANALYSES

The following sensitivity analyses are provided:

- Covariate adjustment (see models below)
- Covariate specification (non-linearities)
- Untransformed PFC independent variable

Models

Models

A: Univariate

B: Adjusted for gestational age.

C: Adjusted for gestational age, maternal age, body mass index, race, parity, smoking, baby gender, height, and net weight gain.

D: Model C + diabetes and hypertension.

E: Model C + maternal education, insurance status, and marital status.

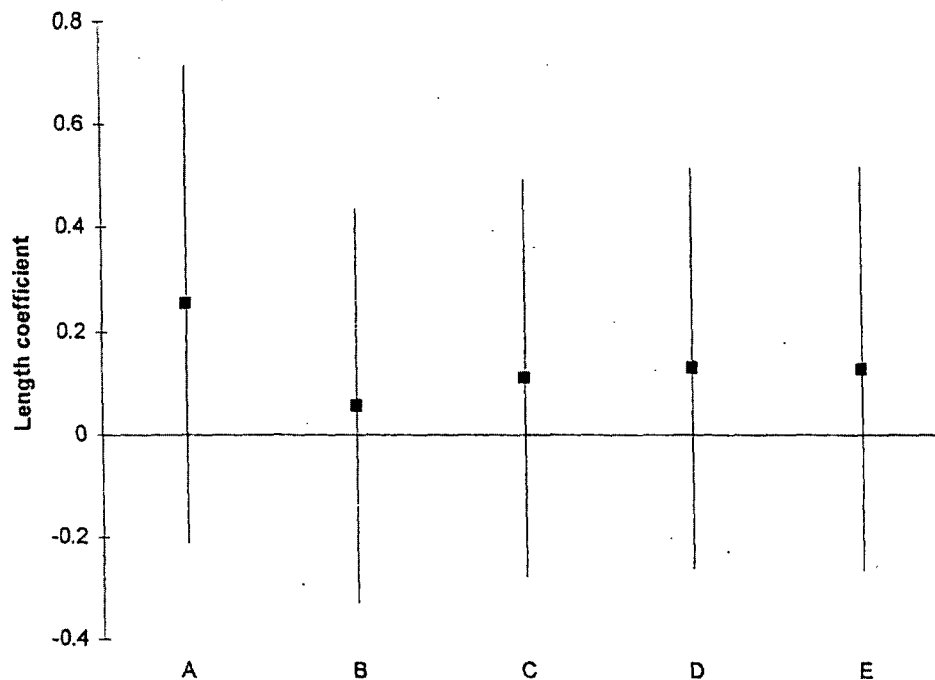


Figure F-1. Coefficients from linear regression of length on $\ln(\text{PFOS})$, with adjustment by different sets of covariates.

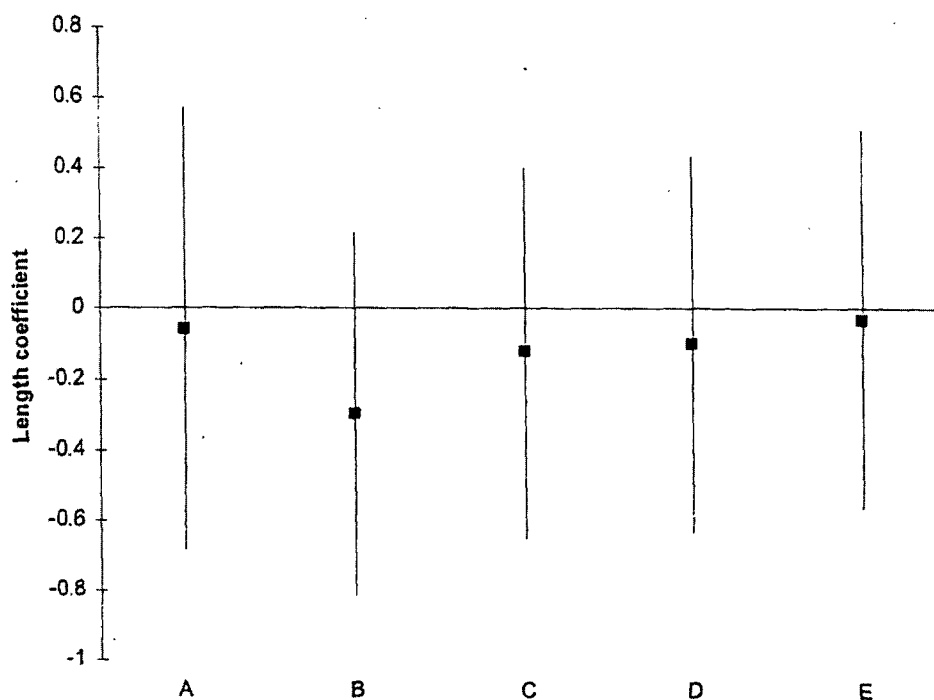


Figure F-2. Coefficients from linear regression of length on $\ln(\text{PFOA})$, with adjustment by different sets of covariates.

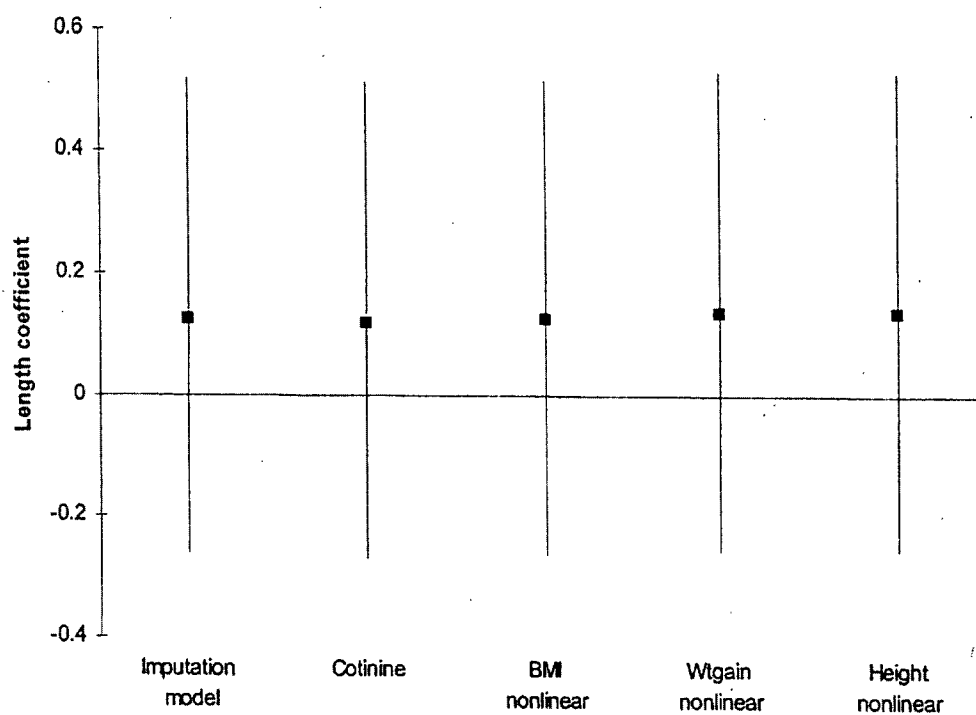


Figure F-3. Coefficients from linear regression of length on $\ln(\text{PFOS})$, with different specifications of potential confounders.

