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Kristin J. Marks	Date

Maternal diet, environmental contaminants, and growth and developmental outcomes in offspring

By

Kristin J. Marks
Doctor of Philosophy

Epidemiology

_____

Terryl J. Hartman, PhD, MPH, RD
Chair

_____

Penelope P. Howards, PhD, MS
Co-Chair

_____

W. Dana Flanders, DSc, MD, MPH, MA
Committee Member

_____

Melissa M. Smarr, PhD
Committee Member

_____

Kate Northstone, PhD, MSc
Committee Member

Accepted:

_____

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

_____
Date

Maternal diet, environmental contaminants, and growth and developmental outcomes in offspring

By

Kristin J. Marks
MPH, Emory University, 2016
BA, Colby College, 2012

Advisors: Terryl J. Hartman, PhD, MPH, RD and Penelope P. Howards, PhD, MS

An abstract of
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Abstract

Maternal diet, environmental contaminants, and growth and developmental outcomes in offspring

By Kristin J. Marks

Exposure to endocrine disrupting chemicals (EDCs) is ubiquitous. Diet, particularly food of animal origin, is considered the most important contributor to persistent EDC exposure. EDC exposure, especially during critical periods of development like the prenatal window, may interfere with the body's endocrine system, which can affect growth and development. Most studies have examined one EDC at a time in relation to disease; however, humans are exposed to many EDCs. By studying mixtures, the human experience can be more closely replicated. This dissertation identified maternal dietary patterns that contributed to persistent EDC exposure (poly- and perfluoroalkyl substances (PFAS), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs)) and investigated the associations of prenatal exposure to persistent EDCs as mixtures with birth size, postnatal body size, and early menarche among girls. First, we identified maternal dietary patterns that are associated with persistent EDC exposure. More frequent consumption of cheese was associated with higher PFAS concentrations, more frequent consumption of white fish and rice with higher PCB concentrations, and more frequent poultry and white fish consumption with higher OCP concentrations. These patterns explained 8% to 20% of the total variance in EDC concentrations. Next, we investigated the association of prenatal exposure to persistent EDCs as a mixture with size at birth and postnatal body size. We found that PFAS, PCB, and OCP mixtures were inversely associated with lower birth weight. Further, we found inverse associations of a 31-chemical persistent EDC mixture with postnatal body size (weight-for-age z-scores) through 19 months driven by early postnatal body size. Lastly, we investigated the association of prenatal exposure to persistent EDCs as a mixture with early menarche (<11.5 vs. ≥11.5 years), and found no association. The results from this dissertation provide insight into maternal diet during pregnancy as a modifiable source of EDC exposure. Moreover, this dissertation estimates the overall effect of prenatal exposure to mixtures of persistent EDCs on growth and developmental outcomes, which gives a more complete estimate of the magnitude of the effects than under the single-chemical paradigm. Results from this work will inform public health strategies designed to improve maternal and child health.

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Chapter 1 Introduction and Overview

Background and Public Health Importance

Diet, particularly food of animal origin, is considered the most important contributor to persistent EDC body burden in the general population (1, 2). Contaminated foods account for up to 90% of total exposure to persistent organic pollutants (3). Given the increasing interest in the association of EDCs with health outcomes, particularly in infants and children (like fetal growth, neurodevelopment, pubertal development, and obesity), it is important to identify modifiable sources of exposure, such as diet. EDCs can cross the placenta, resulting in in utero exposure during critical periods of development, and have been implicated in many outcomes among offspring. To date, most studies have examined one EDC at a time in relation to disease, and it is thought that this has led to inconsistent results on the association between prenatal exposure to EDCs and growth and developmental outcomes like birth weight and age at menarche in offspring. Humans are exposed to many EDCs. By studying combined exposures, or "mixtures," the human experience can be more closely replicated (4). In this context, the National Institute of Environmental Health Sciences (NIEHS) defines an environmental mixture as a combination of three or more independent chemicals or chemical groups (5).

In this dissertation, we first explored the use of dietary pattern analysis to characterize mixtures of persistent EDCs consumed through diet, the main source of exposure to persistent EDCs (3). Pattern analysis has previously been used in populations to identify dietary patterns that contribute to human exposure to persistent EDCs. Significant costs and time could be saved if groups with high EDC contamination could be identified through food frequency questionnaire (FFQ) data in populations without chemical analyses available. Furthermore, with more research, it is possible that these pattern(s) could inform dietary guidelines for pregnant women. To better understand prenatal exposure to EDCs, we identified dietary pattern(s) that contribute to mixtures

of persistent EDCs among pregnant women and investigate associations of mixtures of EDCs with growth and developmental outcomes in offspring through the three aims of this dissertation.

This research should lead to improved understanding of maternal diet during pregnancy as a potential source of EDC exposure and the impact of prenatal exposure to mixtures of persistent EDCs on growth and developmental outcomes. This could lay the groundwork for the opportunity to update dietary guidelines for pregnant women and subsequently improve child health. Further, examining EDCs as mixtures can assist in identifying the most harmful chemicals, predominant sources of exposure, or exposure patterns, which can inform policies surrounding EDC reduction as well as behavior changes at an individual level.

Study Motivation

Humans are exposed to a large number of environmental chemicals across the lifespan, through diet and other pathways. Exposure to these chemicals, especially EDCs, is widespread, multisource, and multi-route (6). Studies indicate that the pattern of exposure in pregnant women and neonates is chronic, low-dose, and involves multiple chemicals rather than chemicals in isolation (7-9). Many chemicals are potentially toxic, but little is known about health effects from exposure to complex mixtures of chemicals (10). By examining chemical mixtures, as opposed to operating under the single-chemical paradigm of previous research, it may be possible to more accurately identify risk factors for outcomes with environmental origins and develop more targeted public health interventions. The same holds true for identifying maternal dietary patterns associated with high EDC concentrations as opposed to individual foods or nutrients: a more complete picture of diet allows for consideration of interactions and identification of foods strongly associated with high EDC concentrations, while being able to make recommendations for a low-EDC diet at a pattern-level. In sum, the motivation for this dissertation was to move beyond the single-chemical or single-food paradigms that have limited previous research of environmental and dietary exposures and to gain a more comprehensive understanding of the associations of

maternal diet, prenatal exposure to persistent EDCs, and growth and developmental outcomes among offspring.

Study Population

This research is based on secondary data analysis from the Avon Longitudinal Study of Parents and Children (ALSPAC) in all aims and uses data from the Norwegian Mother, Father, and Child Cohort Study (MoBa) in the first aim.

Avon Longitudinal Study of Parents and Children (ALSPAC)

ALSPAC is a prospective birth cohort of 14,541 pregnancies. ALSPAC enrolled pregnant women with an expected delivery date between 1 April 1991 and 31 December 1992 from three health districts in the former county of Avon, Great Britain. Information was collected on these parents and children through clinic visits, interviews, and mailed questionnaires. A nested case-control study (n=448) was conducted within ALSPAC to explore associations of prenatal maternal concentrations of suspected EDCs (measured at median 15 weeks gestation) and age at menarche among the daughters. The concentrations of various EDCs were measured at the Centers for Disease Control and Prevention's (CDC) National Center for Environmental Health (NCEH). The study design has been described in detail elsewhere (299). Briefly, from the original base population of 14,062 live births, case and control series were selected from singleton female subjects who had completed at least two puberty staging questionnaires between the ages of 8 and 13 (five possible questionnaires returned). Girls meeting eligibility criteria were ordered according to reported age at menarche. A cut-off of 11.5 years was established as defining 'early' menarche to satisfy sample size and power needed for the case-control study. Eligible cases could complete any two questionnaires in the series, provided that one was completed after menarche, while controls had to complete the 13-year old questionnaire in order to ascertain that menarche had not occurred by the cutoff of 11.5 years.

Norwegian Mother, Father, and Child Cohort Study (MoBa)

MoBa is a prospective population-based pregnancy cohort study which recruited from hospitals and maternity units across Norway from 1999-2008 (357). The cohort includes 109,000 children and 91,000 mothers. Women giving their consent received three questionnaires during pregnancy (including an FFQ), and additional questionnaires after birth. Biological samples were collected from mothers at roughly 17 to 18 weeks gestation (358). MoBa data is supplemented with birth characteristics data from the Medical Birth Registry of Norway (MBRN) (359). A subsample (n=278) of the MoBa cohort mothers had PFAS, PCBs, and OCPs measured through the European Union's Human Early Life Exposome (HELIX) initiative (360). The aim of the HELIX study was to measure and describe multiple environmental exposures during early life (pregnancy and childhood) in a prospective cohort and associate these exposures with molecular omics signatures and child health outcomes. The HELIX study represents a collaborative project across six established and ongoing longitudinal population-based birth cohort studies in six European countries (France, Greece, Lithuania, Norway, Spain, and the UK).

Study Aims

Broad, Long-Term Goal(s): *To investigate the association between persistent endocrine disrupting chemicals (EDCs) as a mixture among pregnant women and their children's growth and development, and to identify maternal dietary pattern(s) that contribute to EDC mixtures in order to inform maternal dietary recommendations (***Figure 1-1***).*

Aim 1: A) Identify maternal dietary pattern(s) that contribute to EDC exposure. We will describe the association of maternal diet (as measured through an FFQ during pregnancy) and serum concentrations of persistent EDCs (poly- and perfluoroalkyl substances (PFAS), organochlorine pesticides (OCPs), and polychlorinated biphenyls (PCBs)) as measured through biomarker indicators in pregnant women. We will identify dietary pattern(s) that contribute to

exposure to a mixture of persistent EDCs in a substudy (n=448 mothers) within the Avon Longitudinal Study of Parents and Children (ALSPAC) and the Norwegian Mother, Father, and Child Cohort Study (MoBa) (n=278 mothers).

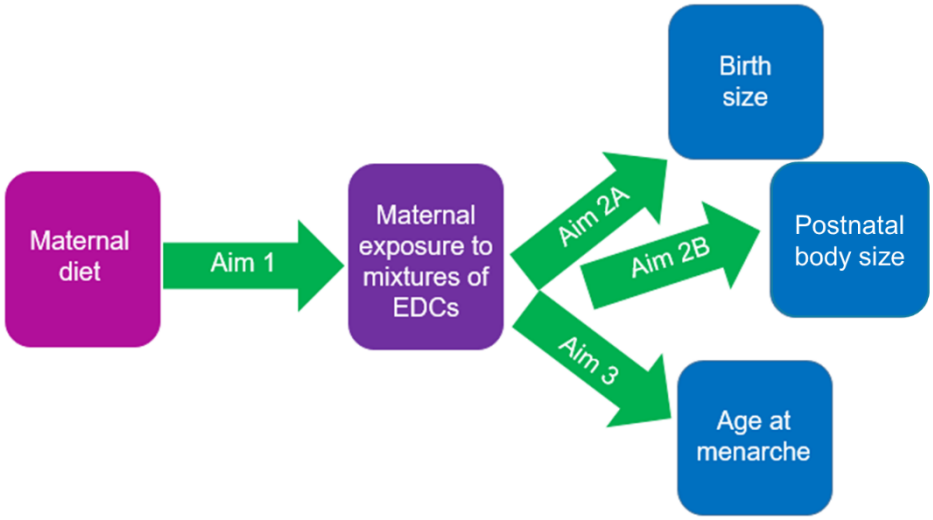

Figure 1-1. Hypothesized causal path relating project aims.

Hypothesis: Diets high in meat, poultry, fish, eggs, and dairy are associated with higher EDC exposure.

Aim 2: Investigate the association of prenatal exposure to persistent EDCs as a mixture with A) size at birth (birth weight, crown to heel length, head circumference, ponderal index) and B) postnatal body size (height and weight at 0, 2, 9, and 20 months) among female offspring. We will assess the association of maternal biomarkers of persistent EDCs as a mixture with size at birth and postnatal body size as documented in medical records and routine child health surveillance.

Hypothesis: High prenatal exposure to persistent EDCs is associated with smaller size at birth.

Aim 3: Investigate the association of prenatal exposure to persistent EDCs as a mixture with age at menarche among female offspring. We will assess the association of maternal biomarkers of persistent EDCs as a mixture with age at menarche as ascertained through questionnaires mailed annually from 8-17 years old.

Hypothesis: High prenatal exposure to persistent EDCs is associated with earlier age at menarche.

Chapter 2 Background and Literature Review

Converging lines of evidence suggest that diet may be an important route of exposure to environmental contaminants. Some potentially harmful diet-associated contaminants, including endocrine disrupting chemicals (EDCs) (see **Appendix A** for list of abbreviations), are pervasive in the environment. Contaminants in foods may come from the application of pesticides to crops, the transport of industrial chemicals in the environment, and chemicals used in food packaging products (11). A number of persistent environmental contaminants tend to accumulate in animals, and are commonly found in meat, poultry, fish, and dairy products. Other contaminants, such as a variety of pesticides, are often found in fruits, vegetables, and other agricultural commodities. Some contaminants in food, such as methylmercury, have naturally occurring and manmade sources. The health risks associated with these contaminants are dependent on both the level of the contaminant in the food as well as the amount of the food consumed by individuals (11).

Given the increasing interest in the association between environmental contaminants and health outcomes, it is important to identify major sources of exposure, such as dietary sources. This information could contribute to our further understanding of the association between dietary exposures and specific health outcomes. Further, this evidence could be used to guide dietary recommendations tailored to prevent disease.

Existing evidence links a variety of foods and food packaging and preparation methods to contaminants. The first section of this review aims to describe contaminants, how they enter the food chain, major dietary sources of the contaminants, and select health effects among infants and children.

Heavy Metals

Methylmercury

Mercury is a naturally occurring element that is released into the environment by a variety of sources such as coal combustion and volcanoes, and mercury may enter bodies of water

through direct release or through emissions to the atmosphere that are subsequently deposited to surface waters. Bacteria in bodies of water convert the deposited mercury into methylmercury (12), which can be absorbed by small aquatic organisms that are then consumed by predators like fish (13). As organisms build up methylmercury in tissue, and as smaller fish are eaten by larger fish, concentrations of methylmercury bioaccumulate, especially in large fish with long lifespans such as sharks and swordfish (14-17) (**Figure 2-1**). Thus, fish are the main dietary source of methylmercury exposure, though exposure varies greatly by type of fish, portion sizes, and frequency of consumption (18).

The Environmental Protection Agency has determined that methylmercury can have neurotoxic and developmental effects in humans (19). Studies show that populations with prenatal exposure to methylmercury through regular consumption of fish have reported subtle detrimental effects on childhood neurological development (20-24). There has been a fair amount of debate on the topic of pregnant women consuming fish: while ingestion of methylmercury in fish may be harmful, there are other compounds naturally present in fish that are extremely beneficial, like omega-3 fatty acids, which contribute to healthy infant and child development (25). Pregnant women are advised to consume dietary sources of omega-3 fatty acids, including fish; however, levels of methylmercury and omega-3 fatty acids vary considerably by species, thus the type of fish, portion size, and frequency of consumption are all important considerations in weighing the health benefits of fish and the extent of methylmercury exposure (25).

Arsenic

Arsenic is a ubiquitous metalloid found in organic and inorganic forms in nature, with various forms of arsenic possessing a range of toxicities. Both inorganic and organic forms of arsenic accumulate in rice (26) through the silicon transport system (27) because arsenous acid, which is the predominant form of arsenic in flooded rice paddies, is indistinguishable from silicic acid to the rice plant. Contaminated drinking water is also a well-recognized source of inorganic arsenic (28), but diet is the primary exposure route for people with limited exposure via drinking

water (29). Seafood, rice, mushrooms, and poultry are considered the primary food sources of arsenic exposure (30-33). Fetal exposure to inorganic arsenic has been associated with low birth weight, increased risk of infection, and higher infant mortality in more highly exposed populations (34-36).

Non-persistent EDCs

Bisphenol A

Bisphenol A (BPA) is an EDC that is used in the inner liners of metallic as well as in polycarbonate plastics often used in food and drink containers (37, 38). The primary route of exposure to BPA is through diet; BPA migrates from food and drink containers, particularly when containers are heated (39-41). At high doses, BPA has demonstrated developmental effects in laboratory animals, though the effects of BPA at lower doses, similar to human exposure levels, are the subject of debate (40, 42-46).

Studies have found that elevated prenatal BPA exposure can increase the risk of low birth weight (47, 48), though results are mixed and may differ by the child's sex (49). Similarly, studies have demonstrated links between prenatal BPA exposure and neurobehavioral outcomes, with stronger associations existing among boys (50, 51). Given the existing literature on BPA, the National Toxicology Program in 2008 determined that there was "some concern" (the midpoint on a five-point scale ranging from "negligible" to "serious" concern) for the effects of BPA on fetuses and infants (40). While there is still some uncertainty about the health effects of BPA at typical exposure levels, several manufacturers have begun phasing out baby products that contain BPA, and several states have banned or limited BPA in food containers and consumer products.

Phthalates

Phthalates are commonly used to increase the flexibility of plastics in a wide array of consumer products, including food packaging (52-55). Phthalates have been found at higher levels in fatty foods such as dairy products, fish, seafood, and oils, as these foods are most likely

to absorb phthalates (55). For example, fast food consumption is positively associated with phthalate concentrations (56). Some phthalates are suspected EDCs; a number of reproductive and developmental effects of phthalates, such as hypospadias and decreased anogenital distance among males, have been found in animal studies (57-66) and epidemiological studies in humans (67-70). Further epidemiological studies of phthalates are needed to assess the effects of phthalates on reproductive toxicity, overweight and obesity, insulin resistance, skeletal anomalies, allergy and asthma, and cancer (71).

Phytoestrogens

Phytoestrogens are estrogenic compounds that occur naturally in plants, with the most common source being soybean products (72), though red clover, flax, licorice, hops, alfalfa, ginseng, and evening primrose oil are also common phytoestrogen-containing foods, among others (73). A litany of health benefits such as decreased risk of osteoporosis, heart disease, breast cancer, and menopausal symptoms (74-78) are frequently attributed to soy foods because they contain phytoestrogens, but there is growing concern that phytoestrogens are also EDCs with the potential to cause adverse health effects (79). As estrogen mimics, it is suspected that phytoestrogens can affect sexual development, including altered pubertal timing. Studies in animal models demonstrate several adverse effects of phytoestrogens on the reproductive system, including premature pubertal onset (80, 81), reduced fertility (82, 83), and altered estrous cyclicity (81, 84). In human studies, prenatal exposure to phytoestrogens (85), soy infant formula use (86-88), and childhood exposure to phytoestrogens (89) have been linked to altered age at menarche.

Atrazines

Atrazine is a commonly used herbicide (90), with the greatest use on corn, sorghum, and sugarcane (91). The European Union (EU) banned it in 2003, though it is still used in over 70 countries globally, including the US (92-94). The general population is most often exposed to

atrazine through contaminated food or drinking water. Through water run-off from crops and lawn applications, atrazine can get into ground and surface waters and contaminate drinking water (91, 94). Atrazine is the most frequently detected pesticide in surface water in the United States (91).

Atrazine exposure has been linked to adverse birth outcomes such as intrauterine growth restriction (95), low birth weight (95, 96), preterm delivery (97), small for gestational age (96, 97), spontaneous abortions (98), and several birth defects (99), specifically abdominal wall defects (100). Animal studies suggest that exposure to atrazine and its metabolites delays the onset of puberty in female and male rats (101-106), though a study of prenatal atrazine exposure in humans found a weak association between diaminochlorotriazine (DACT), an atrazine analyte, and early menarche (107).

Organophosphate pesticides

Agricultural crops are often treated with pesticides to control insects and other pests that might interfere with crop growth. There are different classes of pesticides used on food crops, including organophosphate pesticides. Pesticide residues may remain on crops after they are harvested, and apples, corn, oranges, rice, and wheat are common sources of organophosphate pesticide exposure (11). Studies have reported associations between prenatal organophosphate pesticide exposures and a variety of neurodevelopmental deficits in childhood, including reduced IQ, perceptual reasoning, and memory (108-110). Since 1999, the Environmental Protection Agency has imposed restrictions on the use of certain organophosphate pesticides on certain food crops and around the home, largely due to concerns about potential exposures of children (111-113).

Persistent EDCs

Dioxins

Dioxins are mainly byproducts of industrial practices. They are produced through a variety of incineration processes, including improper municipal waste incineration and burning trash, as

well as through natural processes such as forest fires and volcanoes (114, 115). Exposure to dioxins and dioxin-like compounds is ubiquitous. Strict regulatory controls on major industrial sources of dioxin have reduced emissions into the air by 90% compared to levels in 1987 (116). Today, 90% of human exposure is through food, in particular animal products (117). Because dioxins are absorbed and stored in fat tissue, they accumulate in animals, who eat plants, which contain chemical residues due to contaminated water or soil. Human consumption of animal products is an example of biomagnification, the increasing concentration of a chemical in the tissues of organisms at successively higher levels in a food chain (**Figure 2-1**).

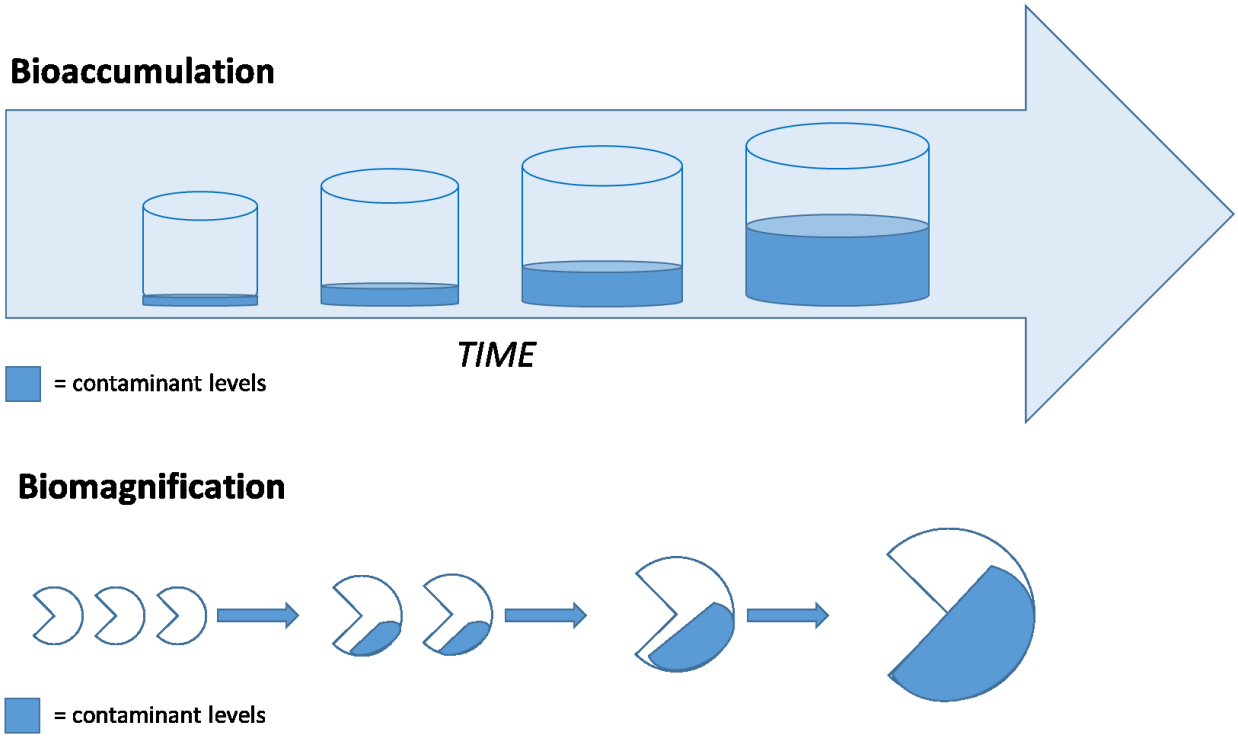

Figure 2-1. Bioaccumulation and biomagnification.
Bioaccumulation and biomagnification are two distinct processes that frequently occur in tandem. Bioaccumulation occurs when contaminants enter the food web by building up in individual organisms, while biomagnification occurs when contaminants move from one trophic level of the food web to the next, thereby increasing in concentration (118).

The dioxin 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is a known carcinogen (119). Dioxin exposure has been linked to a number of other diseases, including type 2 diabetes, ischemic heart disease, and an acne-like skin disease called chloracne, which is a hallmark of

acute dioxin exposure. Further, dioxins can cause developmental problems in children, lead to reproductive and infertility problems in adults, result in miscarriages, damage the immune system, and interfere with endocrine function (116, 117).

Organochlorine pesticides

Organochlorine pesticides (OCPs) were widely used during the boom in industrial production after World War II, when thousands of synthetic chemicals were introduced into commercial use for pest and disease control (120). OCPs have been used as fungicides and as insecticides on agricultural crops and to control insects that carry diseases such as malaria and typhus (121-123). OCPs are lipophilic and bioaccumulate through the food chain (123). Diet, particularly meat, fish, and dairy products, is the primary source of exposure to OCPs (124). Twelve persistent organic pollutants (POPs), including the OCPs dichlorodiphenyltrichloroethane (DDT) and hexachlorobenzene (HCB), were banned globally in a 2001 treaty at the Stockholm Convention on Persistent Organic Pollutants (125).

Exposure to OCPs has been associated with neurological, reproductive, and developmental health effects (121-123). DDT has been found to be associated with pre-term birth, miscarriages, spontaneous abortion, and reduced height in children (123, 126-128). Similarly, one study found β-hexachlorocyclohexane (HCH) exposure to be associated with intrauterine growth retardation in infants of exposed mothers (129). Studies have found HCB exposure increased risk for undescended testis and impaired development of locomotive skills (123).

Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are a group of persistent chemicals used for insulating, lubricating, and building purposes. PCBs were banned in 1979 in the United States, though are still allowed to be used in certain electrical equipment today. Because of their persistent nature, PCBs remain in the environment and accumulate in fat tissue, therefore are often found in foods derived from animals. While fish have higher levels of PCBs, foods with lower PCB levels but

more frequent consumption like meat, dairy, and poultry products also contribute to PCB exposure (130, 131). PCB levels in beef and chicken declined between 2002 and 2008, though levels in turkey and pork remained relatively consistent during those years (132). Exposure to PCB remains widespread (133, 134), though declining environmental levels of PCBs suggest that younger generations are exposed to lower levels of PCBs compared to older generations (130, 135-138).

Through studies of populations that regularly consume fish, prenatal PCB exposure has been found to be associated with adverse effects on children's neurological development and impaired immune response (139-141). Consequently, the Agency for Toxic Substances and Disease Registry has determined that there is substantial data which suggests that PCBs play a role in neurobehavioral alterations observed in newborns and children of women with PCB burdens near background levels (130). Additionally, studies have shown that prenatal PCB exposure is modestly associated with low birth weight (142, 143).

Perfluoroalkyl substances

Perfluoroalkyl substances (PFAS) are used in food packaging and the production of nonstick coatings on cookware (144, 145), among other uses. Some PFAS have already been phased out by the chemical industry, though the persistence of these chemicals means that they will remain in the environment and human bodies for many years. While there are numerous routes of exposure to PFAS, recent studies have identified food consumption as the primary exposure pathway (146, 147). PFAS-treated food-contact packaging, like microwave popcorn bags, may be a source of PFAS exposure (148, 149). As is the case with BPA, heating these materials may cause PFAS to migrate into food. In addition to exposure through containers and packaging, meats may also be contaminated with PFAS due to exposure of source animals to air, water, and feed contaminated with PFAS (149-151). PFAS have also been detected in some plant-based foods (147).

In a Norwegian study of adult men and women, PFAS concentrations in serum were associated with a number of dietary factors: lean fish, fish liver, shrimp, and meats. Fish and shellfish contributed 38% of the estimated dietary intake of PFOA. Further, PFAS levels were associated with age, breastfeeding history, and area of residence (152).

Animal studies have demonstrated the reproductive and developmental toxicity of PFAS (153, 154). Results from human health studies have been mixed, with some studies reporting associations between prenatal PFAS exposure and adverse birth outcomes such as low birth weight (155-158), while other studies have not (159, 160). Further, PFAS exposure has been linked to fecundability among parous women, but it is not clear if the association is causal (161).

Flame Retardants

Flame retardants are used in many applications, including furniture foam, small appliances, and electronic products to slow the ignition and rate of fire growth. There are two common classes of flame retardants: polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyls (PBB). Both PBDEs and PBBs are persistent in the environment, accumulate in fat tissue, and have been found in a variety of foods with high fat content, including fish, meat, poultry, and dairy products as well as breast milk (162-171). Exposure studies have concluded that the presence of PBDEs in house dust and in foods are both important contributors of PBDE exposures for people of all ages, but exposures from house dust are generally greater than those from food (169, 170, 172-177). Of the three forms of PBDEs once used in the United States (pentaBDE, octaBDE, and decaBDE), only the decaBDE form was recently in production. Manufacturers of decaBDE agreed to phase out all uses by the end of 2013 (178). The manufacture of PBBs was stopped in 1976 (179). PBDE toxicity to the developing nervous system as well as endocrine disruption have been identified as areas of potential concern (163, 180-184).

Perchlorate

Perchlorate is a naturally occurring and manmade chemical that is used in the manufacture of fireworks, explosives, flares, and rocket propellant (185, 186). Perchlorate has been detected in surface and groundwater, human breast milk, infant formula, dairy products, and leafy vegetables and other produce (185-197).

Exposure to high doses of perchlorate has been shown to inhibit iodide uptake by the thyroid gland, potentially disrupting thyroid function and leading to reduced production of thyroid hormone (185, 198, 199). Thyroid hormones are important for growth and development of the central nervous system in fetuses and infants (121). Due to the sensitivities of the developing fetus, perchlorate exposures among pregnant women carry the potential for risk of adverse health effects.

Summary

In conclusion, diet is the primary exposure route for a number of environmental contaminants, including EDCs and heavy metals, and an important route for other contaminants (**Table 2-1**). While some progress is being made in recognizing the toxicity of these contaminants and mitigating pregnant mothers' and children's exposure to these chemicals through chemical bans and increased awareness, there is still a great deal unknown about human exposure to these contaminants and the subsequent health effects. Clarifying the relation between environmental contaminants and diet will allow for improved guidance on diet for pregnant women, infants, and children, and in turn, the potential for improved perinatal and pediatric outcomes.

While there are numerous chemicals in foods, this dissertation focuses on OCPs, PCBs, and PFAS. These chemicals are considered EDCs and have long half-lives. Further, diet is the main source of exposure to OCPs, PCBs, and PFAS (unlike perchlorate and BFRs, where the main sources of exposure are water and dust, respectively). Diet is the predominant source of

exposure (>90%) to persistent organic pollutants like OCPs, PCBs, and dioxins (3). This dissertation focuses on OCPs and PCBs since dioxin exposure has decreased by 90% since strict regulatory controls on major industrial sources have reduced emissions since 1987 (116). PFAS, on the other hand, is a class of chemicals that is of rising concern. The two main sources of PFAS exposure are contaminated drinking water and food. Contaminated drinking water is mainly a concern for populations living near military bases and other sites that where firefighting foam has been sprayed and contaminated the groundwater, accounting for up to 75% of total PFAS exposure in these areas (200). Otherwise, diet is the main source of PFAS exposure (accounting for greater than two-thirds of exposure in all but one study). Dust and inhalation typically account for 1-15% of exposure and dermal absorption is <1% (200). In sum, OCPs, PCBs, and PFAS were selected for this dissertation due to their persistence, endocrine disrupting potential, and preponderance in food, a modifiable source of exposure.

Table 2-1. Environmental contaminants commonly found in foods.

Environmental contaminant	Foods	Packaging or preparation	Non-diet sources	Status of use
Heavy metals				
Methylmercury	Fish (concentrations vary greatly by size)		Using or breaking products containing mercury (fever thermometers, novelty jewelry, dental fillings, vaccines, gold mining)	Naturally occurring
Arsenic	Seafood, rice, mushrooms, and poultry		Contaminated groundwater, smoking tobacco products, processing of glass, pigments, textiles, paper, metal adhesives, wood preservatives, and ammunition	Naturally occurring
Non-persistent EDCs				
Bisphenol A (BPA)		Inner lining of metallic food and drink containers; polycarbonate plastics used in food and drink containers; BPA migrates especially when containers are heated	Carbonless paper (e.g., cash register receipts), compact discs, medical devices, plastic toys	Banned in the US and EU for certain purposes (e.g., in baby bottles)

Phthalates	Fatty foods such as dairy products, fish, seafood, and oils	Increases the flexibility of plastics in food packaging	Personal care products, soft plastic/vinyl products (e.g., pacifiers, toothbrushes), vinyl clothing, medical devices	Banned in the US and EU for certain purposes (e.g., in children's products like toys)
Phytoestrogens	Soybean products, red clover, flax, licorice, hops, alfalfa, ginseng, and evening primrose oil			Naturally occurring
Atrazines	Corn, sorghum, sugarcane, and pineapple		Contaminated groundwater, contaminated soil	Banned in the EU
Organophosphate pesticides (OPPs)	Apples, corn, oranges, rice, and wheat		Contaminated groundwater, contaminated soil	Banned in the US for residential use, but allowed for agricultural use. In the EU, 33 OPPs are banned
Persistent EDCs				
Polychlorinated biphenyls (PCBs)	Meat, fish, dairy, and poultry		Hazardous waste sites, indoor air (specifically in locations with older fluorescent lights that still have transformers or ballasts that contain PCBs)	Banned in the US in 1978, and by the Stockholm Convention on Persistent Organic Pollutants in 2001

Perfluoroalkyl substances (PFAS)	Meat, some plant-based products	Food packaging (like microwave popcorn bags); nonstick coatings on cookware	Contaminated groundwater, stain- and water-repellent fabrics, nonstick products, fire-fighting foams	Certain PFAS are no longer manufactured in the United States as a result of phase outs by eight major chemical manufacturers that agreed to eliminate the use of PFOA and PFOA-related chemicals in their products
Organochlorine pesticides (OCPs)	Meat, fish, and dairy		Contaminated groundwater, contaminated soil	Many banned by the Stockholm Convention on Persistent Organic Pollutants in 2001
Brominated flame retardants (BFRs)	Meat, fish, dairy, and poultry		Contaminated dust in indoor environments (because of use in consumer products), contaminated soil	EU banned the use of two classes of flame retardants (PBDEs and PBBs) in electric and electronic devices. Certain US states have banned some PBDEs. PBBs are no longer produced in the US
Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs)	Meat, fish, and dairy		Contaminated groundwater, contaminated soil (dioxins are by-products of industrial processes (e.g., waste incinerators)	Parties must take action to reduce the unintentional releases of PCDDs and PCDFs under the Stockholm Convention on Persistent

		and natural processes (e.g., volcanic eruptions and forest fires))	Organic Pollutants in 2001
Perchlorate	Infant formula, dairy, leafy vegetables and other produce	Contaminated drinking water	Naturally occurring and produced industrially

Diet Scores/Patterns

Given the increasing evidence indicating the pervasiveness of chemicals in food, researchers have begun to develop diet scores to estimate chemical exposure based on food consumption. Such diet scores may be useful to explore associations with health outcomes in large-scale epidemiologic studies without the expense and burden of collecting and measuring multiple biomarkers. For example, researchers in the United States have developed a pesticide residue burden score (PRBS) based on food frequency questionnaire (FFQ) and surveillance data on food pesticide residues in order to characterize dietary exposure over the past year (201). The PRBS and class-specific (organophosphate pesticide and organochlorine pesticide) scores were associated with pesticide biomarkers in a validation study (n=3,679 from the National Health and Nutrition Examination Survey (NHANES)) (202). These scores were successful in ranking individuals by their pesticide residue exposures from fruits and vegetables in a smaller study (n=189) based in Rochester, New York (203).

A similar diet score approach has been taken in Norway with dioxins and PCBs. Estimates of dioxin and PCB intake were calculated by combining food consumption (as collected in semi-quantitative FFQs) and the concentration of dioxins, dioxin-like PCBs, and non-dioxin-like PCBs in foods (the concentrations of dioxins and PCBs in Norwegian food items have been published previously). Analysis of dioxins and PCBs in whole blood allowed for validation of the score (204).

Dietary pattern analysis allows for the examination of overall diet (205). Data-driven pattern analysis has been used in a handful of previous studies among various populations to identify dietary patterns that contribute to exposure to persistent EDCs (**Table 2-2**).

While the vast majority of previous studies have taken a data-driven approach using methods such as principal component analysis or reduced rank regression, one study has examined existing dietary patterns or guidelines in relation to persistent chemical exposure. Among Swedish seniors (n=844), a Mediterranean-like diet was positively associated with levels of several PCBs, trans-nonachlor, and mercury. A low carbohydrate–high protein diet was

positively associated with PCBs 118 and 153, trans-nonachlor, hexachlorobenzene and DDE, mercury, and lead. The World Health Organization (WHO) recommended diet (which includes fruits, vegetables, legumes, nuts, and whole grains and limits free sugars, saturated and trans fats, and salt) was inversely associated with levels of dioxin and lead, and borderline positively with PCB 118 and trans-nonachlor (206).

The majority of previous studies of dietary patterns and persistent EDCs have focused on PCBs as opposed to multiple classes of chemicals. While results on certain foods, such as yogurt, conflict across previous studies of dietary patterns and concentrations of persistent EDCs, a general trend starts to emerge. Diets characterized by high intakes of certain animal-based products are associated with greater concentrations of persistent EDCs among children and adults, including pregnant women. The project described in this dissertation allows us to identify and validate dietary patterns that contribute to the mixture of persistent EDC exposure (PCBs, OCPs, and PFAS) among pregnant women, specifically. The longitudinal data available in this study is rather unique among the mostly biomonitoring studies previously used.

Table 2-2. Studies of dietary patterns associated with biomarkers of persistent endocrine disrupting chemicals (EDCs).

Study	Population	Sample size	Location	Time period	EDCs under study	Analytic methods	Associated foods/food groups						
							Meat (Beef, pork)	Poultry	Fish/ Seafood	Dairy	Eggs	Fruits & Vegetables	Other
OCPs													
Arrebola et al., 2018 (2)	Adults (18–65 years old)	1880	Spain	2009-2010	OCPs	Multivariate linear regression and weighted quantile sum regression			↑	↑			
Boada et al., 2014 (207)	Representative sample (6–75 years old)	1747	Canary Islands	1997-1998	Non-DDT-derivative pesticides	Multivariate models	↑ Rabbit ↓ Sausage, bacon, lard	↑		↑ Cheese ↓ Yogurt	↑		
Lee et al., 2018 (208)	Children (7–9 years old)	188	Korea	2011-2012	OCPs	RRR	↓ Processed meat		↑ Shrimp			↓ Seaweeds	↑ Beverages
PCBs													

Arrebola et al., 2018 (2)	Adults (18–65 years old)	1880	Spain	2009-2010	PCBs	Multivariate linear regression and weighted quantile sum regression			↑		↑			
Boada et al., 2014 (207)	Representative sample (6–75 years old)	1747	Canary Islands	1997-1998	PCBs	Multivariate models	↑ Rabbit	↑		↑ Cream, butter ↓ Dairy desserts	↑			
Eguchi et al., 2017 (209)	Mothers and fathers of infants	mothers: 1477 fathers: 219	Japan	2011-	PCBs	Bayesian linear regression modeling			↑		↑			
Lee et al., 2018 (208)	Children (7–9 years old)	188	Korea	2011-2012	PCBs	RRR			↑	↑ Cheese				
Ravenscroft, Schell, and Akwesane Task Force on the Environment,	Adolescents (10–17 years old)	246	Akwesane Mohawk Nation	1995-2000	PCBs	PCA	↓*	↓*	↑	↑	↓*	↓*	↑ Cereal ↓* Beans, bread	

2018 (210)													
PCDDs, PCDFs, and dl-PCBs													
Arisawa et al., 2011 (211)	Adults (15–73 years old)	1656	Japan	2002-2007	dl-PCBs	PCA			↑	↑			↑ Alcohol
Boada et al., 2014 (207)	Representative sample (6–75 years old)	1747	Canary Islands	1997-1998	dl-PCBs	Multivariate models	↑						
Kvalem et al., 2009 (204)	High consumers of seafood and game and representative sample	high consumers: 111 representative: 73	Norway	2003	PCDDs, PCDFs, PCBs	PCA			↑ Fish liver		↑ Seagull eggs		
Lee et al., 2018 (208)	Children (7–9 years old)	188	Korea	2011-2012	dl-PCBs	RRR	↓ Processed meat		↑	↑ Yogurt		↑ Fruit ↓ Seaweeds	↑ Beverages ↓ Grain
Papadopoulou et al., 2014 (212)	Pregnant women	604	Greece, Spain, United Kingdom,	2006-2010	PCDDs, PCDFs, dl-PCBs	RRR	↑	↑	↑	↑↓			↓ Salty snacks

			Denmark, Norway							
					PFAS					
Arrebola et al., 2018 (2)	Adults (18–65 years old)	1880	Spain	2009-2010	PFAS	Multivariate linear regression and weighted quantile sum regression	↑ Cold cuts	↑	↑	

Abbreviations: PCDDs, polychlorinated dibenzo-*p*-dioxins; PCDFs, polychlorinated dibenzofurans; dl-PCBs, dioxin-like polychlorinated biphenyls; ndl-PCBs, non-dioxin-like- polychlorinated biphenyls; PCBs, polychlorinated biphenyls; PBDE, polybrominated diphenyl ether; OCPs, organochlorine pesticides; PFAS, perfluoroalkyl substances; BPA, bisphenol A; PCA, principal component analysis; RRR reduced rank regression
* At moderate levels

Recommendations and Guidelines for Pregnant Women

Pregnancy is a critical period during which maternal nutrition and lifestyle choices have major influence on mother and child health. Inadequate levels of key nutrients during crucial periods of fetal development may lead to reprogramming within fetal tissues, predisposing the infant to chronic conditions later in life (213). In this section, we will describe lifestyle and dietary guidelines for pregnant women. Components associated with a healthy pregnancy outcome include healthy prepregnancy weight, appropriate weight gain and physical activity during pregnancy, consumption of a wide variety of foods, appropriate vitamin and mineral supplementation, avoidance of alcohol and other harmful substances, and safe food handling (213). While there are a number of recommendations made surrounding healthy eating during pregnancy, few address concerns related to exposure to environmental contaminants in foods.

Lifestyle

Evidence is building that maternal diet and lifestyle choices influence the long-term health of children. For example, prepregnancy adherence to healthful dietary patterns, including the alternate Mediterranean Diet, Dietary Approaches to Stop Hypertension (DASH), and alternate Healthy Eating Index, have been associated with lower risk of gestational diabetes (214), which may be associated with neurodevelopmental problems in children (215). Additionally, insufficient amounts of important nutrients during key periods of fetal development may lead to reprogramming of fetal tissues, predisposing the infant to poor health in areas such as obesity, cardiovascular disease, bone health, cognition, immune function, and diabetes (216).

Maternal weight gain during pregnancy outside the recommended range is associated with increased risk to maternal and child health (217). The Institute of Medicine (IOM) recommends that US women achieve gestational weight gain within the range identified for their prepregnancy body mass index (BMI); for example, the recommended range of total weight gain for normal weight women is 25-35 pounds while the range for overweight women is 15-25 pounds

(217). Pregnant women benefit from eating a variety of foods to meet nutrient needs and consuming sufficient calories to support recommended weight gain (218).

Additionally, physical activity during pregnancy benefits a woman's overall health. In a low-risk pregnancy, moderately intense activity does not increase risk of poor birth outcomes (219). A prenatal nutrition and exercise program, regardless of exercise intensity, has been shown to reduce excessive gestational weight gain in women of normal prepregnancy BMI (220).

In addition to guidelines surrounding energy balance, there is evidence that some substances should be limited during pregnancy, such as alcohol and caffeine. Pregnant women should not consume alcohol; no safe level of alcohol consumption during pregnancy has been established (221). The American College of Obstetricians and Gynecologists (ACOG) advises women who are pregnant or trying to become pregnant to consume no more than 200 mg of caffeine per day (approximately the amount in one 12-oz cup of coffee) (222). Moreover, it is advised that pregnant women avoid energy drinks, which may contain varying amounts of sugar and caffeine, as well as taurine, carnitine, gingko, and ginseng, which have not been studied for safety or are not recommended for use during pregnancy (213). Lastly, there has been limited research addressing the safety of non-nutritive sweeteners during pregnancy (120).

Vitamins and Minerals

Iron deficiency with resultant anemia is the most prevalent micronutrient deficiency worldwide, primarily affecting pregnant or lactating women and young children (223). Iron-deficiency anemia in pregnant women remains high in industrialized nations (17.4%) (224), which underscores the need for iron supplementation during pregnancy. Supplementation with folic acid is also recommended because of its preventive properties against neural tube defects (225). Women who are capable of becoming pregnant should consume 400 μg/day folic acid and pregnant women are advised to consume 600 μg/day folic acid (225).

Vitamin D, calcium, choline, and iodine are other vitamins and minerals of interest during pregnancy. The IOM recommends 600 IU per day of vitamin D to meet the needs of most North American adults, including pregnant women (226), though ongoing research suggests that higher levels of supplementation are safe and effective for improving maternal and infant vitamin D status (227). Similar to vitamin D, the Dietary Reference Intake for calcium in pregnancy is equal to that of nonpregnant women of the same age (226). Choline is important for fetal brain development and is found in many foods, but the majority of women are not achieving the Adequate Intake for pregnancy of 450 mg choline per day (228). Iodine is also important for normal brain development and growth, and iodine requirements increase during pregnancy. The IOM recommends an iodine intake of 150 µg/day before conception, and 220 µg/day for pregnant women (229).

Food that are good sources of these vitamins and minerals, such as meats and dairy, may also be important sources of EDCs. Alternatively, these vitamins and minerals can modify the absorption of environmental chemicals, such as dietary calcium and iron blocking the absorption of lead (230).

Environmental Issues

Pregnant women should limit or avoid certain foods during pregnancy due to the increased risk of foodborne illness during pregnancy and concerns regarding mercury. To prevent foodborne illnesses caused by bacteria like *Listeria monocytogenes, Toxoplasma gondii, Brucella* species*, Salmonella* species*,* and *Campylobacter jejuni* (213)*,* pregnant women should follow general guidelines: 1) rinse all produce thoroughly under running water before eating, cutting, or cooking; 2) wash hands, knives, countertops, and cutting boards after handling and preparing uncooked foods; and 3) avoid all raw and undercooked seafood, eggs, and meat (231). To help prevent listeriosis in particular, it is recommended that pregnant women avoid eating unpasteurized milk and foods made with unpasteurized milk; hot dogs, lunch meats, and cold cuts unless they are

heated until steaming hot just before serving; refrigerated pate and meat spreads, and refrigerated smoked seafood (231).

The adequate intake for total water during pregnancy (through drinking water, beverages, and food) is 3 L/day. This includes approximately 2.3 L (approximately 10 cups) as total beverages (232). Adequate hydration is essential to a healthy pregnancy as a woman accumulates 6 to 9 L of water during gestation.

The nutritional value of seafood is important for fetal growth and development. Intake of omega-3 fatty acids, particularly docosahexaenoic acid, from at least 8 oz of seafood per week for pregnant women is associated with improved infant visual and cognitive development (221). Although prenatal mercury exposure (≥1 μg/g) was found to be associated with a greater risk of attention-deficit hyperactivity disorder-related behaviors, prenatal fish consumption of more than two servings per week was protective of those behaviors (233). Because some types of fish have higher levels of mercury than others, it is recommended that pregnant women choose fish and shellfish such as shrimp, salmon, catfish, and pollock, while avoiding shark, swordfish, king mackerel, marin, orange roughy, and tilefish, and limiting white (albacore) tuna to 6 ounces a week (231, 234). While these recommendations are based on concerns about mercury, the same principals of bioamplification and biomagnification apply, and fish at the top of the food chain would theoretically have higher levels of persistent EDCs (e.g., PCBs) as well. Before eating fish caught in local waters, pregnant women should check for advisories. If there is no advisory in place, pregnant women should eat only one serving, and no other fish that week (234).

Gaps in Recommendations

As described, recommendations for healthy eating during pregnancy address some environmental contaminants such as methylmercury; however, there are no recommendations regarding EDCs. Overall, nutritional advice during pregnancy does not differ substantially from general dietary guidelines for adults. There are guidelines about gestational weight gain (235),

but few specific guidelines exist beyond certain restrictions (alcohol, some types of fish) plus taking a prenatal vitamin with folic acid (236). The Environmental Protection Agency has determined that methylmercury can have neurotoxic and developmental effects in humans (19). Studies reported that populations with prenatal exposure to methylmercury through regular consumption of fish have subtle detrimental effects on childhood neurological development (20-24). This has led to much debate on the topic of fish consumption among pregnant women: while ingestion of methylmercury in fish may be harmful, other compounds, like omega-3 fatty acids, naturally present in fish, are extremely beneficial and contribute to healthy development (25). Similar considerations may have to be made when weighing the potential benefits and harms of foods that are high in EDCs. The dissertation begins to inform what, if any, changes to dietary recommendations should be made in order to limit exposure to EDCs during the critical period of pregnancy.

Prenatal Exposure to EDCs

As stated previously, exposures during pregnancy may be particularly relevant to growth and developmental outcomes in the offspring. There is growing concern that in utero exposure to harmful EDCs may have adverse effects on a developing fetus. Fetal growth and growth and development after birth are mediated by hormones that are released from endocrine glands into the bloodstream and transported to different tissues and organs. For normal growth and development to occur, tissues require specific concentrations of certain hormones at particular times (237). Fetal and infant development are windows especially susceptible to EDCs as they disrupt these hormonal processes.

Studies show that EDCs are commonly found in the placenta and amniotic fluid (238, 239). Exposures during pregnancy are extremely relevant to growth and development since this represents the period of initial organ development, including the brain, endocrine system, and reproductive tract. Furthermore, a fetus is more susceptible to such exposures due to smaller

size, lack of a complete blood-brain barrier, and absence of metabolizing enzymes (240). With biologic plausibility established, studies show that exposure to EDCs during critical windows of vulnerability can lead to adverse birth outcomes and increased risks of disease and disability across the lifespan (241), like altered pubertal development. Recognizing this critical window, researchers have prioritized the study of individual susceptibility across the lifespan to chronic, complex diseases resulting from environmental factors (242).

