THE ROLE OF FLAME RETARDANTS AS ENDOCRINE DISRUPTORS AND THEIR EFFECT ON ADVERSE PREGNANCY OUTCOMES

by

SKARLET GISSEL VELASQUEZ APLICANO

(Under the Direction of José F. Cordero)

ABSTRACT

Background: Preterm birth (gestational age less than 37 weeks), disproportionally impacts pregnancies in Puerto Rico. Organophosphate flame retardants (OPFRs) which are used as flame retardants and plasticizers have widespread exposure. Epidemiologic studies suggest that OPRFs have the capacity to disrupt growth and metabolism through endocrine-related mechanisms of action. The purpose of this dissertation is to (1) Identify knowledge gaps on the role of flame retardants on adverse pregnancy outcomes (APOs) and assess the current knowledge of this association; (2) Identify associations between prenatal exposure to OPFRs with gestational age and birth weight and (3) Identify associations between OPFRs and 9 maternal hormones. **Methods**: For aim 1, a research protocol was developed according to the PRISMA framework to conduct a systematic review on prenatal exposure to OPFRs and its effects on APOs with a focus on preterm birth and low birth weight. For aims 2 &3, data came from the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) pregnancy cohort. OPFRs (>70% LOD) were

measured in maternal urine and collected at two visits. Multivariable linear regression was used to examine the associations between average OPFR levels across 2 time points in pregnancy with gestational duration and birth weight. Linear mixed models were used to assess the effect of 8 OPFRs and 9 serum hormones measured over two time points during pregnancy. **Results:** The systematic review yielded 40 epidemiological studies. Of these studies, only 6 studies examined the associations between OPFRs and preterm birth/low birth weight and 4 studies examined other outcomes of interest **(Aim 1).** No associations were observed between OPFR metabolites and APOs **(Aim 2).** For maternal hormones**,** increased levels of FT4 were associated with BCPP (%Δ:0.25, 95% CI: 0.05, 0.45) and BDCPP (%Δ:0.24, 95% CI: 0.07, 0.41) A decrease in FT4 was associated with DPhP (%Δ: -0.21, 95% CI: -0.37, -0.06). DPhP was associated with a decrease in T4(%Δ: -0.16, 95% CI: -0.31, -0.01) **(Aim 3). Conclusions:** Findings from our study highlight the role of OPFRs as possible endocrine disruptors and the urgent need for more research on emerging flame retardants, such as OPFRs.

INDEX WORDS: Organophosphate esters, endocrine disruption, gestational exposures, preterm birth, pregnancy, Puerto Rico

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DEDICATION

First and foremost, I want to thank Jesus, my Lord and Savior, who makes all things possible. Yet, apart from Him, I can do nothing.

Mami, this would not have been possible without you. You are the reason I am who I am. Thank you for always doing your best and giving me your unconditional love and support. I will always do this for you.

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CHAPTER 1

INTRODUCTION

Project Narrative

Persistent organic pollutants (POPs) are toxic chemicals that adversely affect the health of humans and the environment[1]. There is an increasing public health concern that exposure, even at low-levels, may have adverse health impacts, particularly during fetal, neonatal, and childhood development. Many of these pollutants are considered endocrine disrupting chemicals (EDCs) that interfere with the body's endocrine system. Because of its abundance in the environment and concerns about its' impact on human health, the focus of this study is on one POP of interest: flame retardants, particularly Organophosphate flame retardants (OPFRs). Our study focuses on the role of OPFRs as endocrine disruptors and their effect on adverse pregnancy outcomes (APOs), with a focus on preterm birth (PTB) and low birth weight (LBW). This study also explores maternal hormones at different time points during pregnancy as a possible mechanism linking the effect of these OPFRs to PTB and LBW. Additionally, this study identifies current knowledge and knowledge gaps of the effect of OPFRs on adverse pregnancy outcomes. Results from our study may help to explain the role of exposure to OPFRs and their effects on APOs, a possible mechanism underlying this relationship, and add to the body of evidence of the effect of human exposure to these chemicals.