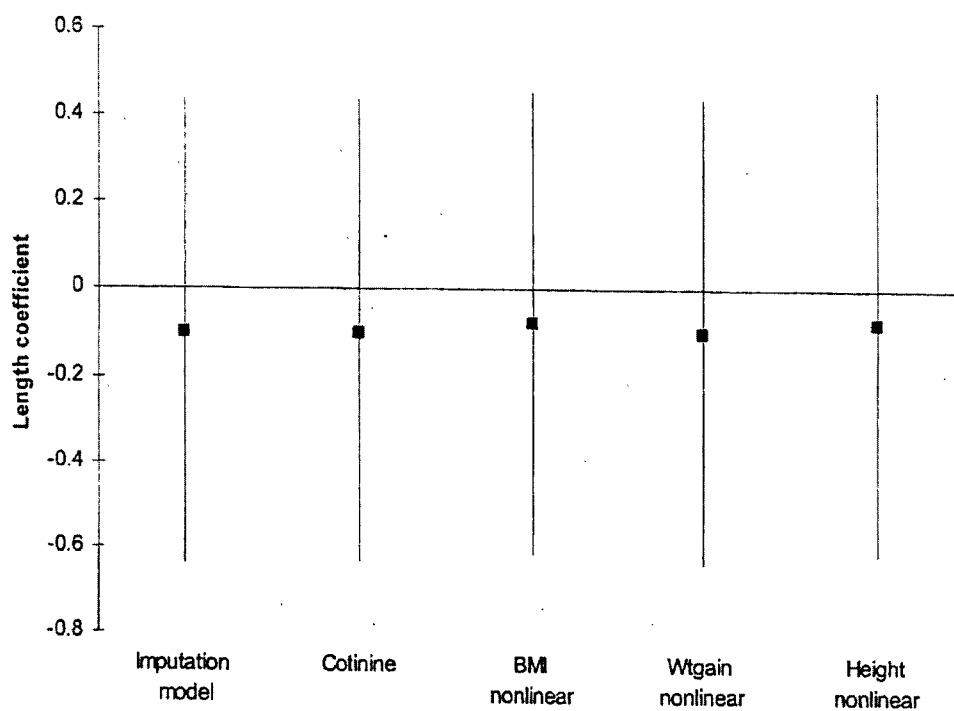


Figure F-4. Coefficients from linear regression of length on $\ln(\text{PFOA})$, with different specifications of potential confounders.

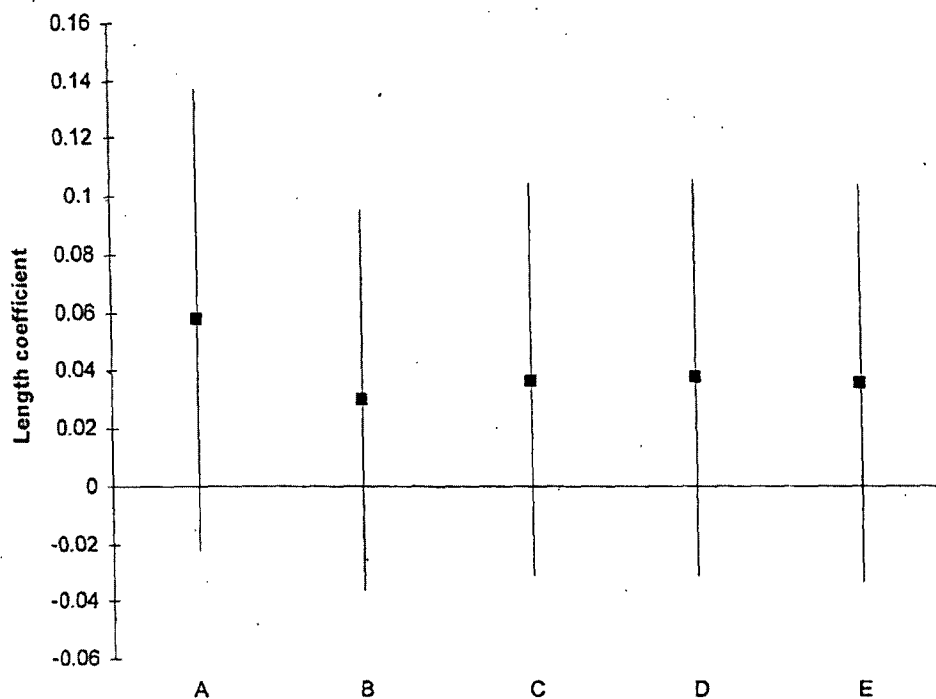


Figure F-5. Coefficients from linear regression of length on PFOS, with adjustment by different sets of covariates.

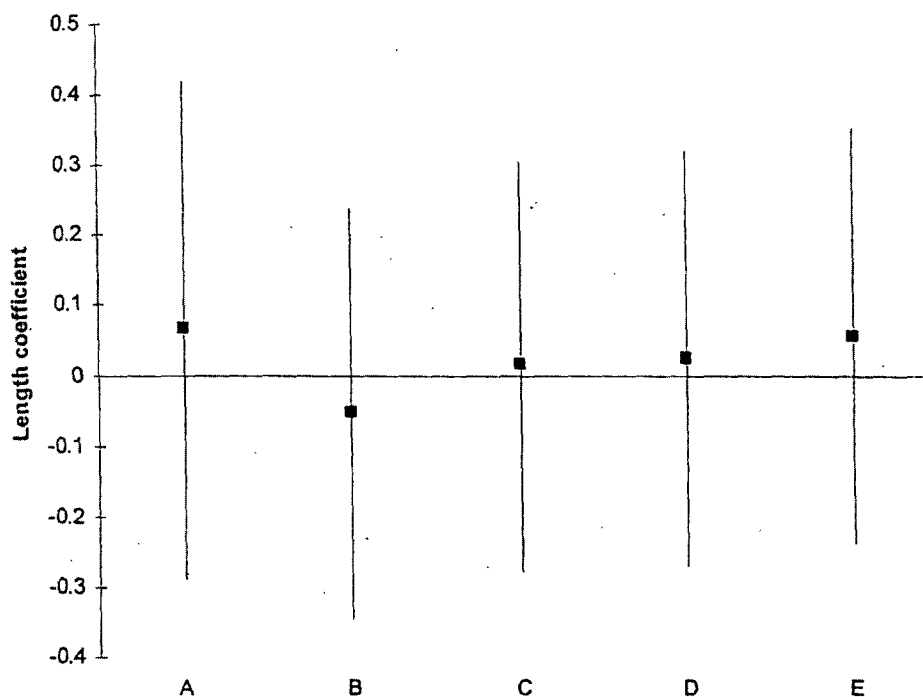


Figure F-6. Coefficients from linear regression of length on PFOA, with adjustment by different sets of covariates.

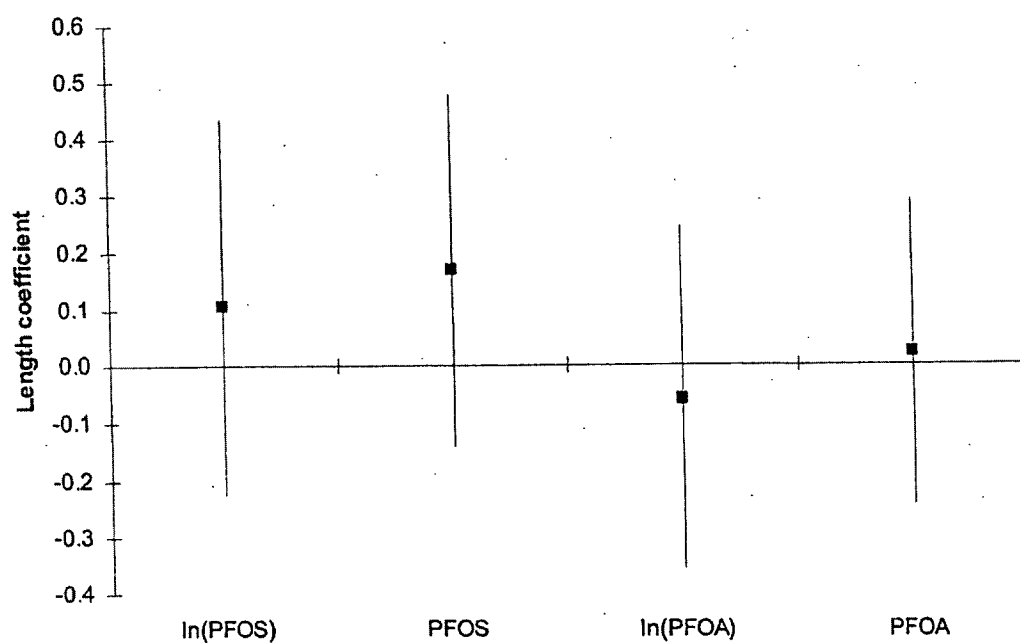


Figure F-7. Adjusted length coefficients associated with a change equal to the IQR, comparing log-linear and linear models.