To assess prenatal exposure to EDCs, maternal concentrations are normally used as a proxy for fetal exposure. Because EDCs like PFAS, PCBs, and OCPs have long half-lives, a biomarker measure is usually representative of fetal exposure throughout pregnancy, though a recent study has shown that maternal persistent EDC concentrations decrease over the course of pregnancy (in part due to transfer of EDCs from mother to fetus) (243). Alternatively, biomarker measures of chemicals with shorter half-lives (e.g., of a day or two), like phytoestrogens or phthalates, may not accurately represent typical exposure. Recently, there has been some concern about reverse causality and confounding in studies of persistent EDCs measured in serum during pregnancy. Such concern stems from the fact that the outcome of interest may affect the measured biomarker concentration and there may be shared biological determinants of the exposure measure and pregnancy outcome (e.g., hemodynamics). This is less of a concern in studies with blood sampled early in pregnancy and studies with a wide range of exposure (244-246). Further, it is important to consider adjusting for age, parity, and previous breastfeeding in studies of prenatal EDC exposure because age is typically associated with higher EDC concentrations, while previous pregnancies and previous breastfeeding result in decreased EDC levels, and all three variables could be associated with the outcome under study.

Size at Birth

Perfluoroalkyl substances

PFAS and size at birth has been extensively studied over the past decade or more. Within the Avon Longitudinal Study of Parents and Children (ALSPAC), associations between prenatal PFOA, PFOS, and PFHxS exposure and birth weight and length have been observed among daughters (247) and associations of prenatal PFOS exposure with birth weight, birth length, and head circumference have been observed among sons (248). Outside of ALSPAC, similar inverse or null results are often reported in other epidemiologic studies of PFAS and birth size. Studies from Japan (n=168) (249), the United States (Maryland) (n=299) (250), Denmark (n=1,400) (251), and Taiwan (n=429) (252) have found evidence of inverse associations of PFOA and PFOS with birth size. An American study (Massachusetts) (n=1,645) found a weakly inverse association of maternal PFNA with birth weight (244). A third American study (Ohio) (n=272) analyzed PFAS as a class using Bayesian Hierarchical Linear Models and found that for a 10-fold increase in chemical concentration, the mean difference in birth weight was -11 g for PFAS (253). In a cohort of 1,250 term singleton infants, Lenters et al. used multi-pollutant models based on elastic net regression and found that two phthalate metabolites, PFOA, and dichlorodiphenyldichloroethylene (DDE) were most consistently predictive of term birth weight. When included in a model with all four exposures, two standard deviation increases in natural log-transformed PFOA, DDE, and one of the phthalate metabolites were associated with lower birth weight, while the other phthalate metabolite was associated with higher birth weight. In particular, PFOA was associated with -43 g (95% CI: -108, 23 g per 1.18 ng/mL) (254). Finally, other studies have found some evidence of modestly inverse or null associations between PFAS and birth weight (159, 255-258).

To summarize, a 2014 meta-analysis by Johnson et al. examined PFOA and birth size, and estimated a 19-g reduction in birth weight with each 1 ng/mL increase in maternal serum PFOA concentrations (259). Estimates for length (-0.06 cm per 1 ng/mL increase) and head

circumference (-0.03 cm per 1 ng/mL increase) were also reported. While these differences may not be considered large at the individual or clinical level, it is important to consider implications at the population level. A relatively modest and subclinical effect size may be associated with substantial population burden if the exposure is prevalent (259, 260), like PFAS.

Polychlorinated biphenyls

Associations of prenatal exposure to PCBs and markers of fetal growth (e.g., birth weight, birth length, head circumference, gestational age at delivery) have been well studied. A meta-analysis of 12 European cohorts (n=7,990) and a pooled study of mother-child dyads from 11 European cohorts (n=9,377) both observed inverse associations between prenatal PCB-153 exposure and birth weight (143, 261). Results from a study of prenatal exposure to PCBs and birth size in mother-daughter dyads of ALSPAC (n=448) are in line with the meta-analytic findings: an inverse association of PCB-118, PCB-153, and PCB-187 and birth weight was observed, though only for the lowest education group (262). While the majority of studies have found an association of prenatal PCB exposure and fetal growth (143, 261-265), there are a few exceptions. In the US Collaborative Perinatal Project (n=1,034), which took place in the early 1960s, Longnecker et al. found no associations between serum concentrations of 11 PCB analytes collected during the third trimester of pregnancy and birth weight or gestational age (266). In a Swedish sample of nulliparous women (n=411), Lignell et al., observed that breast milk concentrations of PCB-138, PCB-153 and PCB-180 assessed within the first month post-delivery were positively associated with birth weight, reporting stronger associations for male infants than females (267). A study based in Ohio (n=272) analyzed PCBs as a class using Bayesian Hierarchical Linear Models and found that for a 10-fold increase in chemical concentration, the mean difference in birth weight was 0.2 g for PCBs (253).

While the majority of studies have seen an inverse association between prenatal PCB exposure and fetal growth markers, namely birth size, there have been some discrepant findings.

These inconsistent findings may be due to a number of factors such as differences in study design, population characteristics, timing or type of sample measured, and overall distribution of exposure. Differences in the PCB analytes examined can lead to varied findings since PCB analytes vary in their ability to bioaccumulate and in level of toxicity (266, 268).

Organochlorine pesticides

Findings regarding the association of OCPs, particularly DDT and DDE, with human fetal growth outcomes are inconsistent. Early studies reported that increased concentrations of DDT and DDE are not associated with infant birth weight (264, 269-274), while later studies have more often found an association with lower birth weight. Other studies of DDT and DDE with adjacent outcomes report associations with lower birth weight (275), small-for-gestational-age (SGA) births (276), and intrauterine growth retardation (IUGR) (277).

As stated, previous studies of DDT and DDE and birth weight, with data collection spanning 1978 to 1999, have found a null association of prenatal DDE exposure and birth weight (264, 269-274). Studies with more recent data collection (2005 and later), and likely lower levels of DDT and DDE, have more often found an association between DDT and DDE with birth weight. An American birth cohort with enrollment between 2005 and 2009 found that among infant girls (n=117), birth weight was lower in association with maternal serum concentrations of DDT (278). A Chinese study (n=81) with enrollment in 2010 found that DDE, DDT, and β-HCH were associated with decreases in infant birth weight. Higher cord serum concentrations of DDT, HCB, and mirex were associated with lower infant birth weight, though associations were not significant, possibly due to small sample size (279). Most recently, in a Spanish study (n=447) that measured OCPs in cord blood in 2016, DDE was found to be associated with higher birth weight, especially among infant girls (280).

While DDT and DDE are the most commonly studied OCPs, there are other OCPs used; studies suggest that hexachlorocyclohexane (HCH) may be associated with birth weight, while

the associations of HCB with birth size outcomes are less clear. A Chinese study (n=1,028) found that cord serum β-HCH was inversely associated with birth weight and ponderal index among infant boys (281). A 2009-2010 Indian study found that there was an inverse correlation between total HCH concentrations across specimen types (maternal blood, cord blood, placenta, and breast milk) and birth weight and length at birth (282). A study of 98 mother-infant pairs in an area polluted with HCB in the period 1997-1999 found that infants born with a small length for gestational age had higher levels of HCB in cord serum than those with adequate length for gestational age (274). A Greek study (n=1,117) found that higher levels of prenatal HCB was inversely associated with birth weight (283). A 2004-2008 Spanish study (n=1,568) focusing on HCB and birth size found no association between maternal HCB concentrations and birth weight, birth length, or SGA (284).

A few studies have examined OCPs as a mixture in relation to birth size. In a cohort of 1,250 term singleton infants, Lenters et al. used multi-pollutant models based on elastic net regression and found that two phthalate metabolites, PFOA, and DDE were most consistently predictive of term birth weight. When included in a model with all four exposures, two standard deviation increases in natural log-transformations of one of the phthalate metabolites, PFOA, and DDE were associated with lower birth weight, while the other phthalate metabolite was associated with higher birth weight. In particular, DDE was associated with -135 g (95% CI: -192, -78 g per 1.82 ng/g lipid) decrease (254). A study based in Ohio (n=272) analyzed OCPs as a class using Bayesian Hierarchical Linear Models and found that for a 10-fold increase in chemical concentration, the mean difference in birth weight was 7 g higher for OCPs (253). The ability to analyze OCPs as a mixture improves upon previous studies which focused on one chemical at a time.

As with other studies of prenatal exposure to persistent EDCs and size at birth, studies of OCPs examined many outcomes (e.g., birth weight, birth length, SGA) in many specimen types

at many time points, potentially leading to the mixed results seen. Namely, earlier studies of OCPs and birth size were more likely to use maternal milk or cord blood, while recent studies have more often used maternal serum. Interestingly, the body burden of OCPs has been declining over recent decades and there seems to be a cohort effect where earlier studies of DDT and DDE and birth weight are most often null, while more recent studies show an effect. Unexpectedly, lower doses of some EDCs may have more potent effects than higher doses (37), which may be the case here. Another possibility is that chemical assays have become more precise in recent years.

Postnatal Body Size

While birth weight is a well-studied outcome in conjunction with prenatal exposure to EDCs, growth trajectories in the years following birth are less studied. The influence of prenatal exposure to EDCs on weight homeostasis may persist after birth. The first two years of life are a particularly important period of change. The greatest variations in rates of weight gain are usually seen in the first two years of life, when infants show accelerated or diminished growth to compensate for intrauterine restraint or enhancement of fetal growth (285). We have focused this discussion on prenatal exposure to persistent EDCs and postnatal body size in infants and toddlers, though studies of growth in school-aged children are briefly summarized.

Perfluoroalkyl substances

Postnatal growth has been examined in a handful of studies, including a 2012 study by Maisonet et al. in girls of the ALSPAC cohort (n=448). In this study, girls born to mothers with higher serum PFAS concentrations (namely PFOA, PFOS, and PFHxS) during pregnancy were smaller at birth. At 20 months, however, girls born to mothers with concentrations of PFOS in the upper tertile weighed 580 g more when compared with those in the lower tertile (247). In a Danish study (n=1,010), PFOS and PFOA concentrations were weakly inversely associated with children's weight and BMI in the first year of life (at age 5 months and 12 months). When stratified by infant sex, the inverse associations with weight and BMI were more pronounced in boys while

there were no clear associations seen for girls (286). In a prospective cohort of women and their children from Cincinnati, Ohio (n=334), inverse associations between prenatal PFAS exposure and child anthropometry (BMI Z-score) from 4 weeks to 2 years old were observed, though no difference in growth rates were seen (255). In a Dutch pregnancy cohort (n=148), PFOA and PFOS were not associated with BMI in the first year of life (287). A prospective cohort of 1,954 singletons from Upstate New York found no association between PFOA and PFOS with early obesity through 3 years of age (288). A Taiwanese study of 223 mothers and their term infants found that certain PFAS were associated with lower average childhood height z-score (at approximately 2, 5, 7, and 11 years of age) (289). Like the Taiwanese study, a handful of other studies have examined prenatal PFAS exposure and anthropometry later in childhood (e.g., 5-9 years of age) (290-293), and generally found that higher PFAS levels were associated with certain measures of adiposity in early school aged children (e.g., percent body fat, waist circumference, rate of BMI gain), but not other measures (e.g., height).

Overall, a wide variety of associations have been observed between PFAS and postnatal body size (positive, inverse, and null). Different findings may be due, in part, to varied timing of anthropometric measurements in the first few years of life and differences by infant sex. With only a handful of studies conducted to date and a variety of outcome measures utilized, it is difficult to see patterns emerging. Additionally, as the outcome measure occurs later in life and further away in time from prenatal exposure, taking into account post-natal exposure to PFAS becomes an important consideration.

Polychlorinated biphenyls and organochlorine pesticides

Five studies have examined prenatal exposure to PCBs and OCPs and infant growth. The largest, which pooled data from seven European birth cohorts, examined estimated prenatal PCB-153 (n=2,487) and DDE (n=1,864) exposure using a validated pharmacokinetic model. Infant growth was characterized as change in weight-for-age Z-score between birth and 2 years of age.

Prenatal DDE exposure was associated with increased infant growth, while there was no association of prenatal PCB-153 exposure with infant growth (294). A Spanish study (n=657) explored whether prenatal exposure to PCBs and OCPs is associated with rapid growth in the first 6 months of life and BMI later in infancy. Prenatal DDE exposure above the first quartile was associated with doubling of the risk of rapid growth among children of normal-weight mothers, but not overweight mothers. DDE was also associated with elevated BMI at 14 months. Other OCPs and PCBs were not associated with rapid growth or elevated BMI (295). In another Spanish study (n=1,285), DDE and HCB were associated with rapid growth in the first 6 months of life and with being overweight at 14 months of age (296). In a Dutch pregnancy cohort, high prenatal DDE exposure in boys (n=56) was associated with low BMI over the first year of life (287). A South African study (n=708) examined prenatal exposure to DDT and body weight and composition at 1 and 2 years old, finding that DDT concentration was positively associated with BMI-for-age, weight-for-height, and weight-for-age among girls but not boys (297). In summary, no studies found an association of PCBs with infant growth measures, while all studies found associations of DDT or DDE with infant growth—three found a positive association and one, the smallest study, found an inverseassociation among boys only. While there appears to be some association of DDT and DDE with rapid growth and infant BMI, there are important effect modifiers to consider such as infant sex and maternal pre-pregnancy BMI.

As was the case with PFAS, a number of studies have examined prenatal PCB and OCP exposure and anthropometry later in childhood (e.g., 4-14 years of age) (298-304). Most studies found an association between some prenatal PCB and OCP (namely DDE and DDT) exposures and various measures of adiposity in school-aged children such as BMI Z-score, overweight/obese status, and waist circumference, while one study in a highly exposed population found no association (304). Interestingly, two studies only found such associations in girls (299,

300), while two other studies (from the same cohort) only found these associations in boys (302, 303).

Pubertal Development and Age at Menarche

Puberty is a crucial period of growth and development. The timing and patterning of pubertal events, such as age at menarche, can provide information on overall health and previous exposures, while potentially forecasting future health outcomes, such as breast cancer (305, 306). The following sections outline findings to date regarding associations of prenatal exposure to persistent chemicals and pubertal development, namely age at menarche.

Perfluoroalkyl substances

The first study to examine prenatal PFAS exposure and age at menarche used data from the British ALSPAC nested case-control study (n=448) of age at menarche (dichotomized at 11.5 years). Serum samples were taken in 1991-1992 and ALSPAC mothers had nearly ubiquitous exposure to most PFAS under study, but prenatal PFAS exposure did not appear to be associated with altered age at menarche of offspring (307). A Danish study (n=343) conducted during a similar time period (1988-1989) found that daughters exposed to higher levels of PFOA prenatally had an age at menarche 5 months (95% confidence interval: 1, 9 months) later than the reference group with lower PFOA (308). Another Danish study (n=1,167) examined prenatal PFAS exposure and pubertal development in girls, with serum collected between 1996 and 2002, and found that prenatal exposure to a number of PFAS (including PFOS, PFHxS, and PFNA) was associated with lower mean age of puberty milestones in girls (Tanner stages for breast and pubic hair development, axillary hair, acne, and menarche). Nonmonotonic associations were observed in girls for PFOS, showing the largest mean age differences in the combined puberty indicator (all pubertal indicators in one model) for the middle tertile of exposure (309). In the C8 cohort of the mid-Ohio Valley focusing on residents near a chemical plant, serum PFOA and PFOS concentrations were associated with delayed menarche among 2,931 girls in a survey conducted

in 2005-2006, though it should be noted that this study was not focused exclusively on prenatal exposure to PFAS, as the other studies discussed were (310).

These studies of PFAS and age at menarche, concentrated in Danish, British, and highly exposed American populations, have mixed results and varied study design, such as different timing of PFAS sample collection and method of ascertaining age at menarche. It should be noted that while PFAS are mixtures of multiple compounds, few studies examine them as such, therefore the role of these compounds as complex mixtures remains largely unknown. This is particularly true in regards to studies of PFAS and age at menarche, of which none have examined PFAS as a mixture (311). A recent commentary in *Environmental Health Perspectives* indicates that mixture methods are needed as the logical next step in studies of EDCs and pubertal timing (312).

Polychlorinated biphenyls

A study of prenatal exposure to PCBs was conducted in a Michigan angler cohort. Maternal concentration of PCB was backward extrapolated, and daughters, who were born between 1950 and 1980, were interviewed between the ages of 20 and 50 about their reproductive health. This study (n=151) found no association between maternal PCB exposure and daughter's age at menarche, though higher PCB concentrations were suggestive of earlier age at menarche. A total PCB concentration was used as opposed to individual congeners (313). Another study (n=327) based in Michigan (following accidental contamination of the food chain with polybrominated biphenyl (PBB)) found that prenatal PCB exposure had no effect on age at menarche or timing of pubertal stages; this study was also unable to differentiate PCBs by congeners (314). In the North Carolina Infant Feeding Study (n=316), prenatal exposure to a summary measure of total PCBs did not alter age at menarche or timing of pubertal stages, though there was a tendency for girls in the highest exposure category (PCB > 4 ppm) to mature earlier (315). In a small Dutch study (n=18), there was no association observed between prenatal

exposure to dioxin-like PCBs and age at menarche (316). A nested case-control study of ALSPAC participants (n=448) examining age at menarche (dichotomized at 11.5 years) found no association of prenatal PCB exposure and age at menarche of daughters (317). The ALSPAC study was able to differentiate by PCB congeners. A Danish pregnancy cohort with follow up on daughters at approximately 20 years of age (n=335) also differentiated between PCB congeners and found no association between prenatal PCB concentrations and age at menarche (318).

All five studies of prenatal exposure to PCBs and age at menarche found no strong association, though some found that higher PCB concentrations were suggestive of earlier age at menarche (313, 315). One of the suspected reasons for the number of null results among PCBs and age at menarche is that total PCB concentrations are often measured as opposed to specific congeners, as was done in three of the five studies (313-315). It is known that various PCB congeners may have different, sometimes antagonistic, effects (319).

Organochlorine pesticides

In the same paper that described prenatal exposure to PCBs and age at menarche in a Michigan angler cohort (n=151), Vasiliu et al. also examined DDE and found that an increase in the prenatal DDE exposure of 15 μg/L reduced age at menarche by 1 year (313). In the North Carolina Infant Feeding Study (n=316), prenatal exposure to DDE did not alter age at menarche or timing of pubertal stages, though there was a tendency for girls in the highest exposure category (DDE > 4 ppm) to mature earlier (315). A Danish pregnancy cohort with follow up on daughters at approximately 20 years of age (n=335) examined prenatal DDE and HCB concentrations and age at menarche, finding no association for either chemical (318). In a nested case-control study within the ALSPAC cohort (n=448), there was no association between prenatal exposure to HCB, β-HCH, Y-HCH, p,p′-DDT, p,p′-DDE, oxychlordane or trans-nonachlor and early menarche (dichotomized at 11.5 years) (320).

Epidemiologic data on the role of organochlorine pesticides and age at menarche are limited. Challenges to such studies include temporal issues, the ability to assess confounding, and a lack of studies that have examined populations with background exposures. Notably, the majority of the studies examined very few OCPs: two of the four studies examined only DDE (313, 315), and another examined DDE and HCB (318), while the ALSPAC study examined seven OCPs (320).

Assessing Health Effects of Environmental Chemical Mixtures

As biomonitoring studies show, humans are exposed to a large number of environmental chemicals across the lifespan, through diet and other pathways. Exposure to these chemicals, EDCs in particular, is widespread, multisource, and multi-route (6). Studies indicate that the pattern of exposure in pregnant women and neonates is chronic, low-dose, and involves multiple chemicals simultaneously rather than individual agents (7-9). Many chemicals are potentially toxic, but little is known about health effects from exposure to complex mixtures of chemicals (10). A mixture is characterized by at least three independent chemicals or chemical groups, indicating exposure to multiple stressors simultaneously. By examining chemical mixtures, as opposed to the old paradigm of one chemical at a time, it may be possible to more accurately identify risk factors for diseases with environmental origins and develop more targeted public health interventions.

For example, multiple EDCs can act through a common mechanism to produce an outcome (such as binding to a particular type of hormone receptor), suggesting that individual chemicals could act together at lower concentrations than the concentration that would be required for each chemical on its own to achieve the same outcome (321). Taking this a step further, different mixtures of EDCs can also produce a common outcome via a common mechanism (321). This has been confirmed in *in vitro* and *in vivo* studies where combinations of

xenoestrogens are able to produce significant effects, even when each individual chemical is present at concentrations below the no-observed-effects levels (NOEL) (322-325). Such a mixture effect was observed in an epidemiological study of breast cancer using a novel biomarker of combined xenoestrogen exposure. A strong and positive association was observed for the mixture and breast cancer, though individual xenoestrogens showed no associations (326). Such a finding casts doubt on the null findings of meta-analyses of breast cancer risk and individual xenoestrogens like DDE (325, 327, 328). Extending this further, these findings suggest that the numerous epidemiological studies of individual chemicals with null findings may have substantially underestimated the risks of exposure to EDCs (329).

There are three main areas of interest in assessing chemical mixtures (330): health effects of individual chemicals within a mixture, interaction between chemicals in a mixture, and health effects of cumulative chemical exposure.

1) Health effects of individual chemicals within a mixture: Epidemiological studies can address the association between individual chemical exposures in a mixture and human health outcomes. Such an approach is needed to identify exposures that are most strongly associated with adverse health outcomes including individual exposures or groups of highly correlated and related exposures with a common source. Results could help guide public health efforts by allowing us to intervene on chemicals that are most likely to be associated with human health (330). There are a number of methods that can be used to quantify the association between individual chemical exposures and human health outcomes, such as Bayesian kernel machine regression, weighted quantile sum regression (WQSR), elastic net, or least absolute shrinkage and selection operator (LASSO) (254, 331). Due to the correlated nature of many environmental pollutants, it is important to adjust for copollutant confounding when trying to identify a single exposure

within a mixture. Additional challenges include multiple comparisons and disentangling the effects of highly correlated copollutants (330).

2) Interactions between chemicals in a mixture: Epidemiological studies can also be used to address whether two or more environmental chemicals have a greater than additive association with the health outcome of interest (330). Identifying interactions is useful to know when planning public health interventions since an intervention aimed at reducing one exposure would likely reduce the effects of other exposures as well. Statistical approaches to identify interaction between chemicals in a mixture include Bayesian kernel machine regression (BKMR) (332, 333). The identification of interactions between chemicals in a mixture is often limited by sample size and the pattern of correlation between exposures, leading to imprecise effect estimates and reduced statistical power. Another challenge is multiple comparisons (330).
3) Health effects of cumulative chemical exposure: A third question epidemiological studies can address is to estimate the association between cumulative chemical exposure and health outcomes. Such an approach would be used to quantify the summary effect of a class or multiple classes of exposure. Unlike the question of interaction, this assumes that joint exposure to the chemicals can be meaningfully condensed into a single summary metric. This approach may be most suitable when the individual components of the mixture act via common biological pathways, when the exposure to individual chemicals is below some threshold of concern (NOEL), and when there are individuals whose aggregate exposure is over this threshold (330). Quantifying the risk of disease from cumulative chemical exposure could help identify exposures that may be amenable to public health interventions (330). Previous approaches to summarize cumulative chemical exposure have included toxic equivalency factors (TEFs), which weight chemicals according to their biological potency (334, 335), but requires toxicological data in order to do so. In addition, simple summary measures like total PCBs have been employed, though

such a measure often reflects the individual component with the highest concentration in the mixture (336) and may not accurately capture the cumulative effect of the mixture if the lower concentration components are more potent than the higher concentration components (330). Furthermore, different health outcomes might require different summary measures or weights to most accurately describe the cumulative exposure to the mixture. Statistically driven approaches, such as principal component analysis (PCA), can identify latent factors that explain the correlation between mixture components, which can then be used as an exposure variable in regression models (337). The derived factors from PCA can be difficult to interpret and may be unique to the population being studied, thus limiting generalizability (330). Other methods, such as weighted quantile sum regression, empirically estimate weights for each chemical, which may be used to create weighted sums of standardized concentrations (331). As discussed, there are a number of challenges to estimating the health effects of cumulative chemical exposure, including verifying the assumption of no interaction, estimating cumulative exposure metrics for specific health outcomes, the availability of toxicological data to create biologically weighted summary measures, and interpretation of results from more complex statistical methods (330).

Challenges to Studying Chemical Mixtures

There has been a call for epidemiological studies to move beyond analyzing the health effects of individual chemicals toward the study of chemical mixtures (329, 330, 338), and this is a priority area for organizations such as the US National Institute of Environmental Health Sciences (NIEHS) (338) and the US National Academy of Sciences (7, 8). However, there are challenges to such studies. Studies of mixtures place a high demand on the quantity and quality of data and require advanced statistical methods, the development of which is an area of ongoing research (10, 330, 332, 339). Statistical challenges include:

Measurement of environmental chemical exposure

Measuring exposure to a large number of chemicals in humans is challenging. First, it is essential to accurately measure individual chemical components of the mixture. Sensitive and specific exposure biomarkers are a common method used to assess chemical exposures. Such biomarkers allow for the direct measures of individual chemical concentrations in a number of biospecimens such as serum, plasma, urine, cord blood, and breast milk (340). While biomarkers of chemical exposures have many strengths and have revolutionized the field of environmental epidemiology, there are some limitations. There can be misclassification of exposures with high within-person variability (e.g., chemicals with short half-lives like bisphenol A), reverse causality due to pharmacokinetic factors related to the outcome under study (245), or the inability for the biomarker to represent exposure during the etiologically relevant time period (further complicated by different half-lives of the biomarkers) (330).

There are also a number of practical issues, such as balancing the financial cost of using biomarker-based approaches to study chemical mixtures with the need for adequate sample size. Further, the volume of biospecimens required to measure biomarkers and the collection and timing of samples (e.g., during pregnancy, at birth) from special populations (e.g., neonates, toddlers) must be considered, particularly in pregnancy cohorts that might aim to study many exposures and outcomes. Lastly, the streetlight effect, a type of observational bias, has previously restricted the number of chemicals studied because only a few chemicals are measured, selected from those known to be of concern and or those for which measurement techniques exist. Recent advances in analytic chemistry, such as nontargeted analyses, allows investigators to broaden their view (330).

Statistical challenges

The possibility of false positive results is often a concern when analyzing a large number of exposures. There are several statistical methods available to reduce type I error rates in studies with many hypotheses, including the Bonferroni correction (341). While this approach can be useful, an over-reliance on significance testing in observational studies where exposures are not randomized and often correlated with another can be problematic (342-345). While hypothesis testing is still used for inference, epidemiologists should also assess the validity, magnitude, and precision of observed associations rather than just the statistical significance of associations (330).

Type II errors can also be a challenge in studies of chemical mixtures. As discussed previously, power to precisely estimate subtle effects between chemicals and health outcomes may be limited by sample size, accuracy of exposure assessment methods (nondifferential exposure misclassification), multicollinearity issues due to high correlations between exposures in the mixture, dependent errors, interactions, and nonlinearity, which can lead to inflated variance estimates and instability of the effect estimates (138, 339, 346-348).

Confounding due to correlated exposures

Confounding due to correlated exposures can exist in studies of chemical mixtures. For example, persistent EDCs like PCBs and OCPs may be correlated with each other and with health outcomes (349). Depending on the magnitude of correlation between the chemicals, such confounding can make identifying the effect of an individual chemical incredibly difficult. Therefore, it is important to understand the patterns of exposure to these chemicals among humans, and whether public health strategies to reduce chemical exposures should target the entire mixture or simply components of it (339).

Related to issues of confounding by coexposures and collinearity is the issue of unmeasured confounding. Approaches to mixture analyses that involve regressing the outcome on several correlated exposures simultaneously can in some cases amplify rather than reduce confounding bias ("coexposure amplification bias") (350). **Figure 2-2** is one of many possible directed acyclic graphs (DAGs) showing coexposure amplification bias when there are two or more exposures and unmeasured or unknown variable(s) present.

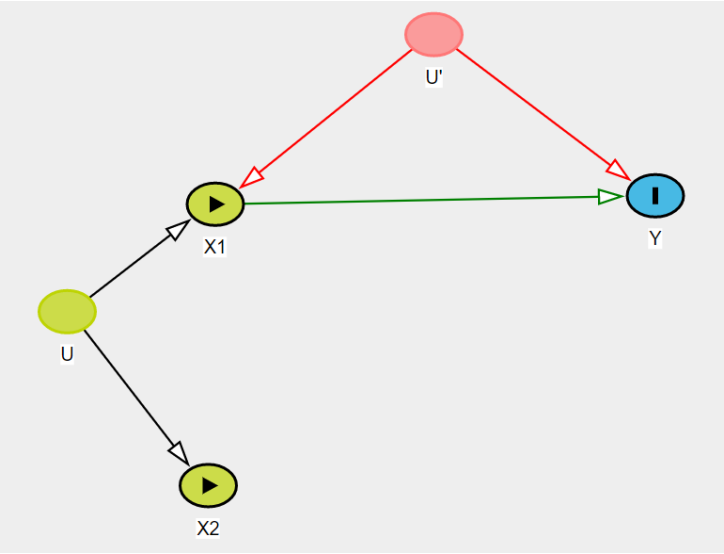

Figure 2-2. Directed acyclic graph (DAG) for coexposure amplification bias. An unknown variable, U', affects the outcome (Y) and one exposure (X1). Adjustment for both exposures (X1, X2) causes bias amplification of the X1-Y association and reversal of the X2-Y association. Example from Weisskopf et al., 2018 (350). Figure created using DAGitty software (351).

Differing degrees of measurement error among exposures

The differing degrees of measurement error among exposures has the potential to bias effect estimates. Persistent chemicals measured during pregnancy are expected to have considerable inter-individual variability (352). Within pregnancy, the timing of exposure assessment is more flexible for persistent chemicals (compared to chemicals with short half-lives such as bisphenol A and phthalates), though there will still be variation within classes of persistent chemicals because of varying half-lives (352). Further, differential placental transfer rates of

chemicals, differential rates of metabolism and bioaccumulation within the fetus across chemicals, and the lower precision measurement of exposures with concentration ranges near detection limits than chemicals with higher concentration range contribute to measurement error (352, 353). These differences between EDCs suggest that exposure may be misclassified to differing degrees for individual chemicals within a mixture as measured during pregnancy. It is recommended that a statistical method that explicitly accounts for differing degrees of measurement error is chosen or the impact of measurement errors on effect estimates is investigated through sensitivity analyses (352).

Identification of important mixtures

The pattern of human exposure to chemicals is complex and multifactorial. Chemicals are often correlated with each other and some combinations of exposures are more likely than others. As the goal is often to identify patterns of exposure that are relevant to health outcomes, some combinations of chemicals may be less relevant if there are few to no individuals with such a pattern of exposure. Therefore, it is informative to rank the importance of these patterns while considering the variability and prevalence of the exposure in the source population, the potential potency of the individual chemical components, and the potential for reducing or mitigating the impact of exposure if adverse health outcomes are identified (354).

Lack of standard methods to evaluate environmental mixtures

As discussed, there are a variety of statistical methods available to address questions regarding mixtures of environmental chemicals (254, 331, 332, 339), yet there is no consensus on standard methods for studying mixtures in epidemiological studies. It is generally recommended to compare results across methods as sensitivity analyses to assess the robustness of results, especially if complementary methods are available (e.g., Bayesian kernel machine regression and weighted quantile sum regression to assess health effects of cumulative

chemical exposure). If differences are observed across methods, it is important to consider the aim and strengths of each method when interpreting results. While a formulaic approach is not recommended by leaders in the field, there is still a need to understand the types of mixture-related questions that epidemiologists can address and the appropriate methods and statistical tools to answer such questions of public health interest (10, 308).

Conclusion

Reproductive, perinatal, and pediatric health is an area that has received substantial attention in the literature on EDCs, and EDCs have been implicated in numerous health outcomes such as birth size, postnatal body size, and age at menarche. However, results have been somewhat inconsistent, perhaps due to the examination of one chemical at a time, as opposed to mixtures of chemicals, which better represent human exposure to environmental chemicals. Additionally, those in the nutrition community are interested in providing recommendations for healthy eating during pregnancy, such as through the Alternative Healthy Eating Index for Pregnancy (AHEI-P) (355, 356), and environmental chemicals could be considered in such recommendations. Given the known toxic effects of certain environmental chemicals found in foods and the developing interest in making healthy eating recommendations during pregnancy, this review has described the role of prenatal diet and environmental exposures in relation to reproductive, perinatal, and pediatric outcomes.

Chapter 3 Maternal dietary patterns during pregnancy and exposure to persistent endocrine disrupting chemicals in two European birth cohorts

Kristin J. Marks[a,b,c], Kate Northstone[d], Eleni Zoumpoulia Papadopoulou[e], Anne Lise Brantsæter[e], Line Småstuen Haug[e], Penelope P. Howards[a], Melissa M. Smarr[f], W. Dana Flanders[a,b], Terryl J. Hartman[a,b]

[a] Department of Epidemiology, Rollins School of Public Health, Emory University, 1518 Clifton Rd NE, Atlanta, Georgia 30322, United States

[b] National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy, Atlanta, Georgia 30341, United States

[c] Oak Ridge Institute for Science and Education, 100 ORAU Way, Oak Ridge, Tennessee 37830, United States

[d] Department of Population Health Sciences, Bristol Medical School, Oakfield House, Oakfield Grove, Bristol BS8 2BN, United Kingdom

[e] Department of Environmental Health, Norwegian Institute of Public Health, P.O. Box 222, NO-0213 Skoyen, Oslo, Norway

[f] Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, 1518 Clifton Rd NE, Atlanta, Georgia 30322, United States

Abstract:

Diet, particularly food of animal origin, is considered the most important contributor to persistent endocrine disrupting chemical (EDC) exposure. This study aims to describe the association between maternal diet during pregnancy and exposure to persistent EDCs using dietary pattern analysis. This study is based on subsamples of the Avon Longitudinal Study of Parents and Children (ALSPAC) (N=422) and the Norwegian Mother, Father, and Child Cohort Study (MoBa) (N=276) which uses data from the Medical Birth Registry of Norway (MBRN). Pregnant women in both studies completed food frequency questionnaires (FFQs) during pregnancy, from which consumption data was grouped into 38 food groups. Both studies collected blood samples during pregnancy and measured maternal concentrations of perfluoroalkyl substances (PFAS), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs) in serum. Dietary patterns were analyzed using reduced rank regression, with serum EDC concentrations as dependent variables, adjusting for energy intake using the residual method. Within ALSPAC, all patterns (PFAS, PCB, and OCP) showed positive factor loading values in meat, poultry, white fish, cheese, and biscuits, and negative factor loading values in baked beans. In MoBa, sausages and burgers (representing processed meats), pasta, and chocolate bars had high positive factor loadings in PCB and OCP dietary patterns, while cheese loaded highly in the PFAS pattern. Across both cohorts, French fries and potato chips showed negative factor loadings, and fresh fruit and vegetables had null factor loadings for the most part. When predictors including maternal education, age at delivery, pre-pregnancy BMI, parity, prenatal smoking, and total energy intake were added to the model, explained variance was as high as 38.4% for PCBs in ALSPAC. In conclusion, animal-based products appear to be the main dietary contributors to persistent EDC concentrations among pregnant women and diet explains more variation in PCB concentrations than for other classes. Future research is needed to guide dietary recommendations for pregnant women.

Keywords: ALSPAC, MoBa, MBRN, endocrine disrupting chemicals, perfluoroalkyl substances, polychlorinated biphenyls, organochlorine pesticides, dietary pattern, pregnancy

Introduction

An endocrine disrupting chemical (EDC) is a chemical that may interfere with the body's endocrine system, potentially producing adverse developmental, reproductive, neurological, and immune effects (357). Persistent EDCs, such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and perfluoroalkyl substances (PFAS), have been used throughout the 20th and 21st centuries for a variety of purposes. These chemicals are typically highly resistant to degradation and tend to bioaccumulate in animals and humans (123, 130, 144). Exposure levels have declined in the general population following many countries banning or severely restricting the production, handling, and disposal of several OCPs and PCBs, as well as certain PFAS. Still, almost all humans have detectable levels of some of these persistent chemicals (124, 358). Moreover, persistent EDCs can cross the placental barrier, allowing for potential fetal exposure, and the quantities of EDCs found in cord serum may be substantial in relation to a developing fetus's size (263, 359-361).

Contaminated air, soil, and water are potential routes of exposure to persistent EDCs, but evidence suggests that diet may be the most important route (1, 204, 362-365). Diet accounts for up to 90% of exposure to persistent organic pollutants like PCBs and OCPs (3). Diet is also the main source of PFAS exposure in the general population, though contaminated drinking water can be the predominant source of PFAS exposure for populations living near military bases and other sites where firefighting foam has been sprayed, causing groundwater contamination (200). Contaminants in foods may come from the application of pesticides to crops, the transport of industrial chemicals in the environment, and chemicals used in food packaging products (11). A number of persistent environmental contaminants tend to accumulate in animals through bioaccumulation and biomagnification, and may commonly be found in meat, poultry, fish, and dairy products (149-152, 365). Other contaminants, such as a variety of pesticides, are often found in fruits, vegetables, and other agricultural commodities (201, 202).

A number of previous studies have evaluated exposure to persistent EDCs by monitoring pollutant levels in foods and estimating tolerable daily intake, such as Haug et al. (2010) and Caspersen et al. (2013) (363, 365). While critical, there is also the need to better understand exposure patterns. Dietary pattern analysis allows for the examination of overall diet (205) and there are two common approaches. Hypothesis-driven patterns are based on pre-existing knowledge and often align with some ideal, such as guidelines for healthy eating. Alternatively, data-driven patterns extract patterns from the population under study using a statistical tool and will lead to a handful of patterns specific to the population, though not all would be considered ideal. Reduced rank regression is a method that integrates these two approaches by identifying dietary patterns associated with selected response variables (e.g., biomarkers of EDC exposure) chosen based on prior knowledge (366, 367).

Data-driven pattern analyses have been used in a handful of previous studies among various populations to identify dietary patterns that contribute to exposure to persistent EDCs, most often dioxins and PCBs (2, 204, 207-212, 368). Previous studies in pregnant women have identified positive associations of fish (209, 212, 368), eggs (209), red and white meat (212), and low-fat dairy (212) with higher concentrations of persistent EDCs. Existing research shows diets characterized by high intakes of certain animal-based products are associated with greater concentrations of persistent EDCs.

Given the increasing interest in the association between environmental contaminants and health outcomes, it is important to identify major sources of exposure, such as dietary sources. This information could contribute to our understanding of the association between dietary exposures and specific health outcomes, as well as guide dietary recommendations tailored to prevent disease. This study aims to identify dietary patterns that contribute to exposure to persistent EDCs (PFAS, PCBs, and OCPs) in two European birth cohorts.

Methods

Study populations

The Avon Longitudinal Study of Parents and Children (ALSPAC)

The Avon Longitudinal Study of Parents and Children (ALSPAC) is an ongoing prospective birth cohort of 14,541 pregnancies. ALSPAC enrolled pregnant women with an expected delivery date between 1 April 1991 and 31 December 1992 from three health districts in the former county of Avon, Great Britain. Information was collected on these parents and children through clinic visits, interviews, and mailed questionnaires. Details on ALSPAC recruitment and study methods have been described elsewhere (369, 370). A substudy was conducted within the ALSPAC that measured prenatal maternal concentrations of various suspected endocrine disrupting chemicals. Details of the substudy are described elsewhere (307). To account for the substudy's design, the sample was weighted.

The study website contains details of all the data that are available through a fully searchable data dictionary and variable search tool (http://www.bris.ac.uk/alspac/researchers/our-data/). We obtained ethical approval for the study from the ALSPAC Ethics and Law Committee, the Local Research Ethics Committees, and the Centers for Disease Control and Prevention (CDC) Institutional Review Board. Mothers provided written informed consent for participation in the study. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004). Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law
Committee at the time.

The Norwegian Mother, Father, and Child Cohort Study (MoBa)

The Norwegian Mother, Father, and Child Cohort Study (MoBa) is a population-based pregnancy cohort study conducted by the Norwegian Institute of Public Health (371). Participants

were recruited from all over Norway from 1999–2008. The participation rate was 41%. The cohort now includes 114,500 children, 95,200 mothers and 75,200 fathers. The current study is based on version 12 of the quality-assured data files released for research. The establishment of MoBa and initial data collection was based on a license from the Norwegian Data protection agency and approval from The Regional Committees for Medical and Health Research Ethics. The MoBa cohort is now based on regulations related to the Norwegian Health Registry Act. The current study was approved by The Regional Committees for Medical and Health Research Ethics (2019/864). The Medical Birth Registry (MBRN) is a national health registry containing information about all births in Norway (372). Blood samples were obtained from both parents during pregnancy and from mothers and children (umbilical cord) at birth (373). Mothers received three questionnaires during pregnancy (including a food frequency questionnaire (FFQ)), and additional questionnaires after birth.

A subsample (n=278) of the MoBa cohort mothers had EDCs measured through the European Union's Human Early Life Exposome (HELIX) initiative (374). The aim of the HELIX study was to measure and describe multiple environmental exposures during early life (pregnancy and childhood) in a prospective cohort and associate these exposures with molecular omics signatures and child health outcomes. The HELIX study represents a collaborative project across six established and ongoing longitudinal population-based birth cohort studies in six European countries (France, Greece, Lithuania, Norway, Spain, and the United Kingdom). The subsample that will be used in the proposed project is composed of the 278 mother-child pairs from MoBa only in order to have access to detailed dietary data.

Exposure assessment

In ALSPAC, maternal diet was assessed using a qualitative FFQ (no portion sizes specified) completed by the women at 32 weeks gestation (**Supplemental Table 3-6**) (375). The ALSPAC FFQ covered the types of foods/drinks typically consumed in the United Kingdom in the early 1990s and was adapted from a validated questionnaire used previously in a neighboring

area and incorporated weighed intake data collected from women in the local area (375, 376). The FFQ contained questions about the typical weekly frequency of consumption of 43 different foods and food groups. The women were asked to tick one of the following options for each food as consumed “nowadays”: never or rarely, once in 2 weeks, 1–3 times per week, 4–7 times per week, more than once a day. The FFQ provides frequency of consumption for foods, total energy intake, and macro- and micronutrients. There were also questions about the types of some foods (e.g., cooking and spreading fats, milk) and about the ways in which foods were prepared and eaten (e.g., whether some or all of the fat was cut off meat, how often food was fried).

Similarly, maternal diet was assessed using a semi-quantitative FFQ completed by the women at 22 weeks gestation in the MoBa study (**Supplemental Table 3-7**) (377). The FFQ asked about the intake of 255 food items and was designed to capture dietary habits and intake of dietary supplements. Respondents were asked to fill in the usual intake of the food items eaten since becoming pregnant. The frequency intervals ranged from never to more than eight times per day. The MoBa FFQ was validated in a subsample of MoBa (n=119) using 4-day weighed food diaries and biological markers as reference methods. Compared with food diaries, the FFQ produced reasonably valid intake estimates and rank pregnant women according to low and high intakes of energy, nutrients, and foods (378). Validation study participants were correctly classified into the same or adjacent quintiles 75%, 59%, and 73% of the time for dairy foods, meats (e.g., beef, pork), and fish/seafood, respectively (378). Comparing across FFQs required condensing foods in the MoBa FFQ (255 items) into groups aligned with the ALSPAC FFQ (43 items) (**Supplemental Table 3-7**). For example, the ALSPAC FFQ lists “Sweets (peppermints, boiled sweets, toffees)” as one item, whereas the MoBa FFQ lists individual varieties separately: caramel, candies licorice; jelly sweets and marshmallow; pastille (regular and sugar-free); and marzipan; each have their own category, and therefore were combined for comparability with ALSPAC.

Mothers were excluded if their estimated energy intake was considered implausible (defined as <500 or >5000 kcal/day). No mothers in ALSPAC had implausible energy intakes and two mothers in MoBa reported >5000 kcal/day and were therefore excluded. Mothers were also excluded if they did not respond to the FFQ. In the ALSPAC sample, we excluded 26 mothers, and in the MoBa sample, we excluded no mothers for this reason.

Outcome assessment

Maternal fasting blood samples were collected from mothers during pregnancy at enrollment in ALSPAC from 1991–1992 at median 15 (interquartile range (IQR): 10–28) weeks gestation, and processed and frozen for later analysis. Maternal serum samples were held in storage facilities at the University of Bristol until they were transferred under controlled conditions and analyzed at the National Center for Environmental Health of the CDC (Atlanta, GA). Laboratory analyses included low- and high-concentration pooled quality control materials, standards, reagent blanks, and study samples.

Within MoBa, non-fasting maternal samples were collected during pregnancy at the routine ultrasound visits offered free of charge at mean 18 (standard deviation: 0.9) weeks gestation and stored in the cohort's biobank (373, 379, 380). All samples were monitored for degradation and contamination. Samples were analyzed for EDCs as part of the HELIX initiative. Chemical assays were conducted in the laboratory at the Department of Environmental Exposure and Epidemiology at the NIPH (374).

Concentrations below the limit of detection (LOD) were imputed by dividing the LOD by the square root of 2 prior to statistical analysis.

<u>Perfluoroalkyl substances</u>

The following PFAS were measured in both cohorts: perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), and perfluorononanoate (PFNA). In ALSPAC, PFAS were measured in serum via on-line solid-phase extraction coupled

to isotope dilution high-performance liquid chromatography-tandem mass spectrometry (381). Limits of detection (LODs) were 0.082 ng/mL (PFNA), 0.10 ng/mL (PFHxS, PFOA) and 0.20 ng/mL (PFOS). In MoBa, PFAS were measured in plasma using online column switching liquid chromatography coupled to a triple quadrupole mass spectrometer (MS) (382). LODs were 0.02 ng/mL for all PFAS under study.

Organochlorine pesticides and polychlorinated biphenyls

The following OCPs were measured: hexachlorobenzene (HCB), dichlorodiphenyldichloroethylene (DDE), and dichlorodiphenyltrichloroethane (DDT). Five PCB congeners were measured: PCB118, PCB138, PCB153, PCB170, and PCB180. In ALSPAC, OCPs and PCBs were measured serum using gas chromatography isotope dilution high resolution mass spectrometry (383). PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration hereafter referred to as PCB138. Within ALSPAC, LODs for PCBs and OCPs are dependent on the size of the sample available, thus an individual LOD was reported for each individual result rather than an overall LOD. In MoBa, OCPs and PCBs were measured in plasma using solid-phase extraction and high-resolution mass spectrometry as described Caspersen et al. (2016), except that gas chromatography-tandem mass spectrometry was used for detection (384). LODs were 0.3 pg/g (DDT, PCB118), 0.61 pg/g (DDE, PCB138, PCB153, PCB170), 0.91 pg/g (PCB180), and 1.52 pg/g (HCB).

Covariates

In both cohorts, covariate information was collected by clinical staff (e.g., gestational age at biological sample collection) or through self-report on questionnaires completed by the mother during or immediately after pregnancy (e.g., maternal education, maternal race). Covariates under consideration include: maternal age (years), maternal pre-pregnancy body mass index (BMI) (kg/m^2), maternal race or nativity (white/nonwhite in ALSPAC, Norwegian-born/not Norwegian-born in MoBa), maternal education (classified as <O-level (ordinary level: required, completed at

age 16), O-level, or > O-level in ALSPAC; no higher education, 1-4 years higher education, or >4 years higher education in MoBa), parity (nulliparous/multiparous), smoking during pregnancy (any/none), physical activity during pregnancy (any/none), gestational age at biological sample collection (weeks; ALSPAC only), and infant sex (male/female; MoBa only).

Statistical analyses

Analyses were restricted to mothers who completed an FFQ during pregnancy with plausible average energy intake (defined as 500–5000 kcal/day) and had measured biomarkers of persistent EDC exposure (n_{ALSPAC}=422; n_{MoBa}=276). Within ALSPAC, each food item was coded according to the frequency of weekly consumption as 0, 0.5, 2, 5.5, or 10 (for no consumption, consumption once every two weeks, 1-3 times/week, 4-7 times/week, or more than once/day, respectively). Within MoBa, mothers could report their frequency of intake by day, week, or month. We used weekly consumption as the common denominator by multiplying daily intake by 7; we coded monthly intake as 0 for 0 times per month, 0.25 for once/month, 0.5 for twice/month, and 0.75 for three times/month. Food groups were analyzed as both absolute frequency of consumption and in relation to total energy intake. For the latter, the average frequency was regressed on average daily intake of total energy (kilocalories), and residuals were added to the mean and used in subsequent analyses to investigate dietary composition independent of total energy intake.

Dietary patterns were obtained by entering the residual frequencies from each food group into a reduced rank regression (RRR) model designed to simultaneously maximize the variability explained in the hypothesized intermediate variables (persistent EDCs: PFAS, PCBs, and OCPs). These EDCs have been associated with growth and developmental outcomes in previous studies, and therefore were considered plausible intermediates on the pathway by which maternal dietary pattern(s) may influence growth and developmental outcomes in offspring. EDCs under study include those that were measured in both ALSPAC and MoBa: PFOA, PFOS, PFHxS, PFNA,

PCB118, PCB138, PCB153, PCB170, PCB180, DDE, DDT, and HCB. Food groups with factor loading values >0.2 or <-0.2 were considered the principal contributors to a dietary pattern.