Specific Aims

Preterm birth (PTB), defined as birth before 37 completed weeks of gestation. is one of the leading causes of neonatal mortality in the world [2]. Babies born too early have higher rates of infant death and disability. Each year, March of Dimes, the nation's leader in maternal and child health, releases a report card that offers a comprehensive overview of the health of mothers and babies across the United States. The report grades the U.S. states, Puerto Rico and 100 cities on preterm birth rates, and includes other information such as infant death, social vulnerability, low-risk cesarean births, etc. According to the 2021 Report Card, the United States remains among the most dangerous developed nations for childbirth with a C- rating. Puerto Rico along with six states (Alabama, Arkansas, Louisiana, Mississippi, South Carolina, and West Virginia) received an F rating on the preterm birth grades. In 2021, the rate of preterm birth in Puerto Rico was 11.6%, higher than the national rate of 10.1%[3]. Although a variety of individual risk factors for preterm birth have been identified, the reasons for these high PTB rates are not completely understood.

Low birth weight (LBW) is defined as a baby born weighing less than 5 pounds, 8 ounces[4]. Having a low weight at birth can cause serious health problems for some babies. LBW is an established risk factor for numerous adverse health outcomes, including increased risk of neonatal and post neonatal morbidity and mortality in adulthood. LBW infants are up to 12 times more likely to die in the perinatal period and have up to a 3-fold higher risk for morbidity from a range of

childhood illnesses, with the risk of disease or death decreasing with increasing birth weight[5].

Gestational age is more predictive of risk of neonatal and childhood mortality than low birth weight, but preterm birth is more difficult to ascertain than birthweight. Although preterm birth is a major reason for a baby being born LBW, LBW is an imperfect surrogate for preterm birth. Mechanisms and risk factors for preterm and for LBW babies may differ despite a substantial proportion of LBW being contributed by preterm births as LBW infants are also a result of intrauterine growth restriction[6] . For this reason, as well as the higher than normal PTB rates in Puerto Rico, the outcomes of interest for this study are PTB and LBW.

Many environmental chemicals deserve investigation because of (1) prevalent exposures, (2) demonstrated reproductive toxicities in animal studies, (3) ability to cross the placenta, and (4) associations with other adverse birth outcomes that may result from related mechanisms[7]. In Puerto Rico, there are 16 superfund sites, mostly in the Northern Karst Region. On the island, the rate of preterm birth is particularly high. In 2011, the island shared the highest rate of preterm birth in the United States with Mississippi at 18%, which is also among the highest rates worldwide. By 2021, this had decreased significantly to 11.6%. However, Puerto Rico still ranks among the locations with higher rates of preterm birth, especially for highly developed countries, and worldwide[8]. Because of the high density of Superfund waste sites located on the island and unknown etiology of preterm birth, identifying contributing environmental contaminant exposures is a priority in the study of PTB and LBW in Puerto Rico.

Flame retardants are chemicals that are applied to materials to prevent the start or slow the growth of fire. They have been used in many consumer and industrial products since the 1970s, to decrease the ability of materials to ignite[9]. Although some of these chemicals, such as polybrominated diphenyl ethers (PBDEs) have been phased out of production, they remain persistent in the environment. PBDEs do not chemically bind to the products to which they are added, so they easily release from these products and get into the air and dust. Since PBDEs have been phased out, some organophosphate flame retardants (OPFRs) and other alternatives have been identified as replacements. OPFRs are used in consumer products such as residential and office furniture, baby products, and electronics. Additionally, they are also used as plasticizers in common consumer products such as nail polish. Human exposure to OPFRs is understood to be ubiquitous and multiple studies have detected OPFR metabolites in the United States and around the world[12]. Therefore, there is a growing concern about OPFRs and how they can affect maternal and child health during pregnancy. In humans, studies have shown that some flame-retardant substances travel through the placenta to the fetus and that PBDEs have been linked to adverse birth outcomes, including preterm birth[10]. Toxicological studies indicate that OPFRs may adversely affect human health, with findings suggesting developmental toxicity, endocrine disruption, and carcinogenicity[11].

It is hypothesized that flame retardant exposure contributes to PTB through endocrine disruption and so may interfere with growth and metabolism. In vitro, OPFRs interfere with the estrogen receptors (ERα and ERβ), androgen receptors (AR), glucocorticoid receptor (GR), pregnane X receptor (PXR), peroxisome

proliferator-activated receptor gamma (PPARγ), and mineralocorticoid receptor (MR), which, in turn, could potentially affect steroidogenesis, growth, development, and metabolic homeostasis[12]. Epidemiologic studies in humans of the endocrine-disrupting and reproductive effects of OPFRs are limited but suggest that OPFRs have the capacity to disrupt growth and metabolism through endocrine-related mechanisms of action. Carignan et al evaluated associations between urinary concentrations of OPFR metabolites and outcomes of in vitro fertilization (IVF) treatment. Their findings suggest that concentrations of some urinary OPFR metabolites are negatively associated with proportions of successful fertilization, implantation, clinical pregnancy, and live birth[13]. Most recently, a study evaluated associations of OPFRs with gestational weight gain (GWG), gestational age at delivery, infant anthropometry, and infant feeding behaviors. Their findings suggest that select OPFRs may affect infant anthropometry and feeding behavior, with the strongest effects observed for BDCPP and DPHP[12].