APPENDIX G
NEWBORN PONDERAL INDEX REGRESSION
SENSITIVITY ANALYSES

The following sensitivity analyses are provided:

- Covariate adjustment (see models below)
- Covariate specification (non-linearities)
- Untransformed PFC independent variable

Models

A: Univariate

B: Adjusted for gestational age.

C: Adjusted for gestational age, maternal age, maternal age squared, body mass index, race, parity, smoking, baby gender, height, and net weight gain.

D: Model C + diabetes and hypertension.

E: Model C + maternal education, insurance status, and marital status.

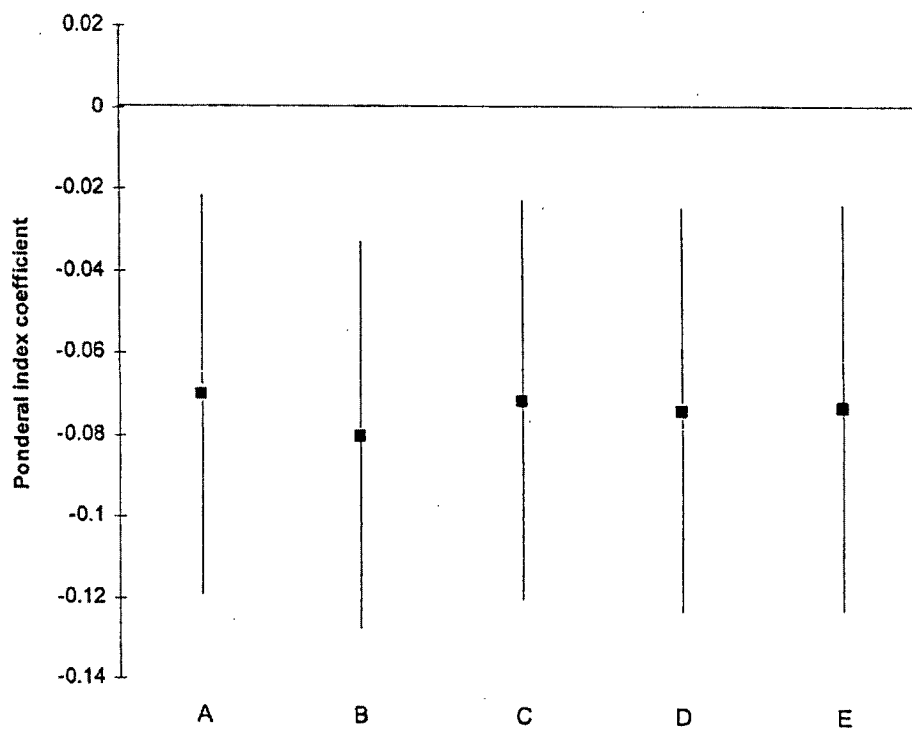


Figure G-1. Coefficients from linear regression of ponderal index on $\ln(\text{PFOS})$, with adjustment by different sets of covariates.

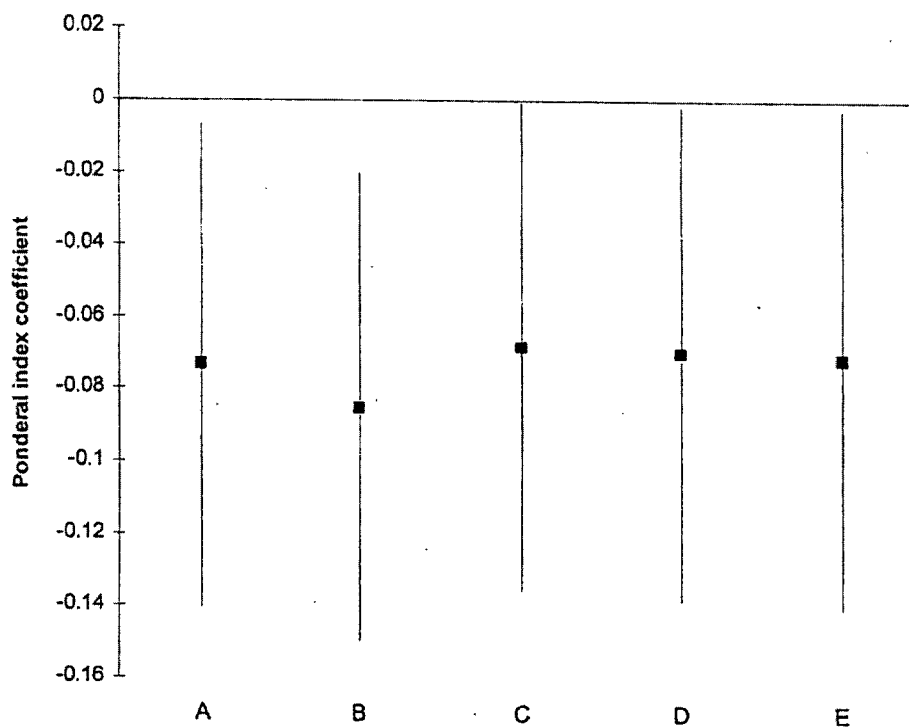


Figure G-2. Coefficients from linear regression of ponderal index on $\ln(\text{PFOA})$, with adjustment by different sets of covariates.

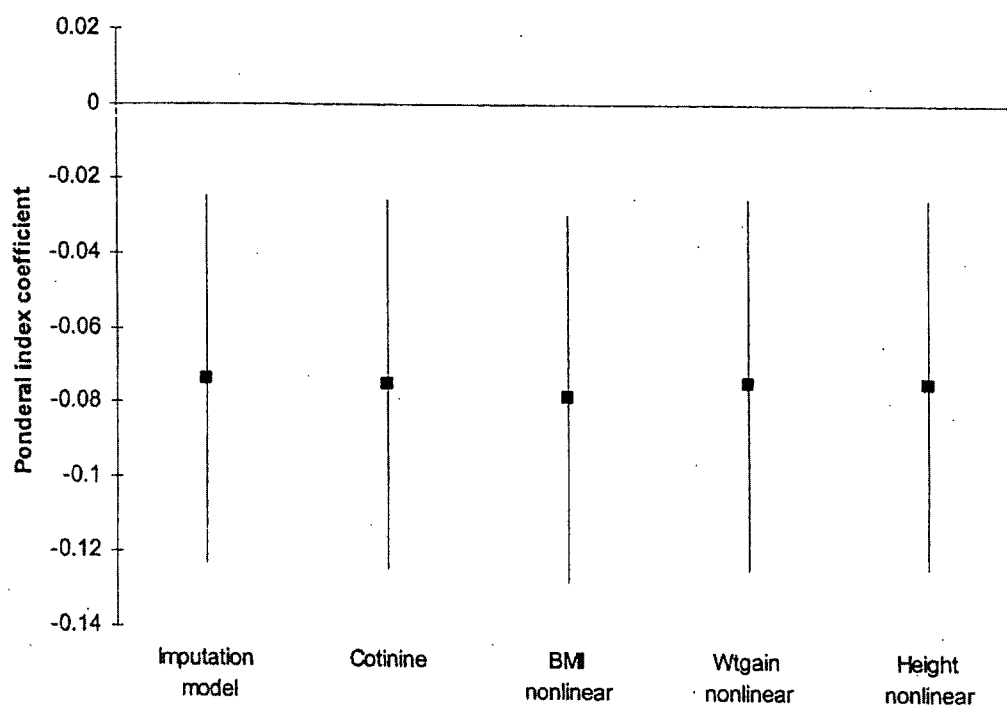


Figure G-3. Coefficients from linear regression of ponderal index on $\ln(\text{PFOS})$ using imputation model, with different specifications of potential confounders.