Pattern score(s) for the dietary patterns were calculated as a continuous measure for each individual, and were also categorized into quartiles to examine nonlinear associations. A higher pattern score, and thus higher quartile, indicates a greater degree of adherence to that dietary pattern. Spearman correlation coefficients (r_s) were estimated between the dietary pattern scores and each EDC. Trends across quartiles were examined using univariate linear regression models with quartile of dietary pattern score as an ordinal predictor for continuous variables. To assess how well the dietary pattern(s) reflect maternal blood EDC concentrations, we performed multiple linear regression analysis, adjusting for maternal age, maternal education, pre-pregnancy body mass index, parity, smoking during pregnancy, gestational age at sample collection, and total energy. Outcomes of these models included serum concentrations of individual EDCs and totals by class (PFAS, PCBs, and OCPs), calculated as the summed total of Z scores within a class.

We conducted a sensitivity analysis to take advantage of the more detailed MoBa FFQ and intake data (grams/day). We ran RRR with 199 food groups where at least 25% of women reported some intake. Next, we excluded all food groups with a variable importance in projection score of <1.0. We report results for 53 to 57 food groups that were consumed by at least 25% of women and were considered important. SAS software 9.4 (Cary, NC) was used for all analyses.

Results

Most ALSPAC mothers were white (98.1%), well-educated (81.7% ordinary level or above), and 30 years or older (43.1%) (**Table 3-1**). The majority of mothers entered pregnancy at a normal BMI (77.8%) and half were nulliparous (49.9%). Few mothers smoked (18.2%) and about half drank alcohol during pregnancy (51.2%). Similarly, 86.6% of MoBa mothers received higher education, 82.2% entered pregnancy at a normal weight, and a little less than half (48.9%)

were nulliparous. Compared to ALSPAC moms, MoBa moms were older (85.1% were 30 years or older) and less likely to smoke (2.6%) or drink during pregnancy (22.8%).

Within the classes of PFAS, PCBs, and OCPs, median concentrations were highest in both cohorts for PFOS, PCB153, and DDE, respectively (**Table 3-2**). Concentrations were consistently higher in ALSPAC than MoBa. PCBs and OCPs showed high correlation within and between the two classes (**Supplemental Figure 3-2**). There was also strong within-class correlation for PFAS in both cohorts.

Median daily total energy intake was 1730 Calories/day (IQR: 1389, 2082) among ALSPAC mothers and 2148 Calories/day (IQR: 1809, 2555) among MoBa mothers (**Table 3-3**), though it should be noted that the MoBa FFQ contained almost six times as many line items. The frequency of consumption for the 38 food groups is presented in **Table 3-3**. The PCB dietary pattern explained a greater amount of total variance in serum EDC concentrations in both ALSPAC (20.3%) and MoBa (13.8%) compared to PFAS and OCP patterns (**Figure 3-1**). Across PFAS, PCB, and OCP energy-adjusted dietary pattern factor loadings in ALSPAC, positive factor loading values were consistently seen for meat, poultry, white fish, cheese, and biscuits, while baked beans had negative factor loading values (**Figure 3-1**). In MoBa, sausages and burgers (representing processed meats), pasta, and chocolate bars had high positive factor loadings in PCB and OCP dietary patterns, while cheese loaded highly in the PFAS pattern. Across all three patterns in both cohorts, fruit and vegetable loadings tended to be similar and close to zero (peas, sweet corn, and broad beans; green leafy vegetables; carrots; other root vegetables; fresh fruit; etc.). Across both cohorts, French fries and potato chips showed negative factor loadings. Findings were similar for absolute (unadjusted for energy) dietary pattern factor loadings (**Supplemental Figure 3-3**).

In our sensitivity analysis within MoBa using 199 food groups and intake data in grams per day (as opposed to frequency per week), we were able to further specify which foods were most strongly associated with EDCs. For example, we observed a strong association of cheese

with PFAS (**Figure 3-1**), and were able to identify low fat hard cheese as the particular type of cheese driving that association (**Supplemental Figure 3-4**). Further, when using this more detailed dietary data, we saw a much higher amount of total variance in serum EDC concentrations explained (as high as 36.0% for PCBs).

Serum EDC concentrations increased linearly with higher dietary pattern scores in ALSPAC and MoBa. The associations of dietary pattern scores and serum EDC concentrations (summed total of Z scores across class) controlling for covariates are shown in **Table 3-4**. In ALSPAC, the PCB dietary pattern, in combination with covariates, explained the most variance (38.4%) out of the three patterns. Across all three EDC classes in ALSPAC, dietary pattern score, maternal age, and parity were important contributors in predicting serum EDC concentration. In MoBa, the PFAS dietary pattern along with covariates explained the most variance (16.4%) out of the three patterns. The dietary pattern score was the only important contributor in predicting serum EDC concentrations within MoBa.

While the three PFAS, PCB, and OCP dietary patterns were derived for their respective chemical class, dietary patterns correlated with many chemicals under study (**Table 3-5)**. For example, PCB and OCP patterns were significantly correlated with chemicals of both classes. Spearman correlations between EDC concentrations and their respective patterns ranged from 0.23–0.48.

Discussion

Using data from two population-based pregnancy cohorts, we identified dietary patterns associated with exposure to persistent EDCs. Meat, white fish, cheese, and biscuits were associated with all three chemical classes in the ALSPAC study. Within MoBa, sausages and burgers, pasta, and chocolate bars were associated with PCBs and OCPs, and cheese was associated with PFAS. The dietary pattern scores for classes of EDCs were positively associated with the EDC concentrations in blood, and each dietary pattern alone accounted for 8% to 20% of EDC concentrations. While patterns were not necessarily similar across cohorts, some

similarities existed, including strongly inverse associations of French fries and potato chips with EDC concentrations, and no association of most fruits and vegetables with EDC concentrations.

Our findings are largely in agreement with previous studies examining dietary patterns associated with persistent chemicals in various populations, two of which examined pregnant women (209, 212). Studies used a variety of methods to ascertain dietary patterns, including reduced rank regression (208, 212), principal component analysis (204, 210, 211), and multivariate modeling (2, 207). Much of the literature on diet patterns and persistent chemicals has focused on PCBs, which makes sense in the context of our study, as diet seems to explain more variation for PCBs than other persistent EDCs (PFAS, OCPs) in both cohorts. Findings show positive associations of PCBs with eggs (2, 207, 209, 211), fish (2, 204, 207-212), meat (207, 212), poultry (207, 212), and dairy (207, 208, 210, 212). Results from ALSPAC are in agreement on fish, meat, poultry, and dairy (cheese). In MoBa, there was a strong association of processed meats and a moderate association of white fish with PCBs. In both ALSPAC and MoBa, the association of PCBs with eggs was not replicated here or in the only other study specifically examining European pregnant women (212), indicating this might be related to pregnancy status or region. Previous studies have identified similar positive associations of OCPs with poultry (207), fish (2, 208), and dairy (specifically cheese) (2, 207), and negative associations with processed meats (207, 208). We found the same in ALSPAC: positive factor loadings for poultry, fish, and cheese, and a negative factor loading for processed meats (sausages, burgers). Within MoBa, we saw positive factor loadings for poultry, fish, and processed meats, but not cheese. One previous study has examined dietary patterns and PFAS, finding positive associations with meat (cold cuts), fish, and eggs (2). In ALSPAC, we found positive associations of PFAS with meat and white fish, but not other fish or eggs, which showed negative factor loadings. In MoBa, the PFAS pattern was largely characterized by a high loading of cheese, with moderate positive loadings of fish and eggs. Overall, diet seems to explain little variation in PFAS serum concentrations (<10%).

Because of the nature of how certain questions were asked within the ALSPAC FFQ, we were unable to include information on milk and fats in our reduced rank regression models. Since the questions on these items were about types of milk or fat normally used instead of frequency of consumption, we examined these items separately in sensitivity analyses using Wilcoxon rank sum tests. We found that those who mainly used butter on bread and vegetables had higher PFOA and PFOS concentrations than those who never used butter for this purpose. Additionally, those who mainly used sunflower oil or similar for frying had higher concentrations of all PCBs under study and HCB, while those who mainly used other vegetable oil for frying had lower concentrations of all PCBs and HCB. Those who reported usually using full fat milk had lower concentrations of all chemicals than those who reported never using full fat milk, while those who reported usually using skimmed milk had higher concentrations of PCBs and OCPs than those who never use skimmed milk. We were able to look at milk and fats in our sensitivity analysis within MoBa. While factor loadings were close to the null for all types of milk, low fat milk consistently showed negative factor loadings. Further, we saw negative factor loadings for butter in both the PCB and OCP patterns; factor loadings for other fats were close to zero. For both milk and fats, it may be important to consider the types of foods they are normally consumed with, such as cereal or coffee for milk and bread or fried foods (e.g., chicken, fish) for fats.

Although our dietary patterns derived using reduced rank regression account for a portion of the variation in serum EDC concentrations and even more when additional characteristics are included in the model, this does not account for the total variance. This may be because there are other routes of exposure that we were unable to account for, such as through inhalation and dermal absorption. Given the R^2 values seen in our study, alternative routes of exposure may be especially likely for PFAS chemicals, which show considerable variations among individuals with regard to the predominant exposure pathway. While ingestion of food and drinks is in general the predominant pathway, house dust ingestion and indoor air inhalation are important contributors for some individuals (385). Furthermore, foods associated with EDCs might not have been

adequately captured by these FFQs, which could have affected the results observed. The large differences in R^2 values between analyses in MoBa of the 38 condensed food groups versus 199 original food groups demonstrates the benefit of having detailed dietary data. Additionally, given their long half-lives, serum concentrations of these persistent chemicals are reflective of long-term exposure, and may not be fully summarized by the FFQs, which reflect diet "nowadays." The R^2 values seen in our study are similar to those reported in a Korean study (n=188) of PCBs and OCPs in children's (7-9 years old) diet by Lee et al., 2018 and to a study of dioxins in the diet of pregnant women from five European countries by Papadopoulou et al., 2014 (208, 212). In the Korean study, dietary pattern scores explained 21.3% of total PCBs while we saw R^2 values of 20.3% and 13.8%. Though their study explained a greater proportion of the variance for total OCPs in blood by dietary pattern score alone (25.0% versus 16.0% in ALSPAC and 8.1% in MoBa), we were able to account for slightly more variance in total OCPs when we added other predictors to the model (32.2% in ALSPAC versus 30.5% in Lee et al., 2018) (208). In the study of pregnant women in Europe, dioxin dietary patterns varied by country and R^2 values ranged from 9.8 to 27.6%.

Pregnancy is a critical period during which maternal nutrition and lifestyle choices have major influence on both mother and child health. Studies show that exposure to EDCs during critical windows of vulnerability can lead to adverse birth outcomes and increased risks of disease and disability across the lifespan (241). Therefore, it is essential to identify modifiable sources of exposure, such as maternal diet, in order to limit fetal exposure to EDCs. Because dietary patterns differ by an individual's food preferences, eating habits, age, gender, and region (386), and are shaped by cultural, environmental, ecological, and technological factors (387), there is a need to conduct studies in various populations, including pregnant women.

In this study, we attempted to compare derived environmental dietary patterns among pregnant women from two European populations. While we expected to see more similarities between the patterns in ALSPAC and MoBa, the results are not completely surprising given that

both diet and exposure to EDCs differ over time and place. Further, there were foods unique to each population that played important roles. For example, there were uniquely British foods that were important contributors to the ALSPAC environmental dietary patterns, namely pies and pasties and baked beans, and we were unable to identify similar foods within the MoBa FFQ. Similarly, the MoBa FFQ included some foods specific to the Norwegian diet, such as certain types of fish and game. Although we saw varied factor loadings between the dietary patterns derived in ALSPAC and MoBa, we were somewhat reassured by the similar loadings for certain groups. For example, we saw positive factor loadings for cheese with PFAS, white fish and rice with PCBs, and poultry and white fish for OCPs. In the future, studies attempting to externally validate environmental dietary patterns should aim to conduct the study within populations from the same geographic area. The need for measurement of persistent EDCs, which can be costly and requires adequate quantities of biological samples, can make such a population difficult to find.

These studies are strengthened by large sample sizes, prospective study designs within population-based birth cohorts, reliable biological measures of a number of persistent EDCs, and extensive covariate data. This research also has limitations. One limitation of this proposed research is the cross-sectional nature of the environmental and dietary data. In some instances, serum samples were taken weeks before the FFQ was administered, though the different timing of dietary and environmental assessments are unlikely to meaningfully affect persistent EDC concentrations since they have long half-lives (388-390). Second, although the study investigated general populations of pregnant women, generalization of the results is hampered by the non-representative nature of the study populations (e.g., more educated, less likely to smoke). There are also limitations relating to the covariate data. Measures such as self-reported smoking and alcohol use during pregnancy are often unreliable and may suffer from social desirability bias. Education was the only proxy for socioeconomic status used in ALSPAC because many women did not report income, and therefore it is possible that socioeconomic status was not completely

captured. Another potential limitation is that diet is not the only source of exposure to EDCs, as evidenced by the reported R^2 values. While diet may account for the majority of persistent chemical exposure in some populations (3), there are other sources of persistent EDCs. Additionally, misreporting of diet is always a possibility. For example, individuals with higher true intake tend to under-report and individuals with lower true intake tend to over-report. Further, detailed information about food preparation, brands, and contextual information about intake is lacking in FFQs, and because an FFQ is composed of a pre-specified food list, an FFQ may not capture every relevant food that is usually consumed by an individual within a specific population. Finally, both diet and exposure to EDCs differ over time and place. For example, concentrations of chemicals are higher in the ALSPAC population than in the MoBa population. The use of two European populations, where data were collected roughly a decade apart and in different European countries, allowed us to explore the feasibility of generalizing dietary patterns.

In conclusion, our study aligns with previous work and identifies a number of animal-based foods as the predominant dietary source of persistent EDC exposure in pregnant mothers. We attempted to identify dietary patterns that associate food groups with persistent EDCs in two European populations from the 1990s and 2000s. While we expected greater similarity between the derived patterns in terms of foods with high loadings, typical diets in the United Kingdom and Norway are quite different and sources of EDCs may be different. Regardless, this type of work is foundational to eventually developing recommendations for pregnant women seeking to limit their exposure to EDCs before and during pregnancy.

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	Energy-adjusted dietary pattern factor loadings					
	PFAS pattern		PCB pattern		OCP pattern	
	ALSPAC	MoBa	ALSPAC	MoBa	ALSPAC	MoBa
R^2	9.6%	9.7%	20.3%	13.8%	16.0%	8.1%
Food Group						
Sausages, Burgers	-0.06	-0.21	-0.20	0.35	-0.12	0.29
Pies, Pasties	0.07	N/A	-0.22	N/A	-0.12	N/A
Meat	0.31	-0.11	0.18	-0.15	0.30	-0.33
Poultry	0.17	-0.03	0.20	-0.03	0.29	0.21
Liver	-0.30	-0.17	0.02	-0.03	-0.06	0.17
White fish	0.16	0.08	0.24	0.09	0.27	0.13
Other fish	-0.16	0.06	0.18	-0.14	0.20	-0.06
Shellfish	0.11	-0.02	0.07	-0.11	0.19	0.01
Eggs, quiche	-0.14	0.10	-0.04	-0.12	-0.01	-0.23
Cheese	0.19	0.38	0.20	0.06	0.13	0.00
Pizza	0.16	-0.13	-0.17	-0.04	-0.19	-0.11
Chips (French fries)	0.06	-0.33	-0.28	-0.32	-0.22	-0.21
Roast potatoes	-0.11	0.05	-0.13	0.07	-0.13	-0.06
Boiled, mashed, jacket potatoes	-0.14	-0.16	0.02	-0.27	-0.09	-0.21
Rice	0.00	0.16	0.17	0.37	0.20	0.06
Pasta	0.22	0.09	0.10	0.30	0.12	0.30
Crisps (potato chips)	0.02	-0.26	-0.31	0.09	-0.24	0.04
Baked beans	-0.14	N/A	-0.30	N/A	-0.37	N/A
Peas, sweet corn, broad beans	0.10	0.18	0.05	-0.01	-0.04	0.08
Green leafy vegetables	0.06	0.02	0.09	-0.03	0.11	-0.06
Other green vegetables	-0.02	0.07	0.22	-0.09	0.27	-0.05
Carrots	0.02	0.06	0.11	0.05	0.06	0.14
Other root vegetables	-0.15	0.14	0.07	0.19	0.08	-0.04
Salad	-0.15	-0.07	0.03	-0.01	0.07	0.25
Fresh fruit	-0.16	-0.07	0.10	0.15	0.10	0.17

Pure juice not in tin	0.12	0.23	0.09	0.20	0.06	0.21
Pudding	0.06	-0.15	0.18	-0.12	0.10	-0.18
Oat cereals	-0.19	-0.23	0.06	-0.02	0.00	0.20
Wholegrain or bran cereals	-0.05	0.12	0.07	-0.12	-0.06	-0.05
Other cereals	0.04	-0.17	-0.11	-0.05	-0.08	0.07
Cakes or buns	-0.02	-0.04	0.25	0.10	0.15	0.01
Crispbreads	0.03	0.02	0.10	-0.17	0.00	-0.14
Biscuits	0.27	0.06	0.22	-0.15	0.19	-0.20
Chocolate bars	0.14	-0.28	-0.08	0.29	0.04	0.32
Pulses (legumes)	-0.46	0.25	0.17	-0.12	0.10	-0.12
Nuts	-0.27	-0.12	0.10	-0.07	0.14	-0.16
Chocolate	0.09	-0.11	0.01	0.19	0.12	0.03
Sweets	0.02	0.27	-0.18	0.20	-0.18	0.08

Figure 3-1. Factor loadings of dietary patterns for exposure to EDCs in serum in the Avon Longitudinal Study of Parents and Children (ALSPAC) and Norwegian Mother, Father, and Child Cohort Study (MoBa) determined through reduced rank regression.
Abbreviations: PFAS, perfluoroalkyl substances; PCBs, polychlorinated biphenyls; OCPs, organochlorine pesticides. Adjusted for energy intake using the residual method. The graded color scale shows green for negative factor loadings (food groups negatively associated with persistent EDCs) and red for positive factor loadings (food groups positively associated with persistent EDCs).

Table 3-1. Characteristics of the Avon Longitudinal Study of Parents and Children (ALSPAC) (N=422) and the Norwegian Mother, Father, and Child Cohort Study (MoBa) (N=276) substudy populations.

	ALSPAC			MoBa	
Characteristic	**N**	**%**	**Characteristic**	**N**	**%**
Maternal race			Maternal country of birth		
White	412	98.1	Norwegian-born	229	84.2
Non-white	8	1.9	Not Norwegian-born	43	15.8
Maternal education[a]			Maternal education[b]		
<O-level	74	18.3	No higher education	34	13.4
O-level	134	33.2	1-4 years higher education	82	32.3
>O-level	196	48.5	>4 years of higher education	138	54.3
Maternal pre-pregnancy BMI, kg/m^2			Maternal pre-pregnancy BMI, kg/m^2		
<25 (under/normal weight)	302	77.8	<25 (under/normal weight)	226	82.2
≥25 (overweight/obese)	86	22.2	≥25 (overweight/obese)	49	17.8
Prenatal smoking			Prenatal smoking		
Any	75	18.2	Any	7	2.6
None	337	81.8	None	264	97.4
Prenatal alcohol use			Prenatal alcohol use		
Any	208	51.2	Any	57	22.8
None	198	48.8	None	193	77.2
Physical activity			Physical activity		
Any	249	65.7	Any	165	61.6
None	130	34.3	None	103	38.4
Maternal age at delivery, years			Maternal age at delivery, years		
<29	240	56.9	<29	41	14.9
≥30	182	43.1	≥30	235	85.1
Child birth order			Child birth order		
First born	201	49.9	First born	135	48.9
Second born or later	202	50.1	Second born or later	141	51.1
Child's sex			Child's sex		
Female	422	100.0	Female	127	46.0
Male	0	0.0	Male	149	54.0

Abbreviations: g, grams; kg/m^2, kilograms per meter-squared

[a] <O-level=none, Certificate of Secondary Education, and vocational education, which are equivalent to no diploma or a GED in the United States. O-levels (ordinary levels) are required and completed at the age of 16. >O-level=A-levels (advanced levels) completed at 18, which are optional, but required to get into university; and a university degree.

[b] No higher education=1-2 years high school, technical high school, 3-year high school general studies, junior college. 1-4 years higher education=Regional technical college, 4-year university degree (Bachelor's degree, nurse, teacher, engineer). >4 years of higher education=University, technical college, more than 4 years (Master's degree, medical doctor, PhD).

Table 3-2. Serum concentrations of persistent endocrine disrupting chemical (EDC) exposure among mothers of the Avon Longitudinal Study of Parents and Children (ALSPAC) (N=422) and the Norwegian Mother, Father, and Child Cohort Study (MoBa) (N=276) during pregnancy.

	ALSPAC			MoBa		
	Median	**Q1**	**Q3**	**Median**	**Q1**	**Q3**
Perfluoroalkyl substances (PFAS) (ng/mL)						
PFOA	3.8	2.9	4.8	2.1	1.4	3.0
PFOS	20.0	15.3	25.3	9.0	6.6	12.9
PFHxS	1.6	1.2	2.2	0.6	0.4	0.9
PFNA	0.49	0.41	0.66	0.50	0.36	0.66
Polychlorinated biphenyls (PCBs) (ng/g lipid)						
PCB118	15.0	10.9	20.8	7.2	5.3	9.8
PCB138[a]	41.8	30.3	54.0	21.4	15.9	30.6
PCB153	64.8	48.3	86.2	44.6	32.9	61.2
PCB170	19.0	14.4	25.2	8.3	5.5	11.0
PCB180	45.6	33.5	60.4	24.5	15.9	32.0
Organochlorine pesticides (OCPs) (ng/g lipid)						
HCB	50.7	38.3	63.5	15.7	12.0	19.7
DDE	318.5	193.5	502.5	56.1	38.4	87.5
DDT	11.0	7.8	16.5	2.1	1.3	3.4

Abbreviations: Q1, quartile 1; Q3, quartile 3; LOD, limit of detection; ng/mL, nanogram per milliliter; ng/g lipid, nanogram per gram lipid

[a] In ALSPAC, PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration referred to as PCB138.

Table 3-3. Summary of daily intake of food groups among mothers of the Avon Longitudinal Study of Parents and Children (ALSPAC) (N=422) and the Norwegian Mother, Father, and Child Cohort Study (MoBa) (N=276) during pregnancy.

	ALSPAC			MoBa		
Food group	**Frequency of Consumption (per day)**			**Frequency of Consumption (per day)**		
	Median	Q1	Q3	Median	Q1	Q3
Sausages, Burgers	0.07	0.00	0.07	0.91	0.46	1.79
Pies, Pasties	0.07	0.00	0.07	N/A	N/A	N/A
Meat	0.29	0.29	0.29	0.43	0.29	0.64
Poultry	0.29	0.07	0.29	0.21	0.14	0.36
Liver	0.00	0.00	0.00	0.50	0.11	0.79
White fish	0.07	0.07	0.29	0.32	0.21	0.50
Other fish	0.07	0.00	0.29	0.36	0.14	0.82
Shellfish	0.00	0.00	0.00	0.07	0.04	0.14
Eggs, quiche	0.29	0.07	0.29	0.21	0.21	0.50
Cheese	0.29	0.29	0.79	1.69	1.08	2.72
Pizza	0.07	0.00	0.07	0.11	0.07	0.11
Chips (French fries)	0.07	0.07	0.29	0.04	0.00	0.09
Roast potatoes	0.07	0.00	0.29	0.04	0.00	0.04
Boiled, mashed, jacket potatoes	0.29	0.29	0.79	0.21	0.09	0.50
Rice	0.07	0.07	0.29	0.21	0.09	0.21
Pasta	0.07	0.07	0.29	0.29	0.18	0.43
Crisps (potato chips)	0.29	0.07	0.29	0.09	0.04	0.21
Baked beans	0.29	0.07	0.29	N/A	N/A	N/A
Peas, sweet corn, broad beans	0.29	0.29	0.29	1.19	0.77	1.78
Green leafy vegetables	0.29	0.07	0.29	0.09	0.04	0.13
Other green vegetables	0.29	0.07	0.29	0.55	0.38	0.98
Carrots	0.29	0.29	0.29	0.30	0.18	0.59
Other root vegetables	0.07	0.00	0.29	0.68	0.39	1.21
Salad	0.29	0.07	0.79	0.88	0.46	1.35
Fresh fruit	0.79	0.79	1.43	2.46	1.58	3.54

Pure juice not in tin	0.29	0.07	0.79	0.88	0.30	1.25
Pudding	0.07	0.07	0.29	0.22	0.13	0.34
Oat cereals	0.07	0.00	0.29	0.09	0.00	0.21
Wholegrain or bran cereals	0.29	0.00	0.79	0.04	0.00	0.21
Other cereals	0.07	0.00	0.29	0.00	0.00	0.00
Cakes or buns	0.29	0.07	0.29	0.25	0.16	0.39
Crispbreads	0.00	0.00	0.07	0.93	0.21	2.00
Biscuits	0.29	0.29	0.79	0.04	0.00	0.09
Chocolate bars	0.29	0.07	0.29	0.09	0.00	0.21
Pulses (legumes)	0.00	0.00	0.07	0.00	0.00	0.00
Nuts	0.00	0.00	0.07	0.09	0.04	0.21
Chocolate	0.07	0.07	0.29	0.21	0.09	0.21
Sweets	0.07	0.00	0.29	0.38	0.18	0.68
Total energy (Calories)	1730	1389	2082	2141	1804	2522

Table 3-4. Associations of dietary pattern scores and summed Z-scores of persistent EDCs in the Avon Longitudinal Study of Parents and Children (ALSPAC) and Norwegian Mother, Father, and Child Cohort Study (MoBa) determined through multiple linear regression analysis.

ALSPAC	**Total PFAS[a]** (n=350)			**MoBa**	**Total PFAS[a]** (n=231)		
	β[d]	**SE**	**p-value**		**β[d]**	**SE**	**p-value**
Dietary pattern score	1.17	0.17	<0.0001	Dietary pattern score	1.39	0.23	<.0001
Non-white race	2.94	1.94	0.13	Not Norwegian-born	0.02	0.58	0.98
Maternal age (years)	0.11	0.04	0.002	Maternal age (years)	0.00	0.07	0.98
Pre-pregnancy BMI (kg/m^2)	0.03	0.04	0.41	Pre-pregnancy BMI (kg/m^2)	0.01	0.07	0.85
Multiparous	-1.52	0.33	<0.0001	Multiparous	0.14	0.45	0.75
> O-level education	-0.13	0.46	0.77	1-4 years higher education	-0.72	0.71	0.31
O-level education	-0.21	0.47	0.66	>4 years higher education	-1.59	0.68	0.02
Smoking	-0.81	0.45	0.07	Smoking	0.20	1.45	0.89
Sample gestation (weeks)	-0.02	0.02	0.31	Male infant	0.62	0.44	0.16
Total Energy (kJ)	0.00	0.00	0.21	Total Energy (kJ)	0.00	0.00	0.93
R^2 (%)	21.6			R^2 (%)	16.4		
	Total PCBs[b] (n=336)				**Total PCBs[b]** (n=239)		

	β[d]	SE	p-value
Dietary pattern score	1.19	0.26	<0.0001
Non-white race	4.86	2.58	0.06
Maternal age (years)	0.46	0.06	<0.0001
Pre-pregnancy BMI (kg/m^2)	-0.08	0.05	0.15
Multiparous	-1.20	0.46	0.01
> O-level education	-1.02	0.64	0.11
O-level education	-1.52	0.65	0.02
Smoking	-0.80	0.64	0.21
Sample gestation (weeks)	0.00	0.02	0.93
Total Energy (kJ)	0.00	0.00	0.98
R^2 (%)	38.4		

	β[d]	SE	p-value
Dietary pattern score	2.07	0.36	<.0001
Not Norwegian-born	-1.11	0.81	0.17
Maternal age (years)	-0.03	0.09	0.73
Pre-pregnancy BMI (kg/m^2)	0.00	0.09	0.96
Multiparous	0.52	0.62	0.40
1-4 years higher education	0.28	0.98	0.78
>4 years higher education	0.39	0.93	0.68
Smoking	-0.47	1.87	0.80
Male infant	-0.14	0.60	0.81
Total Energy (kJ)	0.00	0.00	0.99
R^2 (%)	13.7		

	Total OCPs[c] (n=324)		
	β[d]	**SE**	**p-value**
Dietary pattern score	0.40	0.11	0.004
Non-white race	2.27	1.08	0.04
Maternal age (years)	0.18	0.02	<0.0001

	Total OCPs[c] (n=226)		
	β[d]	**SE**	**p-value**
Dietary pattern score	0.69	0.19	0.0004
Not Norwegian-born	-0.55	0.45	0.22
Maternal age (years)	-0.05	0.05	0.36

Pre-pregnancy BMI (kg/m^2)	0.09	0.02	0.0001	Pre-pregnancy BMI (kg/m^2)	-0.01	0.05	0.87
Multiparous	-0.63	0.20	0.002	Multiparous	0.27	0.33	0.41
> O-level education	-1.13	0.27	<0.0001	1-4 years higher education	0.07	0.54	0.89
O-level education	-0.63	0.28	0.02	>4 years higher education	0.20	0.51	0.69
Smoking	-0.45	0.28	0.10	Smoking	0.53	0.99	0.59
Sample gestation (weeks)	0.00	0.01	0.74	Male infant	-0.25	0.32	0.43
Total Energy (kJ)	0.00	0.00	0.59	Total Energy (kJ)	0.00	0.00	0.68
R^2 (%)	32.6			R^2 (%)	7.6		

Abbreviations: PFAS, perfluoroalkyl substances; PCBs, polychlorinated biphenyls; OCPs, organochlorine pesticides; BMI, body mass index

[a] Total PFAS=summed z-scores of PFOA, PFOS, PFHxS, and PFNA

[b] Total PCBs=summed z-scores of PCB118, PCB138, PCB153, PCB170, and PCB180

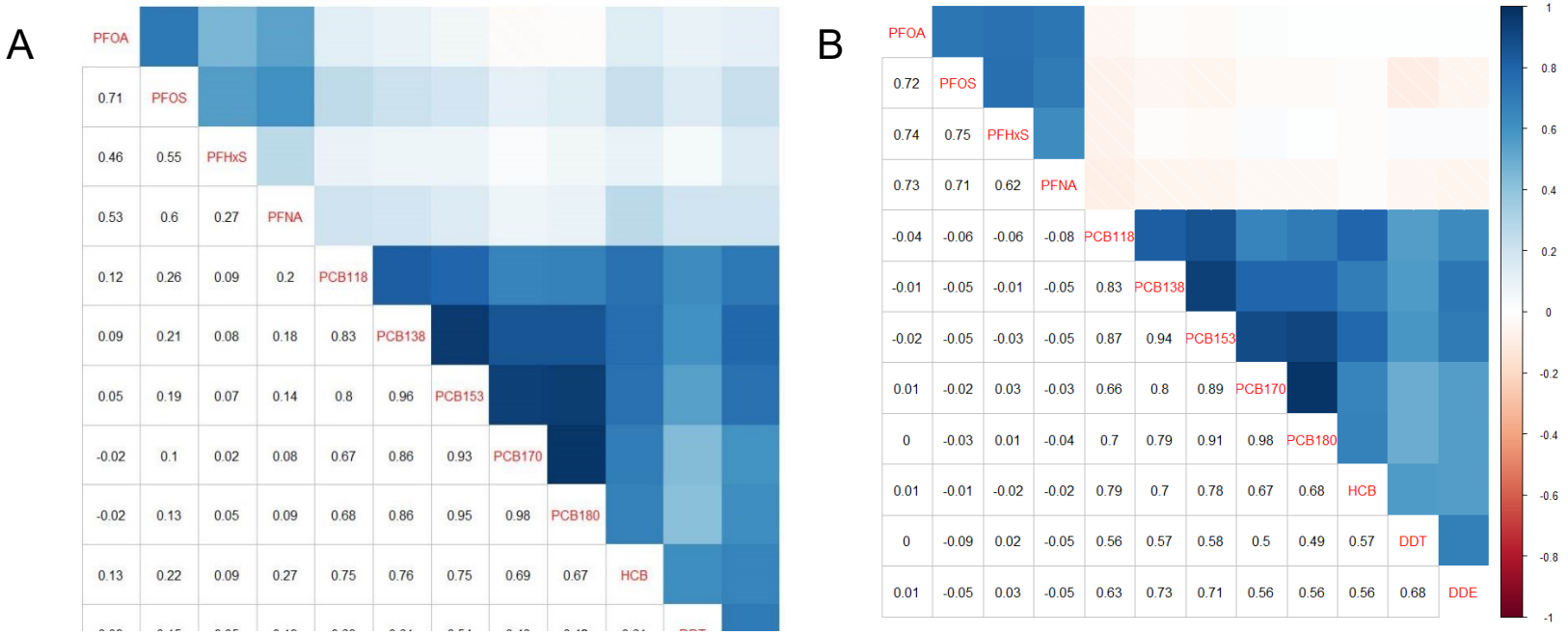
[c] Total OCPs=summed z-scores of DDT, DDE, and HCB

[d] β estimates represent a one-unit change in the independent variable (e.g., dietary pattern score or maternal age) with the respective change (of size β) in the EDC Z-score.

Table 3-5. Spearman correlation coefficients (r_s) between energy-adjusted dietary pattern scores determined using reduced rank regression and persistent endocrine disrupting chemicals in the Avon Longitudinal Study of Parents and Children (ALSPAC) and the Norwegian Mother, Father, and Child Cohort Study (MoBa).

	PFAS pattern		PCB pattern		OCP pattern	
	ALSPAC	**MoBa**	**ALSPAC**	**MoBa**	**ALSPAC**	**MoBa**
PFOA	0.29*	0.30*	0.05	0.06	0.09	0.04
PFOS	0.25*	0.36*	0.12*	0.03	0.15*	-0.02
PFHxS	0.23*	0.38*	0.05	0.05	0.09	-0.04
PFNA	0.24*	0.24*	0.09	0.00	0.13*	-0.02
PCB118	0.22*	0.02	0.41*	0.31*	0.39*	0.27*
PCB138	0.20*	0.04	0.46*	0.32*	0.43*	0.23*
PCB153	0.15*	0.03	0.48*	0.36*	0.42*	0.26*
PCB170	0.09	0.04	0.46*	0.36*	0.39*	0.23*
PCB180	0.09	0.02	0.48*	0.37*	0.40*	0.24*
DDT	0.12*	-0.06	0.28*	0.15*	0.33*	0.29*
DDE	0.16*	0.02	0.41*	0.22*	0.42*	0.27*
HCB	0.23*	0.02	0.36*	0.26*	0.39*	0.30*

* $p<0.05$



Supplemental Figure 3-2. Correlation heatmap of serum concentrations of persistent endocrine disrupting chemicals in women during pregnancy in A) the Avon Longitudinal Study of Parents and Children (N=422) and B) the Norwegian Mother, Father, and Child Cohort Study (MoBa) (N=276). Spearman correlation coefficients presented for untransformed distributions. PCB and OCP concentrations were lipid-adjusted.

	Absolute dietary pattern factor loadings					
	PFAS pattern		PCB pattern		OCP pattern	
	ALSPAC	MoBa	ALSPAC	MoBa	ALSPAC	MoBa
R^2	10.1%	9.5%	20.3%	13.7%	16.0%	8.0%
Food Group						
Sausages, Burgers	-0.15	-0.21	-0.19	0.37	-0.16	0.27
Pies, Pasties	-0.02	N/A	-0.22	N/A	-0.15	N/A
Meat	0.21	-0.11	0.19	-0.11	0.26	-0.33
Poultry	0.10	-0.03	0.21	-0.01	0.27	0.21
Liver	-0.30	-0.18	0.03	-0.01	-0.06	0.16
White fish	0.07	0.08	0.25	0.10	0.24	0.13
Other fish	-0.18	0.06	0.19	-0.13	0.19	-0.06
Shellfish	0.08	-0.02	0.07	-0.09	0.19	0.00
Eggs, quiche	-0.23	0.10	-0.03	-0.12	-0.05	-0.24
Cheese	0.06	0.36	0.20	0.09	0.09	-0.01
Pizza	0.04	-0.14	-0.16	-0.02	-0.22	-0.12
Chips (French fries)	-0.07	-0.34	-0.26	-0.29	-0.26	-0.22
Roast potatoes	-0.19	0.05	-0.13	0.07	-0.17	-0.07
Boiled, mashed, jacket potatoes	-0.24	-0.17	0.03	-0.23	-0.13	-0.22
Rice	-0.04	0.16	0.18	0.38	0.19	0.06
Pasta	0.13	0.08	0.10	0.31	0.09	0.30
Crisps (potato chips)	-0.08	-0.27	-0.31	0.11	-0.28	0.04
Baked beans	-0.23	N/A	-0.29	N/A	-0.40	N/A
Peas, sweet corn, broad beans	0.01	0.17	0.06	0.01	-0.07	0.08
Green leafy vegetables	0.00	0.02	0.09	-0.01	0.09	-0.07
Other green vegetables	-0.07	0.06	0.23	-0.06	0.26	-0.06
Carrots	-0.04	0.06	0.12	0.07	0.03	0.14
Other root vegetables	-0.20	0.14	0.08	0.20	0.06	-0.05
Salad	-0.16	-0.08	0.04	0.00	0.07	0.26
Fresh fruit	-0.19	-0.08	0.10	0.18	0.09	0.15

Pure juice not in tin	0.10	0.21	0.10	0.22	0.06	0.19
Pudding	-0.07	-0.15	0.18	-0.08	0.05	-0.19
Oat cereals	-0.23	-0.24	0.06	0.00	-0.02	0.19
Wholegrain or bran cereals	-0.08	0.12	0.07	-0.10	-0.07	-0.05
Other cereals	-0.03	-0.18	-0.11	-0.03	-0.10	0.07
Cakes or buns	-0.16	-0.04	0.24	0.13	0.09	0.00
Crispbreads	0.01	0.01	0.11	-0.14	-0.01	-0.15
Biscuits	0.12	0.06	0.22	-0.12	0.14	-0.21
Chocolate bars	-0.04	-0.29	-0.06	0.30	-0.03	0.31
Pulses (legumes)	-0.49	0.26	0.18	-0.12	0.08	-0.12
Nuts	-0.29	-0.13	0.11	-0.04	0.13	-0.17
Chocolate	-0.07	-0.11	0.02	0.21	0.05	0.02
Sweets	-0.06	0.25	-0.17	0.22	-0.22	0.07

Supplemental Figure 3-3. Absolute factor loadings of dietary patterns for exposure to EDCs in serum in the Avon Longitudinal Study of Parents and Children (ALSPAC) and the Norwegian Mother, Father, and Child Cohort Study (MoBa) determined through reduced rank regression. Abbreviations: PFAS, perfluoroalkyl substances; PCBs, polychlorinated biphenyls; OCPs, organochlorine pesticides. Not adjusted for energy intake. The graded color scale shows green for negative factor loadings (food groups negatively associated with persistent EDCs) and red for positive factor loadings (food groups positively associated with persistent EDCs).

Dietary pattern factor loadings

	PFAS pattern	PCB pattern	OCP pattern
R^2	29.9%	36.0%	25.1%
Food Group			
Full fat milk	-0.11	0.02	0.02
Low fat milk			-0.17
Skimmed milk	0.11		
Cream whipped	-0.03	-0.32	-0.31
Creme fraiche	0.09		0.06
Creme fraiche light	-0.04		
Yogurt plain	-0.12		
Chocolate milk		0.04	-0.06
Feta cheese		-0.09	-0.16
Hard cheese low fat	0.45		
Cheese other kinds		0.26	
Eggs			-0.17
Hamburger			0.24
Hot dogs	-0.12		
Salami			0.07
Cold cuts medium fat		0.26	
Liver paste			0.12
Low fat cold cuts		0.19	
Panfried chicken turkey	0.26		
Chicken fillet	0.00		
Pasta with meat			0.33
Beef		-0.23	
Pork tenderloin		-0.03	
Bacon			-0.14
Taco	0.11		
Roe spread	0.12		
Shrimps northern		-0.03	
Muesli unsweetened		-0.08	
Crispbread	0.01	-0.08	-0.14
Crackers		0.01	
Waffle	-0.04		
Rice		0.25	
Porridge	0.13		-0.03
Cookies		-0.10	-0.13
Bread wholemeal	0.16	-0.05	-0.07
Muesli sweetened		0.22	0.19
Bread dark		0.06	0.09
Other vegetables		-0.02	-0.05
Aubergine		0.09	0.06

Califlower raw		0.08	0.16
Peas		0.03	-0.11
Carrots raw			0.14
Cabbage raw	-0.02	-0.07	-0.19
Garlic			-0.06
Green mixed salad		-0.06	-0.07
Onions raw	0.03		
Pepper raw		0.22	
Brussels sprouts		0.07	
Lettuce		-0.11	0.09
Mushrooms raw		0.20	0.25
Mushroom fried casseroles	-0.17	0.01	
Tomato		-0.22	-0.14
Frozen vegetables			-0.07
Ketchup	-0.17		
Squash	0.02		
Potatoes			-0.12
Green beans	0.14		-0.03
Mushroom	0.01		-0.03
Other berries	-0.24	0.09	-0.03
Strawberries	-0.24	0.01	-0.02
Apricots dried	0.04	0.01	0.01
Avocado			-0.13
Banana	0.03	-0.09	-0.01
Apple	-0.07		
Peach	0.07	0.15	
Fruit salad fresh	-0.07		
Mango			0.12
Plum			0.13
Pear	0.11		
Raisins	-0.13	-0.11	-0.06
Prunes	-0.11	0.06	-0.01
Almonds	-0.05		-0.11
Olives			-0.04
Peanuts	-0.13		
Orange juice	0.16		0.17
Fruit nectars	0.08		
Peppers casserole	0.11		
Cauliflower cooked	-0.01	-0.16	
Cabbage cooked	0.03		
Swede boiled	0.04	0.00	-0.02
Broccoli cooked	0.23	-0.11	
French fries	-0.20		-0.11
Potatoes creamed		0.05	-0.03

Spread vegetables			0.12
Butter	-0.01	-0.24	-0.25
Soya oil		-0.24	
Margarine soft	-0.16		
Mayo	0.05	0.18	0.19
Dressing		-0.18	
Dressing homemade	0.15		
Dressing with yogurt		0.02	
Marzipan		0.05	
Chocolate plain	-0.07	0.15	
Chocolate filled			0.19
Pastille	0.15		
Pastille sugar free	0.12		-0.08
Candies	0.11	0.13	0.14
Gravy	-0.17	-0.14	-0.12
Salty snacks	-0.12		
Popcorn	-0.04		
Water sparkling		0.02	
Coffee filter		0.06	0.07
Cafe latte	0.18	-0.05	-0.04
Water still	0.00		
Tea green	0.03		

Supplemental Figure 3-4. Absolute factor loadings of dietary patterns for exposure to EDCs in serum in the Norwegian Mother, Father, and Child Cohort Study (MoBa) using intake data and detailed food groups. Dietary patterns were determined through reduced rank regression and include only those food groups that were ever eaten by 25% of pregnant women and had a variable importance in projection score ≥1.0. Abbreviations: PFAS, perfluoroalkyl substances; PCBs, polychlorinated biphenyls; OCPs, organochlorine pesticides. Not adjusted for energy intake. The graded color scale shows green for negative factor loadings (food groups negatively associated with persistent EDCs) and red for positive factor loadings (food groups positively associated with persistent EDCs).

Supplemental Table 3-6. List of foods in the ALSPAC "Your Pregnancy" questionnaire (Section C: Your Diet) administered at 32 weeks gestation.

Foods
Sausages, burgers
Pies, pasties (pork pie, steak/meat pie)
Meat (beef, lamb, pork, ham, bacon)
Poultry (chicken, turkey)
Liver, liver pate, kidney, heart
White fish (cod, haddock, plaice, fish fingers)
Other fish (pilchards, sardines, mackerel, tuna, herring, kippers, trout, salmon)
Shellfish (prawns, crab, cockles, mussels)
Eggs, quiche
Cheese
Pizza
Chips (French fries)
Roast potatoes (cooked in fat)
Boiled, mashed, jacket potatoes
Rice (boiled)
Pasta (spaghetti, pot noodles, lasagna)
Crisps (potato chips)
Fried foods (fried fish, eggs, bacon, chops)[a]
Baked beans
Peas, sweetcorn, broad beans
Cabbage, Brussel sprouts, kale and other green leafy vegetables
Other green vegetables (cauliflower, runner beans, leeks)
Carrots
Other root vegetables (turnip, swede, parsnip)
Salad (lettuce, tomato, cucumber)
Fresh fruit (apple, pear, banana, orange, bunch of grapes)
Tinned juice (including tomato juice)[b]
Pure juice not in tin
Pudding (fruit pie, crumble, cheesecake, milk pudding, mousse, gateaux)
Oat cereals (porridge, Ready Brek, muesli)
Wholegrain or bran cereals (All Bran, Bran Flakes, Weetabix, Wheatflakes, Fruit & Fibre)
Other cereals (Corn-flakes, Rice Krispies, Special K, Frosties)
Cakes or buns (fruit cake, sponge, teacake, buns, doughnut, flapjack, scone, custard, tart, cream cake)
Crispbreads (Ryvita, crackerbread)
Biscuits (digestive, shortcake, Hob Nobs, Rich Tea, Nice, Maries, chocolate biscuits, Penguin, Club, Kit Kat)
Chocolate bars (Mars, Twix, Wispa, Bounty, Creme Egg)
Pulses (dried peas, beans, lentils, chickpeas)
Nuts, nut roast
Bean curd (tofu, miso)[b]
Tahini[b]
Soya 'Meat', T V P, vegeburgers[b]
Chocolate (dairy milk or plain, nut, fruit filled)

Sweets (peppermints, boiled sweets, toffees)

[a] Removed from analyses because it represents a heterogeneous, duplicative group that represents a preparation method instead of particular food

[b] Food groups removed from analyses due to infrequent consumption

Supplemental Table 3-7. Select list of foods in the MoBa "Your Diet" questionnaire administered at 22 weeks gestation mapped to broader food groups.