Very few studies have examined the relationship between flame retardant exposure and adverse pregnancy outcomes. To the best of our knowledge, ours is one of the first studies that looks at exposure of OPFRs in pregnant women and its effect on adverse pregnancy outcomes in Puerto Rico. The primary goal for this dissertation is to identify knowledge gaps on the role of flame retardants on APOs, examine how exposure to OPFRs during pregnancy may contribute to PTB and LBW, and to examine one mechanism through which this possibly occurs. To accomplish this goal, we will use data from the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT), an ongoing prospective cohort study designed to examine

environmental risk factors for PTB in the Northern Karst region of Puerto Rico. We will accomplish the following specific aims:

Aim 1: Identify knowledge gaps on the role of flame retardants on adverse pregnancy outcomes

Sub-aim 1a: Describe the biological mechanism of flame retardants as endocrine disruptors

Sub-aim 1b: Assess the current knowledge of the effects of flame retardants on adverse pregnancy outcomes

Aim 2: Examine associations between prenatal exposure to OPFRs with gestational age and birth weight using the PROTECT cohort

Aim 3: Examine associations between OPFRs and maternal hormones using the PROTECT cohort

CHAPTER 2

LITERATURE REVIEW

Adverse pregnancy outcomes as a significant public health threat

Preterm birth is a global public health concern. Preterm birth is the leading cause of death in children younger than 5 years worldwide.[14] Complications of preterm birth are the single largest direct cause of neonatal deaths, responsible for 35% of the world's 3.1 million deaths a year. Although over 60% of preterm births occur in low to middle income countries, data suggests that high income countries have increasing rates of preterm birth as well.[14] Although the preterm birth rate declined from 10.2% in 2019 to 10.1% in 2020[15], the United States remains among the top 10 countries with the highest numbers of preterm births.[16] Of the 6 million pregnancies in the United States each year, approximately 2.2 million end with miscarriage or stillbirth or are voluntarily terminated, about a half million babies are born prematurely, and about 120,000 babies have birth defects.[17]

Besides preterm birth, low birth weight is also an important determinant of child health as it is associated with greater risk of death, poor health, and disabilities. It is estimated that almost 15% of all births worldwide are low birth weight, representing around 20.5 million births in 2015.[18] Our knowledge and understanding of what causes adverse pregnancy outcomes has greatly increased over the past decades. However, there is still much that is not known. In particular, the role of

environmental exposures in reproductive and infant health is complex and not largely understood[19].

Overview of Preterm Birth

Preterm birth is defined as a birth <37 weeks' gestation.[2] In 2020, preterm birth affected 1 of every 10 infants born in the United States.[15] Preterm birth can be further sub-divided based on gestational age: extremely preterm (<28 weeks), very preterm (28 - <32 weeks) and moderate preterm (32 - <37 completed weeks of gestation).[20] PTB due to preterm labor with cervical dilation or preterm rupture of membranes is classified as "spontaneous." A medically "indicated" preterm birth is when there is induced labor or in which the infant is delivered by cesarean section for maternal or fetal illness. Spontaneous preterm birth is a multi-factorial process, resulting from the interplay of factors causing the uterus to change from quiescence to active contractions and to birth before 37 completed weeks of gestation.[21] The cause of spontaneous preterm labor remains mostly unidentified, however, there are some strong risk factors. Some of these risk factors include age at pregnancy and pregnancy spacing, underlying maternal chronic medical conditions, lifestyle/work related factors, maternal psychological health, and genetics as well as environmental factors.[22] There is an overlap of these risk factors with medically indicated preterm birth. This splitting of PTB phenotypes is one attempt to separate distinct pathophysiologic pathways and patients who may benefit from different prediction, prevention, and treatment strategies.[23]

Overview of Low birth weight (LBW)