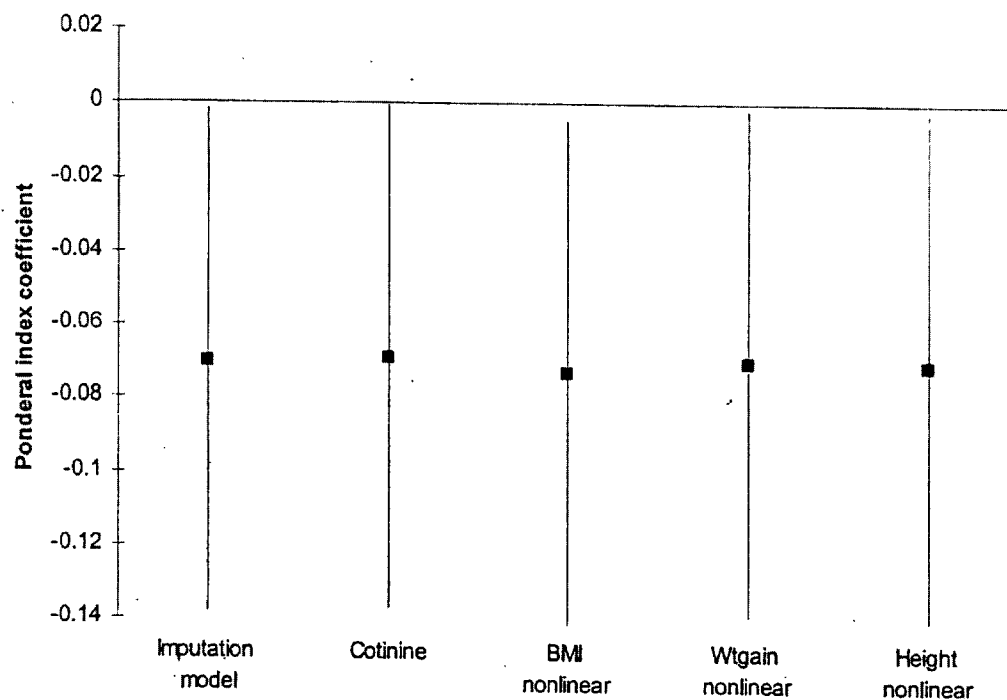


Figure G-4. Coefficients from linear regression of ponderal index on $\ln(\text{PFOA})$ using imputation model, with different specifications of potential confounders.

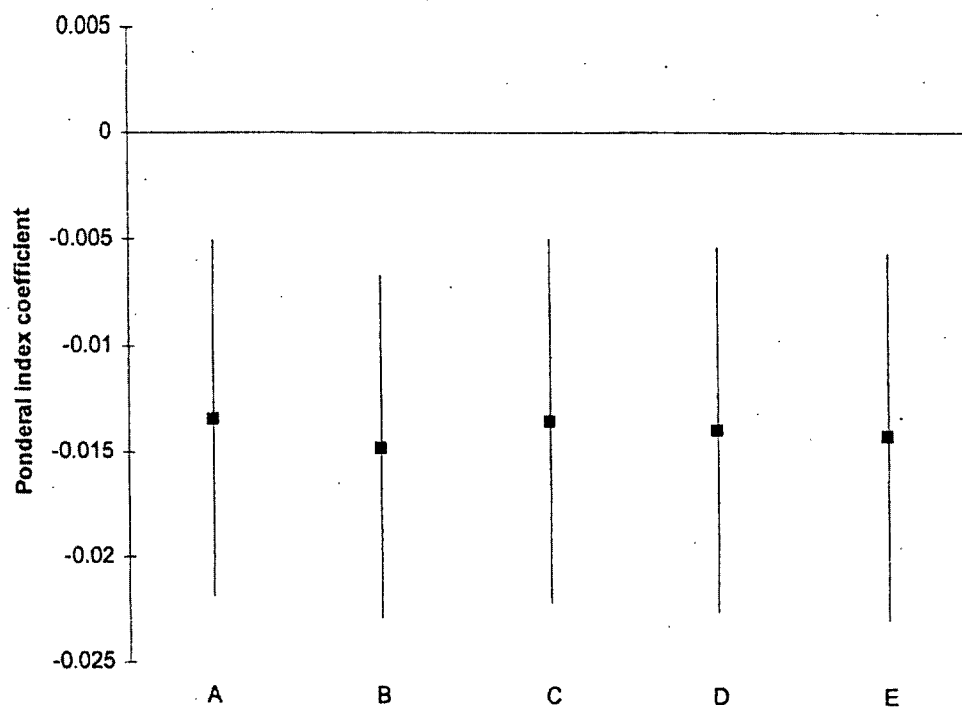


Figure G-5. Coefficients from linear regression of ponderal index on PFOS, with adjustment by different sets of covariates.

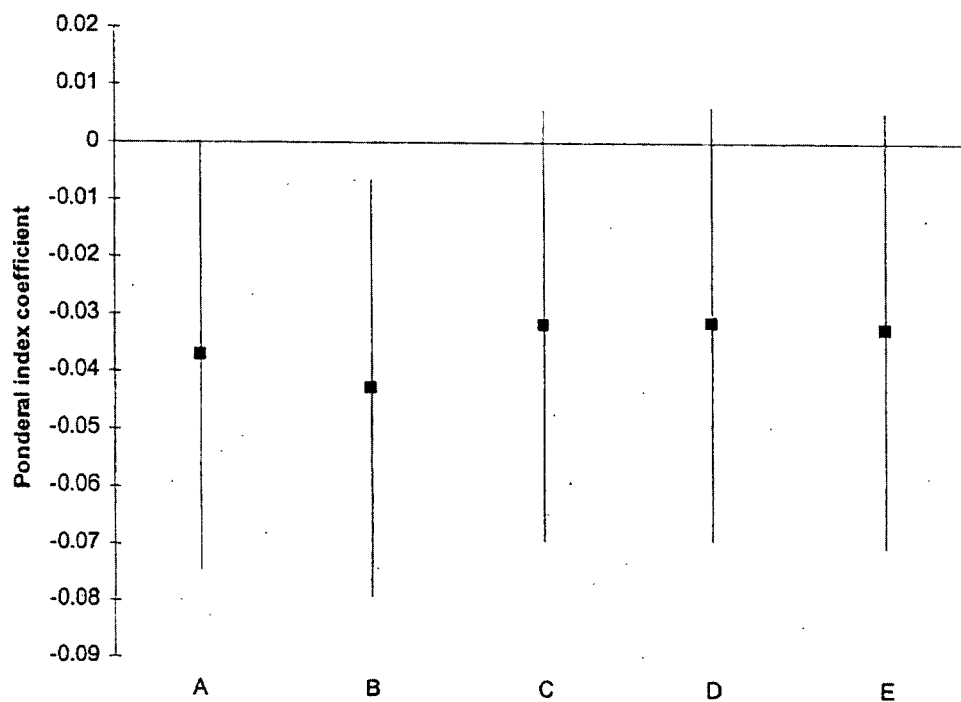


Figure G-6. Coefficients from linear regression of ponderal index on PFOA, with adjustment by different sets of covariates.

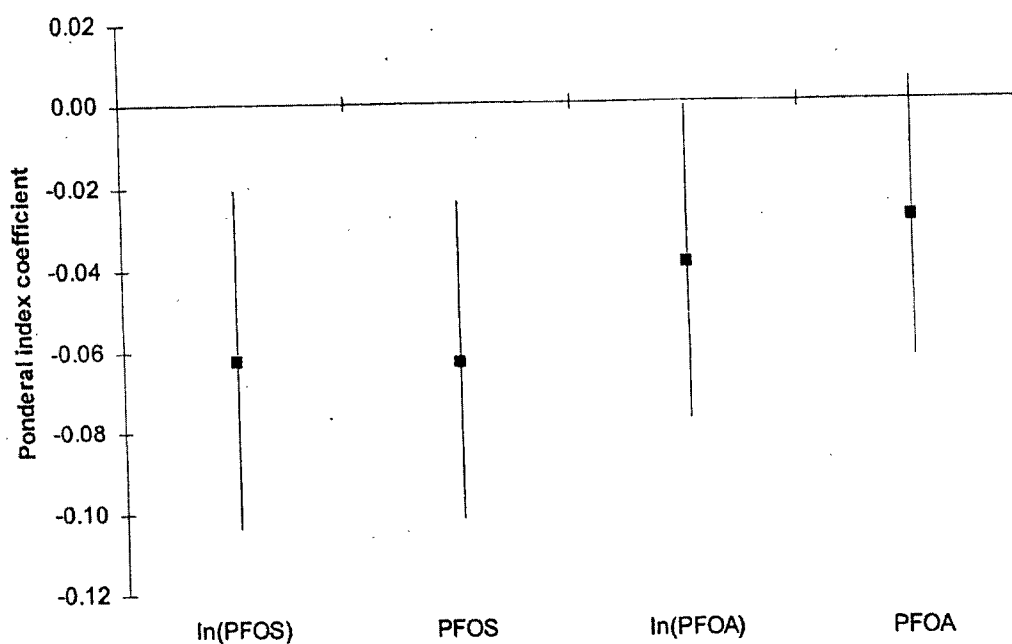


Figure G-7. Adjusted ponderal index coefficients associated with a change equal to the IQR, comparing log-linear and linear models.