Foods	Mapped to Food Group
Meat /pork sausage	Sausages, Burgers
Hot dogs and/or frankfurters	Sausages, Burgers
Chicken and/or turkey sausage	Sausages, Burgers
Hamburger, meat patty	Sausages, Burgers
Low fat cold cuts (ham, roast beef etc.)	Sausages, Burgers
Medium fat cold cuts of lamb, calf etc.	Sausages, Burgers
Salami, Swedish sausage etc.	Sausages, Burgers
Other kinds of cold cut meat	Sausages, Burgers
Bacon	Sausages, Burgers
Cold cuts of turkey, chicken	Sausages, Burgers
N/A	Pies, Pasties
Meatballs, meat loaf	Meat
Minced meat	Meat
Beef and/or veal roast	Meat
Beef (fillet, tenderloin, sirloin, entrecote)	Meat
T-bone steak, beef, and veal	Meat
Beef stew, beef soup	Meat
Pork chop, pork roast, pork schnitzel	Meat
Pork tenderloin, fillet	Meat
Pork loin smoked	Meat
Pork belly bacon, spareribs	Meat
Pork stew	Meat
Lamb roast, lamb sirloin	Meat
Lamb stews (Fårikål etc.)	Meat
Reindeer roast	Meat
Roast of elk, roe deer, fallow deer	Meat
Reindeer patty/reindeer stew	Meat
Patty/ stew of elk, roe / fallow deer	Meat
Chicken fillet, turkey fillet	Poultry
Fried chicken	Poultry
Pan fried/baked/boiled chicken, turkey	Poultry
Chickenschnitzel, nuggets	Poultry
Game (grouse, pheasant etc.)	Poultry
Other poultry (duck, goose, ostrich)	Poultry
Liver paste	Liver
Liver, kidney from beef, pork	Liver
Liver, kidney from lamb	Liver
Liver, kidney from venison	Liver
Black pudding, hashed lungs	Liver
Cod, saithe, haddock, Pollack	White fish
Halibut, plaice, flounder	White fish
Perch, pike, pikecake	White fish
Fish cake, fish pudding, fish balls	White fish
Fish fingers, breaded fish	White fish
Tuna (spread)	White fish
Other kinds of fish (spread)	White fish
Tuna fish	White fish

Other fishes	White fish
Fish casserole, soup	White fish
Roe spread	Other fish
Mackerel/sardine in tomato sauce (spread)	Other fish
Sardine in oil	Other fish
Smoked salmon/trout/mackerel	Other fish
Herring, pickled	Other fish
Svolværpostei (spread of fish liver/roe)	Other fish
Mackerel, herring	Other fish
Salmon, trout	Other fish
Roe	Other fish
Fish liver	Other fish
Shrimp (sandwich)	Shellfish
Crab (sandwich)	Shellfish
Shrimp (hot meals)	Shellfish
Mussels (hot meals)	Shellfish
Crab (hot meals)	Shellfish
Eggs, - fried, boiled, scrambled, omelet	Eggs, quiche
Hard cheese, cream cheese	Cheese
Hard cheese, cream cheese (low fat)	Cheese
Whey cheese goat milk, regular	Cheese
Whey cheese (low fat), spread goat milk	Cheese
Blue cheese (Camembert, Norzola etc.)	Cheese
Feta cheese	Cheese
Other kinds of cheese	Cheese
Cheese with pasta	Cheese
Pizza	Pizza
French fries, fried potatoes	Chips (French fries)
Creamed potatoes, potato casserole	Roast potatoes
Potatoes (boiled, baked, mashed)	Boiled, mashed, jacket potatoes
Rice	Rice
Pasta with meat (Bolognaise Lasagna)	Pasta
Pasta with fish/ mussels/ shrimp	Pasta
Pasta with vegetables	Pasta
Pasta with only tomato sauce/ ketchup	Pasta
Spaghetti, macaroni, noodles	Pasta
Potato chips	Crisps (potato chips)
N/A	Baked beans
Frozen vegetables	Peas, sweet corn, broad beans
Green beans, haricots verts	Peas, sweet corn, broad beans
Peas	Peas, sweet corn, broad beans
Corn, corn on the cob	Peas, sweet corn, broad beans
Pepper (raw or in casserole)	Peas, sweet corn, broad beans
Tomato	Peas, sweet corn, broad beans

Brussels sprouts	Green leafy vegetables
Cabbage (cooked or raw)	Green leafy vegetables
Spinach	Green leafy vegetables
Aubergine	Other green vegetables
Avocado	Other green vegetables
Cauliflower (raw or boiled/ in casseroles)	Other green vegetables
Broccoli (raw or boiled/ in casseroles)	Other green vegetables
Squash (zucchini)	Other green vegetables
Other vegetables	Other green vegetables
Carrots (raw or boiled)	Carrots
Garlic	Other root vegetables
Swede (raw or boiled)	Other root vegetables
Onions	Other root vegetables
Celery	Other root vegetables
Green salad mix in plastic bag	Salad
Lettuce, Chinese cabbage	Salad
Cucumber	Salad
Orange, clementine	Fresh fruit
Banana	Fresh fruit
Grapes	Fresh fruit
Apple	Fresh fruit
Peach, nectarine	Fresh fruit
Grapefruit	Fresh fruit
Strawberries	Fresh fruit
Other berries (blueberries etc)	Fresh fruit
Mango	Fresh fruit
Melon	Fresh fruit
Papaya	Fresh fruit
Plum	Fresh fruit
Pear	Fresh fruit
Other fruits	Fresh fruit
Fruit salad made of fresh fruit	Fresh fruit
Orange juice	Pure juice not in tin
Other fruit juices, nectar	Pure juice not in tin
Pudding (chocolate, creme caramel etc.)	Pudding
Canned fruit, stewed fruit thickened with potato flour	Pudding
Ice cream	Pudding
Water ice sticks, sherbet	Pudding
Vanilla sauce	Pudding
Cream, whipped cream	Pudding
Sweetened muesli, muesli with fruits/ nuts	Oat cereals
Porridge, cream of wheat, rice etc.	Oat cereals
Unsweetened muesli, All-Bran Flakes	Wholegrain or bran cereals
Corn Flakes, Frosties etc.	Other cereals
Waffle	Cakes or buns
Sweet bun	Cakes or buns
Chocolate cake, cream layer cake, etc.	Cakes or buns
Doughnut, sponge cake	Cakes or buns
Danish pastry	Cakes or buns
Fiber bread, fiber crispbread, ryecrisp	Crispbreads
Crispbread, rusk etc.	Crispbreads

Crackers (Cream cracker etc.)	Crispbreads
Cookies	Biscuits
Fancy and filled chocolate	Chocolate bars
Vegetables dishes as main course with beans and/or lentils	Pulses (legumes)
Almonds	Nuts
Peanuts	Nuts
Plain chocolate	Chocolate
Caramel, candies, licorice	Sweets
Jelly sweets, marshmallow	Sweets
Pastille	Sweets
Pastille sugar free	Sweets
Marzipan	Sweets

Chapter 4 Mixtures of prenatal concentrations of persistent endocrine disrupting chemicals and birth size in British girls

Kristin J. Marks[a,b,c], Penelope P. Howards[a], Melissa M. Smarr[d], W. Dana Flanders[a,b], Kate Northstone[e], Johnni H. Daniel[b], Andreas Sjödin[f], Antonia M. Calafat[f], Terryl J. Hartman[a,b]

[a] Department of Epidemiology, Rollins School of Public Health, Emory University, 1518 Clifton Rd NE, Atlanta, Georgia 30322, United States
[b] Division of Environmental Health Science and Practice, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy, Atlanta, Georgia 30341, United States
[c] Oak Ridge Institute for Science and Education, 100 ORAU Way, Oak Ridge, Tennessee 37830, United States
[d] Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, 1518 Clifton Rd NE, Atlanta, Georgia 30322, United States
[e] Department of Population Health Sciences, Bristol Medical School, Oakfield House, Oakfield Grove, Bristol BS8 2BN, United Kingdom
[f] Division Of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy, Atlanta, Georgia 30341, United States

Abstract:
Previous studies of endocrine disrupting chemicals (EDCs) have examined one EDC at a time in association with an outcome; however, humans are exposed to many EDCs. By studying mixtures of EDCs, the human experience can be better approximated. We investigated the association of prenatal exposure to persistent EDCs (per- and polyfluoroalkyl substances (PFAS), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs)) as mixtures with birth size among female offspring in a sub-study of the Avon Longitudinal Study of Parents and Children (ALSPAC) (N=448), based in the United Kingdom in 1991-1992. We quantified concentrations of 52 EDCs in maternal serum collected during pregnancy. Birth weight, crown to heel length, and head circumference were measured at birth; ponderal index and small for gestational age were calculated. We used weighted quantile sum (WQS) regression to examine the association of prenatal concentrations of EDC mixtures with birth size measures for each chemical class separately and for all three classes combined. All mixtures (each chemical class separately and all three together) were inversely associated with birth weight. For example, one-unit higher of the WQS index (representing a one-decile increase in chemical concentrations) for all three classes combined was associated with 74 g (β: -74 g, 95% CI: -118, -29 g) lower birth weight. PCB146, perfluorohexane sulfonate, and 2-(N-ethylperfluorooctanesulfonamido) acetate had the highest weights in WQS regression (weights: 0.19, 0.15, 0.12, respectively). Inverse associations were also seen for length and head circumference. These results suggest prenatal exposure to mixtures of persistent EDCs may affect birth size.

Keywords: ALSPAC, pregnancy, birth weight, endocrine disruptors, per- and polyfluoroalkyl substances, polychlorinated biphenyls, organochlorine pesticides

Introduction

The United States National Institute of Environmental Health Sciences (NIEHS) defines an endocrine disrupting chemical (EDC) as a chemical that may interfere with the body's endocrine system, potentially producing adverse developmental, reproductive, neurological, and immune effects (357). Environmentally persistent EDCs, such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs), and per- and polyfluoroalkyl substances (PFAS), used throughout the 20th and 21st centuries for a variety of purposes, are typically highly resistant to degradation, and tend to bioaccumulate in humans and animals (123, 130, 144). Exposures to PFAS, PCBs, and OCPs have declined in the general population following numerous countries banning or severely restricting the production, handling, and disposal of several OCPs and PCBs, as well as certain PFAS. Still, almost all humans have detectable concentrations of some of these persistent chemicals (124, 358). Moreover, persistent EDCs can cross the placental barrier, allowing for potential fetal exposure, and the quantities of EDCs detected in cord serum may be substantial in relation to a developing fetus's size (263, 359-361).

Birth size is considered a relevant and sensitive marker of prenatal EDC exposure and is an important predictor of future health (391). In the United States, 8.3% of infants are born with low birth weight (<2500 grams) (392), but even slight perturbations in birth weight have been associated with lower intelligence quotient (393) and increased obesity risk (391) later in life. Many previous studies of prenatal exposure to persistent EDCs and birth size suggest persistent EDCs are associated with smaller birth size (143, 155, 156, 244, 247-249, 252, 259, 261-265, 278, 279, 281-283), though others have shown somewhat mixed results (129, 159, 255-258, 264, 266, 267, 269-276, 280, 284). A meta-analysis of maternal perfluorooctanoate (PFOA) exposure and infant birth weight estimated a 19 g reduction in birth weight for each 1 ng/mL increase in maternal serum PFOA concentration; length (−0.06 cm per 1 ng/mL increase) and head circumference (−0.03 cm per 1 ng/mL increase) estimates were also reported (259). While these associations with birth size measures may not be considered large at the individual or clinical

level, it is important to consider implications at the population level. A relatively modest and subclinical effect size may be associated with substantial population burden if the exposure is prevalent, like for PFAS (259). Additionally, PFOA is just one of the many environmental chemicals that could affect birth weight. Examining the cumulative effect of several EDCs may show an even larger effect size than reported in the meta-analysis and other previous studies that examined chemicals individually.

Historically, most studies have examined one EDC at a time in relation to an outcome. Because humans are exposed to many chemicals, as opposed to one chemical in isolation, examining combined exposures or “mixtures” of chemicals would allow for a better approximation of the human experience (4). According to NIEHS, a mixture is a combination of three or more independent chemicals or chemical groups (5). Two previous studies have explored the use of mixture methods in relation to persistent EDCs and birth size, though they used methods that accomplished different objectives. The first study used Bayesian hierarchical linear models, which takes *a priori* defined groups and estimates their overall effect on the outcome. This study reported small or no differences in birth weight by concentration of PFAS, PCBs, or OCPs (394). The second study used elastic net regression, a method aimed at identifying the chemicals that contribute the most to the outcome from a correlated mixture of chemicals. This study found that among PFAS, OCPs, and phthalates, two phthalate metabolites, PFOA, and p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) were most consistently predictive of birth weight among term infants, with PFOA, p,p'-DDE, and one of the phthalates showing inverse associations (254).

While several studies have examined prenatal exposure to persistent EDCs and birth size, few have explored persistent EDC exposure as a mixture. Our aim was to investigate the association of maternal gestational concentrations of mixtures of persistent EDCs (PFAS, PCBs, and OCPs) and birth size measures (weight, crown to heel length, head circumference, ponderal index, small for gestational age) in a sub-study of the Avon Longitudinal Study of Parents and

Children (ALSPAC). Specifically, we aimed to estimate the overall effect of the mixture and identify the chemicals contributing the most to the overall effect within the mixture.

Methods

Study population

The Avon Longitudinal Study of Parents and Children (ALSPAC) is an ongoing prospective birth cohort of 14,541 pregnancies. ALSPAC enrolled pregnant women with an expected delivery date between 1 April 1991 and 31 December 1992 from three health districts in the former county of Avon, Great Britain. Information was collected on parents and children through clinic visits, interviews, and mailed questionnaires. Details on ALSPAC recruitment and study methods have been described elsewhere (369, 370). A nested case–control study was conducted within the ALSPAC cohort to explore associations of prenatal maternal concentrations of various suspected endocrine disrupting chemicals and age at menarche among the daughters. Details of the nested case–control study are described elsewhere (307). Cases were girls that obtained early menarche, defined as menarche prior to 11.5 years of age. The nested case-control study was reweighted to represent the full cohort. The weight for the cases (all girls who attained menarche before 11.5 years) was 1, and the weight for the controls (a random sample of girls who attained menarche at or after 11.5 years) was 15.1.

The study website contains details of all the data that are available through a fully searchable data dictionary and variable search tool (http://www.bris.ac.uk/alspac/researchers/our-data/). We obtained ethical approval for the study from the ALSPAC Ethics and Law Committee, the Local Research Ethics Committees, and the Centers for Disease Control and Prevention (CDC) Institutional Review Board. Mothers provided written informed consent for participation in the study. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004). Informed consent for the use of data

collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law
Committee at the time.

Exposure assessment

At enrollment in 1991-1992, fasting blood samples were collected from mothers at median 15 (interquartile range (IQR): 10–28) weeks gestation. Samples were processed and frozen for future analysis. Maternal serum samples were held in storage at the University of Bristol until they were transferred under controlled conditions and analyzed at the National Center for Environmental Health of the CDC (Atlanta, GA). Laboratory analyses included low- and high-concentration pooled quality control materials, standards, reagent blanks, and study samples. Prior to statistical analysis, concentrations below the limit of detection (LOD) were imputed by dividing the LOD by the square root of 2.

Per- and polyfluoroalkyl substances

We quantified eight PFAS (**Table 4-1**) in serum via on-line solid-phase extraction coupled to isotope dilution high-performance liquid chromatography-tandem mass spectrometry (381). LODs were 0.20 ng/mL (EtFOSAA, PFDA, PFOS), 0.174 (MeFOSAA), 0.10 ng/mL (FOSA, PFHxS, PFOA), and 0.082 (PFNA). Coefficients of variation (CVs) for PFAS were largely below 10%. PFAS detected in greater than 75% of mothers were included in the main analyses.

Organochlorine pesticides and polychlorinated biphenyls

We measured nine OCPs and 35 PCBs (**Table 4-1**) in serum using gas chromatography isotope dilution high resolution mass spectrometry (383). PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration hereafter referred to as PCB138. PCB congeners 196 and 203 also could not be separated and were similarly quantified as a summed concentration hereafter referred to as PCB196. For PCBs and OCPs, LODs are dependent on the size of the sample available, thus an individual LOD was reported for each

individual result instead of an overall LOD. CVs were generally below 10%. PCB and OCP concentrations were adjusted for lipids. As with PFAS, PCBs and OCPs detected in greater than 75% of mothers were included in the main analyses.

Outcome assessment

Birth weight (g) was abstracted from infant medical records. Trained ALSPAC staff measured crown to heel length (cm) using a Harpenden neonatometer (Holtain Ltd., Crymych, United Kingdom) and head circumference (cm) using a lasso tape measure within 24 h of birth (median 1 day, range: 1–14 days). Additional details on birth size measurement and quality control are described elsewhere (395, 396). Low birth weight was considered < 2500 g. Ponderal index was calculated using the following formula: (weight in g/height in cm^3) × 100. A ponderal index of <2.4 was considered low (397). We defined small for gestational age (SGA) as below the 10^{th} percentile of the distribution for birth weight among female infants in the United Kingdom, adjusted for gestational age at birth. We calculated standard deviation scores of weight on the basis of the British growth reference centiles from 1990 (398) with Excel macros provided on the internet (www.healthforallchildren.co.uk). In ALSPAC, the final clinical estimate of the expected date of delivery was abstracted from the obstetric records and used to calculate gestational age at delivery. While we initially intended to examine preterm birth as an outcome, there was inadequate sample size to assess preterm birth (dichotomized at <37 weeks gestation).

Covariates

Covariate information was collected by clinical staff (e.g., gestational age at biological sample collection) or through self-report on questionnaires completed by the mother during or immediately after pregnancy (e.g., maternal education, maternal race). Covariates under consideration included: gestational age at biological sample collection (weeks), maternal age (years), maternal pre-pregnancy body mass index (BMI) (kg/m^2), maternal race (white/nonwhite), maternal education (classified as <O-level (ordinary level: required, completed at age 16), O-

level, or > O-level), parity (nulliparous/multiparous), smoking during pregnancy (any/none), and hours of physical activity (enough to work up a sweat) per week during pregnancy (>0 hours/0 hours).

Statistical analyses

Descriptive analyses were conducted to compare mother-daughter dyad characteristics by median birth weight and select EDCs. The median and IQR, as well as percent below the LOD, were reported for all measured EDCs. Correlations among EDCs were reported using Spearman correlation coefficients (r_s).

The chemical exposures under study were modeled as natural log-transformed continuous variables. First, we ran single-chemical linear regression models to examine independent associations between each chemical and birth weight. Then, we ran multi-chemical linear regression models to examine associations between each chemical in a class (e.g., PFAS) and birth weight, independent of other chemicals in the class (e.g., examining the beta for one chemical adjusting for other chemicals in the class). Sensitivity analyses were conducted comparing birth size among those with versus those without detectable levels.

Bayesian kernel machine regression (BKMR) was used to visualize the exposure-response function and verify assumptions (linearity, no interaction) using the R package *bkmr* (333, 399, 400). Assuming no identification of non-linearity and/or interaction within the mixture through BKMR, weighted quantile sum (WQS) regression was used to estimate associations of maternal EDC mixtures with birth size using the R package *gWQS* (401). Mixtures under study were each chemical class separately (PFAS, PCBs, and OCPs) and all three chemicals classes combined.

WQS regression creates a weighted linear index of correlated predictors that are weighted according to their strength of association with the outcome of interest (402). Specifically, the following equation was used to calculate the weights of *c* set of correlated variables:

$$g(\mu) = \beta_0 + \beta_1 \left(\sum_{i=1}^{c} w_i q_i \right) + z'\varphi$$

The sum term was the index for the ***c*** items, scored into quantiles (denoted q_i), and weights were represented by the sum of w_i. Each w_i was constrained between 0 and 1. Within each bootstrap sample, the w_i were estimated by maximum likelihood and constrained to sum to 1. All covariates were represented by ***z′φ***. Prior to analysis, the data were split into two datasets at random: a training dataset (40%) and validation dataset (60%). Using the training dataset, 100 bootstrap samples were selected and the strength of the associations for each ***c*** item was determined by the beta coefficient. The index was calculated based on the mean w_is across all bootstrap samples and was interpreted as an estimation of the overall mixture effect (331, 402-405).

BKMR was used as a sensitivity analysis to confirm the WQS regression findings. BKMR is a flexible semi-parametric technique that models the combined effects of different chemicals, while allowing for nonlinearity and interactions among chemicals (406). This approach enables the examination of independent effects of mixture members, interactions among them, and the overall mixture effect. Within BKMR, we used hierarchical variable selection, which provided group importance scores (posterior inclusion probabilities (PIPs)) for pre-defined mutually exclusive groups of variables. Further, we estimated the importance of a chemical given that the group containing the chemical was important (conditional PIPs) (333, 399, 400). Within BKMR, we standardized all continuous variables to improve computational efficiency. Currently, the *bkmr* package does not allow for weighting, so we were unable to weight our nested case-control data back to the full cohort, which limits generalizability. SAS software 9.4 (Cary, NC) was used for descriptive analyses. R software 3.5.0 (Vienna, Austria) was used for WQS regression and BKMR analyses.

Results

Descriptive statistics

Most mothers in this subsample of ALSPAC were white (98.1%), well-educated (81.9% completed secondary education or higher), and above the age of 25 (79.3%). The majority of mothers entered pregnancy at a normal BMI (77.9%) and about half were nulliparous (49.6%). Less than one-fifth of mothers reported smoking (18.5%) and about half reported drinking alcohol during pregnancy (50.8%).

PCB153 and p,p'-DDE were highest among women with greater than a secondary education and higher among women who drank alcohol during pregnancy (**Table 4-2**). PCB153 and p,p'-DDE were higher among older women and PFOA was higher among nulliparous women.

Very few infants were born with low birth weight (3.9%); median birth weight was 3413 g (IQR: 3110–3690 g) (data not shown). Median head circumference was 34.5 cm (IQR: 33.7–35.4 cm) and median crown to heel length was 50.4 cm (IQR: 49.2–51.6 cm). Median ponderal index was 2.64 (IQR: 2.50–2.81) and 12.5% of infants had a low ponderal index. Just over 3% of infants were born preterm. Women who entered pregnancy overweight or obese were more likely to have a heavier infant (3560 g versus 3400 g among under-/normal weight mothers), while smokers were more likely to have a lighter infant (3300 g versus 3460 non-smokers) (**Table 4-2**). First-born children were smaller than second born or later children (3320 g versus 3500 g). As expected, infants who were born preterm, who had low ponderal index, and who were SGA weighed less than those who were not.

Of the 52 chemicals measured, 31 were detected in more than 75% of mothers. Certain OCPs were rarely detected (e.g., o,p'-DDT and Mirex were detectable in <2% of samples) and certain PCBs were also rarely detected (e.g., PCB128 and PCB151) (**Table 4-3**). The majority of PFAS were detected in most samples, except PFDA (detected in <3% of samples).

Correlation was high among the subset of chemicals detected among most mothers (detected in >75% of samples) (**Figure 4-1**). Among the 31 chemicals, PCBs and OCPs showed

high correlation between classes, and weak correlations with PFAS. Among PCBs, there was very strong correlation within the class (up to r_s=0.98 between PCB170 and PCB180). Correlation within OCPs was strong as well (as high as r_s=0.82 between HCB and β-HCH). PFAS chemicals exhibited lower correlation within the class; but, were still positively correlated with some strong correlations (up to r_s=0.72 between PFOA and PFOS).

Single- and multi-chemical models

In adjusted single-chemical models of maternal serum concentrations of EDCs with birth weight, most chemicals were inversely associated with birth weight (**Supplemental Table 4-5**). Among PFAS, a 10% higher PFOA concentration was associated with 25 g lower birth weight (β: -25 g, 95% CI: -36, -14 g); PFOS and EtFOSAA were also strongly inversely associated with birth weight. PCB105, PCB138, PCB153, PCB170, PCB180, PCB196, and PCB206 were strongly inversely associated with birth weight, while no OCPs were strongly associated with birth weight. In multi-chemical models, inverse associations persisted for PFOA and EtFOSAA in the model for the PFAS class (**Supplemental Table 4-5**). In the large class of PCBs, PCB172 was positively associated with birth weight when adjusting for all other chemicals in the class. Associations for OCPs were null.

Weighted Quantile Sum Regression

In weighted quantile sum regression models, the WQS indices for mixtures (PFAS, PCBs, OCPs, and all three classes combined) were inversely associated with birth size measures, especially birth weight. For example, one-unit higher of the WQS index (representing a one-decile increase in chemical concentrations) for all three classes combined was associated with 74 g (β: -74 g, 95% CI: -118, -29 g) lower birth weight (**Table 4-4**). PCB146, PFHxS, and EtFOSAA were identified as contributing the most to the WQS index (weights: 0.19, 0.15, and 0.12, respectively). Inverse associations were also seen for head circumference and crown to heel length: one-unit higher of the WQS index for all three classes combined was associated with 0.11 cm (β: -0.11

cm, 95% CI: -0.22, 0.00 cm) smaller head circumference and 0.33 cm (β: -0.33 cm, 95% CI: -0.52, -0.14 cm) shorter crown to heel length. Associations with ponderal index were null for all mixtures under consideration.

Forty infants were considered SGA. Class-specific mixtures of PFAS and OCP chemicals and the mixture consisting of all three classes combined (PFAS, PCBs, and OCPs) showed weak associations with SGA. For the mixture of all three classes combined, one-unit higher of the WQS index (representing a one-decile increase in chemical concentrations) was associated with a 31% higher odds of SGA (odds ratio: 1.31; 95% CI: 0.84, 2.05) (**Table 4-4**).

Bayesian Kernel Machine Regression

In the BKMR model for all three classes combined, PFAS had the highest PIP (0.76), making it the most important group in the mixture. The next most important group was OCPs (PIP: 0.65), followed by PCBs (PIP: 0.61). Within the PFAS, EtFOSAA contributed the most to the model (conditional PIP: 0.68). The next most important chemicals in the PFAS group were PFOA (conditional PIP: 0.18) and PFOS (conditional PIP: 0.10). Within the OCP group, p,p'-DDT had the highest PIP (conditional PIP: 0.41), followed by p,p'-DDE (conditional PIP: 0.32). In the PCB group, PCB153 and PCB180 contributed the most (conditional PIPs: 0.11).

The independent chemical associations all appear relatively linear (**Figure 4-2A**). Few chemicals had slightly positive associations (HCB), some appeared to have negative associations (PFOA, EtFOSAA, PCB138, p,p'-DDT), and others showed no association with birth weight. We observed no interaction among mixture members (**Supplemental Figure 4-3**). We found a weak overall mixture effect, with higher exposure to the mixture associated with lower birth weight (**Figure 4-2B**).

Sensitivity analyses

We conducted a sensitivity analysis to explore differences in birth weight among those with detectable versus those with non-detectable concentrations (coded as a dichotomous

variable) (**Supplemental Table 4-6**). We found that for FOSA, infants born to mothers with detectable concentrations were 120 g smaller (β: -120 g, 95% CI: -218, -23 g) than those with non-detectable concentrations. Similarly, those with detectable concentrations of MeFOSAA, PCB101, and oxychlordane had lower birth weight than those with non-detectable concentrations.

Discussion

In this study, we observed an inverse association of prenatal exposure to mixtures of PFAS, PCBs, and OCPs with birth size, especially birth weight, among British girls using weighted quantile sum regression and Bayesian kernel machine regression. These results support previous findings under the single-chemical paradigm: higher prenatal concentrations of persistent EDCs are associated with lower birth weight, smaller head circumference, and shorter crown to heel length.

Previous studies in the ALSPAC population have examined prenatal concentrations of single EDCs, specifically PFAS (247) and PCBs (262), and birth size. Both studies found inverse associations of prenatal concentrations of EDCs and birth size, as we confirmed here when examining mixtures of these chemicals. The present study examined more chemicals within each class than previous studies and was able to identify important chemicals within each mixture. For example, MeFOSAA, EtFOSAA, PCB146, PCB172, PCB177, and PCB199 were identified as important contributors to the overall mixture effect on birth weight, but were not examined in previously published studies (247, 262).

This is the first study published on the association of OCPs and birth size within ALSPAC, and we found that OCPs were weakly associated with lower birth weight in single-chemical models. In mixture models, OCPs and birth weight were weakly inversely associated, while associations with head circumference, crown to heel length, and ponderal index were null. HCB consistently appeared as one of the most important components in the OCP mixture, but was less impactful in the mixture containing PFAS, PCBs, and OCPs. Few studies have examined prenatal

HCB exposure and birth size: a Greek study reported an inverse association with birth weight(283) while a Spanish study found no association (284).

Previous studies of prenatal exposure to mixtures of persistent EDCs have used different methods with differing goals. One study used elastic net regression to identify the chemicals that contribute the most to the outcome from a correlated mixture of chemicals, and identified PFOA and p,p'-DDE as being inversely associated with birth weight (254). While not their primary goal, WQS regression and BKMR can also identify important chemical contributors within a mixture. In our birth weight analyses using WQS regression (which examined a different mixture of chemicals than Lenters et al., 2016), PFOA was an important contributor to the overall mixture effect, but it was not the most important. In our analyses, p,p'-DDE was not identified as an important contributor. We employed hierarchical variable selection within our BKMR analyses and found that PFAS were the most important group in the overall mixture, followed by OCPs. PFOA was the most important chemical within the PFAS group, and p,p'-DDE was the second most important chemical within the OCP group. The other previously published study of prenatal concentrations of EDC mixtures and birth weight used Bayesian hierarchical linear models, which takes a priori defined groups and estimates their overall effect on the outcome. Woods et al. reported PFAS, PCBs, and OCPs had null or small associations with birth weight, and PFAS were most strongly associated with lower birth weight (394). While our study found differences of a larger effect size, the findings of Woods et al. are in line, in terms of the most important class, with what we found using WQS regression and BKMR in this study: PFAS generally show stronger inverse associations with birth weight than PCBs and OCPs.

Interpreting and comparing the effect sizes observed in this study to previous studies under the single-chemical paradigm is challenging. For example, a meta-analysis of PFOA and birth weight estimated a 19 g reduction in birth weight for each 1 ng/mL increase in maternal serum PFOA concentration (259). In this study, we found a 74 g reduction in birth weight for one-unit higher of the WQS index (representing a one-decile increase in maternal chemical

concentrations) for all three classes of chemicals combined. In the ALSPAC population, a one-decile increase in PFOA concentration is less than 1 ng/mL; for example, going from the 60th to the 80th percentile (two deciles) in PFOA concentrations is approximately a 1 ng/mL difference. Therefore, in our mixture analyses, a 1 ng/mL difference in PFOA, alongside a two decile increase in other chemicals of the mixture, is associated with a roughly 148 g reduction in birth weight, which is almost eight times that of considering PFOA alone (19 g reduction per 1 ng/mL increase). While this is a somewhat crude calculation, it shows the potential magnitude of relatively small changes in chemical concentrations when examined as a mixture.

Birth size is an important predictor of future health. According to the National Center for Health Statistics, 8.3% of American infants born in 2017 weighed less than 2500 g at birth, which is considered low birth weight (407). Low birth weight infants face more immediate health problems than their normal weight counterparts, including difficulty breathing and increased risk of infection (408). Further, infants born with low birth weight may be more likely than infants born at a normal weight to develop certain health conditions later in life such as intellectual and developmental delays, obesity, diabetes, and heart disease (408). Any progress that could be made in reducing the incidence of low birth weight, such as through the reduction of EDC exposure, would have a profound public health impact.

This study has several strengths, including its prospective study design within a population-based birth cohort with frequent and thorough longitudinal data collection. Additionally, we have reliable biomonitoring measurements of more than 50 persistent EDCs collected at median 15 weeks gestation, a number of outcomes measured by health professionals at birth, and extensive covariate data available for mothers and daughters.

This study also has limitations. We only examined mother-daughter dyads in this sub-study that was originally intended to investigate early menarche. There is some evidence to suggest that associations of EDCs and birth size are modified by infant sex (409, 410), so restricting to daughters may be a prudent choice. Additionally, we were unable to weight BKMR

analyses of this nested case-control study back to the full cohort, which limits generalizability. Nevertheless, results of the unweighted BKMR analyses were similar to the WQS results in terms of direction and magnitude of the overall effect of the mixture; and using weights for estimating associations between exposures and outcomes is not considered essential (411-414). Further, while we have detailed covariate data, there is always the possibility that we were not able to completely control for confounding by certain sensitive or self-reported variables, such as smoking, alcohol use, and socioeconomic status. Approaches to mixture analyses that involve regressing the outcome on several correlated exposures simultaneously can in some cases amplify rather than reduce confounding bias ("coexposure amplification bias"), particularly in cases of residual confounding (350). Further, due to the large number of variables used in mixture analyses, we were missing data on roughly 30% of the sub-sample (**Supplemental Figure 4-4**). We compared mother-daughter characteristics for those with complete data included in mixture analyses (n=313) to those in the nested case-control study (n=448) and to the population from which the case-control study was drawn (n=3338) (**Supplemental Table 4-7**). Characteristics were similar across subsets, though low birth weight infants were somewhat underrepresented in the subset with complete data. We also compared unadjusted estimates from the complete data included in mixture analyses (n=313) to the subset without complete covariate data (n=378) and found similar results (β: -40 g, 95% CI: -73, -7 g and β: -43 g, 95% CI: -80, -6 g) (**Supplemental Table 4-8**). Lastly, there is the possibility of reverse causality and confounding because the outcome of interest, birth size, may affect the measured biomarker concentrations and there may be shared determinants, such as hemodynamics, of the biomarker and pregnancy outcome (245). Studies have demonstrated that reverse causality and confounding are less of a concern when the range of concentrations is wide and when blood samples are collected early in pregnancy (244, 246). In our study, 66% of samples were collected in the first half of pregnancy and we adjusted for gestational age (in weeks) of biological sample collection.

In conclusion, we found inverse associations between prenatal concentrations of mixtures of persistent EDCs and birth size, namely birth weight. While this study reaches the same conclusion as previous studies published on this topic under the single-chemical paradigm, it fills a gap relating to mixtures of EDCs and birth size and comes closer to replicating the human experience. Specifically, this study gives a better sense of the overall effect of prenatal exposure to a number of PFAS, PCBs, and OCPs on birth size.

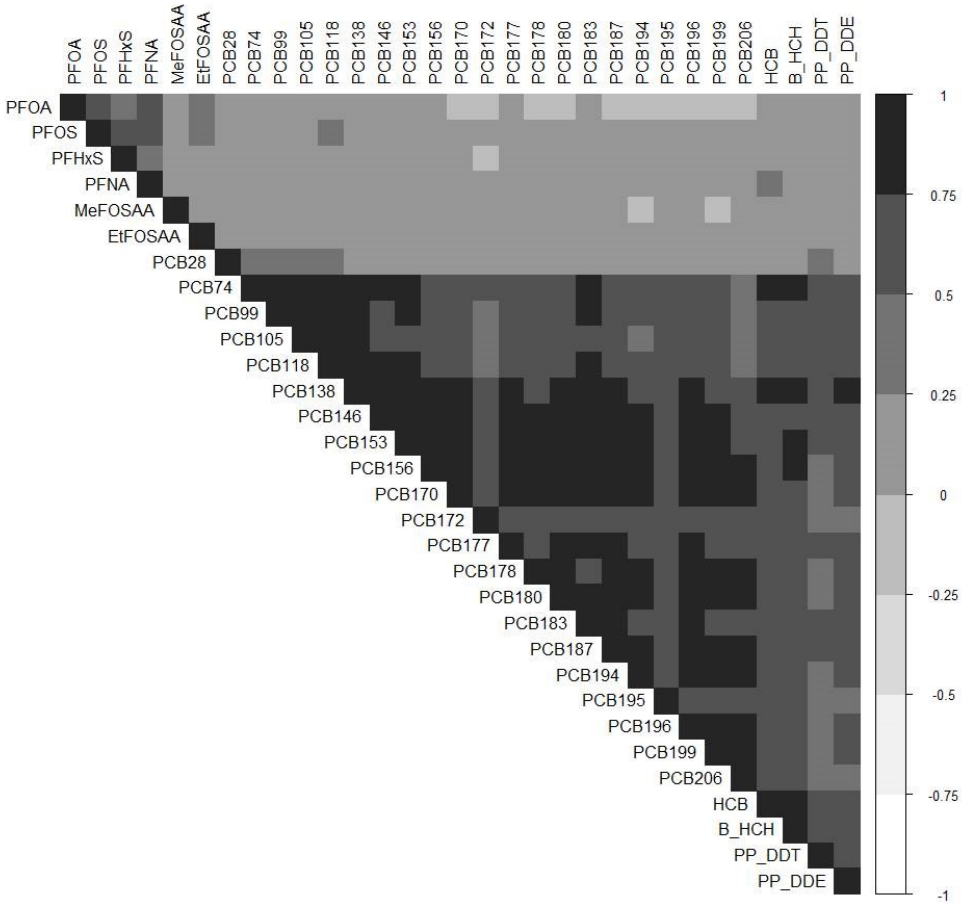


Figure 4-1. Correlation heatmap of serum concentrations of persistent endocrine disrupting chemicals in women during pregnancy in the Avon Longitudinal Study of Parents and Children (N=448). Spearman correlation coefficients presented for per- and polyfluoroalkyl substance (PFAS), polychlorinated biphenyl (PCB), and organochlorine pesticide (OCP) chemicals. PCB and OCP concentrations were lipid-adjusted. Figure created in R (*corrplot*).

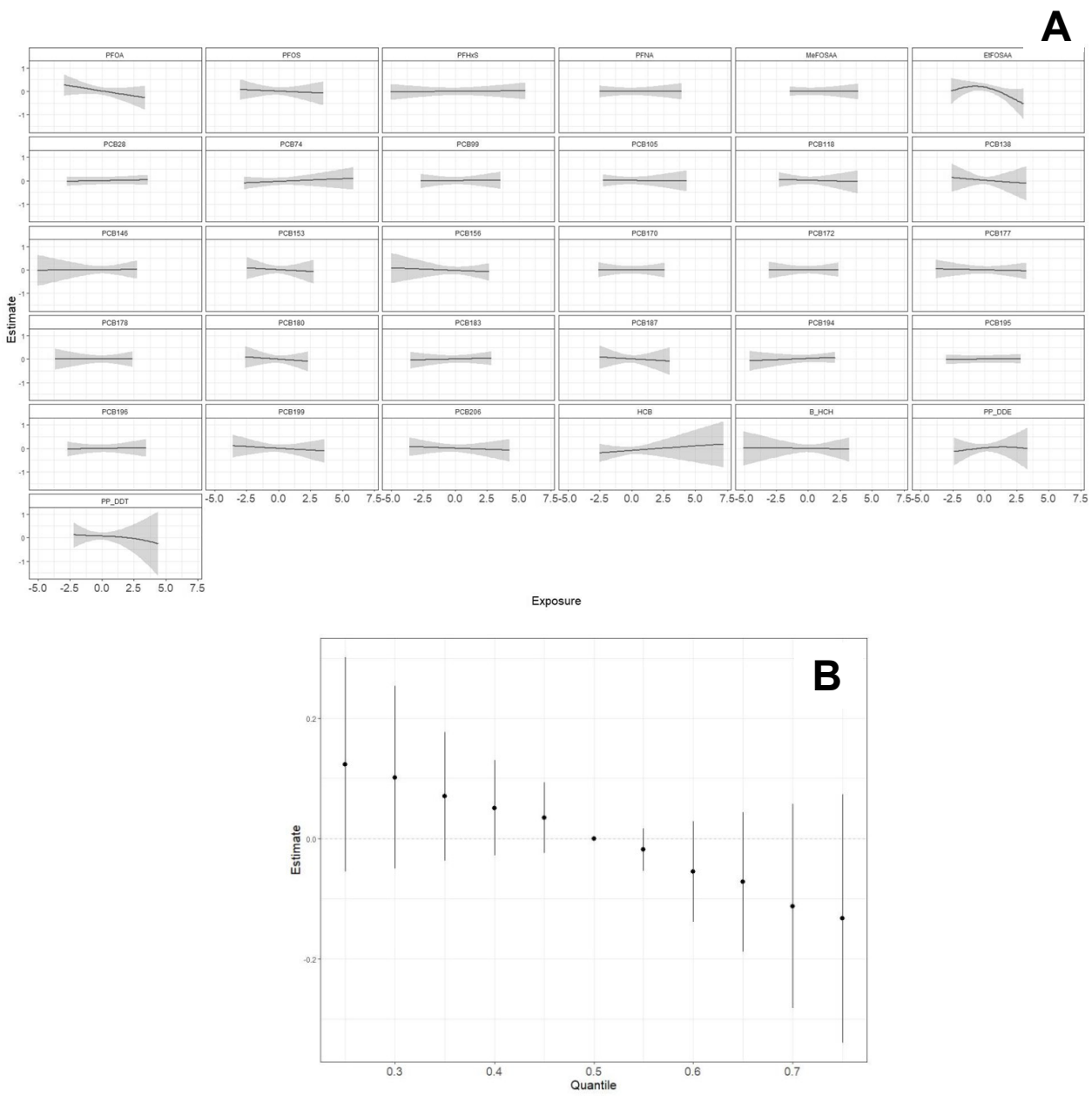


Figure 4-2. Results from Bayesian Kernel Machine Regression. **A** Chemical-specific effect estimates of mixture members on birth weight in ALSPAC mother-daughter dyads (n=313) estimated by BKMR. Single congener associations and 95% credible bands are presented with other chemicals fixed at their median. **B** Overall effect of the mixture on birth weight (estimates and 95% credible intervals). The overall effect is defined as the change in the mean outcome when all the exposures are fixed at a particular quantile as compared to when all the exposures are fixed at the median, with all of the covariates held constant. The model adjusted for maternal education, parity, pre-pregnancy body mass index, age, smoking, and gestational week at sample collection. All chemical concentrations were natural log-transformed and standardized; PCB and OCP concentrations were lipid-adjusted.

Table 4-1. Persistent endocrine disrupting chemicals quantified in maternal serum in the ALSPAC nested case-control study.

Chemical Name	Abbreviated Name
Per- and polyfluoroalkyl substances	
Perfluorooctane sulfonamide	FOSA
2-(N-ethylperfluorooctanesulfonamido) acetate	EtFOSAA
2-(N-methyl-perfluorooctanesulfonamido) acetate	MeFOSAA
Perfluorohexane sulfonate	PFHxS
Perfluorooctane sulfonate	PFOS
Perfluorooctanoate	PFOA
Perfluorononanoate	PFNA
Perfluorodecanoate	PFDA
Polychlorinated Biphenyls	
2,4,4'-trichlorobiphenyl	PCB28
2,2',3,5'-tetrachlorobiphenyl	PCB44
2,2',4,5'-tetrachlorobiphenyl	PCB49
2,2',5,5'-tetrachlorobiphenyl	PCB52
2,3',4,4'-tetrachlorobiphenyl	PCB66
2,4,4',5-tetrachlorobiphenyl	PCB74
2,2',3,4,5'-pentachlorobiphenyl	PCB87
2,2',4,4',5-pentachlorobiphenyl	PCB99
2,2',4,5,5'-pentachlorobiphenyl	PCB101
2,3,3',4,4'-pentachlorobiphenyl	PCB105
2,3,3',4',6-pentachlorobiphenyl	PCB110
2,3',4,4',5-pentachlorobiphenyl	PCB118
2,2',3,3',4,4'-hexachlorobiphenyl	PCB128
2,2',3,4,4',5'-hexachlorobiphenyl and 2,3,3',4,4',6-hexachlorobiphenyl	PCB138-158
2,2',3,4',5,5'-hexachlorobiphenyl	PCB146
2,2',3,4',5',6-hexachlorobiphenyl	PCB149
2,2',3,5,5',6-hexachlorobiphenyl	PCB151
2,2',4,4',5,5'-hexachlorobiphenyl	PCB153
2,3,3',4,4',5-hexachlorobiphenyl	PCB156
2,3,3',4,4',5'-hexachlorobiphenyl	PCB157
2,3',4,4',5,5'-hexachlorobiphenyl	PCB167
2,2',3,3',4,4',5-heptachlorobiphenyl	PCB170
2,2',3,3',4,5,5'-heptachlorobiphenyl	PCB172
2,2',3,3',4',5,6-heptachlorobiphenyl	PCB177
2,2',3,3',5,5',6-heptachlorobiphenyl	PCB178
2,2',3,4,4',5,5'-heptachlorobiphenyl	PCB180
2,2',3,4,4',5',6-heptachlorobiphenyl	PCB183
2,2',3,4',5,5',6-heptachlorobiphenyl	PCB187
2,3,3',4,4',5,5'-heptachlorobiphenyl	PCB189
2,2',3,3',4,4',5,5'-octachlorobiphenyl	PCB194
2,2',3,3',4,4',5,6-octachlorobiphenyl	PCB195
2,2',3,3',4,4',5',6-octachlorobiphenyl and 2,2',3,4,4',5,5',6-octachlorobiphenyl	PCB196-203
2,2',3,3',4,5,6,6'-octachlorobiphenyl	PCB199
2,2',3,3',4,4',5,5',6-nonachlorobiphenyl	PCB206
Decachlorobiphenyl	PCB209

Organochlorine Pesticides	
Hexachlorobenzene	HCB
β-Hexachlorocyclohexane	β-HCH
ϒ-Hexachlorocyclohexane (Lindane)	γ-HCH
Oxychlordane	Oxychlordane
Trans-Nonachlor	Trans-nonachlor
2,2-Bis(4-chlorophenyl)-1,1-dichloroethene	p,p'-DDE
2-(4-chlorophenyl)-2-(2-chlorophenyl)-1,1,1-trichloroethan	o,p'-DDT
2,2-Bis(4-chlorophenyl)-1,1,1-trichloroethan	p,p'-DDT
Mirex	Mirex

Table 4-2. Characteristics of the Avon Longitudinal Study of Parents and Children (ALSPAC) sub-study population (N=448 mother-daughter dyads) by select persistent endocrine disrupting chemicals.

		Birth weight (g)	**PFOA** (ng/mL)	**PCB153** (ng/g lipid)	**p,p'-DDE** (ng/g lipid)
Characteristic	**n (%)[a]**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**	**Median (IQR)**
Maternal race					
White	423 (98.1)	3420 (3140–3700)	3.8 (2.9–4.8)	64.8 (48.6–85.8)	308 (193–490)
Non-white	8 (1.9)	2985 (2530–3740)	2.3 (1.6–2.9)	67.7 (47.4–95.1)	620 (363–1635)
Maternal education[b]					
< O-level	75 (18.1)	3490 (3180–3650)	3.6 (2.8–4.5)	59.7 (45.5–78.6)	298 (184–472)
O-level	140 (33.7)	3420 (3100–3680)	3.7 (2.9–5.0)	55.9 (44.2–72.3)	257 (166–460)
>O-level	200 (48.2)	3400 (3140–3720)	3.9 (2.8–4.8)	74.4 (57.8–95.6)	384 (227–536)
Maternal pre-pregnancy BMI					
<25 kg/m^2 (under/normal weight)	313 (77.9)	3400 (3100–3620)	3.8 (2.8–4.8)	69.0 (51.2–88.1)	329 (194–513)
≥25 kg/m^2 (overweight/obese)	89 (22.1)	3560 (3200–3870)	3.7 (3.0–4.8)	57.2 (44.0–77.6)	306 (211–541)
Prenatal smoking					
Any	79 (18.5)	3300 (2880–3560)	3.4 (2.9–4.4)	59.8 (46.0–74.3)	283 (170–412)
None	348 (81.5)	3460 (3180–3740)	3.8 (2.8–4.9)	65.7 (48.9–87.5)	323 (200–504)
Prenatal alcohol use					
Any	215 (50.8)	3420 (3140–3680)	3.7 (2.8–4.6)	71.5 (49.6–94.3)	352 (218–549)
None	208 (49.2)	3400 (3080–3720)	3.8 (2.9–4.9)	60.5 (46.4–80.8)	278 (176–469)
Physical activity					
Any	252 (65.5)	3420 (3120–3740)	3.8 (2.9–5.0)	65.2 (49.9–88.4)	322 (203–504)
None	133 (34.6)	3390 (3100–3625)	3.7 (2.9–4.7)	66.3 (46.1–85.2)	316 (187–533)
Maternal age at delivery					
<25 years	92 (20.7)	3360 (3070–3605)	3.9 (3.0–4.8)	44.2 (35.0–56.5)	178 (136–292
25–29 years	164 (36.9)	3460 (3100–3760)	3.8 (3.0–4.9)	59.8 (48.1–74.1)	289 (198–422)
≥30 years	189 (42.5)	3420 (3140–3700)	3.6 (2.5–4.6)	81.9 (64.3–105.4)	451 (283–620)
Child birth order					
First born	208 (49.6)	3320 (3015–3580)	4.4 (3.4–5.4)	63.9 (46.3–84.3)	316 (198–513)
Second born or later	211 (50.4)	3500 (3240–3790)	3.1 (2.4–4.0)	66.9 (50.2–87.5)	323 (193–497)
Child birth weight					
<2500 g	17 (3.9)	2220 (2020–2340)	4.1 (3.3–5.6)	74.4 (60.0–102.8)	461 (329–1390)
≥2500 g	423 (96.1)	3430 (3160–3720)	3.7 (2.8–4.8)	63.7 (47.5–84.5)	302 (185–487)
Preterm birth					
<37 weeks	14 (3.1)	2421 (2100–2780)	4.7 (2.8–5.6)	69.7 (64.3–110.4)	299 (226–614)

≥37 weeks	431 (96.9)	3430 (3160–3720)	3.7 (2.8–4.8)	63.6 (47.9–85.4)	311 (188–494)
Ponderal index					
<2.4	47 (12.5)	3080 (2720–3440)	3.4 (2.9–4.6)	60.3 (44.9–87.2)	283 (165–517)
≥2.4	328 (87.5)	3460 (3180–3740)	3.8 (2.8–4.9)	63.7 (48.6–84.5)	311 (194–493)
Small for Gestational Age					
<10th percentile	40 (9.1)	2715 (2570–2920)	4.1 (3.1–4.9)	68.0 (47.6–96.6)	368 (211–513)
≥10th percentile	400 (90.9)	3460 (3200–3740)	3.7 (2.8–4.7)	64.0 (48.1–84.1)	299 (187–491)

Abbreviations: IQR, interquartile range; PFOA, perfluorooctanoate; PCB153, 2,2',4,4',5,5'-hexachlorobiphenyl; p,p'-DDE, 2,2-bis(4-chlorophenyl)-1,1-dichloroethene; g, grams; kg/m^2, kilograms per meter-squared

[a] Missing data not represented

[b] <O-level=none, Certificate of Secondary Education, and vocational education, which are equivalent to no diploma or a GED in the United States. O-levels (ordinary levels) are required and completed at the age of 16. >O-level=A-levels (advanced levels) completed at 18, which are optional, but required to get into university; and a university degree.

Table 4-3. Serum concentrations of persistent endocrine disrupting chemical (EDC) exposure among mothers of the Avon Longitudinal Study of Parents and Children (ALSPAC) during pregnancy (median gestational age at sample collection: 15 weeks) (N=448 mother-daughter dyads).

	Serum concentrations			
	Q1	**Median**	**Q3**	**% <LOD[a]**
Per- and polyfluoroalkyl substances (PFAS) (ng/mL)				
PFOA	2.8	3.7	4.8	0.0
PFOS	15.1	19.8	24.9	0.0
PFHxS	1.2	1.6	2.2	0.2
PFNA	0.41	0.49	0.66	0.2
FOSA	<LOD	0.20	0.30	30.6
MeFOSAA	0.26	0.35	0.65	14.5
EtFOSAA	0.40	0.60	0.90	2.5
PFDA	<LOD	<LOD	<LOD	97.3
Polychlorinated biphenyls (PCBs) (ng/g lipid)				
PCB28	3.5	5.3	8.3	8.7
PCB44	<LOD	1.9	4.0	30.4
PCB49	<LOD	<LOD	1.9	58.3
PCB52	<LOD	3.3	7.6	30.1
PCB66	<LOD	1.6	2.5	30.4
PCB74	8.6	11.1	15.1	0.22
PCB87	<LOD	<LOD	1.7	59.6
PCB99	7.0	9.4	12.1	0.9
PCB101	<LOD	2.2	5.4	30.4
PCB105	2.0	2.9	4.0	7.4
PCB110	<LOD	<LOD	2.8	53.6
PCB118	10.9	14.9	20.6	0.22
PCB128	<LOD	<LOD	<LOD	89.5
PCB138[b]	30.4	41.5	54.0	0.2

PCB146	4.6	6.0	8.1	2.5
PCB149	<LOD	<LOD	1.8	60.9
PCB151	<LOD	<LOD	<LOD	79.5
PCB153	48.3	64.5	85.8	0.0
PCB156	4.8	6.3	8.4	1.8
PCB157	<LOD	1.3	1.9	33.9
PCB167	<LOD	2.0	2.8	26.1
PCB170	14.5	18.9	24.9	0.0
PCB172	1.1	1.9	2.7	23.0
PCB177	2.3	3.1	4.1	8.9
PCB178	1.8	2.7	3.7	14.3
PCB180	33.5	45.2	60.1	0.0
PCB183	4.6	6.2	8.2	3.4
PCB187	8.6	11.3	15.2	1.1
PCB189	<LOD	<LOD	0.7	74.3
PCB194	5.5	7.5	10.4	3.4
PCB195	1.5	2.2	3.0	19.0
PCB196[b]	5.7	7.7	10.5	2.0
PCB199	3.9	5.5	7.8	2.7
PCB206	1.7	2.3	3.2	10.3
PCB209	<LOD	1.5	2.0	27.7
Organochlorine pesticides (OCPs) (ng/g lipid)				
HCB	37.9	50.2	63.4	0.0
β-HCH	34.6	47.1	62.4	1.8
γ-HCH	<LOD	<LOD	<LOD	79.0
Oxychlordane	<LOD	<LOD	4.2	71.9
Trans-nonachlor	<LOD	<LOD	4.6	67.0
p,p'-DDE	193	311	499	0.2
o,p'-DDT	<LOD	<LOD	<LOD	98.4

p,p'-DDT	7.7	11.0	16.2	11.4
Mirex	<LOD	<LOD	<LOD	99.3

Abbreviations: Q1, quartile 1; Q3, quartile 3; LOD, limit of detection; ng/mL, nanogram per milliliter; ng/g lipid, nanogram per gram lipid

[a] The LODs for PFAS were 0.082 ng/mL for PFNA, 0.10 ng/mL for PFOA, PFHxS, and FOSA, 0.174 ng/mL for MeFOSAA, and 0.20 ng/mL for PFOS, EtFOSAA, and PFDA. LODs of OCPs and PCBs are dependent on the sample size and blanks, thus, an individual LOD is reported for each individual result rather than an overall LOD.