Low birth weight is defined as weight at birth < 2500 grams (5.5 pounds).[24] Low birth weight is further categorized into two categories: very low birth weight, (<1500 g) or extremely low birth weight (<1000 g).[24] Low birth weight is a result of preterm birth, intrauterine growth restriction (IUGR), or both.[25] The underlying causes of both PTB and IUGR are multifactorial, and the biological pathways and preventive strategies for these two conditions are quite different. At the population level, the proportion of infants with a low birth weight is an indicator of a multifaceted public health problem that includes long-term maternal malnutrition, ill-health, and poor health care in pregnancy. Neonates with low birth weight have a >20 times greater risk of dying than neonates with birth weight >2500 g. Low birth weight infants are more likely to have long-term neurologic disability, impaired language development, impaired academic achievement, and increased risk of chronic diseases including cardiovascular disease and diabetes.[25] There are also epidemiological factors associated with LBW. Some of these include African American race, age, socioeconomic status, and medical and obstetric risks. These medical and obstetric risks include hypertension/preeclampsia, diabetes, obstetric history, multiple pregnancies, infections, nutrition, behavioral and environmental risks.

Persistent Organic Pollutants

According to the United Nations Environment Programme, persistent organic pollutants are chemicals which have a particular combination of physical and chemical properties such that, once released into the environment, they (1) remain

intact for exceptionally long periods of time, (2) become widely distributed throughout the environment as a result of natural processes involving soil, water, and, most notably air, (3) accumulate in the fatty tissue of living organisms including humans and are found in higher concentrations at higher levels in the food chain, and (4) are toxic to both humans and wildlife.[26] Because they can be transported by wind and water, most POPs in one country can and do affect people and wildlife far from where they are used and released. Due to their toxicity and persistence, several chemicals are targeted by the Stockholm Convention. The Stockholm Convention is a multilateral treaty overseen by the United Nations Environment Programme that mandates that parties who have signed must take administrative and legislative actions to prevent the environmental impacts that POPs pose, both within their jurisdiction and in the global environment. Of interest to this study, several families of brominated flame retardants (BFRs) have been listed as POPs in this convention.[27]

Flame retardants

Flame retardants are chemicals that are applied to materials to prevent the start or slow the growth of fire. They have been used in many consumer and industrial products since the 1970s to decrease the ability of materials to ignite.[9] There are hundreds of different flame retardants. They are often broken down into categories based on chemical structure and properties. In general, flame retardants are grouped based on whether they contain bromine, chlorine, phosphorus, nitrogen, metals, or boron. The National Institute of Environmental Health Sciences (NIEHS) breaks them down into the categories in Table 2.1[9]:

Table 2.1: Flame retardant categories adapted from NIEHS[9]

Category	Description
Brominated flame retardants	• Contain bromine and are the most abundantly used flame retardants • Used in consumer goods such as electronics, furniture, building materials etc.
Tetrabromobisphenol A	• Widely used to make computer circuit boards and electronics • Also used in some textiles and paper • Used as an additive in other flame retardants
Hexabromocyclododecane (HBCD)	• Additive primarily used in polystyrene foam building materials • Primary risk to humans is from leaching out of products and getting into indoor dust • Low levels of HBCD have also been found in some food products
Polybrominated diphenyl ethers (PBDE's)	• Do not chemically bind with the products to which they are added so they easily release and enter air and dust • PBDEs have been found to have associations with lower birth weight/length of children, and impair neurological development
Organophosphate flame retardants (OPFRs)	• Emerging flame retardants due to the phasing out of PBDEs

Widespread exposure to flame retardants

People can be exposed to flame retardants through a variety of ways, including diet, consumer products in the home, car, airplane, and workplace, and household dust. These chemicals can also get into the air, water, and soil during

manufacture. They can also leak from products into dust and into the air. If the contaminated dust gets on the hands, it can then get onto food and into the mouth when food is eaten. Additionally, exposure can happen from uncontrolled burning and dismantling of electronic and electric waste. Though most people are exposed to flame retardants, there are some groups that are more vulnerable to these exposures. Two of these groups are pregnant women and children. Pregnant women are vulnerable due to prenatal exposure having lasting detrimental impacts on children and can cause diseases that show up in childhood. Infants and young children are believed to have a higher exposure to flame retardants when compared with adults because they spend more time indoors, in close proximity to contaminant sources, and engage in frequent hand-to-mouth contact.[28]

Organophosphate Flame retardants (OPFRs)