Different Birth weight for Length Analyses:

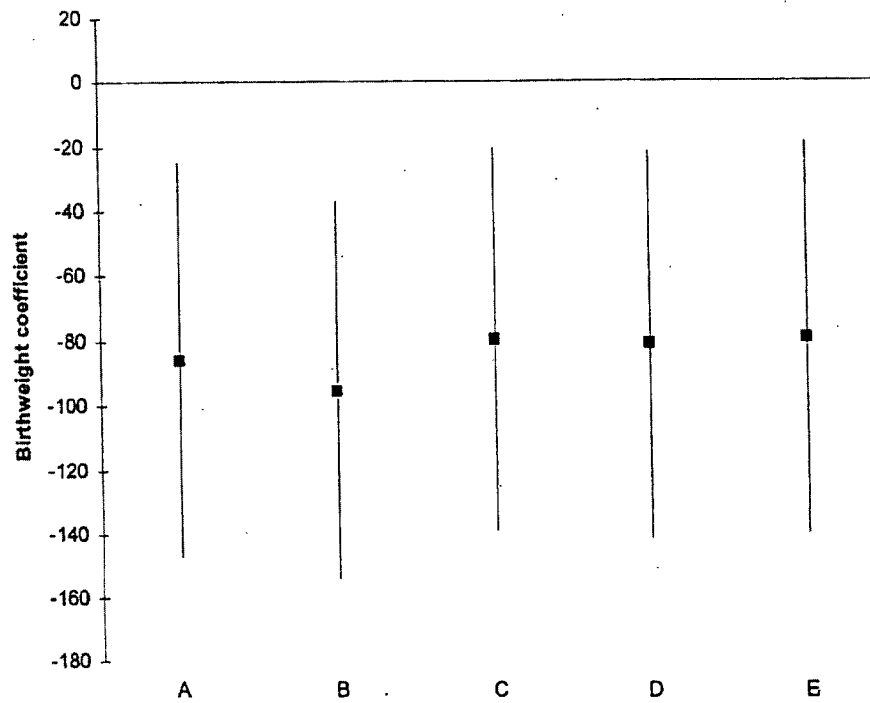


Figure G-8. Coefficients from linear regression of birth weight on ln(PFOS) after adjustment for length, with subsequent adjustment by different sets of covariates.

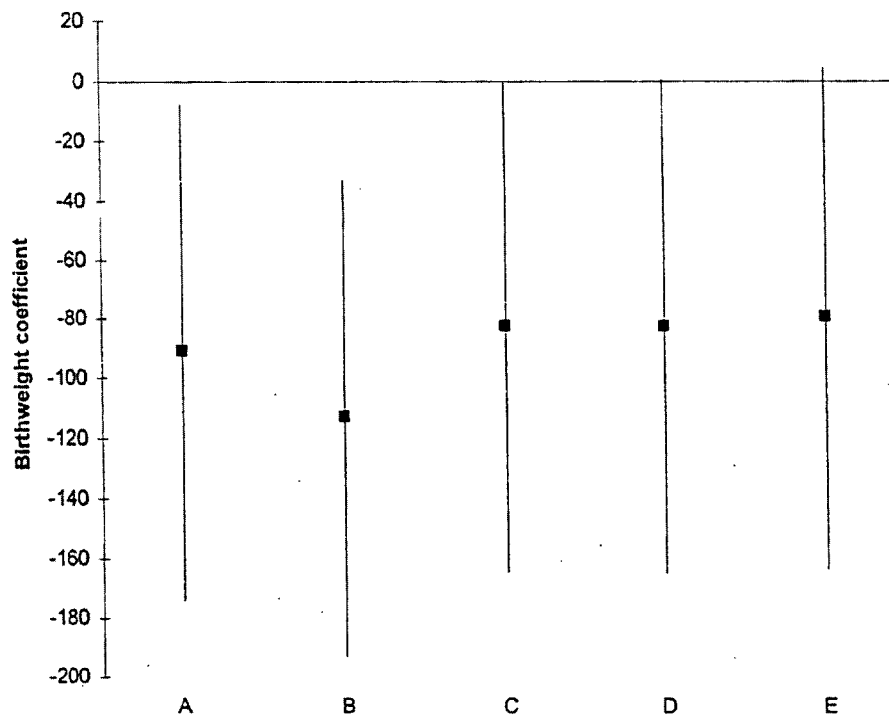


Figure G-9. Coefficients from linear regression of birth weight on ln(PFOA) after adjustment for length, with subsequent adjustment by different sets of covariates.

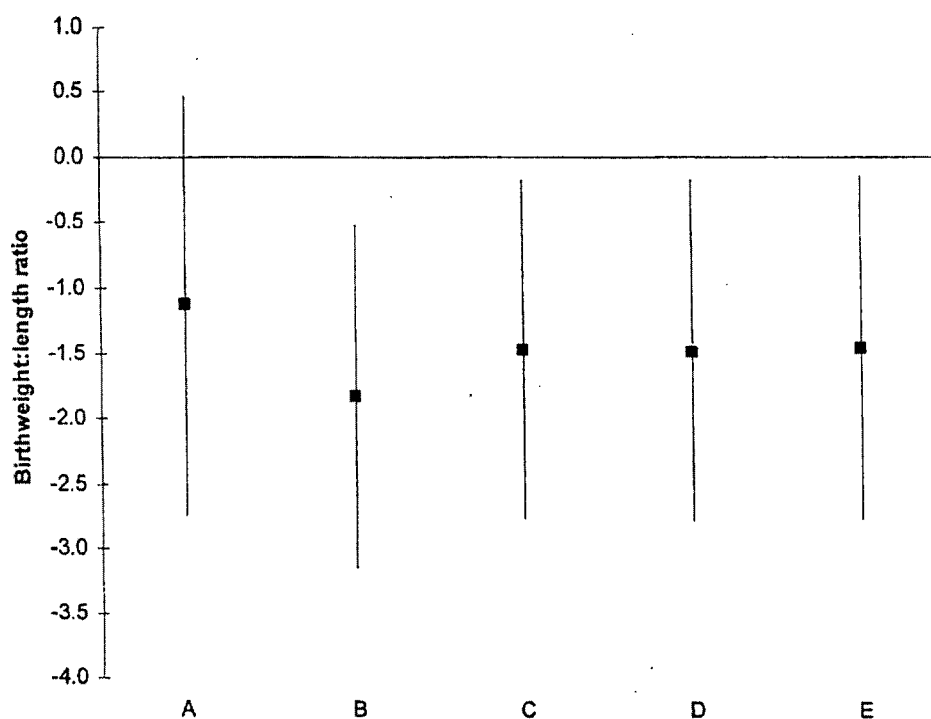


Figure G-10. Coefficients from linear regression of birth weight/length ratio on $\ln(\text{PFOS})$, after adjustment by different sets of covariates.