[b] PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration, referred to as PCB138. Similarly, PCB congeners 196 and 203 could not be separated and were quantified as a summed concentration, referred to as PCB196.

Table 4-4. Adjusted[a] associations of mixtures with accompanying weights of maternal serum concentrations of persistent endocrine disrupting chemical (EDC) exposure with birth size measures in the Avon Longitudinal Study of Parents and Children (ALSPAC) sub-study using weighted quantile sum regression. Estimates represent the change for one-unit higher of the WQS index (representing a one-decile increase in chemical concentrations).

	Birth weight (g)			**Head circumference (cm)**			**Crown to Heel Length (cm)**			**Ponderal Index**			**SGA**		
	β[b]	**95% CI**	**Wt[c]**	**β[b]**	**95% CI**	**Wt[c]**	**β[b]**	**95% CI**	**Wt[c]**	**β[b]**	**95% CI**	**Wt[c]**	**OR[d]**	**95% CI**	**Wt[c]**
PFAS	-46	-84, -7		-0.10	-0.21, -0.01		-0.22	-0.39, -0.04		0.01	-0.02, 0.03		1.19	0.86, 1.64	
PFOA			0.07						0.14			0.04			0.37†
PFOS			0.01						0.02			0.14			0.07
PFHxS			0.35†			0.38†			0.32†			0.01			0.07
PFNA			0.30†			0.12			0.11			0.19			0.14
MeFOSAA			0.11			0.31†			0.27†			0.29			0.16
EtFOSAA			0.16			0.19†			0.13			0.34			0.19†
PCBs	-46	-81, -12		-0.05	-0.15, 0.05		-0.05	-0.21, 0.12		0.00	-0.02, 0.02		1.01	0.73, 1.39	
PCB28						0.08†			0.09†			0.07†			0.01
PCB74						0.01			0.01			0.08†			
PCB99						0.04			0.13†						0.02
PCB105						0.02						0.18†			
PCB118						0.01			0.01			0.01			
PCB138[e]						0.11†			0.02			0.10†			
PCB146			0.06†			0.16†			0.05†			0.02			0.12†
PCB153			0.01			0.05†									
PCB156			0.07†												0.03

PCB170			0.02											
PCB172			0.01			0.01			0.01			0.06†		0.13†
PCB177			0.11†			0.04						0.26†		0.03
PCB178			0.19†									0.06†		0.25†
PCB180			0.01									0.03		0.03
PCB183			0.05†			0.21†			0.51†			0.01		
PCB187			0.18†			0.01						0.07†		0.05†
PCB194						0.02			0.02					
PCB195			0.01			0.08†			0.15†			0.01		
PCB196[e]			0.01			0.01								0.17†
PCB199			0.26†			0.13†						0.02		0.09†
PCB206			0.02			0.01						0.02		0.03
OCPSs	-24	-52, 4		-0.01	-0.08, 0.06		0.04	-0.09, 0.17		-0.01	-0.03, 0.00		1.22	0.86, 1.73	
HCB			0.26†			0.52†			0.48†			0.48†			0.24
β-HCH			0.24			0.07			0.07			0.31†			0.29†
p,p'-DDE			0.19			0.29†			0.28†			0.08			0.25
p,p'-DDT			0.31†			0.12			0.17			0.13			0.22
Overall[f]	-74	-118, -29		-0.11	-0.22, 0.00		-0.33	-0.52, -0.14		-0.01	-0.03, 0.02		1.31	0.84, 2.05	
PFOA			0.03†						0.09†						0.01
PFOS			0.06†												0.05†
PFHxS			0.15†			0.07†			0.12†			0.23†			0.02
PFNA			0.01						0.07†			0.02			0.15†

MeFOSAA	0.07†	0.06†	0.05†	0.05†	0.01
EtFOSAA	0.12†	0.07†	0.10†	0.02	0.07†
PCB28		0.02		0.03	
PCB74					
PCB99		0.01			0.06†
PCB105				0.01	
PCB118				0.03	
PCB138[e]					0.01
PCB146	0.19†	0.08†	0.12†	0.15†	0.01
PCB153		0.01			0.01
PCB156		0.02			0.02
PCB170	0.03				
PCB172	0.06†	0.12†			0.11†
PCB177	0.09†	0.07†	0.01		0.15†
PCB178	0.01	0.06†		0.19†	0.12†
PCB180	0.01	0.07†	0.03†	0.04†	
PCB183		0.01			
PCB187				0.01	
PCB194	0.01		0.07†		0.02
PCB195	0.03	0.20†	0.12†		
PCB196[e]					
PCB199	0.10†	0.03	0.04†	0.05†	0.01

PCB206	0.01	0.01		0.03†	0.05†
HCB		0.04†	0.01		
β-HCH		0.02	0.04†		
p,p'-DDE				0.04†	0.04†
p,p'-DDT	0.02		0.02	0.10†	0.06†

Abbreviations: SGA, small for gestational age; Wt, weight; OR, odds ratio; CI, confidence interval; ng/mL, nanogram per milliliter; ng/g lipid, nanogram per gram lipid

[a] Adjusted for education, parity, pre-pregnancy body mass index, age, smoking, and gestational week at sample collection

[b] β for one-unit higher of the WQS index (representing a one-decile increase in chemical concentrations)

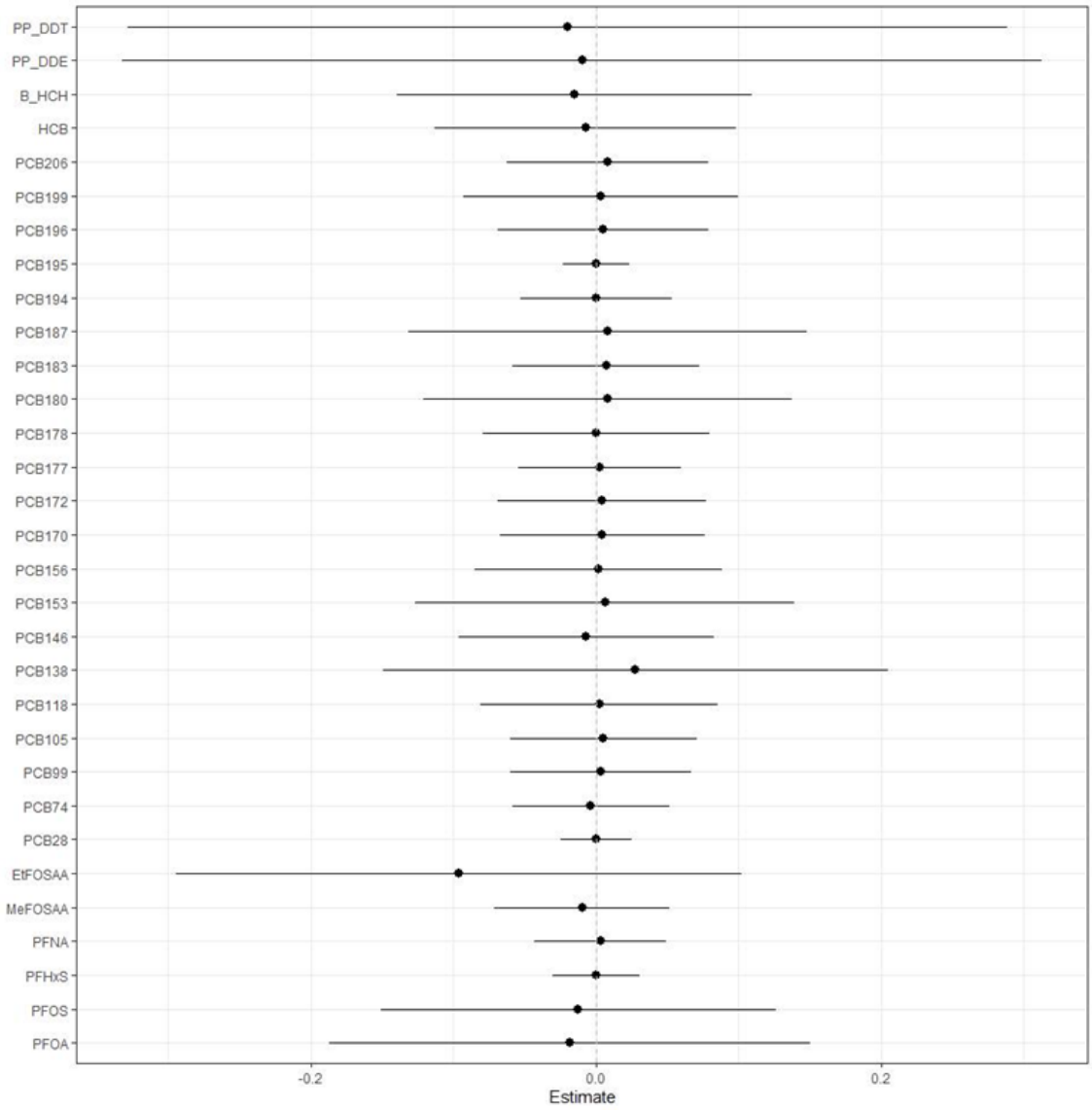
[c] Weights greater than 1/number of chemicals in the mixture are considered significant contributors to the overall mixture effect

[d] OR for one-unit higher of the WQS index (representing a one-decile increase in chemical concentrations)

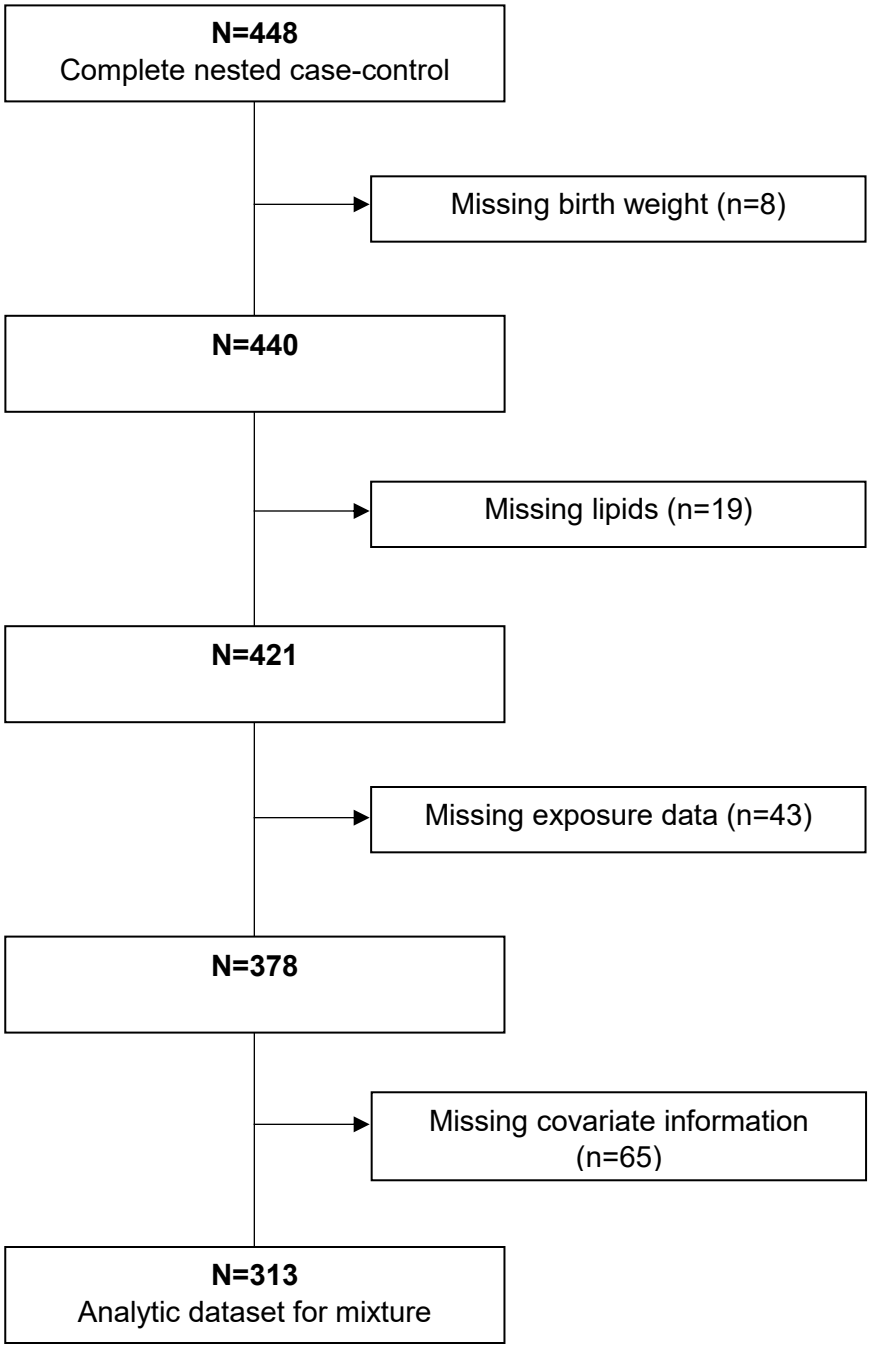
[e] PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration, referred to as PCB138. Similarly, PCB congeners 196 and 203 could not be separated and were quantified as a summed concentration, referred to as PCB196

[f] Overall mixture includes PFAS, PCB, and OCP classes

† Significant contributor to the overall mixture effect (>1/number of chemicals in mixture)



Supplemental Figure 4-3. Interaction terms for individual mixture members and the remaining chemicals in ALSPAC mother-daughter dyads estimated by BKMR (n=313). Each point represents the difference between the effect size of the chemical when all other chemicals are held at their 75th percentiles and the effect size of the same chemical when all other chemicals are held at their 25th percentiles. Range indicates 95% credible interval. Model adjusted for parity, pre-pregnancy BMI, maternal age, education, smoking, and gestational age at sample collection. All chemical concentrations were natural log-transformed and standardized; PCBs and OCPs were lipid adjusted.



Supplemental Figure 4-4. Flowchart depicting missing data in the study of prenatal exposure to mixtures of persistent endocrine disrupting chemicals and birth size in a sub-study of the Avon Longitudinal Study of Parents and Children.

Supplemental Table 4-5. Adjusted[a] single[b]- and multi[c]-chemical associations of maternal serum concentrations of persistent endocrine disrupting chemical (EDC) exposure with birth weight in the Avon Longitudinal Study of Parents and Children (ALSPAC) sub-study using linear regression. Beta estimates represent the change in birth weight for 10% higher chemical concentration.

	Single-chemical models[b]		Multi-chemical models[c]	
	β	**95% CI**	**β**	**95% CI**
Per- and polyfluoroalkyl substances (PFAS) (ng/mL)				
PFOA	-25	-36, -14	-26	-42, -10
PFOS	-18	-29, -6	1	-20, 21
PFHxS	-2	-8, 4	3	-5, 11
PFNA	-7	-17, 3	6	-6, 19
MeFOSAA	-5	-10, 0	-3	-9, 3
EtFOSAA	-14	-21, -7	-9	-17, 0
Polychlorinated biphenyls (PCBs) (ng/g lipid)				
PCB28	-1	-8, 5	2	-7, 11
PCB74	-8	-20, 4	18	-10, 47
PCB99	-4	-14, 5	-12	-54, 29
PCB105	-12	-23, -1	6	-38, 49
PCB118	-2	-11, 8	0	-56, 55
PCB138[d]	-12	-23, -1	-12	-86, 61
PCB146	-7	-17, 3	-10	-30, 10
PCB153	-18	-31, -5	6	-115, 127
PCB156	-9	-21, 2	1	-25, 28
PCB170	-23	-38, -8	-13	-85, 58
PCB172	3	-6, 12	16	2, 30
PCB177	-9	-18, 0	-6	-26, 14
PCB178	-8	-19, 2	6	-12, 24
PCB180	-23	-38, -9	-24	-123, 74
PCB183	-6	-17, 5	-1	-29, 27

PCB187	-10	-21, 1	-20	-62, 21
PCB194	-3	-12, 6	-1	-16, 14
PCB195	-4	-14, 6	-2	-18, 14
PCB196[d]	-19	-34, -5	31	-19, 81
PCB199	-4	-14, 6	8	-14, 30
PCB206	-14	-25, -2	-14	-39, 11
Organochlorine pesticides (OCPs) (ng/g lipid)				
HCB	-4	-16, 9	1	-16, 18
β-HCH	-5	-14, 3	-4	-16, 7
p,p'-DDE	-3	-10, 4	0	-10, 10
p,p'-DDT	-5	-14, 4	-4	-17, 10

Abbreviations: CI, confidence interval; ng/mL, nanogram per milliliter; ng/g lipid, nanogram per gram lipid

[a] Adjusted for parity, pre-pregnancy BMI, maternal age, education, smoking, and gestational age at sample collection.

[b] Single-chemical linear regression models were run to examine independent associations between each chemical and birth weight. Betas represent a change of 10% higher chemical concentration.

[c] Multi-chemical linear regression models were run to examine associations between each chemical in a class (e.g., PFAS) and birth size, independent of other chemicals in the class (e.g., adjusting for other chemicals in the class). Betas represent a change of 10% higher chemical concentration.

[d] PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration, referred to as PCB138. Similarly, PCB congeners 196 and 203 could not be separated and were quantified as a summed concentration, referred to as PCB196.

Supplemental Table 4-6. Sensitivity analysis exploring associations[ab] of detectable versus non-detectable serum concentrations of persistent endocrine disrupting chemicals with birth weight in the Avon Longitudinal Study of Parents and Children (ALSPAC) sub-study using linear regression. Beta estimates represent the change in birth weight for those with detectable concentrations, compared to those with non-detectable concentrations.

	β[c]	95% CI
Per- and polyfluoroalkyl substances (PFAS) (ng/mL)		
PFOA	N/A	
PFOS	N/A	
PFHxS	N/A	
PFNA	N/A	
FOSA	-120	-218, -23
MeFOSAA	-95	-235, 44
EtFOSAA	N/A	
PFDA	N/A	
Polychlorinated biphenyls (PCBs) (ng/g lipid)		
PCB28	62	-98, 223
PCB44	9	-95, 113
PCB49	-13	-108, 83
PCB52	-10	-114, 94
PCB66	82	-21, 186
PCB74	N/A	
PCB87	39	-57, 135
PCB99	N/A	
PCB101	-92	-196, 12
PCB105	44	-122, 210
PCB110	15	-80, 109
PCB118	N/A	
PCB128	48	-115, 211
PCB138[d]	N/A	
PCB146	N/A	

PCB149	51	-43, 146
PCB151	-10	-128, 107
PCB153	N/A	
PCB156	N/A	
PCB157	81	-19, 182
PCB167	10	-97, 117
PCB170	N/A	
PCB172	69	-41, 179
PCB177	62	-120, 244
PCB178	72	-84, 229
PCB180	N/A	
PCB183	N/A	
PCB187	N/A	
PCB189	-41	-140, 58
PCB194	N/A	
PCB195	-29	-157, 99
PCB196[d]	N/A	
PCB199	N/A	
PCB206	-88	-276, 99
PCB209	-28	-143, 88
Organochlorine pesticides (OCPs) (ng/g lipid)		
HCB	N/A	
β-HCH	N/A	
γ-HCH	-28	-137, 81
Oxychlordane	-82	-189, 24
Trans-nonachlor	-5	-118, 107
p,p'-DDE	N/A	
o,p'-DDT	N/A	
p,p'-DDT	61	-85, 208

Mirex	N/A	

Abbreviations: CI, confidence interval; ng/mL, nanogram per milliliter; ng/g lipid, nanogram per gram lipid

[a] Adjusted for parity, pre-pregnancy BMI, maternal age, education, smoking, and gestational age at sample collection

[b] Restricted to those with % <LOD between 5% and 95%

[c] β represents the change in birth weight for those with detectable concentrations, compared to those with non-detectable concentrations (coded as a dichotomous variable)

[d] PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration, referred to as PCB138. Similarly, PCB congeners 196 and 203 could not be separated and were quantified as a summed concentration, referred to as PCB196

Supplemental Table 4-7. Comparison of characteristics of various sub-samples of mother-daughter dyads from the Avon Longitudinal Study of Parents and Children (ALSPAC) population. The nested case-control study (N=448) was drawn from cohort daughters who were enrolled at puberty (N=3338). Complete data for mixture analyses was available for 313 mother-daughter dyads.

	Enrolled at Puberty N=3338	Nested Case-Control N=448	Complete Data N=313
Characteristic	**n (%)[a]**	**n (%)[a]**	**n (%)**
Maternal race			
White	3050 (98.3)	423 (98.1)	308 (98.4)
Non-white	54 (1.7)	8 (1.9)	5 (1.6)
Maternal education[b]			
< O-level	549 (18.2)	75 (18.1)	53 (16.9)
O-level	1083 (35.9)	140 (33.7)	104 (33.2)
>O-level	1385 (45.9)	200 (48.2)	156 (49.8)
Maternal pre-pregnancy BMI			
<25 kg/m^2 (under/normal weight)	2379 (80.8)	313 (77.9)	243 (77.6)
≥25 kg/m^2 (overweight/obese)	566 (19.2)	89 (22.1)	70 (22.4)
Prenatal smoking			
Any	2677 (13.3)	79 (18.5)	47 (15.0)
None	410 (86.7)	348 (81.5)	266 (85.0)
Prenatal alcohol use			
Any	1585 (51.5)	215 (50.8)	155 (50.3)
None	1491 (48.5)	208 (49.2)	153 (49.7)
Physical activity			
Any	1901 (67.2)	252 (65.5)	184 (65.0)
None	929 (32.8)	133 (34.6)	99 (35.0)
Maternal age at delivery			
<25 years	473 (14.8)	92 (20.7)	54 (17.3)
25–29 years	1275 (40.0)	164 (36.9)	122 (39.0)
≥30 years	1439 (45.2)	189 (42.5)	137 (43.8)
Child birth order			
First born	1469 (48.0)	208 (49.6)	156 (49.8)
Second born or later	1593 (52.0)	211 (50.4)	157 (50.2)
Child birth weight			
<2500 g	134 (4.3)	17 (3.9)	8 (2.6)
≥2500 g	3010 (95.7)	423 (96.1)	305 (97.4)

Abbreviations: g, grams; kg/m^2, kilograms per meter-squared

[a] Missing data not represented

[b] <O-level=none, Certificate of Secondary Education, and vocational education, which are equivalent to no diploma or a GED in the United States. O-levels (ordinary levels) are required and completed at the age of 16. >O-level=A-levels (advanced levels) completed at 18, which are optional, but required to get into university; and a university degree.

Supplemental Table 4-8. Unadjusted[a] associations of mixtures with accompanying weights of maternal serum concentrations of persistent endocrine disrupting chemical (EDC) exposure with birth size measures in the Avon Longitudinal Study of Parents and Children (ALSPAC) sub-study using weighted quantile sum regression. Estimates represent the change for one-unit higher of the WQS index (representing a one-decile increase in chemical concentrations).

	Unadjusted (N=378)			**Unadjusted (N=313)**		
	Subset with missing covariate information			Subset with complete covariate information		
	Birth weight (g)			**Birth weight (g)**		
	β[b]	**95% CI**	**Weight[c]**	**β[b]**	**95% CI**	**Weight[c]**
Overall[d]	-43	-80, -6		-40	-73, -7	
PFOA			0.44†			0.40†
PFOS						0.02
PFHxS			0.02			
PFNA			0.01			0.02
MeFOSAA			0.03			0.01
EtFOSAA			0.28†			0.08†
PCB28			0.01			
PCB74						0.01
PCB99			0.01			
PCB105						
PCB118						
PCB138[e]			0.01			0.15†
PCB146			0.02			
PCB153			0.01			0.03†
PCB156			0.04†			0.10†
PCB170						0.02
PCB172			0.04†			0.01
PCB177						0.02
PCB178			0.01			
PCB180			0.02			0.03

PCB183	0.01	0.01
PCB187		
PCB194		0.03
PCB195	0.01	0.02
PCB196[e]		
PCB199	0.01	
PCB206	0.01	
HCB	0.02	0.04†
β-HCH	0.01	0.01
p,p'-DDE	0.01	
p,p'-DDT		

Abbreviations: SGA, small for gestational age; OR, odds ratio; CI, confidence interval; ng/mL, nanogram per milliliter; ng/g lipid, nanogram per gram lipid

[a] Adjusted for gestational week at sample collection, for which we had complete data

[b] β for one-unit higher of the WQS index (representing a one-decile increase in chemical concentrations)

[c] Weights greater than 1/number of chemicals in the mixture are considered significant contributors to the overall mixture effect

[d] Overall mixture includes PFAS, PCB, and OCP classes

[e] PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration, referred to as PCB138. Similarly, PCB congeners 196 and 203 could not be separated and were quantified as a summed concentration, referred to as PCB196

† Significant contributor to the overall mixture effect (>1/number of chemicals in mixture)

Chapter 5 Mixtures of prenatal concentrations of persistent endocrine disrupting chemicals and postnatal body size in British girls

Kristin J. Marks[a,b,c], Penelope P. Howards[a], Melissa M. Smarr[d], W. Dana Flanders[a,b], Kate Northstone[e], Johnni H. Daniel[b], Andreas Sjödin[f], Antonia M. Calafat[f], Terryl J. Hartman[a,b]

[a] Department of Epidemiology, Rollins School of Public Health, Emory University, 1518 Clifton Rd NE, Atlanta, Georgia 30322, United States
[b] Division of Environmental Health Science and Practice, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy, Atlanta, Georgia 30341, United States
[c] Oak Ridge Institute for Science and Education, 100 ORAU Way, Oak Ridge, Tennessee 37830, United States
[d] Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, 1518 Clifton Rd NE, Atlanta, Georgia 30322, United States
[e] Department of Population Health Sciences, Bristol Medical School, Oakfield House, Oakfield Grove, Bristol BS8 2BN, United Kingdom
[f] Division Of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy, Atlanta, Georgia 30341, United States

Abstract:
Endocrine disrupting chemical (EDC) exposure is ubiquitous. EDC exposure during critical windows of development, such as the prenatal period, may interfere with the body's endocrine system, affecting growth. Previous human studies have examined one EDC at a time in relation to infant growth. By studying mixtures, the human experience can be better approximated. We investigated the association of prenatal exposure to persistent EDCs (per- and polyfluoroalkyl substances (PFAS), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs)) as mixtures with postnatal body size among female offspring in a sub-study of the Avon Longitudinal Study of Parents and Children (N=425), based in the United Kingdom (UK). We quantified 52 EDCs in maternal serum collected during pregnancy. Weight and height measures at 0, 2, 9, and 19 months were obtained by health professionals as part of the routine child health surveillance program in the UK. We used Bayesian kernel machine regression with a random intercept to examine the association of prenatal concentrations of EDC mixtures with longitudinal postnatal body size measures for each EDC class separately (PFAS, PCBs, and OCPs) and for all three classes combined. The mixtures representing six PFAS (n=347) and all three classes combined (31 chemicals) (n=301) were inversely associated with postnatal body size. Perfluorooctanoate and perfluorooctane sulfonate were the most important chemicals among PFAS. Holding all 31 EDCs in the three classes combined mixture at the 75th percentile compared to the 50th percentile was associated with 0.15 lower weight-for-age z-score (estimate: -0.15, 95% credible interval: -0.26, -0.03). Weak inverse associations were also seen for height-for-age and BMI-for-age scores. These results suggest that prenatal exposure to mixtures of persistent EDCs, especially PFAS, may affect postnatal body size.

Keywords: ALSPAC, endocrine disrupting chemicals, per- and polyfluoroalkyl substances, polychlorinated biphenyls, organochlorine pesticides, early childhood growth, postnatal body size

Introduction

Endocrine disrupting chemicals (EDCs) may interfere with the body's endocrine system, potentially producing adverse developmental, reproductive, neurological, and immune effects (357). Environmentally persistent EDCs are normally highly resistant to degradation, tend to bioaccumulate in animals and humans, and have been used throughout the 20th and 21st centuries for a variety of purposes (123, 130, 144). Exposure to several persistent EDCs have declined in the general population following several countries banning or severely restricting the production, handling, and disposal of numerous polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs), as well as certain per- and polyfluoroalkyl substances (PFAS). Still, almost all humans have detectable serum concentrations of some of these persistent chemicals (124, 358). Moreover, many persistent EDCs can cross the placental barrier, enabling potential fetal exposure, and the amount of EDCs found in cord serum may be substantial in relation to a developing fetus's size (263, 359-361).

While birth weight is a well-studied outcome in relation to prenatal exposure to EDCs (select references: (155, 156, 244, 259, 261, 265, 266)), growth in the years following birth has been studied less, though the influence of prenatal exposure to EDCs on growth may persist after birth. The first two years of life are a particularly important period of change as the greatest variations in rates of weight gain are usually seen during this time when infants show accelerated or diminished growth to compensate for intrauterine restraint or enhancement of fetal growth (285).

Postnatal growth has been examined in a few previous studies with regard to prenatal EDC exposure. No studies have found an association of PCBs with growth measures in the first few years following birth (287, 294-296). All studies to examine OCPs found associations of dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE), or hexachlorobenzene (HCB) with early growth: four studies found a positive association (294-297) and one small study found an inverse association (287). Studies of PFAS and growth through age

3 have been especially mixed, with some studies finding children with higher prenatal PFAS exposure to be heavier in the years after birth (247), while other studies found lower body mass index (BMI) postnatally (255, 286) or no association (287, 288).

Because only a few studies have focused on growth after birth, and the available studies have used a variety of outcome measures (e.g., height, weight, BMI z-score, rapid growth), it is difficult to see patterns emerging across chemical classes. Previous studies have only examined one EDC at a time in relation to growth, and it is thought that this could have led to inconsistent conclusions on their effects on health. Because humans are exposed to many chemicals, as opposed to any one chemical in isolation, examining combined exposures or "mixtures" of chemicals allows for a better approximation of the human experience (4). Typically, a mixture is defined as a combination of three or more independent chemicals or chemical groups (5).

Our aim was to investigate the association of maternal gestational concentrations of persistent EDCs (PFAS, PCBs, and OCPs) and postnatal body size (weight-, height-, and BMI-for-age scores) at 0, 2, 9, and 19 months using a mixtures approach.

Methods

Study population

The Avon Longitudinal Study of Parents and Children (ALSPAC) is an ongoing prospective birth cohort of 14,541 pregnancies. ALSPAC enrolled pregnant women with an expected delivery date between 1 April 1991 and 31 December 1992 from three health districts in the former county of Avon in the United Kingdom (UK). Information was collected on parents and children through clinic visits, interviews, and mailed questionnaires. Details on ALSPAC recruitment and study methods have been described elsewhere (369, 370). The present analysis uses exposure data from a nested case–control study that was established within the ALSPAC cohort to explore associations of prenatal maternal concentrations of various suspected EDCs and age at

menarche among the daughters. Details of the nested case–control study (N=448) are described elsewhere (307). Where possible, the nested case-control study data were reweighted in analyses to represent the full cohort.

The ALSPAC website contains details of all available data through a fully searchable data dictionary and variable search tool (http://www.bris.ac.uk/alspac/researchers/our-data/). We obtained ethical approval for the study from the ALSPAC Ethics and Law Committee, the Local Research Ethics Committees, and the Centers for Disease Control and Prevention (CDC) Institutional Review Board. Mothers provided written informed consent for participation in the study. Consent for biological samples has been collected in accordance with the Human Tissue Act (2004). Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.

Exposure assessment

Fasting blood samples were collected from mothers during pregnancy at enrollment in 1991–1992 at a median of 15 (interquartile range (IQR): 10–28) weeks gestation. Samples were processed and frozen for later analysis. Maternal serum samples were held in storage facilities at the University of Bristol until they were transferred under controlled conditions and analyzed at the National Center for Environmental Health of the CDC (Atlanta, GA) in 2009-2010. Laboratory analyses included low- and high-concentration pooled quality control materials, standards, reagent blanks, and study samples. Concentrations below the limit of detection (LOD) were imputed by dividing the LOD by the square root of 2 prior to statistical analysis. The persistent EDCs detected in greater than 75% of mothers were included in the main analyses.

Per- and polyfluoroalkyl substances

Eight PFAS were quantified (**Table 5-1**) in serum via on-line solid-phase extraction coupled to isotope dilution high-performance liquid chromatography-tandem mass spectrometry (381). LODs are presented in **Table 5-1**. Coefficients of variation (CVs) were largely below 10%.

Organochlorine pesticides and polychlorinated biphenyls

We quantified nine OCPs and 35 PCBs (**Table 5-1**) in serum using gas chromatography isotope dilution high resolution mass spectrometry (383). PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration hereafter referred to as PCB138. Similarly, PCB congeners 196 and 203 could not be separated and were quantified as a summed concentration hereafter referred to as PCB196. LODs for PCBs and OCPs are dependent on the size of the sample available, thus an individual LOD was reported for each individual result rather than an overall LOD. CVs were generally below 10%. PCB and OCP concentrations were adjusted for lipids.

Outcome assessment

Birth weight (g) was abstracted from infant medical records. Trained ALSPAC staff measured crown to heel length (cm) using a Harpenden neonatometer (Holtain Ltd., Crymych, UK) and head circumference (cm) using a lasso tape measure within 24 hours of birth. Weight and height at roughly 2, 9, and 19 months were obtained by health professionals as part of the routine child health surveillance program in the UK. These weight and height measurements were extracted from health visitor records, which form part of standard child care in the UK. We calculated standard deviation (SD) scores (z-scores) of weight, height, and BMI on the basis of the British growth reference centiles for females from 1990 (398) with Excel macros provided on the internet (www.healthforallchildren.co.uk). The final clinical estimate of the expected date of delivery was abstracted from the obstetric records and used to calculate gestational age at birth.

Covariates

Covariate information was collected by clinical staff or through self-report in questionnaires completed by the mother during or immediately after pregnancy. Covariates under examination included gestational age at biological sample collection, age at measurement, maternal age at delivery, maternal pre-pregnancy BMI, maternal education, parity, and smoking during pregnancy.

Statistical analyses

The analytic dataset included daughters who had EDC measurements at three or four time points (N=425; 23 excluded). Descriptive analyses were conducted to report mother-daughter dyad characteristics and body size measures across time points. The persistent EDCs under study were modeled as natural log-transformed continuous variables. We ran single-chemical linear regression models to examine independent associations between each chemical and weight-for-age z-score at 19 months. We also ran multi-chemical linear regression models to examine associations between each chemical in a class (e.g., PFAS or PCBs) and weight-for-age score at 19 months, independent of other chemicals in the class (e.g., adjusting for other chemicals in the class). To account for the study design, we reweighted the sample to represent the full cohort of girls. All models adjust for parity (categorized as nulliparous/multiparous), pre-pregnancy BMI (kg/m^2, linear), maternal age at delivery (years, linear), maternal education (categorized as <O-level (ordinary level: required, completed at age 16), O-level, or > O-level), prenatal smoking (categorized as any/none), and gestational age at sample collection (categorized as ≤20 weeks or >20 weeks).

We also analyzed the data using the multiple measurement time points and accounting for clustering within child. We used single-chemical linear mixed models to examine independent associations between each chemical and weight-for-age z-scores while accounting for the longitudinal nature of the data. We also used multi-chemical linear mixed models to examine associations between each chemical in a class and weight-for-age scores, independent of other

chemicals in the class. The full linear mixed models included the same covariates as the models for the outcomes at 19 months plus a time variable representing the age (in months, initially included as linear and quadratic terms) when weight measurements were taken (roughly 0, 2, 9 or 19 months). The linear mixed-effects models also included an intercept, a subject-specific random intercept and a random slope for age at the measurement (as a linear term) (**Supplemental Methods**, Models 1 and 2). As before, we reweighted the sample to represent the full cohort. This full model was used to test for the necessity of including the quadratic term for age at measurement and the random-effects (random intercept and random slope for age at measurement in months) and to assess various random-effects covariance structures for the R matrix (autoregressive(1), compound symmetric, Toeplitz, Toeplitz with two bands, unstructured, and variance components). We also considered models that included an interaction between age at measurement and EDC concentrations (see **Supplemental Methods** for details on model selection). Our final model included a subject-specific random intercept with a Toeplitz with two bands covariance structure for the R matrix (**Supplemental Methods**, Model 2).

To estimate how prenatal mixtures are associated with repeated measures of postnatal size-for-age scores, we used Bayesian kernel machine regression (BKMR). BKMR is a flexible approach for estimating the joint health effects of exposure to multiple concurrent risk factors (333). BKMR allowed us to evaluate the association of the EDC mixture with postnatal body size measures (collected at 0, 2, 9, and 19 months), accounting for the correlated nature of the data through use of a random intercept (specified using the *id* argument within the main argument *kmbayes* of the R package *bkmr*) (400). Within BKMR, we used hierarchical variable selection when examining multiple classes of chemicals (i.e., PFAS, PCBs, and OCPs) in the same mixture, which provided group importance scores (posterior inclusion probabilities (PIPs)) for pre-defined mutually exclusive groups of variables. Further, we estimated the importance of a chemical given that the group containing the chemical was important (conditional PIPs) (333, 399, 400). Currently, the *bkmr* package does not allow for weighting, so we were unable to reweight our

nested case-control data back to the full cohort, which limits generalizability. We ran the Markov Chain Monte Carlo (MCMC) sampler for 10,000 iterations. Natural log-transformed EDC concentrations were centered and scaled by the standard deviation. Mixtures under study included each chemical class separately (PFAS, PCBs, and OCPs) and all three chemicals classes combined. These models included the same covariates used in the linear mixed effects models but continuous covariates were centered and scaled.

In sensitivity analyses, we considered BKMR models that excluded the observation at birth, and using weight-for-age scores that were adjusted for gestational age (see **Supplemental Methods).** We also did sensitivity analyses using weighted quantile sum (WQS) regression to construct a weighted index estimating the mixture effect associated with all predictor variables on weight-for-age at each measurement time point in separate models (331, 402). WQS regression is a complementary method to BKMR and can also estimate the overall effect of a mixture, while identifying the most important chemicals within the mixture.

SAS software 9.4 (Cary, NC) was used for descriptive analyses and single- and multi-chemical models. R software 3.5.0 (Vienna, Austria) was used for WQS regression and BKMR analyses.

Results

Descriptive statistics

There were 425 ALSPAC mother-daughter dyads with 3 or 4 postnatal body size measures (**Supplemental Figure 5-4**), of these, 354 had weight measurements at 19 months. From the 425 ALSPAC mother-daughter dyads with ≥3 measures, 347 had complete covariate data. An additional 46 mother-daughter dyads were missing information on lipids, PCBs, or OCPs, bringing the analytic dataset for mixture analyses of all three chemical classes to 301 dyads.

ALSPAC mothers were predominantly higher-educated, non-smokers who were 25 years or older at the time of delivery (**Table 5-2**). On average, daughters were 3.39 kg (SD: 0.50 kg) at

birth and 11.54 kg (SD: 1.41 kg) at 19 months of age (**Table 5-3**). To examine characteristics of mother-daughter dyads, we stratified the sample by weight-for-age scores at 19 months (n=354) (**Table 5-2**). Daughters born to mothers with under-/normal weight pre-pregnancy BMI were more likely to have z-scores <0 at 19 months (81.3%) compared to z-scores >1 (71.3%). A lower birth weight (<2800 g) was more frequently observed among daughters with an z-score <0 at 19 months (22.0%), compared to those with an z-score >1 (percent suppressed due to small cell size).

Median serum concentrations (and interquartile ranges) of PFAS, PCBs, and OCPs are presented in **Supplemental Table 5-5**. Of the 52 chemicals measured, 31 were detected in more than 75% of mothers. Correlation was high among the 31 chemicals (**Figure 5-1**), with strong intra- and inter-class correlation among PCBs and OCPs.

Single- and multi-chemical models

Weight-for-age scores at 19 months

Supplemental Table 5-6 shows the results of single- and multi-chemical models for weight-for-age scores at roughly 19 months of age. While single-chemical associations were generally weak, they were consistent in direction: all chemicals were positively associated with weight-for-age scores in single-chemical models. For example, a 10% higher PCB199 concentration was associated with 0.03 higher (95% confidence interval (CI): 0.00, 0.05) z-score for weight-for-age.

Weight-for-age scores at 0, 2, 9, and 19 months

Table 5-4 shows results from single- and multi-chemical mixed models for weight-for-age scores at 0, 2, 9, and 19 months old with a random intercept. While associations were weak in single-chemical mixed models, they were consistent in direction: almost all chemicals were inversely associated with weight-for-age scores (with the exception of PCB172). In the multi-chemical PFAS mixed model, 10% higher PFOA was inversely associated with weight-for-age

scores (β: -0.03, 95% CI: -0.07, 0.01). In the multi-chemical PCB mixed model, 10% higher PCB74 was positively associated with weight-for-age scores (β: 0.07, 95% CI: 0.01, 0.13), while 10% higher PCB118 was inversely associated with weight-for-age scores (β: -0.09, 95% CI: -0.19, 0.01).

Bayesian Kernel Machine Regression

All three classes combined and weight-for-age scores

In the BKMR model with random intercept for the mixture of all three classes combined and weight-for-age scores at 0, 2, 9, and 19 months, OCPs had the highest posterior inclusion probability (PIP) (0.61), making it the most important group in the mixture (PIP_{PCBs}: 0.60 and PIP_{PFAS}: 0.54). Within the OCP group, β-HCH and p,p'-DDT had the highest PIPs (conditional PIP: 0.32 and 0.30, respectively). In the PCB group, PCB105 and PCB118 contributed the most (conditional PIP: 0.11 and 0.09, respectively). Within the PFAS class, PFOS and PFOA contributed the most to the model (conditional PIPs: 0.27 and 0.24, respectively).

Within the BKMR model, the independent natural log-transformed chemical associations all appeared relatively linear with the exception of β-HCH (**Figure 5-2**). Some chemicals had slightly positive associations (PFNA, PCB74, PCB105, PCB183, PCB194, HCB), some appeared to have negative associations (PFOA, PCB118, PCB153, p,p'-DDT), and the remainder showed no association with weight-for-age z-scores. We found an overall mixture effect, with higher exposure to the mixture associated with lower weight-for-age z-scores (**Figure 5-3A**). Holding all persistent EDCs at the 75th percentile compared to the median was associated with 0.15 lower weight-for-age z-score (estimate: -0.15, 95% credible interval: -0.26, -0.03). Comparing all persistent EDCs at the 75^{th} percentile to the 25^{th} percentile was associated with 0.27 lower weight-for-age z-score (estimate: -0.27, 95% credible interval: -0.42, -0.11).

All three classes combined and height- and BMI-for-age scores

In both the height- and BMI-for-age models, PCBs had the highest posterior inclusion probability (PIP) (PIP_{height}: 0.54 and PIP_{BMI}: 0.45). In the height-for-age model, PCB153 contributed the most (conditional PIP: 0.17) to the mixture of all three classes combined, while PCB178 contributed the most (conditional PIP: 0.14) to the mixture in the BMI-for-age model. While associations were null, the overall mixture effect for all three classes combined and height-for-age (**Figure 5-3B**) and BMI-for-age (**Figure 5-3C**) z-scores showed slight inverse associations.

Class-specific mixtures and weight-for-age scores

BKMR models were run for each chemical class individually (i.e., separate models for PFAS, PCBs, and OCPs). In the PFAS model, PFOA and PFOS had the highest PIPs (PIPs: 0.57 and 0.54, respectively). We found an overall mixture effect for PFAS, with higher exposure to the mixture associated with lower weight-for-age z-scores (**Supplemental Figure 5-5A**). Similarly, in the PCB model, we found an inverse association of exposure to the mixture with weight-for-age z-scores (**Supplemental Figure 5-5B**), although it was less precisely estimated. In the OCP model, there was no overall mixture effect; median exposure to the OCP mixture was associated with the lowest weight-for-age z-scores (**Supplemental Figure 5-5C**).

Sensitivity analyses

We conducted sensitivity analyses to examine the association between the overall mixture of chemicals and weight-for-age scores at various postnatal time points (separate time points, as opposed to all time points together in a mixed model). Using both WQS regression and BKMR, we found an inverse association of the overall mixture with birth weight (**WQS: Supplemental Table 5-7; BKMR: Supplemental Figure 5-6A**), which has previously been reported in ALSPAC (415), as well as weight-for-age scores at 2 months (**WQS: Supplemental Table 5-7; BKMR: Supplemental Figure 5-6B**). We found no association between the mixture of 31 chemicals and

weight-for-age scores at 9 or 19 months (**WQS: Supplemental Table 5-7; BKMR: Supplemental Figure 5-6C&D**). See **Supplemental Methods** for further sensitivity analysis results.

Discussion

In this study of prenatal exposure to mixtures of persistent EDCs and postnatal body size at four time points through 19 months among British girls, we found an inverse association between a 31-chemical mixture and weight-for-age z-scores. This association seems to be driven by early postnatal weight-for-age. Further, weakly inverse associations were seen for height-for-age and BMI-for-age z-scores. These results suggest that prenatal exposure to mixtures of persistent EDCs may affect postnatal body size. We found that holding all 31 EDCs in the mixture at the 75th percentile compared to the 50th percentile was associated with 0.15 lower weight-for-age z-score (estimate: -0.15, 95% credible interval: -0.26, -0.03). At mean values for 19 months of age, a 0.15 lowering of the weight-for-age z-score corresponds to 0.18 kg lower weight (estimate: -0.18 kg, 95% credible interval: -0.31, -0.03 kg), which is a 1.6% decrease (estimate: -1.6%, 95% credible interval: -2.7, -0.3%).

Previous studies under the single-chemical paradigm have identified no association between prenatal PCB exposure and postnatal growth (change in weight-for-age Z-score between birth and 2 years (294), rapid growth in the first 6 months of life (295), and elevated BMI at 14 months (295). We observed an imprecise but inverse association of a mixture of 21 PCB congeners with weight-for-age scores through 19 months. An important difference between previous studies and this study is the use of mixture methods: it is possible that previous studies of PCBs and postnatal growth and body size have underestimated the risks of exposure to PCBs by examining only one congener at a time.

We observed no association between a four-chemical mixture of OCPs and weight-for-age scores. Previous studies of OCPs and postnatal growth measures under the single-chemical paradigm have noted positive associations between prenatal exposure to DDE (294-296), DDT (297), and HCB (296) and a variety of infant growth measures (change in weight-for-age z-score

(294), BMI/overweight status (295, 296), BMI-for-age, weight-for-height, and weight-for-age (297)) within the first one to two years of life. We saw no association of the four-chemical OCP mixture with weight-for-age scores through 19 months using BKMR: low (e.g., 25th percentile) or high (e.g., 75th percentile) concentrations of the mixture were associated with higher weight-for-age scores than median concentrations. In our single-chemical models at 19 months, there were weakly positive associations for all OCPs and weight-for-age scores at 19 months, with β-HCH having the strongest association of the four.

A number of studies from Denmark (n=1,010), Ohio (n=334), and Taiwan (n=223) have found inverse associations (255, 286, 289) of prenatal PFAS exposure (mainly PFOA and PFOS) and postnatal growth measures (weight (286), height (289), BMI (255, 286)) at various time points in the first two years of life. Other studies from the Netherlands (n=148) and New York (n=1,954) have found no association (287, 288). We found an inverse association between the six-chemical PFAS mixture and weight-for-age scores through 19 months, and PFOA and PFOS were the most important contributors.

A previous study in the ALSPAC population examined prenatal concentrations of three PFAS (PFOA, PFOS, and PFHxS) and postnatal body size (247). The study found inverse associations of prenatal concentrations of PFOA, PFOS, and PFHxS with birth weight, while those in the highest tertile of prenatal PFOS concentrations were 364 g heavier at 20 months than those in the lowest tertile. In addition to PFOA, PFOS, and PFHxS, the present study examined PCBs, OCPs, and additional PFAS (PFNA, MeFOSAA, and EtFOSAA) using a mixtures approach. We saw results similar to Maisonet et al. (2012) in the present study: there were strong inverse associations with birth weight, and most chemicals were weakly associated with higher weight-for-age scores at 19 months in single- and multi-chemical models. While we observed an overall inverse association between prenatal exposure to mixtures of persistent EDCs and weight-for-age scores at four time points through 19 months, especially for PFAS mixtures, the association shrinks following birth and is null by 19 months. This finding fits with previous ALSPAC studies

that have examined prenatal PFAS and PCB exposure with body fatness at age 9 and have largely found no association (293, 416).

This is the first study to examine prenatal exposure to EDCs and postnatal body size using a mixtures approach. A few studies have examined prenatal exposure to mixtures of EDCs and birth size (254, 394, 415), though the influence of prenatal exposure to EDCs on weight may persist after birth (247, 255, 286, 287, 294-297), and the first two years of life are a particularly important period of change (285). There is mounting evidence that both prenatal and infant growth are associated with future obesity risk; rapid infant growth and obesity in infancy are associated with a higher risk of obesity later in life (417, 418).

This study has several strengths, including its prospective study design within a population-based birth cohort with frequent and thorough longitudinal data collection. Additionally, we have reliable biomonitoring measurements of more than 50 persistent EDCs collected at median 15 weeks gestation, several outcomes measured by health professionals at birth and during infancy, and extensive covariate data available for mothers and daughters.