Due to the environmental and health concerns of brominated flame retardants, such as polybrominated diphenyl ethers (PBDEs), organophosphorus flame retardants (OPFRs) emerged. They are used as flame retardant additives in plasticizers, foams, hydraulic fluids, anti-foam agents, coatings for electronic components/devices, furniture, textile, electronics, construction, vehicle, and petroleum industries. The increased use of OPFRs has caused concerns regarding their adverse effects on the environment, animal, and human health. In addition, studies have shown that indoor air and dust have concentrations of OPFRs higher than that of PBDEs.[29] Because OPFRs are frequently present as additives rather than chemically bonded to materials, they are easily released to different environmental compartments via volatilization,

leaching and/or abrasion.[28] A major concern is the potential carcinogenic, neurological effects, and endocrine disruption of these compounds.[29] Another issue of concern is that many of these OPFRs were rapidly marketed due to the need for rapid PBDE substitution, however, their environmental behavior and toxicological effects were not properly assessed.[28]

OPFRs as endocrine disruptors

The endocrine system regulates key interrelated functions in the body, including reproduction, early development, as well as metabolic and neurologic processes. Chemicals or chemical mixtures that can cause adverse health effects by perturbing any aspect of hormone action are defined as endocrine disrupting chemicals (EDCs).[30] The endocrine system is composed of glands of an organism that secrete hormones directly into the circulatory system which are carried to a distant target organ. The major endocrine glands include the pineal gland, pituitary gland, pancreas, ovaries, testes, thyroid gland, parathyroid gland, gastrointestinal tract, adrenal glands, and hypothalamus. EDCs cause endocrine diseases that are characterized by dysregulated hormone releases of the endocrine system.[31] UNEP has established a list of 45 EDCs or potential EDCs by reviewing evidence from multiple sources, a list that includes some flame retardants. However, it is likely that this list is incomplete due to many more chemicals showing endocrine disrupting activities in humans and experimental animals.

Replacement flame retardants, such as OPFRs, are a good example of chemicals of emerging concern. After the banning of PBDEs, there has been an

increase of replacement flame retardants to assure compliance with flammability standards. Organic flame retardants used as replacements include brominated, organophosphate, and chlorinated (some of which are also OPFRs) flame retardants.[29] As a result, the replacement flame retardants are now detected in indoor air and dust, in the environment, biota, the food chain, as well as human samples.[31] Although there is clear evidence of increasing population exposure, there is little or no information regarding the effects on human health for many replacement flame retardants, but the few studies available suggest that some of them can induce adverse outcomes.[31]

Biological Mechanism of EDCs

EDCs may act on nuclear receptors by mimicking the endogenous hormone and activating the receptor (agonists), or by inhibiting the effect of the hormone (antagonists).[32] Nuclear receptors are ligand-inducible transcription factors that specifically regulate the expression of target genes involved in metabolism, development, and reproduction.[32] Their primary function is to mediate the transcriptional response in target cells to hormones, such as the sex steroids, adrenal steroids, vitamin D3, and thyroid and retinoid hormones, in addition to a variety of other metabolic ligands. Forty-eight nuclear receptors are known to exist in humans, and these proteins comprise the single largest family of metazoan transcription factors, the nuclear receptor superfamily. Experimental studies suggest that PBDEs affect transactivation via nuclear receptors such as estrogen receptors (ERs), androgen receptors (AR), and pregnane X receptor (PXR). For OPFRS, it remains unclear whether this toxin has any potential nuclear receptor activity. However,

studies show that OPFRs are endocrine-disrupting compounds that may interfere with these receptors. In vitro, OPFRs interfere with the estrogen receptors (ERα and ERβ), androgen receptor (AR), glucocorticoid receptor (GR), pregnane X receptor (PXR), peroxisome proliferator-activated receptor gamma (PPARγ), and mineralocorticoid receptor (MR), which, in turn, could potentially affect steroidogenesis, growth, development and metabolic homeostasis.[12] Several studies have reported sex-dependent effects of OPFR exposure on the hypothalamic-pituitary-gonad (HPG) axis. For example, adult zebrafish exposed to TDCPP for 14 days showed elevated serum levels of estradiol (E2) and testosterone (T) in both males and females. For males, the E2/T ratio was slightly elevated, while females showed an E2/T ratio decrease. Changes in serum hormone levels corresponded with increased mRNA expression of CYP17 and CYP19A, enzymes involved in sex steroid synthesis.[33] In addition, TDCPP has been implicated in dysregulation of the thyroid hormone system. In one human epidemiological study, high concentrations of TDCPP in house dust were associated with decreased T4 levels in a cohort of men.[34] Various classes of hormones are potential targets for OPFR disruption and could subsequently have negative effects on pregnancy as described below:

Hormones

Thyroid hormones*:* Thyroid hormone balance is important for maintaining normal physiological processes in humans and its disruption may bring adverse effects on human health. During pregnancy, maternal thyroid hormone balance is an important

factor for normal fetal development especially during early pregnancy when the fetal thyroid gland is not mature.[35] This requires an increase in maternal thyroid hormone production to provide for both the mother and baby. The thyroid gland releases T4, which circulates in blood bound to thyroxin-binding globulin. It is measured in its unbound fraction (free T4) and in total quantities (total T4). Once free T4 reaches target tissues, local enzymes convert it into its biologically active form, triiodothyronine (T3). Thyroid-stimulating hormone (TSH) stimulates the thyroid to produce more T4 when levels drop, and it is regulated directly and indirectly through levels of T4 and T3.[36] Clinically, both maternal hypothyroidism and hyperthyroidism during pregnancy have been associated with a small, but significant increased risk of preterm birth.[37] In vitro, tests showed that exposure to OPFRs may result in agonistic activity to thyroid hormone nuclear receptors and enhancement of thyroxine (T4) binding to human transthyretin, which all lead to thyroid balance disruption. Wang et al. showed that treating zebrafish with tris(1,3-dichloro-2-propyl) phosphate, the parent compound of BDCIPP, caused decreased transcription of genes related to the hypothalamic-pituitary-thyroid axis.[38] In contrast, limited epidemiological data are available in human studies. Urinary DPHP was found to be associated with an increase in total T4, which was less significant in males.[36] Another study in China investigated these associations in pregnant women and newborns. They observed associations between maternal urinary DNBP levels during pregnancy and increased TSH concentrations in newborns and also between cross-sectional maternal urinary DPHP levels during pregnancy and maternal TSH levels.[39] Growing evidence suggests the transplacental potential of OPFRs and their binding affinity to

transthyretin contributes to this process. This may in consequence disrupt normal transplacental processes of thyroid hormones. The causation for the observed associations can be complex but the results emphasized the links between OPFR exposure and thyroid hormone balance during the gestational period, when fetal development is susceptible to consequences of hormone disruption. PBDEs, which are structurally similar to thyroid hormones, are reported to interfere with thyroid hormone signaling by altering the binding of hormones to thyroid receptors.[38]

Testosterone*:* During the first 9 weeks of pregnancy the corpus luteum and, to a lesser extent, the maternal ovary and the adrenal cortex, contribute to circulating concentrations of maternal estradiol, estrone, and progesterone. After this period, the placenta becomes the predominant source of maternal steroids.[40] Women with polycystic ovarian syndrome or other hyperandrogenic conditions that are characterized by higher levels of testosterone have been shown to be associated with preterm birth.[41] Elevated testosterone levels are associated with in utero growth restriction, development of gestational diabetes, and preeclampsia.[41]

Progesterone and estriol: Progesterone is largely produced by the corpus luteum until about 10 weeks of gestation.[35] In early pregnancy, the maternal levels of 17 a-hydroxyprogesterone rise, marking the activity of the corpus luteum. Progesterone is important in suppressing the maternal immunologic response to fetal antigens, thereby preventing maternal rejection of the trophoblast. It is an essential hormone in the process of reproduction as it induces secretory changes in the lining of the uterus

and is essential for a successful implantation of the embryo.[35] Several studies have used progesterone and related steroids in an attempt to prevent spontaneous miscarriage and to increase the embryo implantation rates in assisted reproduction programs.[40] Similarly, a major estrogen formed in pregnancy is estriol. Estriol is not secreted by the ovary of non-pregnant women, but it makes up more than 90% of the estrogen in the urine of pregnant women.[42] Many EDCs display estrogenic activity and interfere with normal estrogen signaling, which is mediated by two estrogen receptors (ERs): Era and ERb. EDCs that target ER signaling can modify genomic and nongenomic ER activity through direct interactions with ERs, indirectly through transcription factors such as the aryl hydrocarbon receptor (AhR), or through modulation of metabolic enzymes that are critical for normal estrogen synthesis and metabolism.[43]