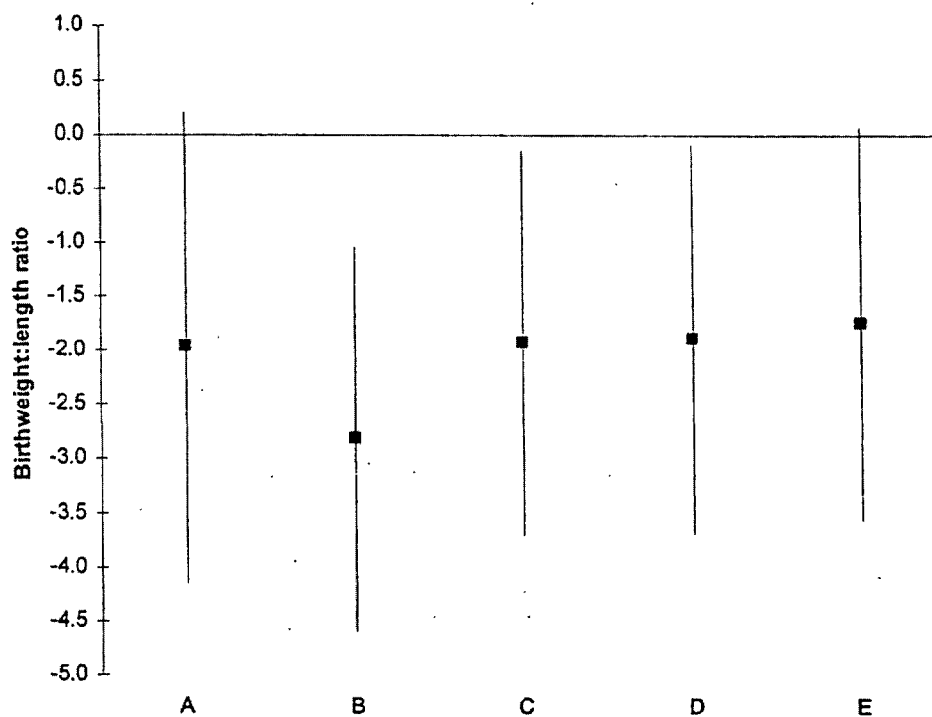


Figure G-11. Coefficients from linear regression of birth weight/length ratio on $\ln(\text{PFOA})$, after adjustment by different sets of covariates.

APPENDIX H

**RELATIONSHIPS BETWEEN PFOS OR PFOA AND
SERUM LIPIDS**

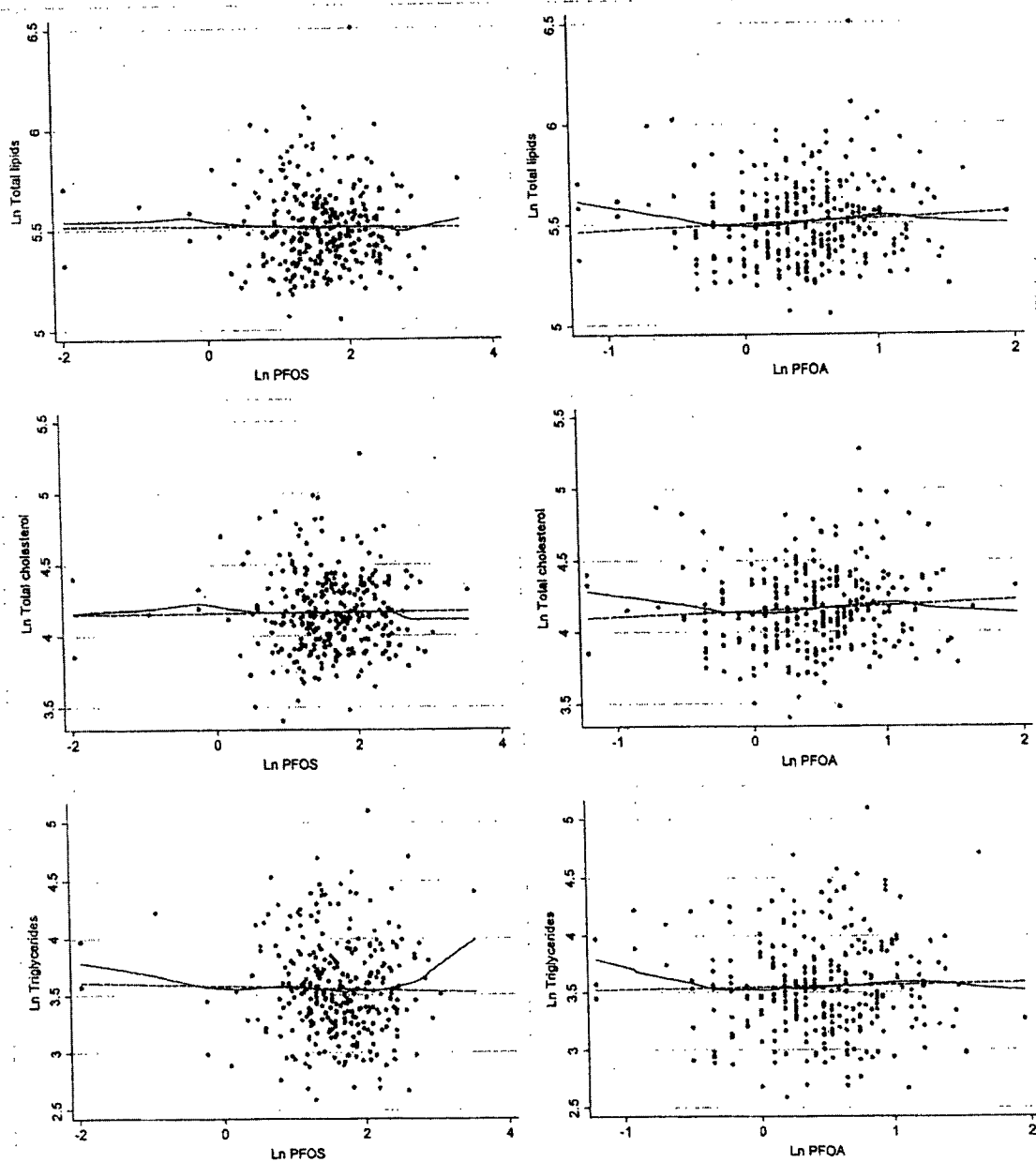


Figure H-1. Association between total serum lipids, total cholesterol, and triglycerides and PFOS and PFOA.

* Solid line based on non-parametric smoothing function. Dashed line denotes predicted linear regression fit.

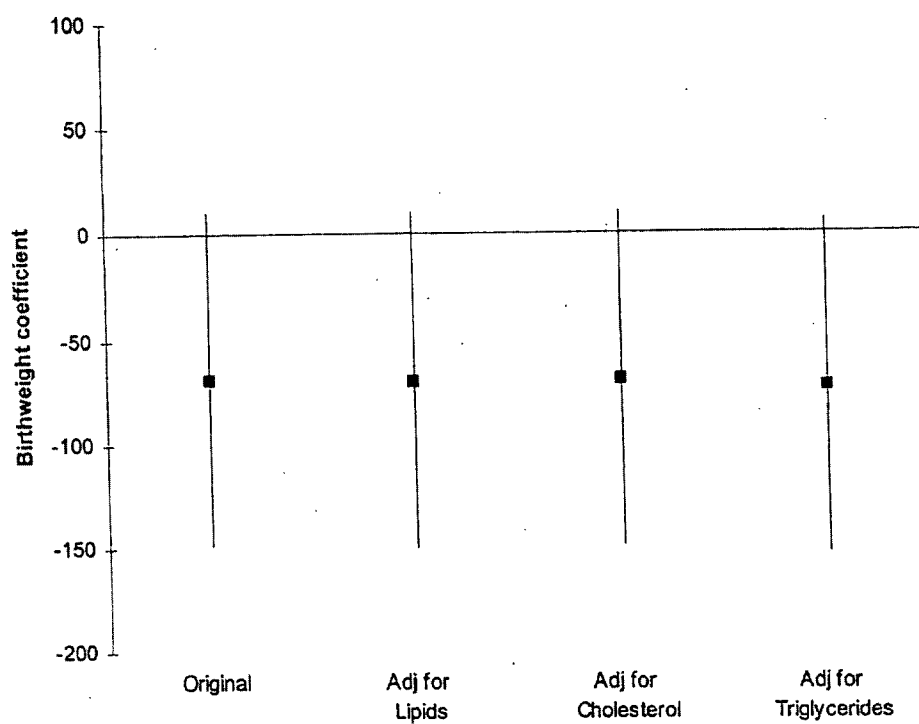


Figure H-2. Coefficients from linear regression of birth weight on $\ln(\text{PFOS})$, before and after adjustment for lipids.