This study also has limitations. We only examined mother-daughter dyads in this sub-study, which was originally intended to investigate early menarche. There is some evidence to suggest that associations of EDCs and some growth outcomes are modified by infant sex (286, 287, 297), so restricting to daughters is a reasonable choice, though results cannot be generalized to male infants. Another limitation in generalizability is that because we centered and scaled exposure variables, what appears to be the most important in this population might not be the same as what would appear to be the most important in a different population with a different distribution of exposures. Further, while we have detailed covariate data, there is always the possibility that we were not able to completely control for confounding by certain sensitive or self-reported variables, such as smoking and socioeconomic status, or possible other unmeasured covariates. Approaches to mixture analyses that involve regressing the outcome on several correlated exposures simultaneously can in some cases amplify rather than reduce confounding

bias (“coexposure amplification bias”), particularly in cases of residual confounding (350). Our results could be biased if mother-daughter dyads with missing data were different from mother-daughter dyads included in the analyses. Mean values of body size outcomes and maternal characteristics for girls included in BKMR analyses were similar to the girls enrolled in the overall cohort, suggesting that selection bias is an unlikely explanation for our results. Additionally, we were unable to examine postnatal exposure to EDCs and its impact on infant growth, but this is an important question for future research.

Another limitation is that we were unable to weight BKMR analyses of this nested case-control study back to the full cohort, which limits generalizability. Nevertheless, results of the unweighted BKMR analyses were similar to weighted single- and multi-chemical mixed model results in terms of direction of effect. In the multi-chemical mixed model for PFAS, PFOA was most strongly inversely associated with weight-for-age scores; in the BKMR model for weight-for-age, PFOA was identified as one of the most important PFAS and was inversely associated with weight-for-age scores. Similarly, in the multi-chemical mixed model for PCBs, PCB118 was strongly inversely associated with weight-for-age scores, while PCB74 and PCB105 were positively associated with weight-for-age scores. Results of the BKMR model showed this as well: PCB118 was one of the most important PCBs in the mixture and had an inverse association with weight-for-age, while PCB74 and PCB105 showed positive associations. We were able to weight the WQS models in our sensitivity analyses examining each measurement time point individually (the R package, *gWQS*, does not yet allow for the analysis of correlated data). We found similar results to the equivalent BKMR models: the mixture of 31 chemicals is inversely associated with weight-for-age at birth and 2 months, but the associations substantially weaken at 9 and 19 months.

In conclusion, we found an inverse association between prenatal exposure to a mixture of persistent EDCs (PFAS, PCBs, OCPs) and longitudinal postnatal body size through 19 months as measured through weight-for-age z-scores. The observed association appears to be driven by

early postnatal body size. The association of the 31-chemical EDC mixture with height-for-age and BMI-for-age scores was also inverse, but weaker than what was observed for weight-for age scores.

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Disclosures

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the CDC. Use of trade names is for identification only and does not imply

endorsement by the CDC, the Public Health Service, or the U.S. Department of Health and Human Services.

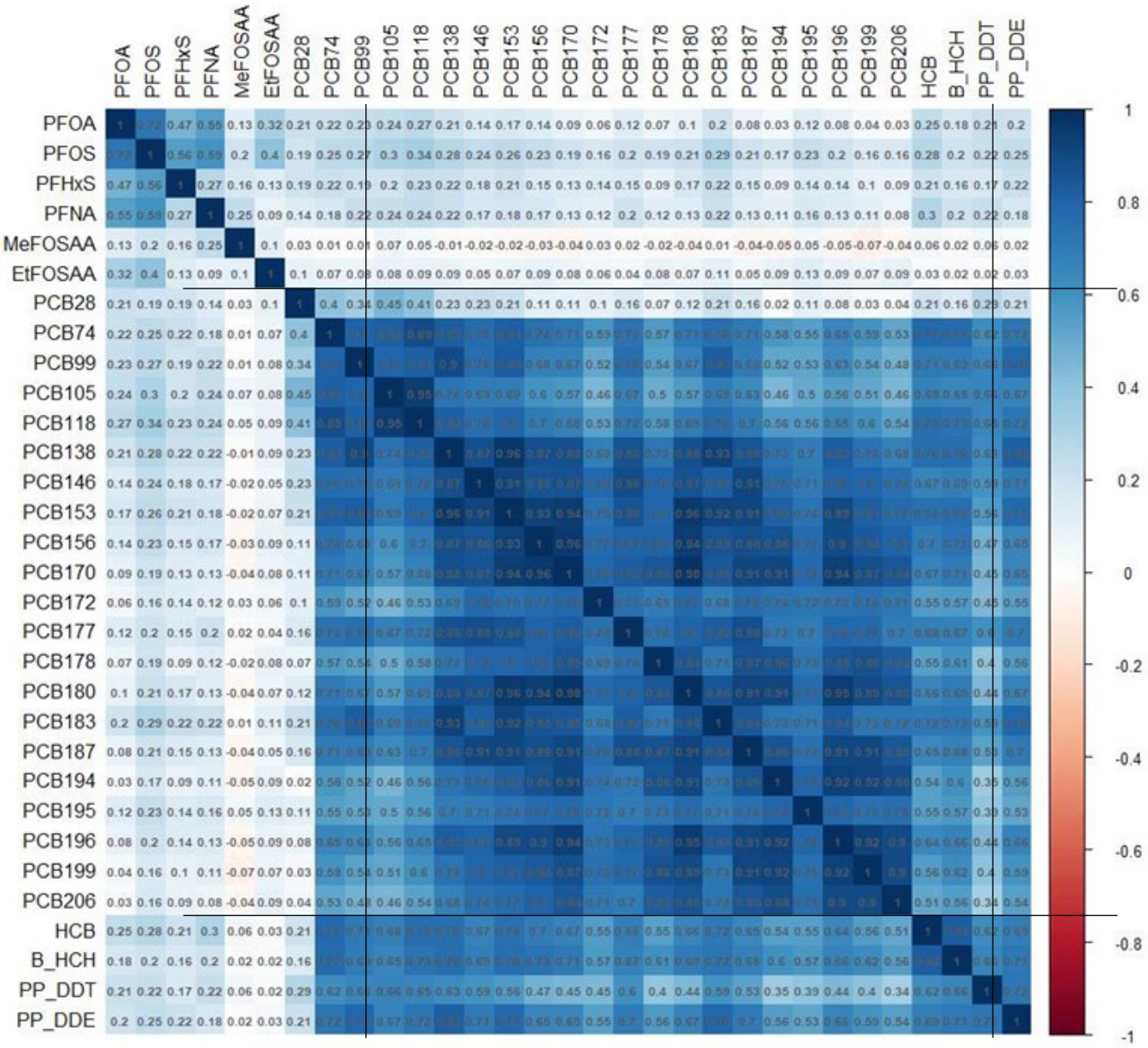

PFOA
PFOS
PFHxS
PFNA
MeFOSAA
EtFOSAA
PCB28
PCB74
PCB99
PCB105
PCB118
PCB138
PCB146
PCB153
PCB156
PCB170
PCB172
PCB177
PCB178
PCB180
PCB183
PCB187
PCB194
PCB195
PCB196
PCB199
PCB206
HCB
B_HCH
PP_DDT
PP_DDE
1
0.8
0.6
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1

Figure 5-1. Correlation heatmap of serum concentrations of persistent endocrine disrupting chemicals across women during pregnancy in the Avon Longitudinal Study of Parents and Children (N=425).
Spearman correlation coefficients presented for untransformed distributions, sectioned according to per- and polyfluoroalkyl substances (PFAS), polychlorinated biphenyl (PCB), and organochlorine pesticide (OCP) group membership. PCB and OCP concentrations were lipid-adjusted.

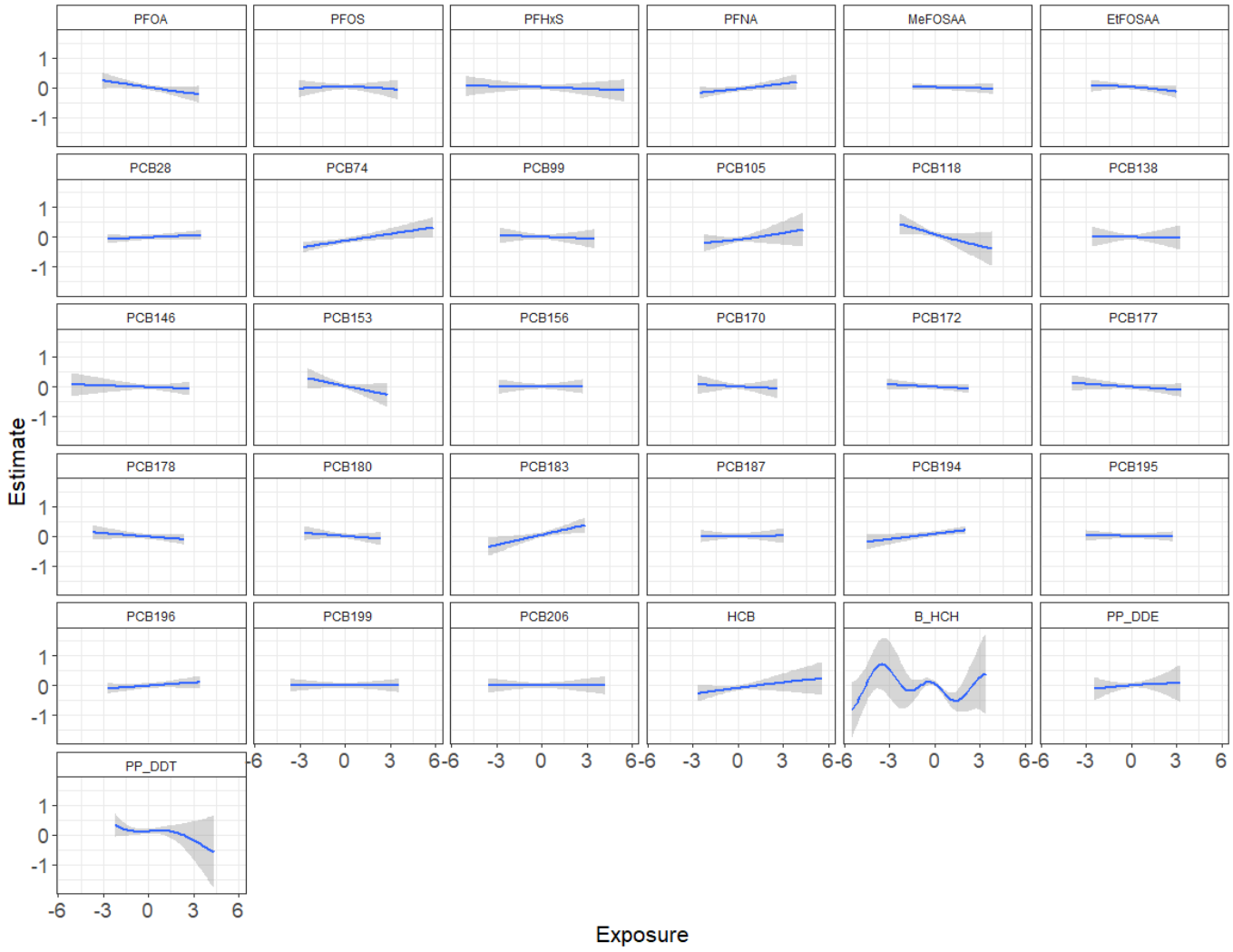


Figure 5-2. Chemical-specific effect estimates of mixture members on weight-for-age z-scores in ALSPAC mother-daughter dyads estimated by BKMR (n=301).
Single chemical associations and 95% credible bands are presented with other chemicals fixed at their median. The model adjusted for maternal education, parity, pre-pregnancy body mass index, maternal age at delivery, prenatal smoking, age at measurement,

and gestational week at sample collection. All chemical concentrations were natural log-transformed and standardized; PCB and OCP concentrations were lipid-adjusted.

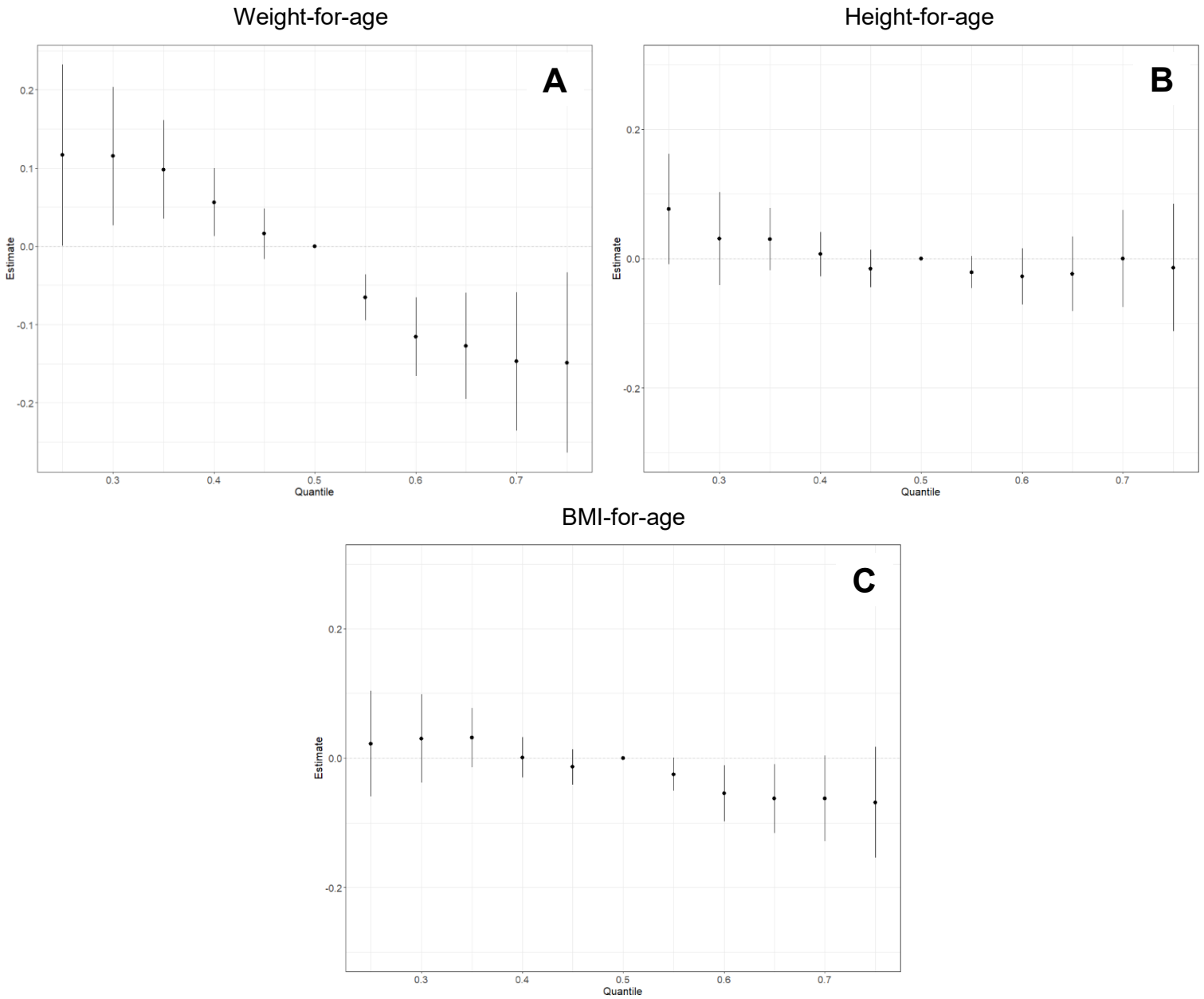


Figure 5-3. Overall effect of the 31 chemical mixture on postnatal body size-for-age z-scores.

A Overall effect of the mixture on weight-for-age z-scores (estimates and 95% credible intervals), comparing the outcome when all exposures are at a particular quantile to the median (n=301). **B** Overall effect of the mixture on height-for-age z-scores (estimates and 95% credible intervals), comparing the outcome when all exposures are at a particular quantile to the median (n=300). **C** Overall effect of the mixture on body mass index (BMI)-for-age z-scores (estimates and 95% credible intervals), comparing the outcome when all concentrations are at a particular quantile to the median (n=300). Bayesian kernel machine regression models adjusted for maternal education, parity, pre-pregnancy body mass index, maternal age at delivery, prenatal smoking, age at measurement, and gestational age at sample collection, and used a random intercept to account for repeated measures at 0, 2, 9, and 19 months. All chemical concentrations were natural log-transformed and standardized; PCB and OCP concentrations were lipid-adjusted.

Table 5-1. Persistent endocrine disrupting chemicals measured in maternal serum in the ALSPAC nested case-control study.

Chemical Name	Abbreviated Name	Limit of Detection
Per- and Polyfluoroalkyl Substances		
Perfluorooctanoate	PFOA	0.10 ng/mL
Perfluorooctane sulfonate	PFOS	0.20 ng/mL
Perfluorohexane sulfonate	PFHxS	0.10 ng/mL
Perfluorononanoate	PFNA	0.082 ng/mL
2-(N-methyl-perfluorooctanesulfonamido) acetate	MeFOSAA	0.174 ng/mL
2-(N-ethyl-perfluorooctanesulfonamido) acetate	EtFOSAA	0.20 ng/mL
Perfluorooctane sulfonamide	FOSA	0.10 ng/mL
Perfluorodecanoate	PFDA	0.20 ng/mL
Polychlorinated Biphenyls		
2,4,4'-trichlorobiphenyl	PCB28	Individual[a]
2,2',3,5'-tetrachlorobiphenyl	PCB44	Individual[a]
2,2',4,5'-tetrachlorobiphenyl	PCB49	Individual[a]
2,2',5,5'-tetrachlorobiphenyl	PCB52	Individual[a]
2,3',4,4'-tetrachlorobiphenyl	PCB66	Individual[a]
2,4,4',5-tetrachlorobiphenyl	PCB74	Individual[a]
2,2',3,4,5'-pentachlorobiphenyl	PCB87	Individual[a]
2,2',4,4',5-pentachlorobiphenyl	PCB99	Individual[a]
2,2',4,5,5'-pentachlorobiphenyl	PCB101	Individual[a]
2,3,3',4,4'-pentachlorobiphenyl	PCB105	Individual[a]
2,3,3',4',6-pentachlorobiphenyl	PCB110	Individual[a]
2,3',4,4',5-pentachlorobiphenyl	PCB118	Individual[a]
2,2',3,3',4,4'-hexachlorobiphenyl	PCB128	Individual[a]
2,2',3,4,4',5'-hexachlorobiphenyl & 2,3,3',4,4',6-hexachlorobiphenyl	PCB138-158	Individual[a]
2,2',3,4',5,5'-hexachlorobiphenyl	PCB146	Individual[a]
2,2',3,4',5',6-hexachlorobiphenyl	PCB149	Individual[a]
2,2',3,5,5',6-hexachlorobiphenyl	PCB151	Individual[a]
2,2',4,4',5,5'-hexachlorobiphenyl	PCB153	Individual[a]
2,3,3',4,4',5-hexachlorobiphenyl	PCB156	Individual[a]
2,3,3',4,4',5'-hexachlorobiphenyl	PCB157	Individual[a]
2,3',4,4',5,5'-hexachlorobiphenyl	PCB167	Individual[a]
2,2',3,3',4,4',5-heptachlorobiphenyl	PCB170	Individual[a]
2,2',3,3',4,5,5'-heptachlorobiphenyl	PCB172	Individual[a]
2,2',3,3',4',5,6-heptachlorobiphenyl	PCB177	Individual[a]
2,2',3,3',5,5',6-heptachlorobiphenyl	PCB178	Individual[a]
2,2',3,4,4',5,5'-heptachlorobiphenyl	PCB180	Individual[a]
2,2',3,4,4',5',6-heptachlorobiphenyl	PCB183	Individual[a]
2,2',3,4',5,5',6-heptachlorobiphenyl	PCB187	Individual[a]
2,3,3',4,4',5,5'-heptachlorobiphenyl	PCB189	Individual[a]
2,2',3,3',4,4',5,5'-octachlorobiphenyl	PCB194	Individual[a]
2,2',3,3',4,4',5,6-octachlorobiphenyl	PCB195	Individual[a]
2,2',3,3',4,4',5',6-octachlorobiphenyl & 2,2',3,4,4',5,5',6-octachlorobiphenyl	PCB196-203	Individual[a]
2,2',3,3',4,5,6,6'-octachlorobiphenyl	PCB199	Individual[a]
2,2',3,3',4,4',5,5',6-nonachlorobiphenyl	PCB206	Individual[a]

Decachlorobiphenyl	PCB209	Individual[a]
Organochlorine Pesticides		
Hexachlorobenzene	HCB	Individual[a]
β-Hexachlorocyclohexane	β-HCH	Individual[a]
Y-Hexachlorocyclohexane (Lindane)	γ-HCH	Individual[a]
Oxychlordane	Oxychlordane	Individual[a]
Trans-Nonachlor	Trans-nonachlor	Individual[a]
2,2-Bis(4-chlorophenyl)-1,1-dichloroethene	p,p'-DDE	Individual[a]
2-(4-chlorophenyl)-2-(2-chlorophenyl)-1,1,1-trichloroethan	o,p'-DDT	Individual[a]
2,2-Bis(4-chlorophenyl)-1,1,1-trichloroethan	p,p'-DDT	Individual[a]
Mirex	Mirex	Individual[a]

[a]LODs for PCBs and OCPs are dependent on the size of the sample available, thus an individual LOD was reported for each individual result rather than an overall LOD. The range of individual LODs for most PCBs under study was 0.40–6.00 ng/g lipid. The range of individual LODs for OCPs was 2.10–30.20 ng/g lipid.

Table 5-2. Characteristics of the Avon Longitudinal Study of Parents and Children (ALSPAC) sub-study population by weight-for-age z-score at 19 months.

	Distribution by weight-for-age z-score					
Characteristic	**<0 n=134**		**0-1 n=134**		**>1 n=86**	
	n	**%**	**n**	**%**	**n**	**%**
Maternal education[a]						
< O-level	23	18.4	26	21.0	14	17.5
O-level	39	31.2	48	38.7	24	30.0
>O-level	63	50.4	50	40.3	42	52.5
Maternal pre-pregnancy BMI, kg/m^2						
<25 (under/normal weight)	100	81.3	91	76.5	57	71.3
≥25 (overweight/obese)	23	18.7	28	23.5	23	28.8
Prenatal smoking						
Any	22	17.2	31	24.0	18	21.4
None	106	82.8	98	76.0	66	78.6
Physical activity						
Any	76	65.5	70	60.3	57	73.1
None	40	34.5	46	39.7	21	26.9
Maternal age at delivery, years						
<25	26	19.4	32	23.9	17	19.8
25–29	44	32.8	61	45.5	31	36.0
≥30	64	47.8	41	30.6	38	44.2
Child birth order						
First born	62	48.1	73	57.9	35	42.7
Second born or later	67	51.9	53	42.1	47	57.3
Birth weight, g						
<2800	29	22.0	9	6.8	--[b]	--[b]
≥2800	103	78.0	124	93.2	81	--[b]

Abbreviations: BMI, body mass index; kg/m^2, kilograms per meter-squared; g, grams

[a] <O-level=none, Certificate of Secondary Education, and vocational education, which are equivalent to no diploma or a GED in the United States. O-levels (ordinary levels) are required and completed at the age of 16. >O-level=A-levels (advanced levels) completed at 18, which are optional, but required to get into university; and a university degree.

[b] Suppressed due to small cell sizes

Table 5-3. Mean (standard deviation) weight, height, and body mass index (BMI) across four time points in the Avon Longitudinal Study of Parents and Children (ALSPAC) sub-study population (N=425 mother-daughter dyads).

	n	**Age (months)** Mean (SD)	n	**Weight (kg)** Mean (SD)	**Weight z-score** Mean (SD)	n	**Height (cm)** Mean (SD)	**Height z-score** Mean (SD)	n	**BMI (kg/m^2)** Mean (SD)	**BMI z-score** Mean (SD)
Birth	424	0 (0)	417	3.39 (0.50)	-0.05 (1.09)	363	50.3 (2.16)	0.06 (1.15)	356	13.4 (1.26)	0.20 (1.05)
2 months	406	1.71 (0.30)	402	4.82 (0.62)	-0.01 (1.04)	361	57.0 (2.37)	0.47 (1.15)	357	14.9 (1.41)	-0.40 (1.16)
9 months	392	9.17 (0.94)	383	8.89 (1.00)	0.23 (1.04)	332	71.6 (2.60)	0.49 (1.10)	323	17.5 (1.48)	-0.04 (1.11)
19 months	358	19.39 (3.14)	354	11.54 (1.41)	0.32 (1.01)	287	83.2 (3.94)	0.33 (1.06)	283	16.8 (1.53)	0.06 (1.12)

Table 5-4. Adjusted[a] single[b]- and multi[c]-chemical associations of maternal serum concentrations of persistent endocrine disrupting chemicals (EDCs) and weight-for-age z-score at 0, 2, 9 and 19 months in the Avon Longitudinal Study of Parents and Children (ALSPAC) sub-study (N=347 mother-daughter dyads) using a linear mixed model with a random intercept. Beta estimates represent the change in weight-for-age z-scores for 10% higher chemical concentrations[d].

	Single-chemical models[be]		**Multi-chemical models[ce]**	
	β	**95% CI**	**β**	**95% CI**
Per- and polyfluoroalkyl substances (PFAS) (ng/mL)				
PFOA	-0.03	-0.06, 0.00	-0.03	-0.07, 0.01
PFOS	-0.02	-0.05, 0.01	-0.01	-0.06, 0.04
PFHxS				
PFNA			0.01	-0.03, 0.05
MeFOSAA				
EtFOSAA				
Polychlorinated biphenyls (PCBs) (ng/g lipid)				
PCB28				
PCB74			0.07	0.01, 0.13
PCB99			-0.05	-0.13, 0.03
PCB105	-0.01	-0.04, 0.01	0.06	-0.03, 0.14
PCB118			-0.09	-0.19, 0.01
PCB138[f]	-0.01	-0.04, 0.01		
PCB146			-0.01	-0.04, 0.02
PCB153	-0.02	-0.05, 0.01	0.03	-0.21, 0.27
PCB156			-0.02	-0.14, 0.09
PCB170	-0.02	-0.06, 0.01	0.02	-0.16, 0.20
PCB172	0.01	-0.02, 0.04	0.03	-0.02, 0.08
PCB177			-0.02	-0.07, 0.03
PCB178			0.01	-0.03, 0.05
PCB180	-0.03	-0.06, 0.01	-0.12	-0.36, 0.12
PCB183			0.05	-0.02, 0.12

PCB187			-0.03	-0.13, 0.06
PCB194				
PCB195				
PCB196[f]	-0.01	-0.05, 0.02	0.08	-0.02, 0.18
PCB199			0.02	-0.02, 0.07
PCB206	-0.01	-0.04, 0.01	-0.04	-0.09, 0.02
Organochlorine pesticides (OCPs) (ng/g lipid)				
HCB			-0.02	-0.06, 0.01
β-HCH			0.01	-0.01, 0.04
p,p'-DDE				
p,p'-DDT				

Abbreviations: CI, confidence interval; ng/mL, nanogram per milliliter; ng/g lipid, nanogram per gram lipid

[a] Adjusted for parity, pre-pregnancy BMI, maternal age at delivery, education, prenatal smoking, age at measurement, and gestational age at sample collection.

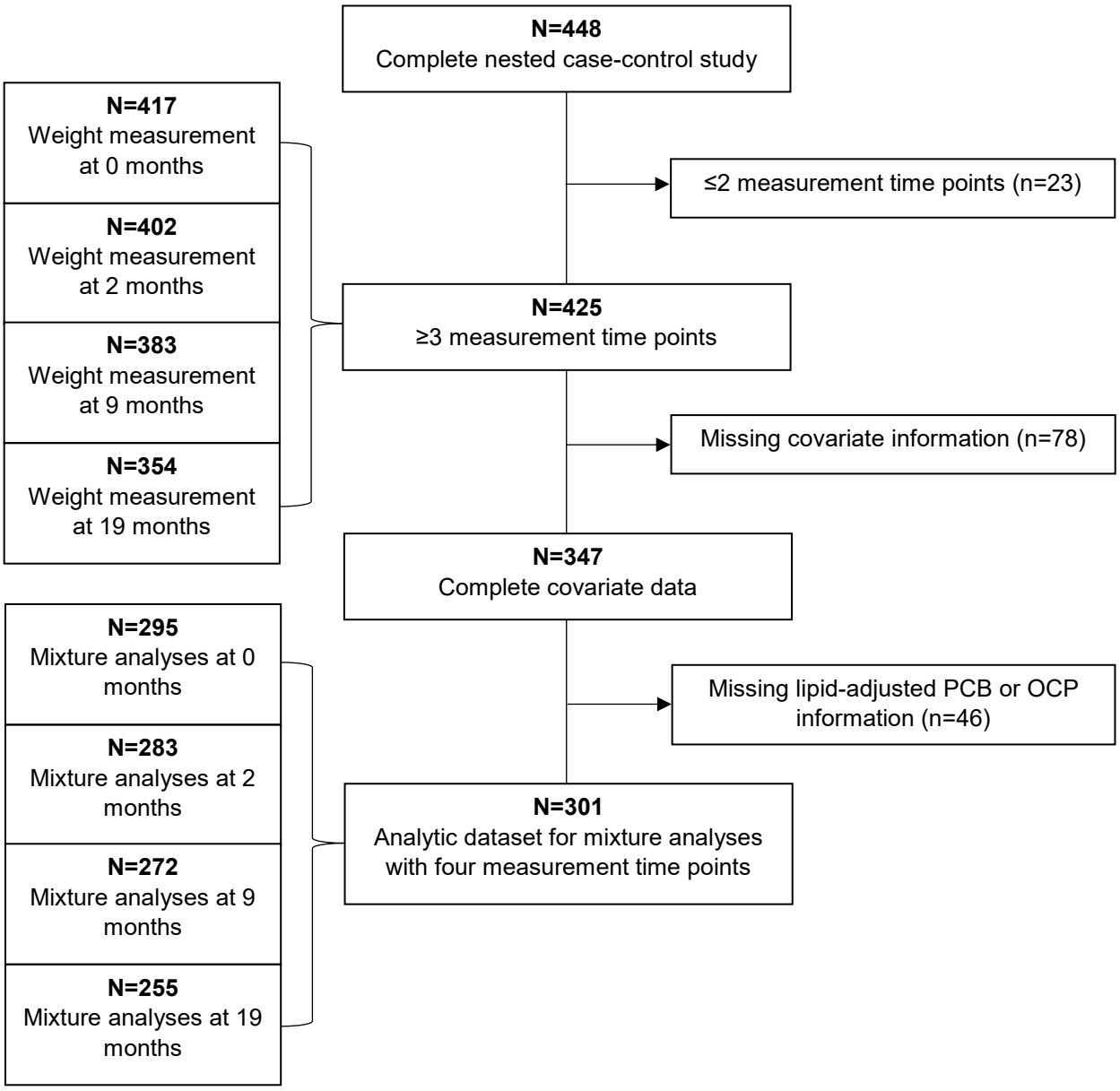
[b] Single-chemical linear regression models were run to examine associations between each chemical and weight-for-age z-scores. Betas represent a change of 10% higher chemical concentrations.

[c] Multi-chemical linear regression models were run to examine associations between each chemical in a class (e.g., PFAS) and weight-for-age z-scores, independent of other chemicals in the class (i.e., adjusting for other chemicals in the class). Betas represent a change of 10% higher chemical concentrations.

[d] 10% change in chemical concentrations calculated as $\beta*\ln(1.1)$

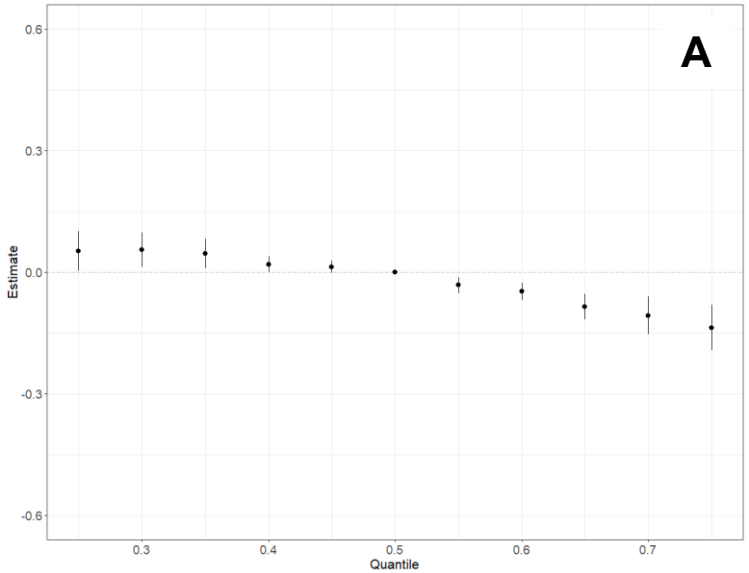
[e] Only β values $\geq|0.01|$ are displayed.

[f] PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration, referred to as PCB138. Similarly, PCB congeners 196 and 203 could not be separated and were quantified as a summed concentration, referred to as PCB196.

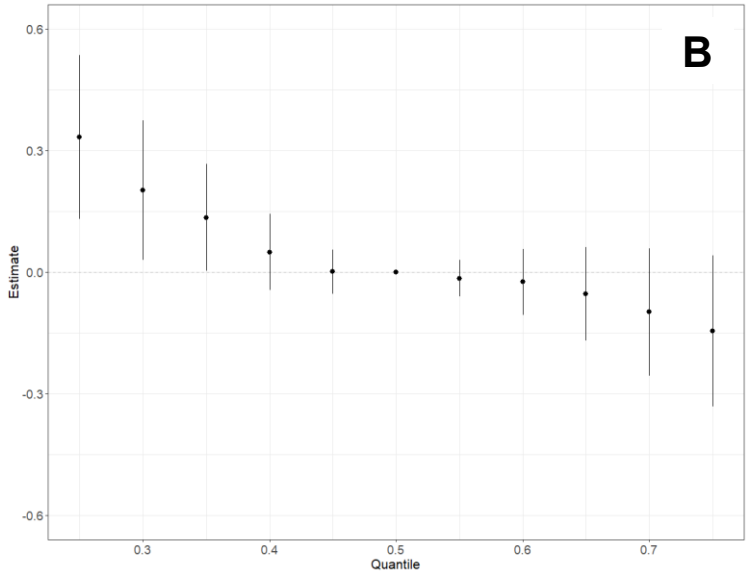


Supplemental Figure 5-4. Flowchart depicting sample size in various models in the study of prenatal exposure to mixtures of persistent endocrine disrupting chemicals and postnatal body size in a sub-study of the Avon Longitudinal Study of Parents and Children.

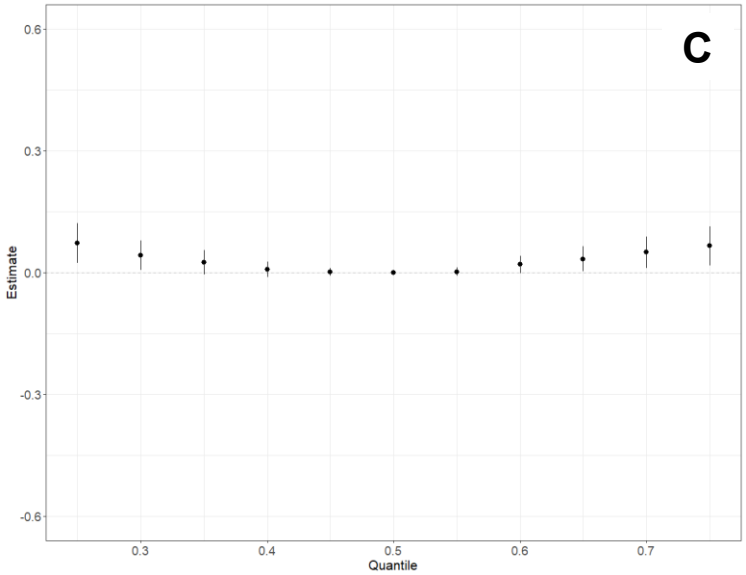
PFAS



PCBs

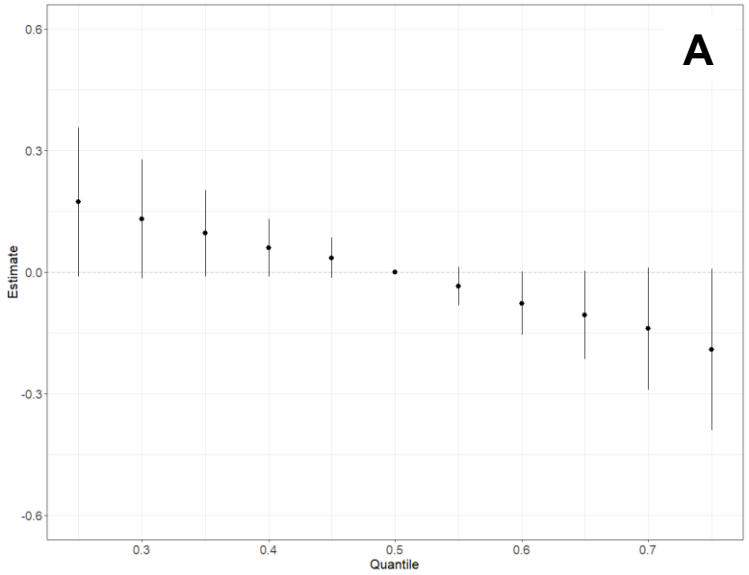


OCPs

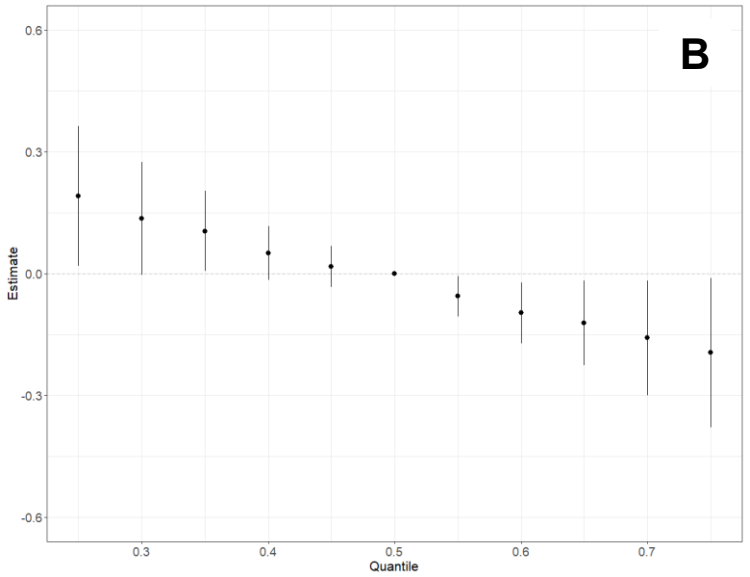


Supplemental Figure 5-5. Overall effect of the class-specific chemical mixtures on weight-for-age z-scores. **A** Overall effect of the PFAS mixture on weight-for-age z-scores (estimates and 95% credible intervals), comparing the outcome when all exposures are at a particular quantile to the median (n=347). **B** Overall effect of the PCB mixture on weight-for-age z-scores (estimates and 95% credible intervals), comparing the outcome when all concentrations are at a particular quantile to the median (n=308). **C** Overall effect of the OCP mixture on weight-for-age z-scores (estimates and 95% credible intervals), comparing the outcome when all concentrations are at a particular quantile to the median (n=319). Bayesian kernel machine regression models adjusted for maternal education, parity, pre-pregnancy body mass index, maternal age at delivery, prenatal smoking, age at measurement, and gestational week at sample collection, and used a random intercept to account for repeated measures at 0, 2, 9, and 19 months. All chemical concentrations were natural log-transformed and standardized; PCBs and OCPs were lipid adjusted.

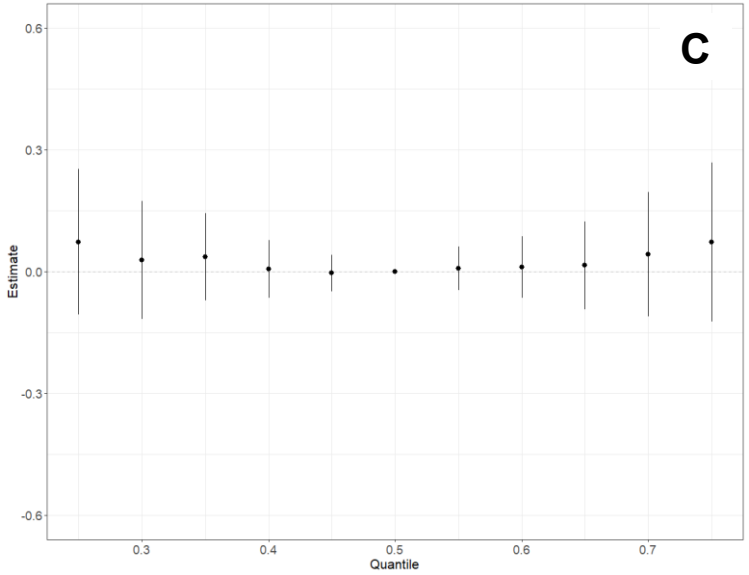
Birth



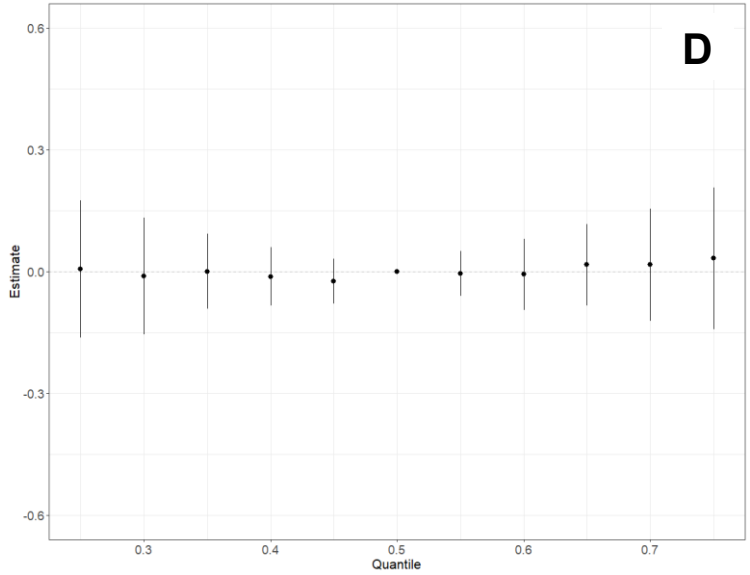
2 months



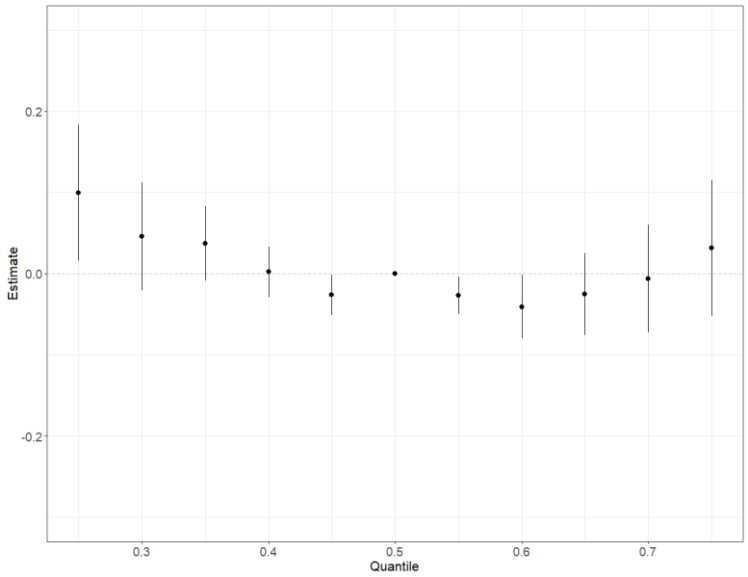
9 months



19 months



Supplemental Figure 5-6. Overall effect of the 31 chemical mixture on weight-for-age z-scores at the four measurement time points. **A** Overall effect of the mixture (all three classes combined) on weight-for-age z-scores at birth (estimates and 95% credible intervals), comparing the outcome when all exposures are at a particular quantile to the median (n=295). **B** Overall effect of the mixture (all three classes combined) on weight-for-age z-scores at 2 months (estimates and 95% credible intervals), comparing the outcome when all concentrations are at a particular quantile to the median (n=283). **C** Overall effect of the mixture (all three classes combined) on weight-for-age z-scores at 9 months (estimates and 95% credible intervals), comparing the outcome when all concentrations are at a particular quantile to the median (n=272). D Overall effect of the mixture (all three classes combined) on weight-for-age z-scores at 19 months (estimates and 95% credible intervals), comparing the outcome when all concentrations are at a particular quantile to the median (n=255). Bayesian kernel machine regression models adjusted for maternal education, parity, pre-pregnancy body mass index, maternal age at delivery, prenatal smoking, and gestational week at sample collection. All chemical concentrations were natural log-transformed and standardized; PCBs and OCPs were lipid adjusted.



Supplemental Figure 5-7. Overall effect of the 31 chemical mixture on weight-for-age z-scores (estimates and 95% credible intervals) excluding the birth weight measurements, comparing the outcome when all concentrations are at a particular quantile to the median (n=301).
The Bayesian kernel machine regression model adjusted for maternal education, parity, pre-pregnancy body mass index, maternal age at delivery, prenatal smoking, age at measurement, and gestational week at sample collection, and used a random intercept to account for repeated measures at 2, 9, and 19 months. All chemical concentrations were natural log-transformed and standardized; PCB and OCP concentrations were lipid-adjusted.

Supplemental Table 5-5. Serum concentrations of persistent endocrine disrupting chemicals (EDCs) among mothers of the Avon Longitudinal Study of Parents and Children (ALSPAC) during pregnancy (median gestational age at sample collection: 15 weeks) (N=425 mother-daughter dyads.

	Serum concentrations			
	Median	**Q1**	**Q3**	**% <LOD[a]**
Per- and polyfluoroalkyl substances (ng/mL)				
PFOA	3.7	2.8	4.8	0.0
PFOS	19.6	15.0	25.0	0.0
PFHxS	1.6	1.2	2.2	0.2
PFNA	0.49	0.41	0.66	0.2
FOSA	0.20	<LOD	0.30	30.9
MeFOSAA	0.35	0.26	0.70	13.9
EtFOSAA	0.70	0.40	0.90	2.4
PFDA	<LOD	<LOD	<LOD	97.4
Polychlorinated biphenyls (PCBs) (ng/g lipid)				
PCB28	5.5	3.5	8.4	8.7
PCB44	1.9	<LOD	4.0	29.7
PCB49	<LOD	<LOD	1.9	57.7
PCB52	3.3	<LOD	7.7	29.4
PCB66	1.6	<LOD	2.5	30.8
PCB74	11.0	8.6	15.2	0.2
PCB87	<LOD	<LOD	1.7	59.5
PCB99	9.4	7.0	12.2	0.9
PCB101	2.3	<LOD	5.5	29.9
PCB105	2.8	2.0	3.9	7.5
PCB110	<LOD	<LOD	2.8	53.4
PCB118	14.8	10.8	20.4	0.2
PCB128	<LOD	<LOD	<LOD	89.4
PCB138_158[b]	41.2	30.2	53.8	0.2

PCB146	6.0	4.6	8.1	2.6
PCB149	<LOD	<LOD	1.9	60.7
PCB151	<LOD	<LOD	<LOD	78.8
PCB153	64.3	48.1	85.5	0.0
PCB156	6.3	4.8	8.3	1.4
PCB157	1.3	<LOD	1.9	33.9
PCB167	2.0	<LOD	2.9	26.1
PCB170	18.8	14.4	24.8	0.0
PCB172	1.9	1.1	2.7	22.8
PCB177	3.0	2.3	4.1	8.7
PCB178	2.7	1.8	3.7	14.4
PCB180	45.1	33.3	60.1	0.0
PCB183	6.1	4.6	8.1	3.5
PCB187	11.2	8.6	15.1	1.2
PCB189	<LOD	<LOD	0.7	74.6
PCB194	7.4	5.5	10.4	3.5
PCB195	2.2	1.5	2.9	19.1
PCB196_203[b]	7.6	5.7	10.5	2.1
PCB199	5.5	3.9	7.7	2.6
PCB206	2.3	1.7	3.2	10.6
PCB209	1.5	<LOD	2.0	27.5
Organochlorine pesticides (OCPs) (ng/g lipid)				
HCB	50.0	37.8	62.7	0.0
β-HCCH	46.8	34.5	60.8	1.4
γ-HCCH	<LOD	<LOD	<LOD	79.5
Oxychlordane	<LOD	<LOD	4.2	72.2
Trans-nonachlor	<LOD	<LOD	4.5	67.5
p,p'-DDE	311	190	493	0.2
o,p'-DDT	<LOD	<LOD	<LOD	98.4

p,p'-DDT	10.9	7.7	16.1	11.8
Mirex	<LOD	<LOD	<LOD	99.3

Abbreviations: Q1, quartile 1; Q3, quartile 3; LOD, limit of detection; ng/mL, nanogram per milliliter; ng/g lipid, nanogram per gram lipid

[a] The LODs for PFAS were 0.082 ng/mL for PFNA, 0.10 ng/mL for PFOA, PFHxS, and FOSA, 0.174 ng/mL for MeFOSAA, and 0.20 ng/mL for PFOS, EtFOSAA, and PFDA. Detection limits for analytes of OCPs and PCBs are dependent on the sample size and blanks, thus, an individual limit of detection is reported for each individual result rather than an overall limit of detection.

[b] PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration, referred to as PCB138. Similarly, PCB congeners 196 and 203 could not be separated and were quantified as a summed concentration, referred to as PCB196.