Corticotropin releasing hormone (CRH): CRH plays a key role in feto-maternal communication by orchestrating and integrating a series of neuroendocrine, immune, metabolic, and behavioral responses. It also regulates neural networks involved in maternal behavior and this determines efficiency of maternal care and neonate interactions.[44] CRH is thought to play a major role in the timing of labor and has been shown to be associated with preterm birth in human studies.[41]

CHAPTER 3

DISSERTATION METHODS

Study Population

Data for this dissertation comes from the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) cohort. PROTECT study participants were recruited in Puerto Rico's North Karst area, at two collaborating hospitals, Manati Medical Center, and Arecibo's Cayetano Coll y Toste Hospital, and five health clinics located in Camuy, Lares, Morovis, Quebradillas and Ciales.[45] Eligible participants were pregnant women receiving prenatal care services at participating clinics or hospitals with a gestational age of less than 20 weeks. Furthermore, participants had to intend to deliver at one of the two collaborating hospitals, have an age of 18 - 40 years old, reside in a municipality in the northern Karst region of the island, did not use oral contraceptives for at least 3 months prior to becoming pregnant, did not use in vitro fertilization to become pregnant, and were free of known medical or obstetrical complications, including diabetes. At the time of enrollment, an initial screening form collecting demographic characteristics and to estimate date of last menstrual period was collected. Following this, women were invited to participate in three study visits. These visits targeted for 20 ± 2 weeks, 24 ± 2 weeks, and 28 ± 2 weeks gestation. During these visits, detailed data on medical, social, and environmental factors were collected as well as biological samples. At delivery, pregnancy outcomes along with birth weight and other newborn measurements were also collected. All women provided informed consent and the Institutional Review

Board at the University of Puerto Rico, University of Georgia, Northeastern University, and University of Michigan approved the PROTECT study.

Women were included in the analytic sample for the second aim if they had complete flame retardant measurements for visit 1 and 3 and complete birth outcome data. The analytic sample for the third aim included pregnant women with all flame retardant measurements and at least one hormone measurement (Visit 1 or Visit 3).

Aim 1

Objective

Identify knowledge gaps on the role of flame retardants on APOs, describe the biological mechanism of flame retardants as endocrine disruptors, and assess the current knowledge of the effects of flame retardants on adverse pregnancy outcomes

METHODS

Study Design

A research protocol was developed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework

Search Strategy

A search strategy was developed with the advice of a professional librarian. We conducted a systematic literature search in PubMed, Embase, Web of Science, and Cochrane for studies describing the association of flame retardants (particularly OPFRs and Polybrominated Diphenyl Ethers) and PTB as well as LBW. Search

themes were combined using the Boolean operators ('AND', 'OR', and '*'). The Medical Subject Headings (MeSH) 'Organophosphates', 'Flame retardants', 'endocrine disruptors', 'Premature Birth', 'Infant, Low Birth Weight' were combined for the searches. All flame retardants measured in the PROTECT cohort were included in the search strategy (Table 3.1). The complete search term for each database is detailed in Appendix A.

Inclusion Criteria

For the purpose of this review, only empirical and epidemiological studies were assessed for inclusion. The included articles must be peer reviewed studies. Studies involving pregnant women assessed at any stage of pregnancy and pregnant women and offspring dyads were considered eligible. In order to measure flame retardant exposure, it must have been measured individually during pregnancy, using validated bio monitoring methods.