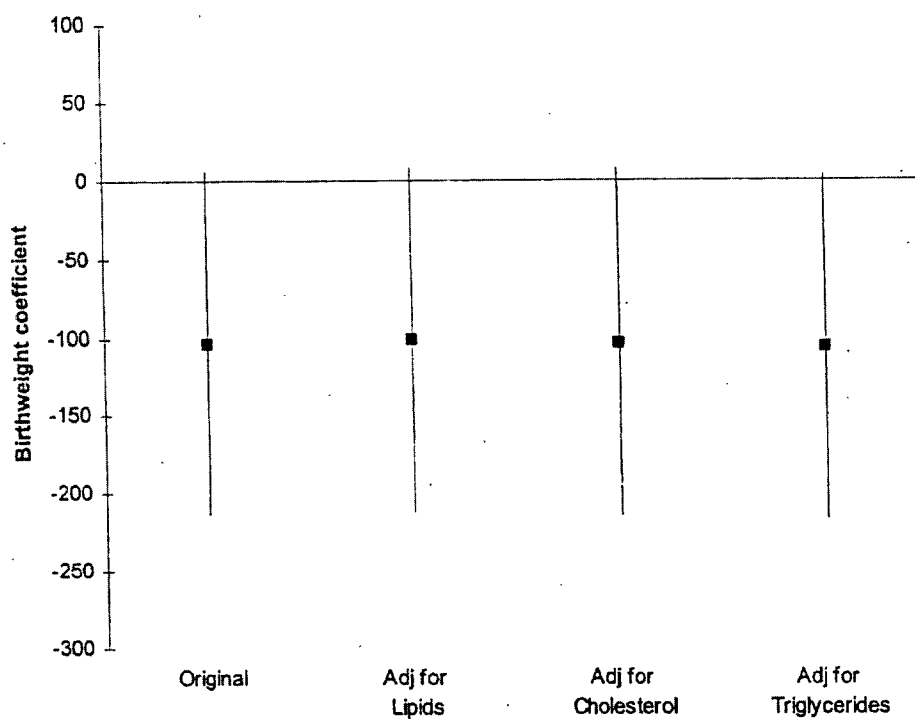


Figure H-3. Coefficients from linear regression of birth weight on $\ln(\text{PFOA})$, before and after adjustment for lipids.

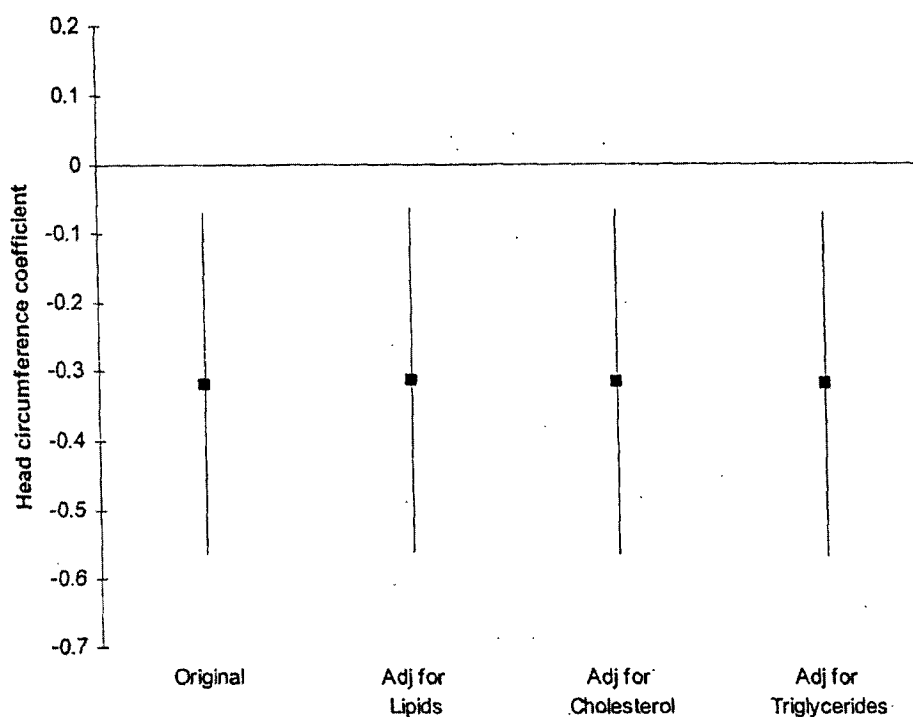


Figure H-4. Coefficients from linear regression of head circumference on $\ln(\text{PFOS})$, before and after adjustment for lipids.

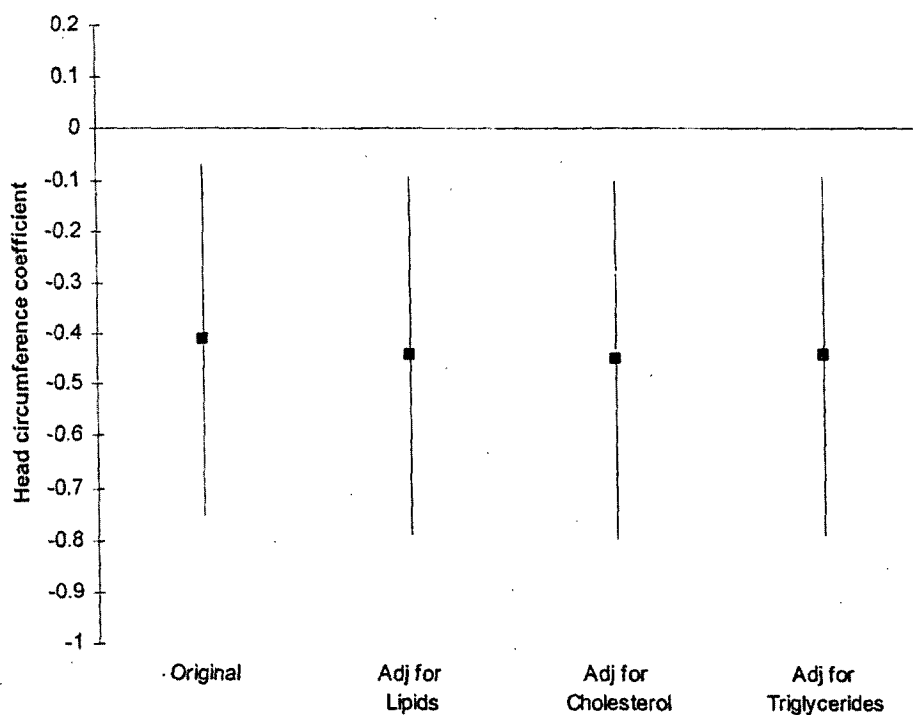


Figure H-5. Coefficients from linear regression of head circumference on $\ln(\text{PFOA})$, before and after adjustment for lipids.

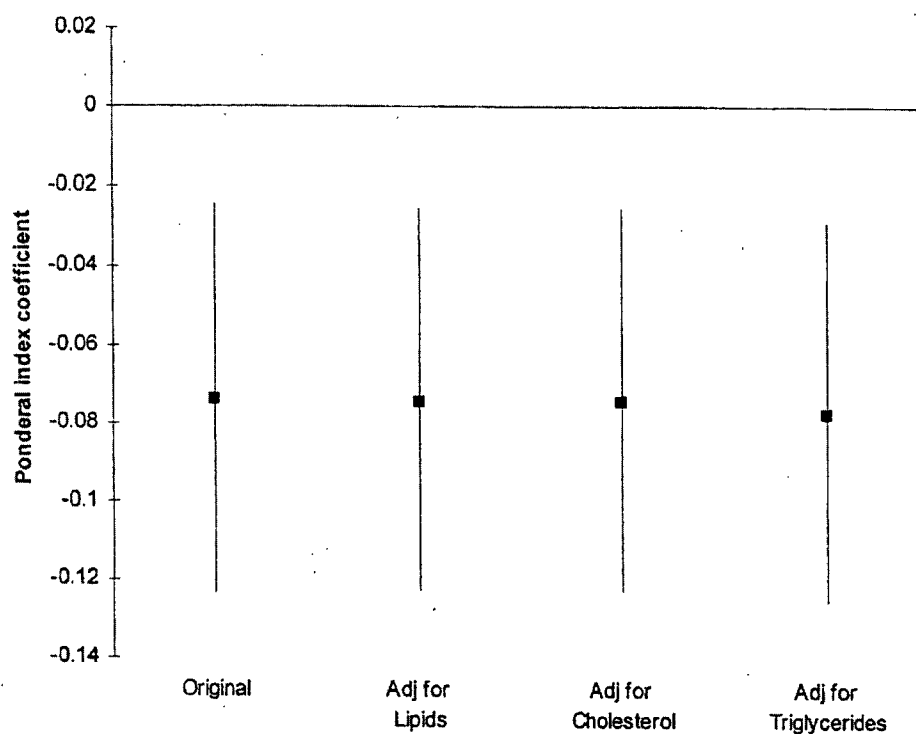


Figure H-6. Coefficients from linear regression of ponderal index on $\ln(\text{PFOS})$, before and after adjustment for lipids.