Supplemental Table 5-6. Adjusted[a] single[b]- and multi[c]-chemical associations of maternal serum concentrations of persistent endocrine disrupting chemicals (EDCs) and weight-for-age z-score at 19 months in the Avon Longitudinal Study of Parents and Children (ALSPAC) sub-study (N=296 mother-daughter dyads).
Beta estimates represent the change in weight-for-age z-score for 10% higher chemical concentrations[d].

	Single-chemical models[be]		Multi-chemical models[ce]	
	β	**95% CI**	**β**	**95% CI**
Per- and polyfluoroalkyl substances (PFAS) (ng/mL)				
PFOA			-0.02	-0.07, 0.03
PFOS			-0.02	-0.07, 0.04
PFHxS			0.01	-0.01, 0.03
PFNA	0.01	-0.01, 0.04	0.02	-0.01, 0.06
MeFOSAA				
EtFOSAA			0.01	-0.01, 0.03
Polychlorinated biphenyls (PCBs) (ng/g lipid)				
PCB28				
PCB74	0.03	-0.01, 0.06	0.11	0.04, 0.19
PCB99			-0.01	-0.13, 0.11
PCB105			0.05	-0.08, 0.18
PCB118			-0.10	-0.26, 0.06
PCB138[f]			0.12	-0.11, 0.35
PCB146			0.01	-0.04, 0.06
PCB153			-0.26	-0.61, 0.10
PCB156	0.02	-0.01, 0.06	0.05	-0.09, 0.20
PCB170			-0.07	-0.31, 0.17
PCB172	0.01	-0.01, 0.03		
PCB177			-0.01	-0.07, 0.04
PCB178	0.02	-0.01, 0.05	0.02	-0.03, 0.07
PCB180			0.02	-0.26, 0.30
PCB183	0.01	-0.02, 0.04	0.02	-0.11, 0.14

PCB187	0.01	-0.02, 0.04	-0.10	-0.21, 0.02
PCB194	0.01	-0.01, 0.03	-0.02	-0.06, 0.02
PCB195	0.01	-0.01, 0.04		
PCB196[f]	0.03	-0.01, 0.07	0.16	0.01, 0.31
PCB199	0.03	0.00, 0.05	0.03	-0.03, 0.08
PCB206	0.02	-0.01, 0.05		
Organochlorine pesticides (OCPs) (ng/g lipid)				
HCB	0.01	-0.02, 0.05	-0.03	-0.08, 0.01
β-HCH	0.02	0.00, 0.05	0.04	0.01, 0.07
p,p'-DDE				
p,p'-DDT				

Abbreviations: CI, confidence interval; ng/mL, nanogram per milliliter; ng/g lipid, nanogram per gram lipid

[a] Adjusted for parity, pre-pregnancy BMI, maternal age at delivery, education, prenatal smoking, and gestational age at sample collection.

[b] Single-chemical linear regression models were run to examine associations between each chemical and weight-for-age z-scores. Betas represent a change of 10% higher chemical concentrations.

[c] Multi-chemical linear regression models were run to examine associations between each chemical in a class (e.g., PFAS) and weight-for-age z-scores, independent of other chemicals in the class (i.e., adjusting for other chemicals in the class). Betas represent a change of 10% higher chemical concentrations.

[d] 10% change in chemical concentrations calculated as $\beta*\ln(1.1)$

[e] Only β values $\geq|0.01|$ are displayed

[f] PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration, referred to as PCB138. Similarly, PCB congeners 196 and 203 could not be separated and were quantified as a summed concentration, referred to as PCB196.

Supplemental Table 5-7. Adjusted[a] associations of mixtures with accompanying weights of maternal serum concentrations of persistent endocrine disrupting chemicals (EDCs) with weight-for-age z-scores at varying measurement time points in the Avon Longitudinal Study of Parents and Children (ALSPAC) sub-study (N=301 mother-daughter dyads) using weighted quantile sum regression.
Estimates represent the change in weight-for-age z-score for one-unit higher of the WQS index (representing a one-decile increase in all chemical concentrations).

	0 months n=295			**2 months** n=283			**9 months** n=272			**19 months** n=255		
	β[b]	**95% CI**	**Weight[c]**	**β[b]**	**95% CI**	**Weight[c]**	**β[b]**	**95% CI**	**Weight[c]**	**β[b]**	**95% CI**	**Weight[c]**
Overall[d]	-0.19	-0.31, -0.08		-0.11	-0.24, 0.01		-0.04	-0.13, 0.05		-0.04	-0.15, 0.07	
PFOA			0.02			0.11†						0.03†
PFOS						0.02			0.05†			0.07†
PFHxS			0.04†			0.01						0.13†
PFNA			0.02			0.06†			0.01			0.09†
MeFOSAA			0.04†			0.26†			0.09†			0.01
EtFOSAA			0.19†			0.12†			0.01			0.03
PCB28			0.01			0.03†						0.01
PCB74			0.04†						0.01			0.01
PCB99			0.01			0.02						0.05†
PCB105												
PCB118												0.02
PCB138[e]												0.09†
PCB146			0.08†			0.03†			0.02			0.07†

PCB153	0.09†			0.16†
PCB156		0.03		0.01
PCB170				0.01
PCB172	0.04†	0.02		0.01
PCB177	0.01	0.02		
PCB178	0.02		0.13†	0.02
PCB180	0.03	0.01	0.04†	0.07†
PCB183				
PCB187		0.02	0.01	0.01
PCB194		0.01	0.02	0.06†
PCB195	0.02	0.01	0.04†	0.02
PCB196[e]			0.01	
PCB199	0.19†	0.12†	0.09†	
PCB206	0.02	0.01	0.46†	
HCB	0.01	0.06†		0.01
β-HCH	0.01	0.01		
p,p'-DDE	0.03	0.01		0.01
p,p'-DDT	0.09†	0.01		

Abbreviations: CI, confidence interval

[a] Adjusted for education, parity, pre-pregnancy body mass index, maternal age at delivery, prenatal smoking, and gestational week at sample collection

[b] β for one-unit higher of the WQS index (representing a one-decile increase in chemical concentrations)

[c] Weights greater than 1/number of chemicals in the mixture are considered significant contributors to the overall mixture effect; in this case, a weight over 0.032 (1/31).

[d] Overall mixture includes PFAS, PCB, and OCP classes
[e] PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration, referred to as PCB138. Similarly, PCB congeners 196 and 203 could not be separated and were quantified as a summed concentration, referred to as PCB196
† Significant contributor to the overall mixture effect (>1/number of chemicals in mixture)

Supplemental methods

Linear mixed-effects models for weight-for-age scores at 0, 2, 9, and 19 months

We explored use of an age^2 term in our mixed models to represent the quadratic nature of childhood growth. Most models with the age^2 term did not converge, and of those that converged, the age^2 term was not significant and therefore not included.

We also examined potential interaction between the EDC concentrations and age at measurement (as a continuous variable (age in months) and as a categorical variable (0, 2, 9, or 19 months)). We did not observe interaction with age at measurement for the vast majority of EDCs under study and therefore did not include interaction terms in our models.

We assessed the need for including a random slope for age at the measurement in the model (**Model 1**). Model fit for the mixed single- and multi-chemical models was improved with the addition of a random slope for age at measurement (as indicated by a smaller AIC fit statistic), but fixed effect estimates did not notably differ from the random intercept only models (**Model 2**). Therefore, for comparison with BKMR models where only a random intercept is possible, we have presented single- and multi-chemical model results from the random intercept only models (**Table 5-4**).

We explored a variety of covariance structures for the R matrix, including autoregressive(1), compound symmetric, Toeplitz, Toeplitz with two bands, unstructured, and the default structure (variance components). Some correlation structures led to receipt of error messages and lack of convergence (compound symmetric, Toeplitz, and unstructured). Of those that converged (autoregressive(1), Toeplitz with two bands, and the default structure (variance components), models with the Toeplitz with two bands R matrix consistently produced the lowest AIC, indicating best model fit. Therefore, we used Toeplitz with two bands R matrix in all proc mixed models.

Model 1: Random intercept and slope (full) model:

$E(\text{Weight-for-age}_{ij}) = (\beta_0 + b_{0i}) + \beta_1\text{EDC}_{ij} + \beta_2\text{MatAge}_{ij} + \beta_3\text{Edu1}_{ij} + \beta_4\text{Edu2}_{ij} + \beta_5\text{Smoke}_{ij} + \beta_6\text{Parity}_{ij} + \beta_7\text{BMI}_{ij} + \beta_8\text{SampleGA}_{ij} + (\beta_9 + b_{9i})\text{MeasurementAge}_{ij} + e_{ij}$

Weight-for-age: continuous z-score for weight-for-age

EDC (continuous): EDC of interest, measured during the prenatal period (log-transformed continuous variable)

MatAge (continuous): Maternal age at delivery in years

Edu (3-level categorical using dummy variables): Maternal education; classified as <O-level (ordinary level: required, completed at age 16), O-level, or > O-level

Smoke (categorical): Prenatal smoking; any smoking or no smoking

Parity (categorical): Parity; nulliparous or multiparous

BMI (continuous): Pre-pregnancy body mass index (kg/m^2)

SampleGA (categorical): Gestational week at serum sample collection; ≤20 weeks or >20 weeks

MeasurementAge (continuous): Age at anthropometric measurement in months

where b_{0i} represents the random intercept for subject i, and where b_{9i} represents a random slope for the variable MeasurementAge for subject i, $b_{0i} \sim N(0, \sigma_s^2)$ and $b_{9i} \sim N(0, \sigma_M^2)$

j=1, 2, 3, 4

i=1,2,…n

e_{ij} =errors, assumed to be $N(0, \sigma^2)$ …

Model 2: Random intercept (final) model:

$E(\text{Weight-for-age}_{ij}) = (\beta_0 + b_{0i}) + \beta_1\text{EDC}_{ij} + \beta_2\text{MatAge}_{ij} + \beta_3\text{Edu1}_{ij} + \beta_4\text{Edu2}_{ij} + \beta_5\text{Smoke}_{ij} + \beta_6\text{Parity}_{ij} + \beta_7\text{BMI}_{ij} + \beta_8\text{SampleGA}_{ij} + \beta_9\text{MeasurementAge}_{ij} + e_{ij}$

Weight-for-age: continuous z-score for weight-for-age

EDC (continuous): EDC of interest, measured during the prenatal period

MatAge (continuous): Maternal age at delivery in years

Edu (3-level categorical using dummy variables): Maternal education; classified as <O-level (ordinary level: required, completed at age 16), O-level, or > O-level

Smoke (categorical): Prenatal smoking; any smoking or no smoking

Parity (categorical): Parity; nulliparous or multiparous

BMI (continuous): Pre-pregnancy body mass index (kg/m^2)

SampleGA (categorical): Gestational week at serum sample collection; ≤20 weeks or >20 weeks

MeasurementAge (continuous): Age at anthropometric measurement in months

where b_{0i} represents the random intercept for subject i, $b_{0i} \sim N(0, \sigma_s^2)$

j=1, 2, 3, 4

i=1, 2,....n

e_{ij} = errors, assumed to be $N(0, \sigma^2)$

Sensitivity analyses results

In sensitivity analyses, we used BKMR to model the mixture of all three classes combined in relation to weight-for-age scores at 2, 9, and 19 months (excluding birth weight measures) using a random intercept (**Supplemental Figure 5-7**). Excluding weight at birth from the analysis showed attenuation of the inverse association seen in **Supplemental Figure 5-7** (estimate for 75th percentile compared to the 25th percentile: -0.07 (95% credible interval: -0.20, 0.06) versus -0.27 (95% credible interval: -0.42, -0.11)). As in the model including birth weight, PCBs and OCPs had the highest PIPs (PIP_{PCBs}: 0.58 and PIP_{OCPs}: 0.57). Within the PCB class, PCB118 contributed the most to the model (conditional PIP: 0.16) and within the OCP class, p,p'-DDE (conditional PIP: 0.33) contributed the most. Similar to the model including birth weight measures, the most important PFAS was PFOS (conditional PIP: 0.23).

Further, we conducted a sensitivity analysis using BKMR with a random intercept to examine the mixture of 31 chemicals with weight-for-age scores adjusted for gestational age at birth (n=301). Adjustment of the weight-for-age z-scores for gestational age at birth had minimal impact on the overall effect estimates. Holding all persistent EDCs at the 75th percentile compared to the 25th percentile was associated with 0.20 lower weight-for-age z-score (estimate: -0.20, 95% credible interval: -0.34, -0.07), compared to a 0.27 lower weight-for-age z-score (estimate: -0.27, 95% credible interval: -0.42, -0.11) in models not adjusting for gestational age.

Chapter 6 Prenatal exposure to mixtures of persistent endocrine disrupting chemicals and early menarche in a population-based cohort of British girls

Kristin J. Marks[a,b,c], Penelope P. Howards[a], Melissa M. Smarr[d], W. Dana Flanders[a,b], Kate Northstone[e], Johnni H. Daniel[b], Antonia M. Calafat[f], Andreas Sjödin[f], Michele Marcus[a,d], Terryl J. Hartman[a,b]

[a] Department of Epidemiology, Rollins School of Public Health, Emory University, 1518 Clifton Rd NE, Atlanta, Georgia 30322, United States

[b] Division of Environmental Health Science and Practice, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy, Atlanta, Georgia 30341, United States

[c] Oak Ridge Institute for Science and Education, 100 ORAU Way, Oak Ridge, Tennessee 37830, United States

[d] Gangarosa Department of Environmental Health, Rollins School of Public Health, Emory University, 1518 Clifton Rd NE, Atlanta, Georgia 30322, United States

[e] Department of Population Health Sciences, Bristol Medical School, Oakfield House, Oakfield Grove, Bristol BS8 2BN, United Kingdom

[f] Division Of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention, 4770 Buford Hwy, Atlanta, Georgia 30341, United States

Abstract:

Exposure to endocrine disrupting chemicals (EDCs) is ubiquitous. EDC exposure, especially during critical periods of development like the prenatal window, may interfere with the body's endocrine system, which can affect growth and developmental outcomes such as puberty. Most studies have examined one EDC at a time in relation to disease; however, humans are exposed to many EDCs. By studying mixtures, the human experience can be more closely replicated. We investigated the association of prenatal exposure to persistent EDCs (poly- and perfluoroalkyl substances (PFAS), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs)) as mixtures with early menarche among female offspring in a nested case-control study within the Avon Longitudinal Study of Parents and Children (ALSPAC) (N=448) recruited in the United Kingdom in 1991–1992. Concentrations of 52 EDCs were quantified in maternal serum samples collected during pregnancy. Daughter's age at menarche was ascertained through mailed questionnaires sent annually. We used weighted quantile sum (WQS) regression to examine the association between prenatal exposure to multiple EDCs and early menarche (<11.5 (n=218) vs. ≥11.5 years (n=230)) for each chemical class separately (PFAS, PCBs, and OCPs) and for all three classes combined. Models were adjusted for maternal age at menarche, maternal education, parity, pre-pregnancy body mass index, maternal age at delivery, prenatal smoking, and gestational week at sample collection. WQS regression models showed null associations between the WQS indices for EDC mixtures (PFAS, PCBs, OCPs, and all three classes combined) and early menarche. For instance, the odds ratio for early menarche for one-unit higher of the WQS index (representing a one-decile increase in chemical concentrations) for all three classes combined was 1.01 (95% CI: 0.79, 1.28). Results suggest the overall effect of prenatal exposure to persistent EDC mixtures is not associated with early menarche.

Keywords: puberty, menarche, poly- and perfluoroalkyl substances, polychlorinated biphenyls, organochlorine pesticides, ALSPAC

Introduction

Puberty is a critical phase of development and growth. The timing and patterning of milestones during puberty can offer insight into general health and earlier exposures, while possibly forecasting later health (305, 306), like breast cancer (419). Menarche, which is a girl's first menstrual period, has been a frequently utilized marker of pubertal development because of its clearly observable occurrence and accurate recall even years later (420-422).

On average, age at menarche has gotten younger since the end of the 19th century (423, 424) and earlier occurrence of secondary sexual characteristics has also been observed (425). Current estimates of mean age at menarche (12.4 years) are close to one year younger than the mean age at menarche of women born in the 1920s (13.3 years); further, decreases in average age at menarche have been seen across races and ethnicities in the United States (426). There are a number of factors potentially contributing to this trend of altered pubertal timing and patterning, including improvements in nutrition, a higher prevalence of childhood obesity, and exposure to endocrine disrupting chemicals (EDCs) (314, 427-429).

The National Institute of Environmental Health Sciences (NIEHS) defines an EDC as a chemical that may interfere with the body's endocrine system and produce adverse developmental, immune, neurological, and reproductive effects in humans (357). Environmentally persistent EDCs, such as poly- and perfluoroalkyl substances (PFAS), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs), are often very resistant to degradation, likely to bioaccumulate in living organisms, and have been used throughout the 20th and 21st centuries for a variety of purposes (123, 130, 144). Many countries have banned or severely limited the production, handling, and disposal of several PCBS and OCPs and certain PFAS. While exposure appears to have declined in the general population, nearly every human has detectable concentrations of some of these chemicals (124, 358). Further, biologically persistent EDCs are able to cross the placental barrier, leading to potential fetal exposure, and the amount of EDCs found in cord serum may be substantial relative to fetal size (263, 359-361, 430). EDCs in

maternal and cord sera are strongly correlated with each other (431). EDC concentrations tend to be higher in maternal serum than cord serum, and characteristics such as parity potentially influence the transfer of EDCs from mother to fetus (432). Maternal age, pre-pregnancy body mass index (BMI), and parity are often predictive of maternal serum concentrations of EDCs (432, 433).

Prior studies of prenatal exposure to persistent EDCs and age at menarche have shown mixed results. Previous examinations of prenatal PFAS exposure and age at menarche have shown no association (307), earlier menarche (309), and later menarche (308). Prenatal PCB exposure was not associated with age at menarche in previous studies (314, 316, 318), though some observed weak associations with early menarche (313, 315). Previous studies of prenatal OCP exposure and age at menarche have shown both null results (318, 320) and associations with earlier menarche, namely for dichlorodiphenyldichloroethylene (DDE) (313, 315).

Most studies to date have examined one EDC at a time in relation to health outcomes, and this may have led to inconsistent results in the association between prenatal exposure to EDCs and growth and developmental outcomes in offspring. Because humans are exposed to many EDCs, the human experience can be more closely replicated by studying combined exposures, or “mixtures” (4). In this context, NIEHS defines an environmental mixture as a combination of three or more independent chemicals or chemical groups (5).

While there have been a number of studies examining prenatal exposure to persistent EDCs and age at menarche, none have examined persistent EDCs as a mixture. Our aim was to investigate the association of maternal gestational concentrations of 52 persistent EDCs (PFAS, PCBs, and OCPs) and prospectively collected age at menarche data in a nested case-control study of a population-based birth cohort.

Methods

Study population

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a continuing prospective birth cohort following 14,541 pregnancies. Pregnant women from three health districts in the former county of Avon, Great Britain were enrolled in ALSPAC. To be enrolled, women needed to have an expected delivery date between 1 April 1991 and 31 December 1992. ALSPAC collected information on parents and children through clinic visits, interviews, and mailed questionnaires. Details on study recruitment and methods have previously been described (369, 370). A nested case-control study (N=448) was conducted to investigate associations of prenatal concentrations of EDCs and early menarche among the daughters. The nested case-control study design has previously been described in detail (307). Briefly, from the original base population of 14,062 live births, cases and controls were selected from singleton daughters who had completed at least two (out of five possible) puberty staging questionnaires between 8 and 13 years old. A cut-off of 11.5 years was selected to define 'early' menarche. To be eligible, cases had to complete at least two questionnaires, with one needing to be completed after menarche. Controls had to return the 13-year old questionnaire to establish that menarche had not occurred before the cutoff of 11.5 years.

The study website contains details of all the data that are available through a fully searchable data dictionary and variable search tool (http://www.bris.ac.uk/alspac/researchers/our-data/). Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee, the Local Research Ethics Committees, and the Centers for Disease Control and Prevention (CDC) Institutional Review Board. Consent for biological samples was collected in accordance with the Human Tissue Act (2004). Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.

Exposure assessment

Maternal fasting blood samples were collected from mothers during pregnancy. They were enrolled in 1991–1992 at median 15 (interquartile range (IQR): 10–28) weeks gestation, and processed and frozen for later analysis. Maternal serum samples were stored at the University of Bristol until they were transferred under controlled conditions and analyzed at the CDC National Center for Environmental Health (Atlanta, GA). Laboratory analyses included low- and high-concentration pooled quality control materials, standards, reagent blanks, and study samples. Concentrations below the limit of detection (LOD) were imputed by dividing the LOD by the square root of 2 prior to statistical analysis. EDCs detected in greater than 75% of mothers were included in the main analyses.

Poly- and Perfluoroalkyl substances

Eight PFAS were quantified (**Table 6-1**) in serum via on-line solid-phase extraction coupled to isotope dilution high-performance liquid chromatography-tandem mass spectrometry (381). LODs were 0.082 (PFNA), 0.10 ng/mL (FOSA, PFHxS, PFOA), 0.174 (MeFOSAA), and 0.20 ng/mL (EtFOSAA, PFOS, PFDA). Coefficients of variation (CVs) were generally below 10%.

Organochlorine pesticides and polychlorinated biphenyls

Nine OCPs and 35 PCBs were measured (**Table 6-1**) in serum using gas chromatography isotope dilution high resolution mass spectrometry (383). PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration hereafter referred to as PCB138. Similarly, PCB congeners 196 and 203 could not be separated and were quantified as a summed concentration hereafter referred to as PCB196. LODs for PCBs and OCPs are dependent on the size of the sample available, thus an individual LOD was reported for each individual result rather than an overall LOD. CVs were generally below 10%.

Outcome assessment

A ‘Growing and Changing’ questionnaire was used to collect information on pubertal development. The questionnaire was mailed annually to participants between the ages of 8–17 years (1999–2008), except in the year 2003 due to funding constraints. Menarche status was reported by the parent or child. If it had occurred, month and year of occurrence was reported and used to calculate age at menarche.

Covariates

Covariate information was collected by clinical staff or through self-report on questionnaires completed by the mother during or immediately after pregnancy. Covariates under consideration include: gestational age at biological sample collection (weeks), maternal age at delivery (years), maternal pre-pregnancy BMI (kg/m^2), maternal race ethnicity (white/nonwhite), maternal education (defined as <ordinary level (O-level: required and completed at 16 years of age), O-level, or > O-level), parity (nulliparous/multiparous), smoking during pregnancy (any/none), hours of physical activity (enough to work up a sweat) per week during pregnancy (>0 hours/0 hours), and maternal age at menarche (years). Breastfeeding the index child (yes/no and duration) was considered as an effect modifier.

Statistical analyses

Descriptive analyses were conducted to compare mother-daughter dyad characteristics by case-control status using chi-square tests. Wilcoxon rank sum tests were utilized to compare median EDC concentrations by case-control status.

The chemical concentrations under study were modeled as natural log-transformed continuous variables. Per the nested case-control study design, age at menarche was dichotomized as early (≤11.5 years; cases) versus not early menarche (>11.5 years; controls) (307). All models were adjusted for maternal age at menarche, maternal education, parity, pre-pregnancy body mass index, maternal age at delivery, prenatal smoking, and gestational week at

sample collection and included all participants with complete data on relevant exposures and covariates (**Supplemental Figure 6-4**).

First, we ran single-chemical logistic regression models to examine independent associations between each chemical and early menarche. Next, we ran multi-chemical logistic regression models to examine associations between each chemical in a class (e.g., PFAS) and early menarche, independent of other chemicals in the class (e.g., adjusting for other chemicals in the class). Sensitivity analyses were conducted comparing the odds of early menarche among those with versus without detectable concentrations.

Bayesian kernel machine regression (BKMR) was used to visualize the exposure-response function and verify assumptions using the R package *bkmr* (333, 399, 400). In the case of no identification of non-linearity and or interaction within the mixture through BKMR, weighted quantile sum (WQS) regression was used to estimate associations of maternal EDC mixtures with early menarche using the R package *gWQS* (401). Mixtures under study included each chemical class separately (PFAS, PCBs, and OCPs) and all three chemicals classes combined.

WQS regression allows for the creation of a weighted linear index of correlated predictors that are weighted by their strength of association with the outcome of interest (402). Specifically, the equation seeks to calculate the weights of ***c*** set of correlated variables:

$$g(\mu) = \beta_0 + \beta_1 \left(\sum_{i=1}^{c} w_i q_i \right) + z'\varphi$$

The sum term is the index for ***c*** items, scored into quantiles (denoted q_i), and weights are signified by the sum of w_i. Each w_i is constrained between 0 and 1. All covariates are represented by ***z'φ***. Before analysis, the data are randomly split into two datasets: a training dataset (40%) and a validation dataset (60%). Bootstrap samples (n=100) are selected using the training dataset, and the strength of the associations for each ***c*** item is determined by the beta coefficient (402). The index is calculated based on the average w_is across all bootstrap samples and is readily interpretable as an estimation of the total mixture effect. WQS regression focuses inference in

one direction through constrained optimization of the beta parameter. Effects can be estimated in both directions by running two models constraining in the positive and negative directions (331, 402-405). We created WQS indices in both directions to explore potentially disparate directions of effect across persistent EDCs on age at menarche.

Bayesian kernel machine regression (BKMR) was also used as a sensitivity analysis to confirm the WQS regression findings. BKMR is a flexible semi-parametric technique that models the combined effects of different chemicals, while also allowing for nonlinearity and interactions among chemicals (406). This approach allows for the examination of independent effects of mixture members, interactions among them, and the overall mixture effect. We used hierarchical variable selection to obtain group importance scores (posterior inclusion probabilities (PIPs)) for pre-defined mutually exclusive groups of variables. Additionally, we estimated the importance of a chemical given that the group that contained the chemical was important (conditional PIPs) (333, 399, 400). Within BKMR, we standardized all continuous variables to improve computational efficiency. SAS software 9.4 (Cary, NC) was used for descriptive analyses. R software 3.5.0 (Vienna, Austria) was used for WQS regression and BKMR analyses.

Results

Descriptive statistics

The study sample consisted of predominantly white mothers (>97%) who achieved secondary levels of education or higher (81.9%) (**Table 6-2**). About half of mothers were nulliparous (49.6%) and most were 25 years or older (79.3%). Some mothers smoked during pregnancy (18.5%) and the majority were physically active during pregnancy (≥1 hour per week) (65.5%). Mothers of cases were more likely to be non-white (3.3% among case mothers versus <2.2% among control mothers) and to have experienced early menarche (between 8–11 years) themselves (32.5% versus 15.2%). Additionally, case mothers were more likely to enter pregnancy at an overweight or obese BMI (≥25 kg/m^2) (29.4% versus 15.1%).

Of the 52 chemicals measured, 31 chemicals were detected in greater than 75% of mothers. Certain OCPs were very rarely detected (<2% of samples >LOD) (e.g., o,p′-DDT and Mirex) and certain PCBs were also rarely detected (e.g., PCB128 and PCB151) (**Table 6-3**). The majority of PFAS were detected in most samples, except for PFDA (<3% of samples >LOD).

Correlation among the 31 chemicals was high (**Figure 6-1**). Overall, PCBs and OCPs showed high inter-class correlation, while PFAS were less correlated with PCBs and OCPs. Among PCBs, there was strong intra-class correlation (up to $r_{Spearman}$=0.98 between PCB170 and PCB180). Correlation within OCPs was also strong (as high as $r_{Spearman}$=0.82 between HCB and β-HCH). PFAS exhibited lower intra-class correlation but were still positively correlated with some strong correlations (up to $r_{Spearman}$=0.72 between PFOA and PFOS).

Single- and multi-chemical models

Few differences were observed in chemical concentration by case-control status. PCB180, which was detected in all samples, was higher among controls than cases (median 47.1 versus 44.0 ng/g lipid) (**Table 6-3**). Similarly, PCB170 was also higher among controls than cases (19.8 versus 18.1 ng/g lipid). No differences were observed by case-control status among PFAS or OCPs. In adjusted single-chemical models, no PFAS or OCPs were associated with early menarche (**Table 6-4**). PCB180, PCB196, and PCB206 were inversely associated with early menarche. For example, the odds ratio for early menarche for 10% higher PCB180 was 0.93 (95% CI: 0.87, 1.00).

In the multi-chemical PFAS model, 10% higher EtFOSAA was associated with 5% higher odds of early menarche (OR: 1.05, 95% CI: 1.01, 1.10) when adjusting for all other PFAS (**Table 6-4**). In the multi-chemical PCB model, PCB172 and PCB187 were associated with higher odds of early menarche, while PCB177 and PCB206 were associated with lower odds of early menarche when all PCBs were in the model. With 21 chemicals in the multi-chemical PCB model, some estimates were highly imprecise, exhibiting very wide confidence intervals. Null

associations were observed for all OCPs in the multi-chemical OCP model, though β-HCH appeared somewhat protective (OR: 0.96, 95% CI: 0.91, 1.02).

Weighted Quantile Sum Regression

Weighted quantile sum regression models showed null associations between the indices for mixtures (PFAS, PCBs, OCPs, and all three classes combined) and early menarche (**Table 6-4**). For instance, the odds ratio for early menarche for one-unit higher of the WQS index (representing a one-decile increase in chemical concentrations) for all three classes combined was 1.01 (95% CI: 0.79, 1.28). Being breastfed did not modify the association of prenatal exposure to persistent EDC mixtures and early menarche.

Bayesian Kernel Machine Regression

In sensitivity analyses using the BKMR model for all three classes combined, OCPs had the highest PIP (0.66), making it the most important group in the mixture. The next most important group was PCBs (PIP: 0.53), followed by PFAS (PIP: 0.49). The independent chemical associations all appear fairly linear (**Figure 6-2**). Some chemicals had slightly positive associations (PFHxS, EtFOSAA), some appeared to have negative associations (MeFOSAA, PCB206, β-HCH), but most showed no association with early menarche. We observed no interaction among mixture members (**Supplemental Figure 6-5**) and did not find an overall mixture effect (**Figure 6-3**).

Sensitivity analyses

We conducted a sensitivity analysis to explore differences in early menarche status among those with detectable concentrations versus those with non-detectable concentrations (**Supplemental Table 6-5**). We found that daughters born to women with detectable concentrations of PCB189 were less likely to experience early menarche (OR: 0.45, 95% CI: 0.26, 0.78) than those with non-detectable concentrations. Some other differences were seen for other chemicals: those with MeFOSAA, PCB177, PCB178, and PCB206 concentrations above the LOD

were less likely to experience early menarche, while those with detectable FOSA concentrations were more likely to experience early menarche, but these estimates were imprecise.

Discussion

In this study, we examined the association of prenatal exposure to multiple PFAS, PCBs, and OCPs (as individual classes and collectively) and early menarche (<11.5 years) among British girls, and mostly observed null associations. We employed WQS regression and BKMR to accomplish this, and results from these two methods were largely in agreement. This study responds to a recent call for research to evaluate the combined effects of exposure to EDCs on pubertal timing (312).

Previous studies in this ALSPAC population examined prenatal exposure to single EDCs and early menarche, specifically for PFAS (307) and OCPs (320). Neither study found an effect of prenatal exposure to EDCs on early menarche, as we confirmed here when examining mixtures of these chemicals using WQS and BKMR. The null findings from ALSPAC are in agreement with many studies published for these EDCs under the single-chemical paradigm (307, 308, 313-316, 320), though two previous studies found an association between DDE and early menarche (313, 315). The DDE and early menarche association was not replicated in our study in single-chemical analyses nor was DDE identified as an important component in mixture analyses using WQS and BKMR. Within ALSPAC, this is the first study to report on the association of PCBs and early menarche, and we found that certain PCBs, including PCB180, decreased the odds of early menarche in single-chemical models. In mixture models, associations of PCBs and early menarche were null.

While there has been previous work on the topic of persistent EDCs (modeled as single chemicals) and early menarche, there is motivation for a study using a mixtures approach. Because it is thought that several EDCs can operate through a common mechanism to affect an outcome, it seems reasonable that individual EDCs could act together at lower concentrations than the concentration that would be required for each chemical to achieve the same outcome on

its own (321). This idea has been shown in *in vitro* and *in vivo* studies where mixtures of EDCs are able to produce significant effects, even when each individual EDC is present at concentrations below the no-observed-effects levels (322-325). For example, a mixture effect was observed in an epidemiological study of breast cancer using a novel biomarker of combined EDC exposure. A positive association was seen between the mixture and breast cancer, yet individual EDCs showed no associations with breast cancer (326). These results suggest that the numerous studies of single EDCs with null findings may have considerably underestimated the risks of exposure to EDCs (329, 352), which is why we have re-analyzed data within this ALSPAC nested case-control study of early menarche using mixture methods.

Twelve persistent organic pollutants including PCBs, DDT, and HCB were banned or limited globally in a 2004 treaty at the Stockholm Convention on Persistent Organic Pollutants (POPs), and HCH was one of nine pollutants added in a 2009 amendment (125). In the years since, global monitoring of POPs has increased (434, 435). Human PCB and OCP concentrations have decreased among the general population in recent decades and concentrations among ALSPAC mothers (1990–1992) were higher than was last seen when the National Health and Nutrition Examination Survey (NHANES) measured these chemicals in Americans (2003–2004) (134). PFAS concentrations were higher in Americans than ALSPAC mothers the first time NHANES examined PFAS (1999–2000), but Americans' PFAS concentrations have since dropped below concentrations of ALSPAC mothers (124). Like PCBs and OCPs, there has been a downward trend for a number of PFAS (436); however, newer PFAS formulations have replaced them.

Our study is strengthened by its prospective design within a population-based birth cohort. Further, the frequent and thorough longitudinal data collection over a long follow up period allows us to examine exposures during pregnancy with distal outcomes such as pubertal development. Thirdly, we have utilized reliable biomarker indicators of exposure to over 50 persistent EDCs, allowing us to examine some less commonly studied chemicals as part of chemical mixtures.

Further, our study is enriched by the extensive covariate data available within ALSPAC. Lastly, our mixtures approach using multiple complementary methods (WQS regression, BKMR) allowed us to better replicate the human experience of exposure to multiple chemicals. Strengths of these mixture methods include their robustness to multicollinearity due to correlated exposures, dimensionality reduction, and ability to estimate mixture health effects while identifying important mixture components.

This study also has some limitations. The size of this sub-sample (n=448) of ALSPAC is modest. Due to the study design, we are unable to examine the association between prenatal mixtures of persistent EDCs and late age at menarche; there are a limited number of girls in the study with both measured maternal EDC concentrations and menarche at or after 14 years old (n=24). We are unable to account for persistent EDC exposure following birth, which could also influence pubertal development. Additionally, there may be confounding by unmeasured or poorly measured covariates, such as SES. Approaches to mixture analyses that involve regressing the outcome on several correlated exposures simultaneously can in some cases amplify rather than reduce confounding bias (“coexposure amplification bias”) (350). Further, due to the large number of variables (many with some missing data) used in mixture analyses, we were missing data on roughly one-third of the sub-sample (**Supplemental Figure 6-4**). We compared mother-daughter characteristics for those in the analytic dataset used for mixture analyses (n=284) to those in the nested case-control study (n=448) and to the population from which the case-control study was drawn (n=3338) (**Supplemental Table 6-6**). Characteristics were similar across subsets; while we saw a higher proportion of mothers with an earlier maternal age at menarche and overweight/obese pre-pregnancy BMI in the nested case-control study and analytic data compared to those enrolled at puberty, this was expected due to the relation of these factors with case status. There is the potential for misclassification of daughter’s age at menarche because it was self-reported annually between the ages of 8 and 17, though this is unlikely to be a concern given the close proximity of the event to the time of

reporting and generally good recall of this outcome (420-422). Lastly, the sub-sample used in the present study differed from the original base population of ALSPAC in a few ways. Mothers in our sub-sample were more highly educated and older than mothers in the original ALSPAC cohort. These differences are somewhat expected given that to be included in this sub-sample, children still had to be involved in the study during puberty. Largely, nonparticipation and loss to follow-up are more common among the less healthy and less advantaged (437-443).

We found no association between prenatal exposure to mixtures of persistent EDCs and early menarche status. This study fills a knowledge gap relating to prenatal exposure to mixtures of EDCs and puberty and comes closer to replicating the human experience by accounting for low-level exposure to many chemicals.

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Disclosures

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

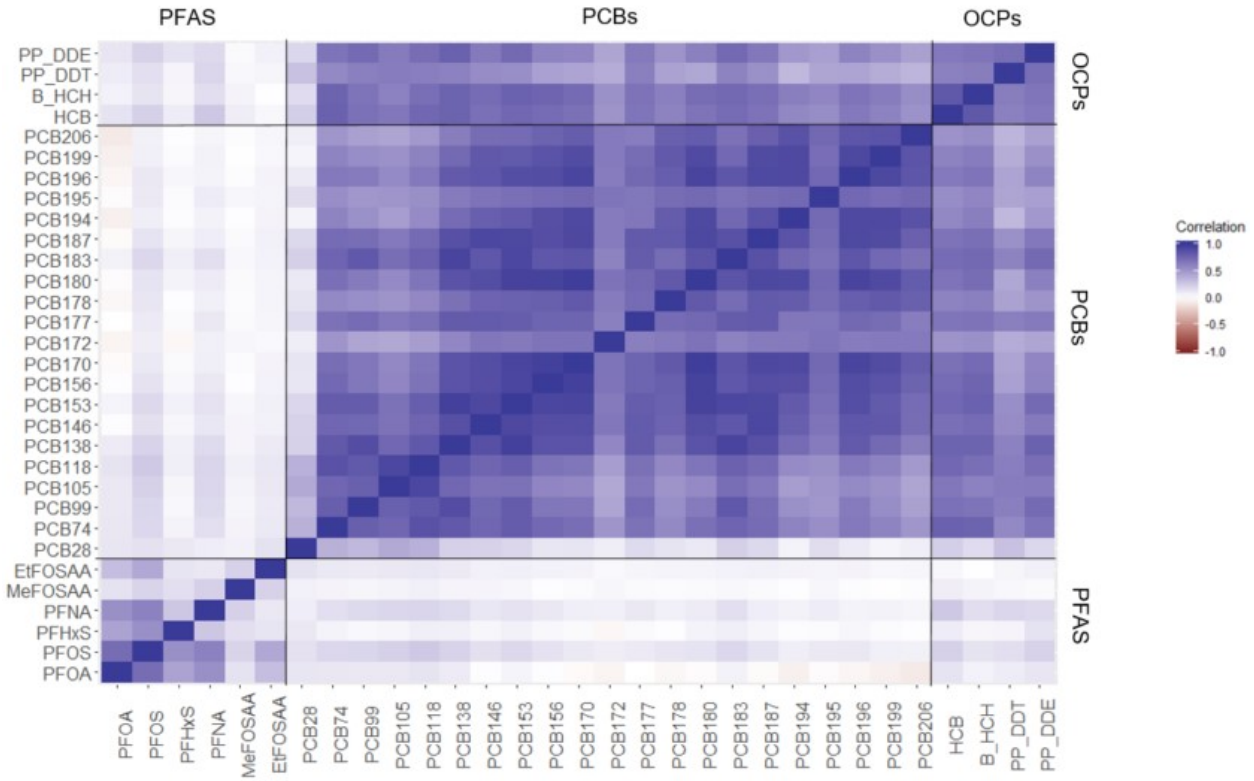


Figure 6-1. Correlation heatmap of serum concentrations of persistent endocrine disrupting chemicals in women during pregnancy in the Avon Longitudinal Study of Parents and Children (N=448). Spearman correlation coefficients presented for untransformed distributions, sectioned according to per- and polyfluoroalkyl substance (PFAS), polychlorinated biphenyl (PCB), and organochlorine pesticide (OCP) group membership. PCB and OCP concentrations were lipid-adjusted.

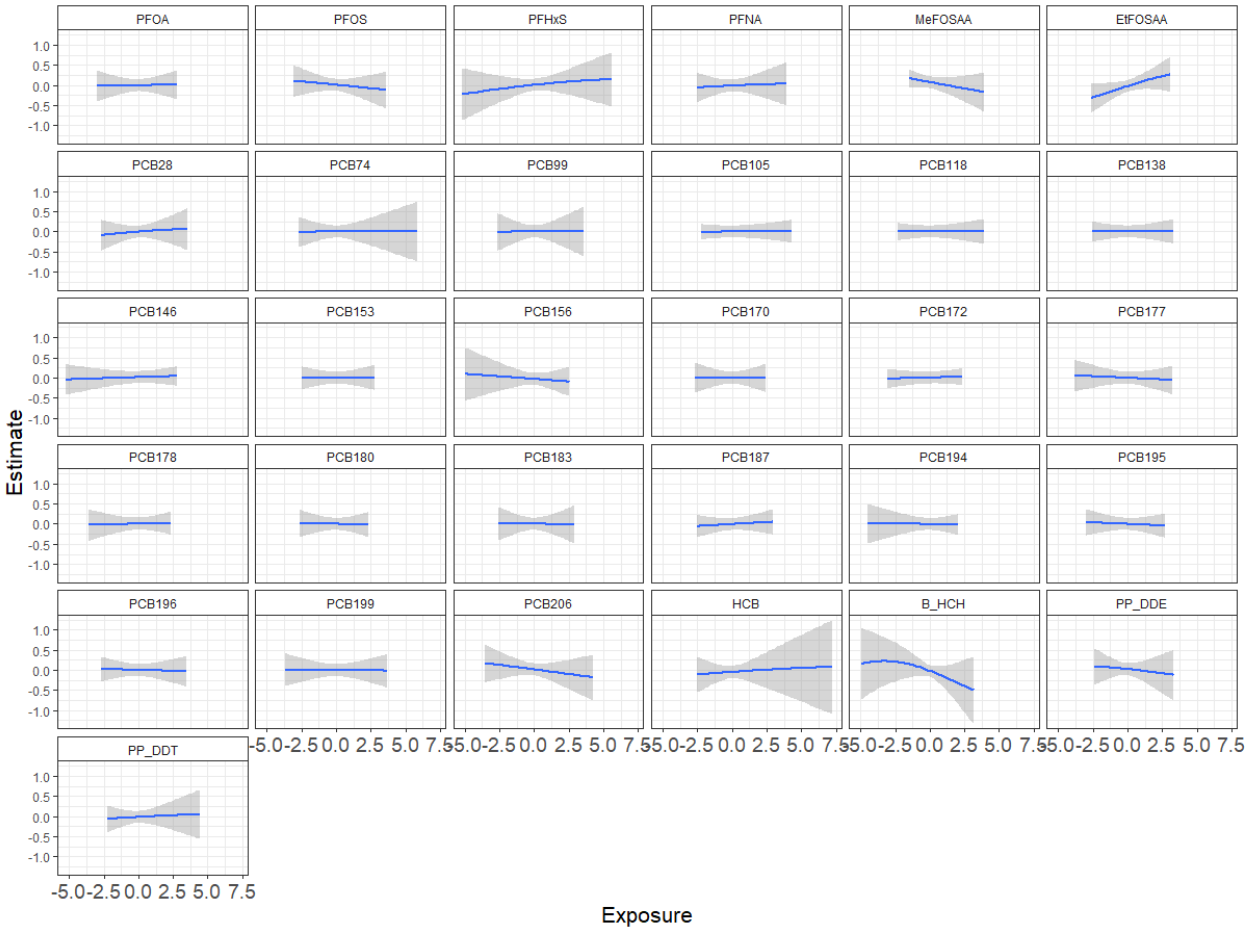


Figure 6-2. Chemical-specific effect estimates of mixture members on early menarche in ALSPAC mother-daughter dyads estimated by Bayesian kernel machine regression (N=284). Single chemical associations and 95% credible bands are presented with other chemicals fixed at their median. The model adjusted for maternal age at menarche, education, parity, pre-pregnancy body mass index, maternal age at delivery, prenatal smoking, and gestational week at sample collection. All chemical concentrations were natural log-transformed and standardized; PCB and OCP concentrations were lipid-adjusted.

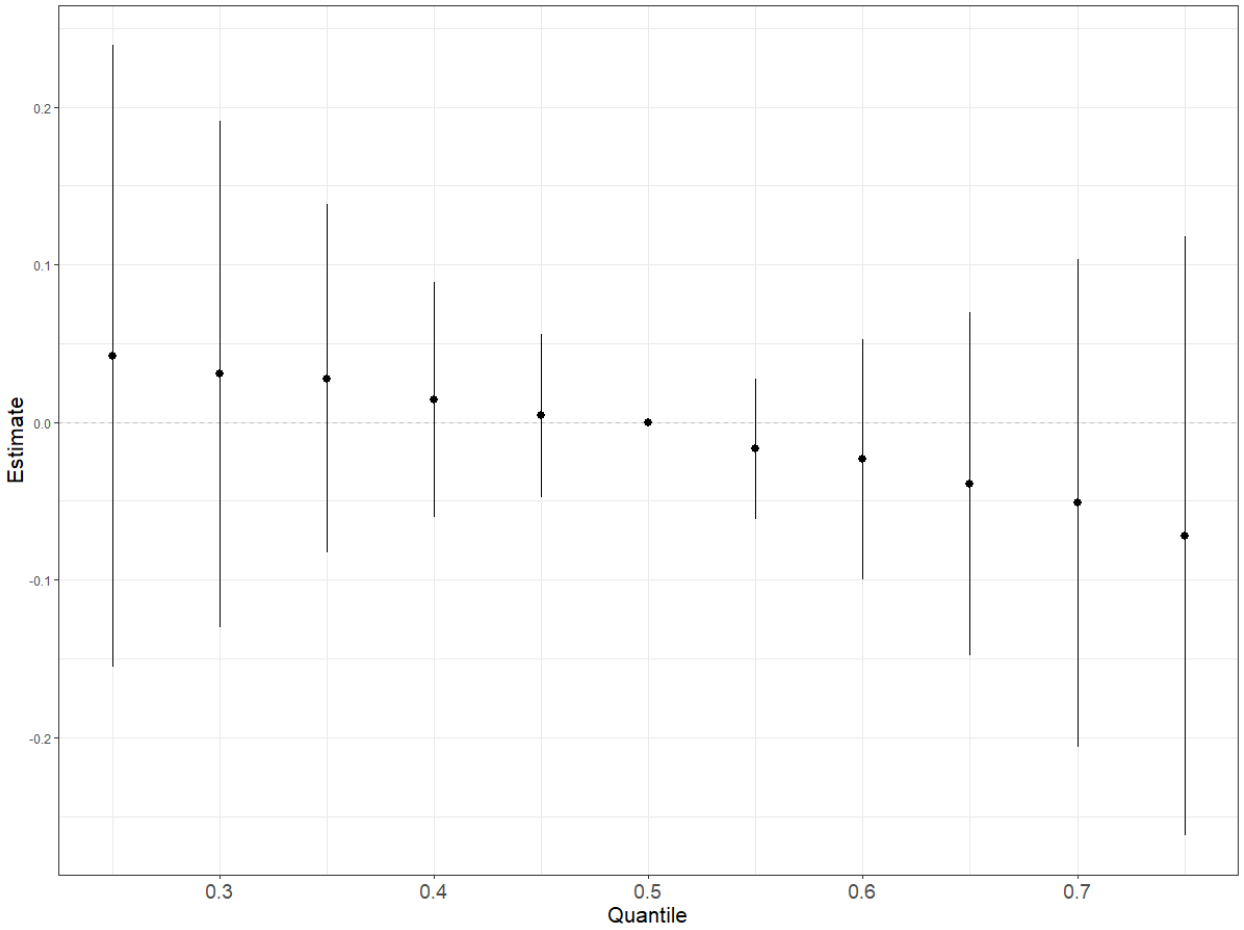


Figure 6-3. Overall effect of the mixture on early menarche status (estimates and 95% credible intervals), comparing the outcome when all exposures are at a particular quantile to the median (N=284). The model adjusted for maternal age at menarche, education, parity, pre-pregnancy body mass index, maternal age at delivery, prenatal smoking, and gestational week at sample collection. All chemical concentrations were natural log-transformed and standardized; PCB and OCP concentrations were lipid-adjusted.

Table 6-1. Persistent endocrine disrupting chemicals measured in maternal serum in the ALSPAC nested case-control study (N=448).

Chemical Name	Abbreviated Name
Per- and Polyfluoroalkyl Substances	
Perfluorooctane sulfonamide	FOSA
2-(N-ethylperfluorooctanesulfonamido) acetate	EtFOSAA
2-(N-methyl-perfluorooctanesulfonamido) acetate	MeFOSAA
Perfluorohexane sulfonate	PFHxS
Perfluorooctane sulfonate	PFOS
Perfluorooctanoate	PFOA
Perfluorononanoate	PFNA
Perfluorodecanoate	PFDA
Polychlorinated Biphenyls	
2,4,4'-trichlorobiphenyl	PCB28
2,2',3,5'-tetrachlorobiphenyl	PCB44
2,2',4,5'-tetrachlorobiphenyl	PCB49
2,2',5,5'-tetrachlorobiphenyl	PCB52
2,3',4,4'-tetrachlorobiphenyl	PCB66
2,4,4',5-tetrachlorobiphenyl	PCB74
2,2',3,4,5'-pentachlorobiphenyl	PCB87
2,2',4,4',5-pentachlorobiphenyl	PCB99
2,2',4,5,5'-pentachlorobiphenyl	PCB101
2,3,3',4,4'-pentachlorobiphenyl	PCB105
2,3,3',4',6-pentachlorobiphenyl	PCB110
2,3',4,4',5-pentachlorobiphenyl	PCB118
2,2',3,3',4,4'-hexachlorobiphenyl	PCB128
2,2',3,4,4',5'-hexachlorobiphenyl and 2,3,3',4,4',6-hexachlorobiphenyl	PCB138-158
2,2',3,4',5,5'-hexachlorobiphenyl	PCB146
2,2',3,4',5',6-hexachlorobiphenyl	PCB149
2,2',3,5,5',6-hexachlorobiphenyl	PCB151
2,2',4,4',5,5'-hexachlorobiphenyl	PCB153
2,3,3',4,4',5-hexachlorobiphenyl	PCB156
2,3,3',4,4',5'-hexachlorobiphenyl	PCB157
2,3',4,4',5,5'-hexachlorobiphenyl	PCB167
2,2',3,3',4,4',5-heptachlorobiphenyl	PCB170
2,2',3,3',4,5,5'-heptachlorobiphenyl	PCB172
2,2',3,3',4',5,6-heptachlorobiphenyl	PCB177
2,2',3,3',5,5',6-heptachlorobiphenyl	PCB178
2,2',3,4,4',5,5'-heptachlorobiphenyl	PCB180
2,2',3,4,4',5',6-heptachlorobiphenyl	PCB183
2,2',3,4',5,5',6-heptachlorobiphenyl	PCB187
2,3,3',4,4',5,5'-heptachlorobiphenyl	PCB189
2,2',3,3',4,4',5,5'-octachlorobiphenyl	PCB194
2,2',3,3',4,4',5,6-octachlorobiphenyl	PCB195
2,2',3,3',4,4',5',6-octachlorobiphenyl and 2,2',3,4,4',5,5',6-octachlorobiphenyl	PCB196-203
2,2',3,3',4,5,6,6'-octachlorobiphenyl	PCB199
2,2',3,3',4,4',5,5',6-nonachlorobiphenyl	PCB206
Decachlorobiphenyl	PCB209

Organochlorine Pesticides	
Hexachlorobenzene	HCB
β-Hexachlorocyclohexane	β-HCH
Y-Hexachlorocyclohexane (Lindane)	γ-HCH
Oxychlordane	Oxychlordane
Trans-Nonachlor	Trans-nonachlor
2,2-Bis(4-chlorophenyl)-1,1-dichloroethene	p,p'-DDE
2-(4-chlorophenyl)-2-(2-chlorophenyl)-1,1,1-trichloroethan	o,p'-DDT
2,2-Bis(4-chlorophenyl)-1,1,1-trichloroethan	p,p'-DDT
Mirex	Mirex

Table 6-2. Characteristics of the Avon Longitudinal Study of Parents and Children (ALSPAC) nested case-control study population (N=448 mother-daughter dyads).