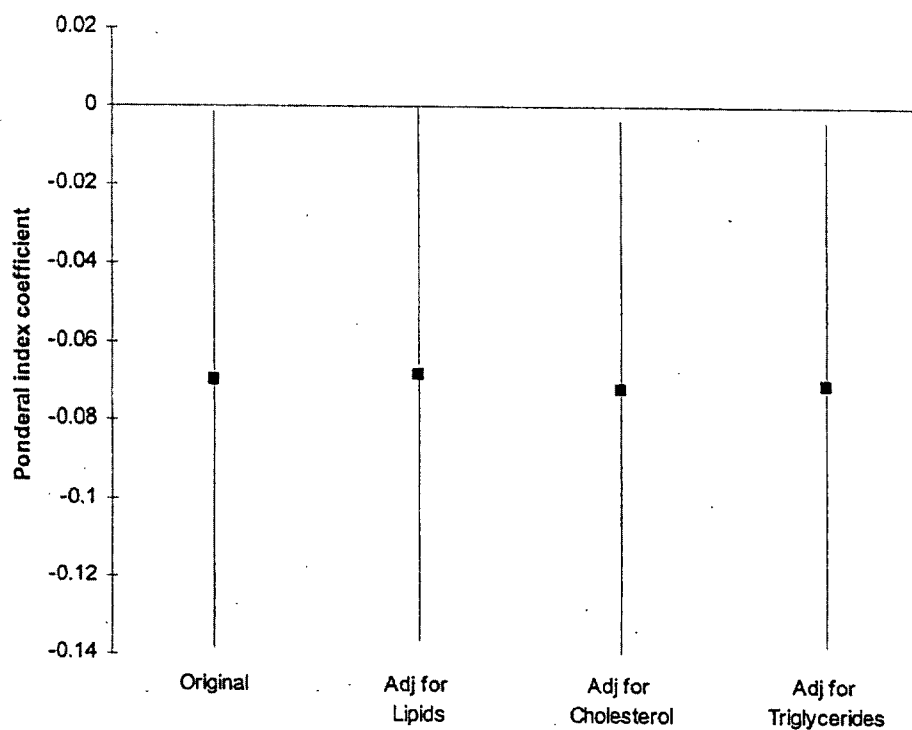


Figure H-7. Coefficients from linear regression of ponderal index on $\ln(\text{PFOA})$, before and after adjustment for lipids.

CURRICULUM VITAE

BENJAMIN J. APELBERG**bapelber@jhsphe.edu**

Birthplace: Baltimore, MD

10/10/1975

EDUCATION**Johns Hopkins Bloomberg School of Public Health, Baltimore, MD**

Doctor of Philosophy, Epidemiology

Dissertation Topic: Fetal exposure to perfluorinated compounds and associations with weight and size at birth.

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Master of Health of Science, Epidemiology

Thesis Topic: Systematic review - Exposure to pets and risk of asthma and asthma-like symptoms.

Certificate awarded in Risk Sciences and Public Policy.

Georgia Institute of Technology, Atlanta, GA

Bachelor of Science, Biology

RELEVANT EXPERIENCE

Institute for Global Tobacco Control

11/2003-present

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

Research Assistant

- Developing a model to estimate the impacts of tobacco control policies on future mortality patterns in the U.S. and Japan.
- Developing an individual risk prediction tool for smokers to examine the impact of continued smoking and quitting on their mortality risk from lung cancer and cardiovascular disease.
- Writing a monograph chapter on the future health implications of tobacco control policies in the U.S.

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD**Teaching Assistant**

Quantitative Methods in Risk Assessment

1/2005-3/2005

Epidemiology and Policy

6/2004

Intermediate Epidemiology

10/2003-12/2003

Occupational Epidemiology

3/2000-5/2000

Abt Associates Inc., Bethesda, MD

7/2000-7/2003

Senior Analyst

- Analyzed the potential health impacts of regulations promulgated by the Environmental Protection Agency (EPA).
- Performed health risk analyses for various EPA offices.
- Created analytical tools to examine the relationships between environmental pollutants and health risk.
- Managed clients, assisted in the recruitment of new employees, and participated in proposal writing.

Pew Environmental Health Commission, Baltimore, MD
Research Assistant

6/1999-5/2000

- Co-authored reports on birth defects and childhood asthma, which highlighted the need for a public health tracking system.
- Collected data on prevalence rates of birth defects, performed literature searches to identify risk factors, and evaluated the effectiveness of state-level birth defect tracking systems.
- Analyzed childhood asthma prevalence rates from national survey data.

PROFESSIONAL ACTIVITIES

American Public Health Association, Member
International Society for Environmental Epidemiology, Member

AWARDS

Student Travel Support Fund in the Department of Epidemiology, 2006
Doctoral Thesis Research Fund, 2005
Genevieve M. Matanoski Fund in Epidemiology, 2005
Department of Epidemiology Tuition Support, 2003-2006

PUBLICATIONS

Apelberg B.J., Buckley, T.J., White R.H. Socioeconomic and racial disparities in cancer risk from air toxics in Maryland. *Environ Health Perspect* 2005 Jun;113(6):693-9.

McCubbin D.R., **Apelberg B.J.**, Roe S., Divita F. Jr. Livestock ammonia management and particulate-related health benefits. *Environ Sci Technol* 2002 Mar 15;36(6):1141-6

Apelberg B., Aoki Y., Jaakkola J. Systematic review: Exposure to pets and risk of asthma or asthma-like symptoms. *J Allergy Clin Immunol* 2001 Mar;107(3):455-60

Goldman L.R., Locke P., **Apelberg B.**, Koduru S. Attack Asthma: Why America needs a public health defense system to battle environmental threats. Pew Environmental Health Commission, Baltimore, MD, 2000.

Goldman L.R., **Apelberg B.**, Koduru S., Sorian R. Healthy from the Start: Why America needs a better system to track and understand birth defects and the environment. Pew Environmental Health Commission, Baltimore, MD, 1999.

PRESENTATIONS

Apelberg B.J., Calafat A.M., Herbstman J.B., Witter F.R., Halden R.U., Kuklennyik Z., Heidler J., Needham L.L., Goldman L.R. Distribution and determinants of perfluorinated compounds in cord blood serum in Baltimore, Maryland. Poster presentation at the *International Conference on Environmental Epidemiology and Exposure*. September 2-6, 2006.

Apelberg B.J., Buckley, T.J., White R.H. Socioeconomic and racial disparities in cancer risk from air toxics in Maryland. Presentation at the *Society for Risk Analysis 2004 Annual Meeting*. December 5, 2004.

Litt J.S., Tran N.L., Malecki K., Neff R., Resnick B.A., Burke, T.A., **Apelberg B.J.**, Wismann A., Nachman K. Identifying priority health conditions, environmental data, and infrastructure needs: A synopsis of the Pew environmental health tracking project. Presentation at the *APHA 132nd Annual Meeting and Exposition*. November 10, 2004

Apelberg B.J., Cunningham K., Mudarri D., Heil M. Asthma related cost savings through asthma management plans and environmental trigger avoidance. Presentation at *Indoor Air 2002 – The 9th International Conference on Indoor Air Quality and Climate*. June 30 - July 5, 2002.

McCubbin D.R., **Apelberg B.J.**, Roe S., Divita F. Jr. Livestock ammonia management and particulate-related health benefits. Presentation at the *2nd International Nitrogen Conference*. October 14-18, 2001.

CONTAINS NO CBI