Characteristic[ab]	Menarche <11.5 years Cases (n=218) n (%)	Menarche ≥11.5 years Controls (n=230) n (%)
Maternal race		
White	205(96.7)*	218(--)[c]*
Non-white	7(3.3)*	<5(--)[c]*
Maternal education[d]		
< O-level	43(21.0)	32(15.2)
O-level	67(32.7)	73(34.8)
>O-level	95(46.3)	105(50.0)
Maternal age at menarche, years		
8–11	63(32.5)*	30(15.2)*
≥12	131(67.5)*	168(84.8)*
Maternal pre-pregnancy BMI, kg/m^2		
<25 (under/normal weight)	139(70.6)*	174(84.9)*
≥25 (overweight/obese)	58(29.4)*	31(15.1)*
Prenatal smoking		
Any	45(21.4)	34(15.7)
None	165(78.6)	183(84.3)
Physical activity		
Any	123(66.5)	129(64.5)
None	62(33.5)	71(35.5)
Maternal age at delivery, years		
<25	44(20.4)	48(21.0)
25–29	83(38.4)	81(35.4)
≥30	89(41.2)	100(43.7)
Child birth order		
First born	110(53.9)	98(45.6)
Second born or later	94(46.1)	117(54.4)
Child birth weight, g		
<2500	7(3.3)	10(4.4)
≥2500	208(96.7)	215(95.6)
Breastfeeding		
Any	164(80.8)	174(80.2)
None	39(19.2)	43(19.8)
Gestational age at sample, weeks		
<20	147(67.4)	150(65.2)
≥20	71(32.6)	80(34.8)

Abbreviations: g, grams; kg/m^2, kilograms per meter-squared

[a] Compared using chi-square tests (or Fisher's exact test, where appropriate)

[b] Percentages are among mothers with non-missing data for each characteristic. Data were missing on maternal race (n=17, 3.8%), maternal education (n=33, 7.4%), maternal age at menarche (n=56, 12.5%), maternal pre-pregnancy BMI (n=46, 10.3%), prenatal smoking (n=21,

4.7%), physical activity (n=63, 14.1%), maternal age at delivery (n=3, 0.7%), child birth order (n=29, 6.5%), child birth weight (n=8, 1.8%), and breastfeeding (n=28, 6.3%). Gestational age at sample data were complete (n=0, 0.0%).

[c] Counts and percents suppressed due to small cell sizes

[d] <O-level=none, Certificate of Secondary Education, and vocational education, which are equivalent to no diploma or a GED in the United States. O-levels (ordinary levels) are required and completed at the age of 16. >O-level=A-levels (advanced levels) completed at 18, which are optional, but required to get into university; and a university degree.

* Cases and controls are significantly different ($p<0.05$)

Table 6-3. Serum concentrations of persistent endocrine disrupting chemicals among mothers of the Avon Longitudinal Study of Parents and Children (ALSPAC) during pregnancy by age at menarche of their daughters (N=448 mother-daughter dyads).

	Menarche <11.5 years Cases				Menarche ≥11.5 years Controls			
	Q1	Median	Q3	% <LOD[a]	Q1	Median	Q3	% <LOD[a]
Per- and polyfluoroalkyl substances (PFAS) (ng/mL)								
PFOA	2.9	3.85	5.0	0.0%	2.7	3.6	4.7	0.0%
PFOS	15.4	19.5	24.8	0.0%	14.6	20.0	24.9	0.0%
PFHxS	1.3	1.7	2.2	0.0%	1.2	1.6	2.2	0.4%
PFNA	0.41	0.57	0.66	0.5%	0.41	0.49	0.66	0.0%
FOSA	<LOD	0.1	0.3	29.8%	<LOD	0.2	0.3	31.3%
MeFOSAA	0.26	0.35	0.61	17.9%	0.26	0.35	0.70	11.3%
EtFOSAA	0.4	0.7	1.0	2.8%	0.4	0.6	0.9	2.2%
PFDA	<LOD	<LOD	<LOD	97.7%	<LOD	<LOD	<LOD	97.0%
Polychlorinated biphenyls (PCBs) (ng/g lipid)								
PCB28	3.6	5.6	8.4	7.8%	3.5	5.2	8.1	9.6%
PCB44	<LOD	1.8	4.0	29.8%	<LOD	2.0	3.9	30.9%
PCB49	<LOD	<LOD	1.8	60.6%	<LOD	<LOD	1.9	56.1%
PCB52	<LOD	3.1	7.7	30.3%	<LOD	3.4	7.2	30.0%
PCB66	<LOD	1.6	2.6	29.4%	<LOD	1.6	2.5	31.3%
PCB74	8.4	11.1	15.1	0.5%	8.6	11.1	15.2	0.0%
PCB87	<LOD	<LOD	1.5	60.6%	<LOD	<LOD	1.8	58.7%
PCB99	6.6	9.4	11.9	0.9%	7.2	9.3	12.2	0.9%
PCB101	<LOD	2.2	5.1	33.0%	<LOD	2.2	5.9	27.8%
PCB105	2.0	3.0	4.1	6.4%	2.0	2.8	3.9	8.3%
PCB110	<LOD	<LOD	2.3	54.1%	<LOD	<LOD	2.9	53.0%
PCB118	10.7	15.2	20.7	0.0%	10.9	14.8	20.4	0.0%
PCB128	<LOD	<LOD	<LOD	87.2%	<LOD	<LOD	<LOD	91.7%

PCB138[b]	30.2	40.5	52.5	0.5%	30.9	43.5	54.3	0.0%
PCB146	4.6	5.9	8.1	2.8%	4.6	6.0	8.1	2.2%
PCB149	<LOD	<LOD	1.7	63.8%	<LOD	<LOD	2.0	58.3%
PCB151	<LOD	<LOD	<LOD	80.3%	<LOD	<LOD	<LOD	78.7%
PCB153	48.1	62.1	85.5	0.0%	48.7	68.2	86.0	0.0%
PCB156	4.6	6.0	8.1	1.4%	5.0	6.6	8.5	2.2%
PCB157	<LOD	1.3	1.8	34.4%	<LOD	1.4	2.0	33.5%
PCB167	0.5	2.0	2.9	24.8%	<LOD	2.1	2.8	27.4%
PCB170	14.0	18.1	24.6	0.0%	14.7	19.8	25.8	0.0%
PCB172	1.1	1.9	2.7	21.6%	<LOD	2.0	2.7	24.4%
PCB177	2.3	3.0	4.2	8.7%	2.4	3.1	4.1	9.1%
PCB178	1.8	2.7	3.6	15.6%	1.9	2.8	3.9	13.0%
PCB180	31.6	44.0	59.3	0.0%	36.0	47.1	61.8	0.0%
PCB183	4.6	6.1	8.0	2.3%	4.7	6.3	8.2	4.4%
PCB187	8.4	10.9	15.8	0.9%	9.0	11.5	14.9	1.3%
PCB189	<LOD	<LOD	<LOD	79.4%	<LOD	<LOD	1.0	69.6%
PCB194	5.3	7.3	10.0	3.7%	5.7	7.8	10.8	3.0%
PCB195	1.3	2.1	2.9	20.6%	1.6	2.3	3.0	17.4%
PCB196[b]	5.3	7.4	10.0	2.3%	6.0	7.9	10.7	1.7%
PCB199	3.7	5.1	7.5	2.8%	4.2	5.7	8.0	2.6%
PCB206	1.6	2.2	3.0	10.6%	1.8	2.5	3.3	10.0%
PCB209	<LOD	1.4	1.9	30.7%	<LOD	1.6	2.1	24.8%
Organochlorine pesticides (OCPs) (ng/g lipid)								
HCB	37.4	50.7	63.7	0.0%	38.3	50.0	63.4	0.0%
β-HCH	33.5	45.3	59.9	1.8%	37.3	47.5	63.2	1.7%
γ-HCH	<LOD	<LOD	<LOD	80.7%	<LOD	<LOD	<LOD	77.4%
Oxychlordane	<LOD	<LOD	3.5	74.3%	<LOD	<LOD	4.6	69.6%
Trans-nonachlor	<LOD	<LOD	4.7	66.5%	<LOD	<LOD	4.6	67.4%
p,p'-DDE	184	314	522	0.5%	200	310	484	0.0%

o,p'-DDT	<LOD	<LOD	<LOD	98.6%	<LOD	<LOD	<LOD	98.3%
p,p'-DDT	7.4	11.3	16.5	10.6%	8.0	10.5	16.1	12.2%
Mirex	<LOD	<LOD	<LOD	100.0%	<LOD	<LOD	<LOD	98.7%

Abbreviations: Q1, quartile 1; Q3, quartile 3; LOD, limit of detection; ng/mL, nanogram per milliliter; ng/g lipid, nanogram per gram lipid

[a] The LODs for PFAS were 0.082 ng/mL for PFNA, 0.10 ng/mL for PFOA, PFHxS, and FOSA, 0.174 ng/mL for MeFOSAA, and 0.20 ng/mL for PFOS, EtFOSAA, and PFDA. LODs of OCPs and PCBs are dependent on the sample size and blanks, thus, an individual LOD is reported for each individual result rather than an overall LOD.

[b] PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration hereafter referred to as PCB138. Similarly, PCB congeners 196 and 203 could not be separated and were quantified as a summed concentration hereafter referred to as PCB196.

Table 6-4. Adjusted[a] associations of maternal serum concentrations of persistent endocrine disrupting chemicals with early age at menarche of the Avon Longitudinal Study of Parents and Children (ALSPAC) nested case-control study (N=448 mother-daughter dyads), modeled as a mixture using weighted quantile sum regression and in single- and multi-chemical models using logistic regression.

	Mixture OR[b] (95% CI)	**Average Weight[cd]**	**Mixture OR[b] (95% CI)**	**Average Weight[cd]**	**Single-chemical OR[e] (95% CI)**	**Multi-chemical OR[f] (95% CI)**
	Positive constraints[g]		**Negative constraints[g]**			
Per- and polyfluoroalkyl substances (PFAS) (n=331)	0.96 (0.81, 1.13)		0.95 (0.81, 1.12)			
PFOA		0.08		0.04	1.02 (0.96, 1.09)	1.04 (0.95, 1.13)
PFOS		0.23			1.01 (0.94, 1.07)	0.93 (0.82, 1.04)
PFHxS		0.39			1.01 (0.97, 1.05)	1.02 (0.98, 1.07)
PFNA		0.09		0.22	1.00 (0.95, 1.05)	1.03 (0.96, 1.10)
MeFOSAA		0.03		0.72	0.98 (0.96, 1.01)	0.97 (0.94, 1.00)
EtFOSAA		0.19		0.02	1.03 (0.99, 1.07)	1.05 (1.01, 1.10)
Polychlorinated biphenyls (PCBs) (n=292)	0.90 (0.75, 1.09)		0.93 (0.78, 1.10)			
PCB28		0.22		0.02	1.01 (0.97, 1.04)	1.00 (0.95, 1.05)
PCB74		0.05			0.97 (0.92, 1.02)	0.98 (0.86, 1.12)
PCB99		0.02		0.01	0.99 (0.94, 1.04)	0.94 (0.75, 1.17)
PCB105		0.30			1.00 (0.94, 1.06)	1.13 (0.89, 1.45)

PCB118		0.02			0.99 (0.95, 1.04)	0.89 (0.66, 1.21)
PCB138[h]		0.01			0.97 (0.92, 1.02)	1.00 (0.68, 1.46)
PCB146		0.03			1.00 (0.95, 1.05)	0.99 (0.85, 1.17)
PCB153					0.96 (0.90, 1.02)	1.29 (0.71, 2.37)
PCB156		0.01			0.96 (0.91, 1.01)	1.01 (0.88, 1.15)
PCB170				0.01	0.94 (0.87, 1.00)	1.01 (0.68, 1.51)
PCB172		0.30			1.02 (0.97, 1.06)	1.15 (1.05, 1.26)
PCB177		0.01			0.98 (0.93, 1.02)	0.89 (0.80, 0.99)
PCB178				0.08	0.97 (0.92, 1.02)	1.01 (0.92, 1.11)
PCB180				0.02	0.93 (0.87, 1.00)	0.69 (0.40, 1.18)
PCB183				0.02	0.97 (0.92, 1.03)	0.92 (0.74, 1.15)
PCB187					0.99 (0.93, 1.04)	1.37 (1.08, 1.75)
PCB194		0.01		0.33	0.97 (0.93, 1.02)	1.02 (0.94, 1.11)
PCB195		0.02			0.96 (0.91, 1.01)	0.94 (0.86, 1.03)
PCB196[h]					0.92 (0.86, 0.99)	1.04 (0.79, 1.38)
PCB199				0.06	0.96 (0.91, 1.01)	0.97 (0.86, 1.09)
PCB206				0.46	0.92 (0.87, 0.98)	0.85 (0.73, 0.98)
Organochlorine pesticides (OCPs) (n=302)	0.98 (0.85, 1.12)		1.02 (0.90, 1.18)			
HCB		0.47	0.02		0.97 (0.92, 1.03)	1.01 (0.93, 1.09)
β-HCH		0.27	0.42		0.96 (0.92, 1.00)	0.96 (0.91, 1.02)

p,p'-DDE		0.21	0.05		0.98 (0.94, 1.01)	0.98 (0.93, 1.03)
p,p'-DDT		0.05	0.51		0.98 (0.93, 1.03)	1.02 (0.95, 1.10)
Overall mixture (PFAS, PCBs, OCPs) (n=284)	1.01 (0.79, 1.28)		0.93 (0.76, 1.14)			
PFOA		0.04		0.03		
PFOS		0.01		0.03		
PFHxS		0.07		0.01		
PFNA		0.07		0.02		
MeFOSAA		0.04		0.09		
EtFOSAA		0.31		0.01		
PCB28		0.05		0.06		
PCB74				0.11		
PCB99				0.06		
PCB105		0.07				
PCB118						
PCB138[h]				0.02		
PCB146		0.03				
PCB153						
PCB156		0.01		0.01		
PCB170						
PCB172		0.01		0.04		

PCB177		
PCB178	0.13	
PCB180		0.01
PCB183		
PCB187	0.01	
PCB194	0.01	0.02
PCB195	0.04	
PCB196[h]		0.01
PCB199	0.06	
PCB206	0.01	0.14
HCB	0.01	0.01
β-HCH		0.02
p,p'-DDE		0.27
p,p'-DDT	0.01	0.01

Abbreviations: OR, odds ratio; CI, confidence interval

[a] Adjusted for maternal age at menarche, education, parity, pre-pregnancy body mass index, maternal age at delivery, prenatal smoking, and gestational week at sample collection

[b] The odds ratio for early menarche for one-unit higher of the WQS index (representing a one-decile increase in chemical concentrations)

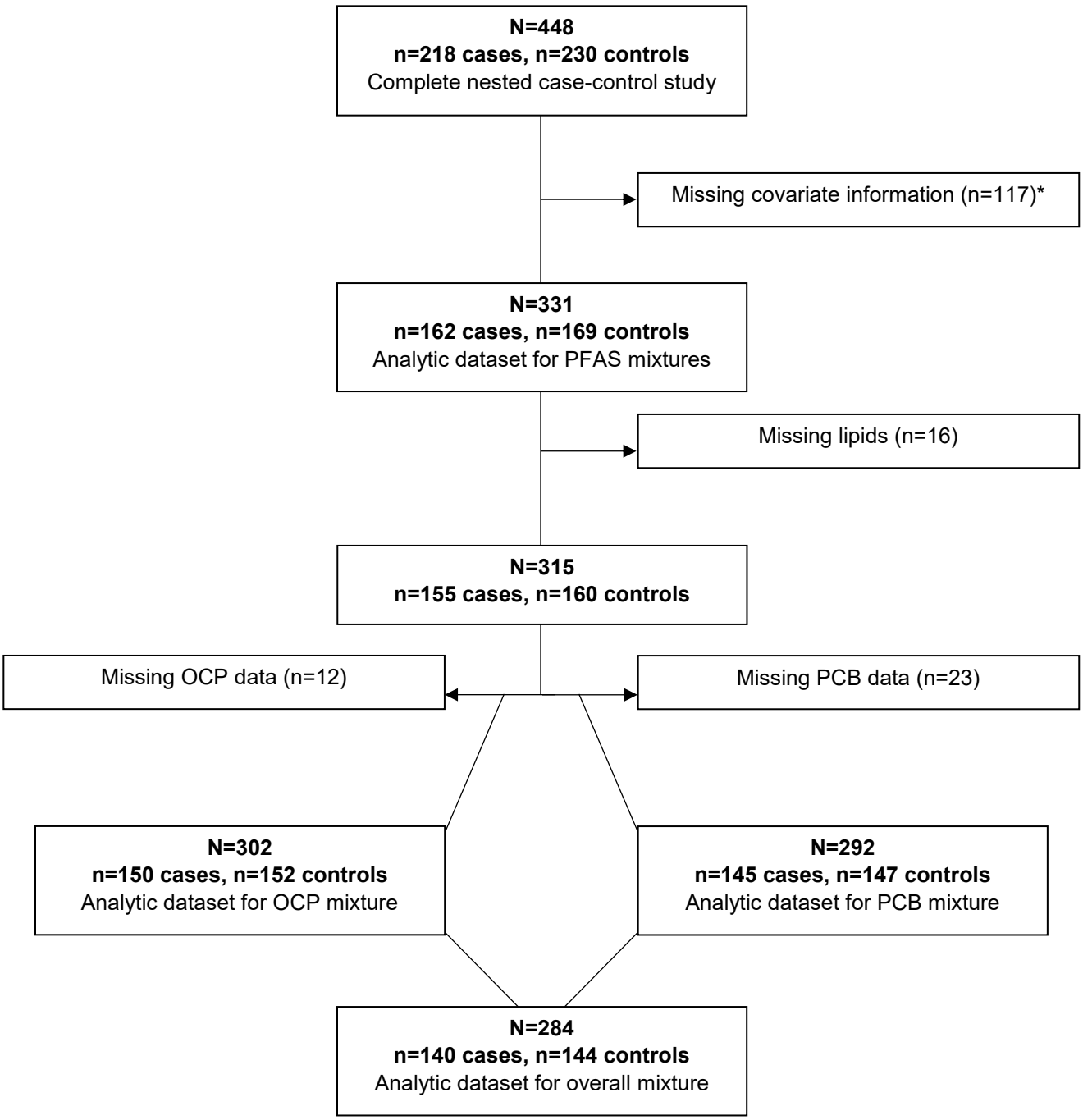
[c] Weights greater than 1/number of chemicals in the mixture are considered significant contributors to the overall mixture effect

[d] Weights may not add to 1 due to rounding

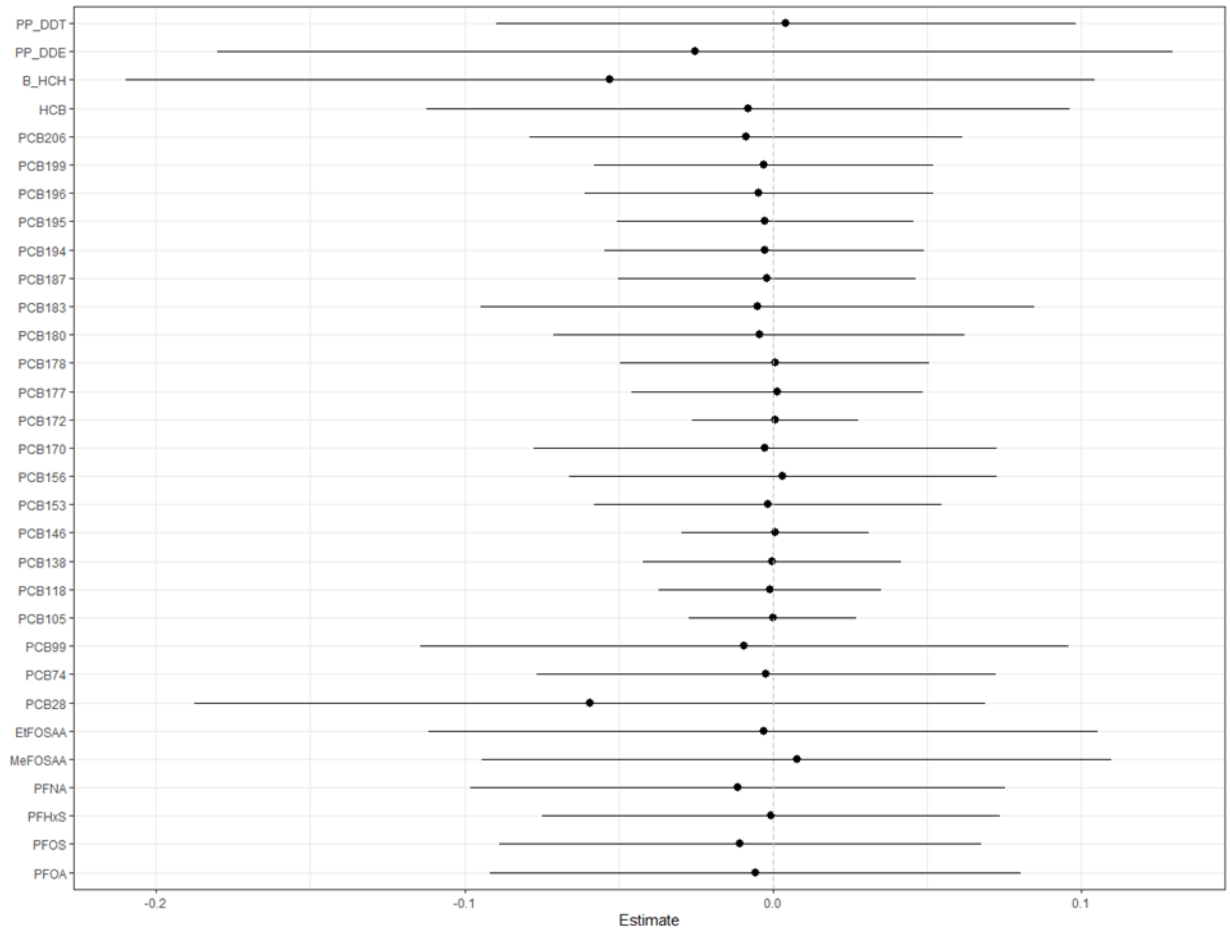
[e] Single-chemical logistic regression models were run to examine independent associations between each chemical and early menarche. Odds ratios represent a change of 10% higher chemical concentrations.

[f] Multi-chemical logistic regression models were run to examine associations between each chemical in a class (e.g., PFAS) and early menarche, independent of other chemicals in the class (e.g., adjusting for other chemicals in the class). Odds ratios represent a change of 10% higher chemical concentrations.

[g] WQS regression focuses inference in one direction through constrained optimization of the beta parameter; effects were estimated for both directions by running separate models constraining in the positive and negative directions.
[h] PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration hereafter referred to as PCB138. Similarly, PCB congeners 196 and 203 could not be separated and were quantified as a summed concentration hereafter referred to as PCB196.



Supplemental Figure 6-4. Flowchart depicting missing data in the study of prenatal exposure to mixtures of persistent endocrine disrupting chemicals and early menarche in a nested case-control study of the Avon Longitudinal Study of Parents and Children.
* Data were missing on maternal education (n=33, 7.4%), maternal age at menarche (n=56, 12.5%), maternal pre-pregnancy BMI (n=46, 10.3%), prenatal smoking (n=21, 4.7%), maternal age at delivery (n=3, 0.7%), and child birth order (n=29, 6.5%).



Supplemental Figure 6-5. Interaction terms for individual mixture members and the remaining chemicals in ALSPAC mother-daughter dyads estimated by Bayesian kernel machine regression (N=284). Each point represents the difference between the effect size of the chemical when all other chemicals are held at their 75th percentiles and the effect size of the same chemical when all other chemicals are held at their 25th percentiles. Range indicates 95% credible interval. Model adjusted for maternal age at menarche, parity, pre-pregnancy BMI, maternal age at delivery, education, smoking, and gestational age at sample collection. All chemical concentrations were natural log-transformed and standardized; PCBs and OCPs were lipid adjusted.

Supplemental Table 6-5. Sensitivity analysis exploring associations of detectable versus below the limit of detection serum concentrations of persistent endocrine disrupting chemicals with early menarche in the Avon Longitudinal Study of Parents and Children (ALSPAC) nested case-control study (N=448 mother-daughter dyads).

	OR (95% CI)[abc]
Perfluoroalkyl substances (PFAS) (ng/mL)	
PFOA	N/A
PFOS	N/A
PFHxS	N/A
PFNA	N/A
FOSA	1.46 (0.88, 2.42)
MeFOSAA	0.53 (0.27, 1.04)
EtFOSAA	N/A
PFDA	N/A
Polychlorinated biphenyls (PCBs) (ng/g lipid)	
PCB28	1.27 (0.55, 2.95)
PCB44	1.02 (0.61, 1.70)
PCB49	0.75 (0.46, 1.21)
PCB52	1.00 (0.60, 1.67)
PCB66	1.06 (0.63, 1.78)
PCB74	N/A
PCB87	0.75 (0.46, 1.23)
PCB99	N/A
PCB101	0.77 (0.46, 1.29)
PCB105	1.78 (0.73, 4.36)
PCB110	1.04 (0.65, 1.67)
PCB118	N/A
PCB128	0.94 (0.42, 2.11)
PCB138[d]	N/A
PCB146	N/A

PCB149	0.76 (0.47, 1.24)
PCB151	0.81 (0.45, 1.46)
PCB153	N/A
PCB156	N/A
PCB157	0.88 (0.53, 1.47)
PCB167	1.20 (0.69, 2.08)
PCB170	N/A
PCB172	1.11 (0.63, 1.95)
PCB177	0.63 (0.27, 1.45)
PCB178	0.57 (0.28, 1.16)
PCB180	N/A
PCB183	N/A
PCB187	N/A
PCB189	0.45 (0.26, 0.78)
PCB194	N/A
PCB195	0.74 (0.40, 1.35)
PCB196[d]	N/A
PCB199	N/A
PCB206	0.52 (0.22, 1.24)
PCB209	0.69 (0.40, 1.20)
Organochlorine pesticides (OCPs) (ng/g lipid)	
HCB	N/A
β-HCH	N/A
γ-HCH	0.82 (0.46, 1.45)
Oxychlordane	0.62 (0.35, 1.07)
Trans-nonachlor	0.84 (0.48, 1.46)
p,p'-DDE	N/A
o,p'-DDT	N/A
p,p'-DDT	0.84 (0.40, 1.78)

Mirex	N/A

Abbreviations: OR, odds ratio; CI, confidence interval; ng/mL, nanogram per milliliter; ng/g lipid, nanogram per gram lipid

[a] Adjusted for maternal age at menarche, parity, pre-pregnancy BMI, maternal age at delivery, education, prenatal smoking, and gestational age at sample collection

[b] Restricted to those with % <LOD between 5% and 95%

[c] OR represents the odds of early menarche for those with concentrations above the LOD, compared to those with concentrations below the LOD

[d] PCB congeners 138 and 158 could not be separated and were quantified as a summed concentration hereafter referred to as PCB138. Similarly, PCB congeners 196 and 203 could not be separated and were quantified as a summed concentration hereafter referred to as PCB196.

Supplemental Table 6-6. Comparison of characteristics of various sub-samples of mother-daughter dyads from the Avon Longitudinal Study of Parents and Children (ALSPAC) population. The nested case-control study (N=448) was drawn from cohort daughters who were enrolled at puberty (N=3338). Complete analytic data for mixture analyses was available for 284 mother-daughter dyads.

	Enrolled at Puberty N=3338	**Nested Case-Control N=448**	**Analytic Data N=284**
Characteristic	**n (%)[a]**	**n (%)[a]**	**n (%)**
Maternal race			
White	3050 (98.3)	423 (98.1)	179 (98.2)
Non-white	54 (1.7)	8 (1.9)	5 (1.8)
Maternal education[b]			
< O-level	549 (18.2)	75 (18.1)	48 (16.9)
O-level	1083 (35.9)	140 (33.7)	95 (33.5)
>O-level	1385 (45.9)	200 (48.2)	141 (49.6)
Maternal age at menarche, years			
8–11	537 (19.0)	93 (23.7)	63 (22.9)
≥12	2282 (81.0)	299 (76.3)	219 (77.1)
Maternal pre-pregnancy BMI			
<25 kg/m^2 (under/normal weight)	2379 (80.8)	313 (77.9)	221 (77.1)
≥25 kg/m^2 (overweight/obese)	566 (19.2)	89 (22.1)	65 (22.9)
Prenatal smoking			
Any	2677 (13.3)	79 (18.5)	44 (15.5)
None	410 (86.7)	348 (81.5)	240 (84.5)
Maternal age at delivery			
<25 years	473 (14.8)	92 (20.7)	48 (16.9)
25–29 years	1275 (40.0)	164 (36.9)	109 (38.4)
≥30 years	1439 (45.2)	189 (42.5)	127 (44.7)
Child birth order			
First born	1469 (48.0)	208 (49.6)	142 (50.0)
Second born or later	1593 (52.0)	211 (50.4)	142 (50.0)

Abbreviations: g, grams; kg/m^2, kilograms per meter-squared

[a] Missing data not represented

[b] <O-level=none, Certificate of Secondary Education, and vocational education, which are equivalent to no diploma or a GED in the United States. O-levels (ordinary levels) are required and completed at the age of 16. >O-level=A-levels (advanced levels) completed at 18, which are optional, but required to get into university; and a university degree.

Chapter 7 Summary of Findings and Future Research

Summary of Findings

Findings from Aim 1

Given the increasing interest in the association between environmental contaminants and health outcomes, it is important to identify major sources of exposure, such as dietary sources. Using data from two population-based pregnancy cohorts, we identified dietary patterns associated with exposure to persistent EDCs (PFAS, PCBs, and OCPs). Meat, white fish, cheese, and biscuits were associated with all three chemical classes in the ALSPAC study. In MoBa, sausages and burgers (representing processed meats), pasta, and chocolate bars had high positive factor loadings in PCB and OCP dietary patterns, while cheese loaded highly in the PFAS pattern. Across both cohorts, French fries and potato chips showed negative factor loadings, and fresh fruit and vegetables had null factor loadings for the most part. The dietary pattern scores derived for the classes of EDCs were associated with maternal EDC concentrations; closer adherence to the derived environmental dietary patterns was associated with higher EDC concentrations. Each dietary pattern alone explained 8% to 20% of EDC concentrations; up to 38% of the variance was explained when the dietary patterns were modeled alongside race, maternal age at delivery, pre-pregnancy BMI, parity, maternal education, prenatal smoking, gestational age at sample collection, and total energy.

Our study aligns with previous work by identifying a number of animal-based foods as the predominant dietary sources of persistent EDC exposure. We attempted to compare dietary patterns from ALSPAC and MoBa. Ultimately, there were differences in which foods contributed the most to a pattern within each cohort, but there were some similarities; we saw positive factor loadings for cheese with PFAS, white fish and rice with PCBs, and poultry and white fish with OCPs in both cohorts. This work across populations is foundational to potentially developing

guidance for pregnant women seeking to limit their exposure to EDCs before and during pregnancy.

Findings from Aim 2A

While many studies have examined prenatal exposure to persistent EDCs and birth size, few have explored these associations considering persistent EDC exposure as a mixture. Our aim was to investigate the association of maternal gestational concentrations of mixtures of persistent EDCs (PFAS, PCBs, and OCPs) and birth size measures (weight, crown to heel length, head circumference, ponderal index, and small for gestational age). Specifically, we aimed to estimate the overall effect of the mixture and identify the chemicals within the mixture contributing the most to the overall effect. We observed an inverse association of prenatal exposure to mixtures of PFAS, PCBs, and OCPs with birth size, especially birth weight, among British girls using weighted quantile sum (WQS) regression and Bayesian kernel machine regression (BKMR). One-unit higher of the weighted quantile sum index (representing a one-decile increase in chemical concentrations) for all three classes combined (PFAS, PCBs, and OCPs) was associated with 74 g (β: -74 g, 95% CI: -118, -29 g) lower birth weight. These results support previous findings under the single-chemical paradigm: higher prenatal concentrations of many persistent EDCs are associated with lower birth weight, smaller head circumference, and shorter crown to heel length (56). While this study reaches the same conclusion as many previous studies published on this topic under the single-chemical paradigm, it fills a gap relating to mixtures of EDCs and birth size and comes closer to replicating the human experience. Specifically, this study gives a better sense of the overall effect of prenatal exposure to a number of PFAS, PCBs, and OCPs on birth size. In crude calculations, we see that the overall effect of the 31-chemical mixture is almost eight times that of considering a single chemical (PFOA) alone.

Findings from Aim 2B

While many studies have examined birth weight in association with prenatal EDC exposure, fewer have investigated postnatal body size and none have taken a mixtures approach. In this aim, we examined the association of maternal gestational concentrations of persistent EDCs (PFAS, PCBs, and OCPs) and postnatal body size (weight-, height-, and BMI-for-age scores) at 0, 2, 9, and 19 months using a mixtures approach. Using BKMR with a random intercept, we found an inverse association between a 31-chemical mixture and weight-for-age z-scores through 19 months of age. This association seems to be driven by early postnatal body size. Further, weak inverse associations were seen for height- and BMI-for-age z-scores and there was an inverse association for the mixture of six PFAS with weight-for-age score. We found that holding all 31 EDCs in the mixture at the 75th percentile compared to the 50th percentile was associated with 0.15 lower weight-for-age z-score (estimate: -0.15, 95% credible interval: -0.26, -0.03). At mean values for 19 months of age, a 0.15 lowering of the weight-for-age z-score corresponds to 0.18 kg lower weight (estimate: -0.18 kg, 95% credible interval: -0.31, -0.03 kg). In general, the first two years of life are a particularly important period of change as the greatest variations in rates of weight gain are usually seen during this time when infants show accelerated or diminished growth to compensate for intrauterine restraint or enhancement of fetal growth.

Findings from Aim 3

Exposure to EDCs, including prenatally, may contribute to altered timing and patterning of pubertal development. Previous studies of age at menarche under the single-chemical paradigm have shown mixed results, including weak associations with early menarche (313, 315). Given these weak associations seen for single EDCs, it makes sense to examine persistent EDCs as an overall mixture in relation to age at menarche because there may be a cumulative effect of a number of EDCs. In this study, we examined the association of prenatal exposure to multiple PFAS, PCBs, and OCPs (as individual classes and collectively) and early menarche (<11.5 years)

among British girls, and observed null associations. We employed WQS regression and BKMR to accomplish this, and results from these two methods were largely in agreement. The odds ratio for early menarche for one-unit higher of the WQS index (representing a one-decile increase in chemical concentrations) for all three classes combined was 1.01 (95% CI: 0.79, 1.28). Nonetheless, this study fills a knowledge gap relating to prenatal exposure to mixtures of EDCs and pubertal outcomes and comes closer to replicating the human experience by accounting for low-level exposure to many chemicals.

Overall Findings

Overall, we attempted to look collectively at dietary and environmental exposures through use of dietary pattern analysis and mixture methods, respectively. First, we identified dietary patterns that contribute to persistent EDC exposure. These EDC dietary patterns were largely characterized by consumption of certain animal products, which have a tendency to bioaccumulate persistent EDCs. Further, we found that each dietary pattern alone accounted for 8% to 20% of variation in EDC concentrations. Though diet is not the only source of exposure, it is a modifiable source of exposure and therefore is worthy of further study as a potential site of action to lessen exposure to persistent EDCs.

Knowing that diet is a major source of persistent EDC exposure in pregnant women (209, 212, 444), we examined the associations of prenatal exposure to persistent EDCs as a mixture with growth and developmental outcomes in female offspring in later aims. Generally speaking, we saw stronger associations of prenatal exposure to persistent EDCs with outcomes closer to birth. We observed substantially lower birth weights for those with higher prenatal exposure to persistent EDCs. In our analysis of prenatal exposure to persistent EDCs and postnatal body size, we saw associations weaken with greater time since birth. In the last aim, we examined a more distal outcome, pubertal development. We examined prenatal exposure to mixtures of persistent EDCs and early menarche (<11.5 years) and found no association. While this was not completely

unexpected based on previous studies of menarche, there was motivation for a study using a mixtures approach. Because it is thought that multiple EDCs can act through a common mechanism to produce an outcome, it seems reasonable that individual EDCs could act synergistically at lower concentrations (321). Although this was not observed in our study of prenatal exposure to EDCs and age at menarche, we did see chemicals acting together in our study of birth size: the overall effect of the 31-chemical mixture was almost eight times that of considering a single chemical alone.

Strengths and Limitations

Strengths

Our studies are strengthened by their prospective design within population-based birth cohorts. In particular, ALSPAC has a rather large sample size for studies of this kind. Further, the frequent and thorough longitudinal data collection over a long follow up period (over 25 years) allows us to examine exposures during pregnancy with distal outcomes such as pubertal development. Thirdly, we have utilized reliable biomarker indicators of exposure to over 50 persistent EDCs, allowing us to examine some less commonly studied chemicals as part of chemical mixtures. Further, our study is enriched by the extensive covariate data available within ALSPAC and MoBa. Specific to aim 1, we compared dietary patterns associated with EDCs across two European cohorts and were able to take advantage of MoBa's rich FFQ data. In aims 2 and 3, our mixtures approach using multiple complementary methods (WQS regression, BKMR) allowed us to better replicate the human experience of exposure to multiple chemicals. Strengths of these mixture methods include their robustness to multicollinearity due to correlated exposures, dimensionality reduction, and ability to estimate mixture health effects while identifying important mixture components.

Limitations in design

One limitation of this research is the cross-sectional nature of the environmental and dietary data. In some instances, serum samples were taken weeks before the FFQ was administered. While the FFQ was supposed to reflect usual consumption “nowadays,” it is possible there was variation in diet during pregnancy (e.g., due to morning sickness or improved diet during pregnancy). The different timing of dietary and environmental assessments are unlikely to affect persistent EDCs since they have long half-lives (388-390).

Another limitation is that the sub-sample used in this study differed from the overall ALSPAC cohort on some factors. For example, mothers in our sample were more likely to be highly educated and older than mothers in the overall ALSPAC cohort. These differences are unsurprising given that to be selected for this sub-sample, children still had to be engaged with the study during puberty (completing two or more puberty questionnaires). Generally, nonparticipation and loss to follow-up tends to be more pronounced among the less advantaged and less healthy (437-443).

We are only able to examine mother-daughter dyads from ALSPAC. Studies have found that EDCs affect the functioning of both androgen and estrogen receptors in utero. Because sex hormones have differential roles by gender on fetal development, EDCs may therefore have differential effects on fetal development (aim 2) (445). The EDC measures needed to perform parallel analyses among mother-son dyads are not available in ALSPAC.

Lastly, we were unable to weight certain analyses to account for the nested case-control study design. In aims not utilizing case-control status (aims 1 and 2), we were unable to reweight back to the full cohort to represent the correct distribution of cases and controls within certain methods. For example, analyses were appropriately weighted (weight of 15.1 for controls and weight of 1 for cases) in all linear and logistic regression models and in mixture analyses using weighted quantile sum (WQS) regression, but we were unable to use weights in reduced rank regression (RRR) (aim 1) and Bayesian kernel machine regression (BKMR) (aim 2A and aim 2B).

Our inability to weight RRR and BKMR analyses of this nested case-control study back to the full cohort limits generalizability. Nevertheless, when comparisons were possible, results of the unweighted analyses were similar to weighted linear regression and WQS regression results in terms of direction of effect. Further, using weights for estimating associations between exposures and outcomes is not considered essential under these circumstances (411-414).

Limitations in ability to control for confounding

There are also limitations relating to the covariate data and control for confounding. Self-reported smoking and alcohol use during pregnancy may suffer from social desirability bias. Education was the only proxy for socioeconomic status used in ALSPAC because many women did not report income; it is possible that there is residual confounding by income.

There is also the problem of missing covariate data. Data were missing on maternal education (n=33, 7.4%), maternal age at menarche (n=56, 12.5%), maternal pre-pregnancy BMI (n=46, 10.3%), prenatal smoking (n=21, 4.7%), maternal age at delivery (n=3, 0.7%), and child birth order (n=29, 6.5%) within ALSPAC. Combined with missing data on lipids and exposures, mixture analyses in aims 2 and 3 were run with roughly two-thirds to three-quarters of the nested case-control study. Currently, the ability to use multiple imputation is not built into packages (*gWQS*, *BKMR*) for mixtures procedures within R. We compared mother-daughter characteristics for those in the analytic dataset used for mixture analyses (n=284, at the smallest) to those in the nested case-control study (n=448) and to the population from which the case-control study was drawn (n=3338). Characteristics were similar across subsets; while we saw a higher proportion of mothers with an earlier maternal age at menarche and overweight/obese pre-pregnancy BMI in the nested case-control study and analytic data compared to those enrolled at puberty, this was expected due to the relation of these factors with case status.

In studies of persistent EDCs measured in serum during pregnancy, there is some concern about bias because fetal growth (which is strongly correlated with the outcome of interest in aim

2A, birth size) may affect the measured biomarker level and there may be shared biological determinants of the exposure measure and fetal growth and birth size (e.g., hemodynamics). This is less of a concern in studies, like ours, with blood sampled early in pregnancy (median 15 weeks gestation) and studies with a wide range of exposure (246). We also adjusted for gestational age at sample collection in all analyses.

Tying many of these aforementioned issues together is the concept of coexposure amplification bias in aims 2 and 3. A key feature of environmental mixtures is that some components can be highly correlated, raising the issues of confounding by coexposure and collinearity. Attention has recently been paid to the impact of residual confounding due to unmeasured or unknown variables. A 2018 paper by Weisskopf, Seals, and Webster examines the potential amplification of such biases when correlated exposure variables are included in regression models using DAGs to describe different scenarios involving residual confounding (350). The authors found that approaches to the analysis of mixtures that involve regressing the outcome on several exposures simultaneously can in some cases amplify, rather than reduce, bias due to confounding. The problem of bias amplification worsened with stronger correlation between mixture components or when more mixture components were included in the model. The authors recommend steps for minimizing possible bias amplification in the design and analysis of epidemiologic studies of multiple correlated exposures, particularly in studies when biomarkers of exposure are used, including identification and control of the unknown confounders and/or the use of instrumental variables or proxy exposures that approach instrumental variables (350). There is strong correlation between most PCBs and OCPs in the ALSPAC sample, though less correlation with PFAS. Through the use of DAGs and our access to extensive lists of available covariates, we attempted to identify and control as many confounders as possible.

Limitations due to misclassification

There is some potential for misclassification of the outcome in aim 3, age at menarche, such as the completion of the questionnaire by a parent unaware of the child's menarche status, or issues in the parent or child's recall of the month and year menstruation began. Previous studies have suggested moderate to high recall ability of age at menarche among adult women; it is expected that adolescent girls would have better recall due to proximity to the event (420-422).

Additionally, in aim 1, misreporting of diet is always a possibility. For example, individuals with higher true intake tend to under-report and individuals with lower true intake tend to over-report. Further, detailed information about food preparation, brands, and contextual information about intake is lacking, and because an FFQ is composed of a pre-specified food list, an FFQ may not capture every food that is usually consumed by an individual within a specific population.

Lastly, in simulation studies of WQS regression that we completed before undertaking this work, we found limited power to detect the chemicals that contribute the most to the outcome of interest (the “bad actors”), a secondary aim of WQS regression. We simulated a dataset of N=450 and seven correlated exposure variables to estimate power using WQS regression. Briefly, we found that estimated power for detecting small to moderate changes in the birth weight (25 to 50 g) (based on published mean and standard deviation values) was reasonably good under most scenarios. In addition to estimating the overall effect of a mixture, WQS regression can also be used to identify chemicals that contribute the most to the outcome based on weights. In our simulated example with seven chemical exposures, the two chemicals that we set to be “bad actors” were correctly identified as the most important chemicals 65% and 48% of the time. Given the high possibility of WQS regression missing identification of important chemicals while incorrectly naming other chemicals as important, we have not placed much emphasis on the identification of important chemical contributors through WQS in our analyses. Further, if

identifying the most important chemicals within a mixture was our primary goal, we would likely want to use a variable selection method such as LASSO or elastic net regression.

Limitations in generalizability

One limitation of this research is that both diet and exposure to EDCs differ over time and place. For example, levels of certain chemicals are higher in the ALSPAC population than in contemporary populations, while other chemicals have increased in use since 1991-1992. The addition of MoBa data, collected roughly a decade later and in a different European country, allowed us to explore the feasibility of generalizing this dietary pattern outside of the ALSPAC population. Specific characteristics of populations might change the predominant sources of exposure.

Future Research

In conclusion, the results from this dissertation provide insight into 1) maternal diet as a source of persistent EDC exposure and 2) the impact of prenatal exposure to mixtures of persistent EDCs on growth and developmental outcomes. This work may begin to inform dietary guidelines for pregnant women and women considering pregnancy in order to minimize prenatal exposure to persistent EDCs. While animal products were associated with persistent EDCs in both cohorts, there was a lack of consistency across associations with specific animal-based food groups, and our results suggest that any attempt to provide guidance may need to be specific to a region's dietary preferences and sources of EDCs. Further, analyzing EDCs as a mixture allowed for the estimation of the overall effect of prenatal EDC exposure, as opposed to the previously limited single-chemical approach. Additionally, we were able to identify the most important class(es) of EDCs in regards to a given outcome. Both overall effect estimation and toxic agent identification have the potential to positively impact and improve maternal and child health.

Ideally, future studies in this area of research will examine more modern populations and next-generation persistent chemicals. Modern populations would likely have lower levels of certain legacy chemicals (e.g., PCBs and OCPs), but higher levels of "newer" chemicals that have replaced previous chemicals of concern (e.g., short-chain PFAS instead of long-chain PFAS). Similarly, it would be worthwhile to examine exposures in populations in parts of the world that have been less studied in regards to EDC exposure, such as Africa, South America, and parts of Asia. Patterns of chemical use and therefore chemical exposures in these populations may differ from those in well-studied European, North American, and East Asian populations.

Future studies, like aim 1, that hope to understand dietary exposures to persistent chemicals, and non-persistent chemicals especially, need a better way to assess exposure through packaging and preparation. For example, we saw an association of pizza with PFAS in ALSPAC (but not PCBs or OCPs), which we suspect is due to PFAS chemicals being found in nonstick packaging, but we cannot be sure this is the reason for the association. Future studies will want to discern whether foods are fast food, frozen meals, or packaged foods, and prepared in nonstick pans or plastic containers heated in the microwave, for example. Currently, few FFQs ask detailed questions about packaging and preparation methods, which could be important sources of EDCs exposure, particularly non-persistent chemicals.

Mixtures approaches, as used in aims 2 and 3, are a new frontier in environmental epidemiology. Future studies should take advantage of the current and developing methods to consider mixtures of environmental chemicals, as opposed to single chemicals, in relation to health outcomes. There are a number of outcomes that were studied under the single-chemical paradigm that should be re-examined using mixtures approaches. So far, mixtures approaches have been used in studies of prenatal exposure to EDCs and outcomes such as birth weight (253, 254), thyroid hormones (446), and neurodevelopment (447), but there are additional outcomes worthy of investigation using a mixtures approach, including childhood body fatness, genitourinary conditions like hypospadias, and markers of pubertal development. Moreover, leveraging the long

follow up of studies like ALSPAC, prenatal, childhood, and/or adulthood exposures could be studied as mixtures in conjunction with adult reproductive outcomes, such as daughters' fertility, pregnancies, and timing of menopause. In the long term, breast cancer would be an outcome of interest due to its endocrine-sensitive nature.

Additionally, there are a number of approaches to examining mixtures. In aims 2 and 3, this dissertation aimed to estimate the overall effect of mixtures on the outcomes of interest, but mixture methods can also be used to identify patterns of exposure, interactions among chemicals, or the most toxic agents in a mixture. While we did not see any evidence of interactions among EDCs in our studies, there may be interactions among different chemicals in other populations that would be worthy of exploration. Further, our study was limited in its ability to identify the most important chemicals within a mixture; future studies could use variable selection methods (LASSO or elastic net regression) to identify the chemicals within a mixture that contribute the most to the outcome.

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