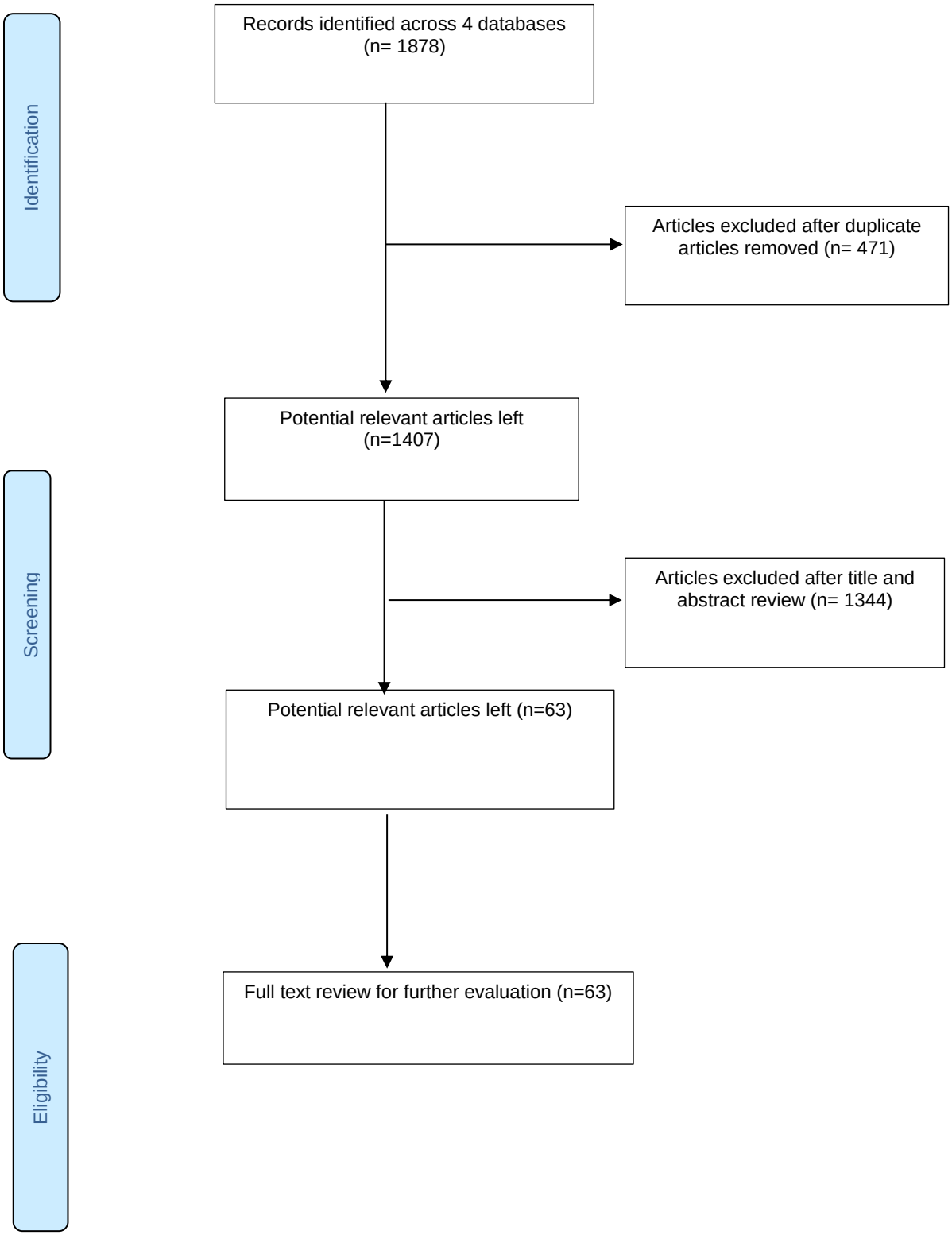
Exclusion Criteria

Studies were excluded if they did not have quantitative exposures, were performed on animals, and were not written in English.

Study Selection Process

Endnote software was used for the management of the articles and to de-duplicate before the review was started. Two investigators (S.M. and A.G.) independently screened the titles and abstracts of potential articles. Discrepancies were resolved by two investigators (S.V. and M.C.). Four authors (A.G., Y.B., and A.P.) independently examined the full text articles and extracted the necessary information using a pre-specified form that includes information such as type of flame retardant and concentration, level of detection, date of publication, study design, outcomes and types of measurement, participant demographics, as well as information for risk of bias assessment. Studies that did not meet the defined eligibility criteria were excluded and the reason for exclusion was recorded. The number of articles retrieved, as well as the number of studies excluded, were documented by a flow diagram of the study selection process (Figure 1). Included studies were assessed for quality and data abstraction.

Figure 3.1: Preliminary Study Selection Process



Strategy for data synthesis

During preliminary searches, we observed that there are only a small number of studies that examine the primary exposure-outcome relationship of interest. Therefore, we did not foresee being able to conduct a meta-analysis. A narrative synthesis of the findings of the included studies was conducted structured around the main exposures and outcomes.

Outcomes

The primary outcomes of interest are preterm birth and low birth weight. Preterm birth is defined as gestational age <37 weeks. Low birth weight is defined as an infant born weighing less than 5lbs 8oz or 2500 grams. Other adverse pregnancy outcomes will include fetal deaths, early pregnancy losses, and spontaneous abortions.

Measures of Effect

The effect measures examined will depend on the study design and how the analysis was conducted. All effect measures will be examined as long as the main outcomes follow the definitions stated above.

Quality Assessment

Each article will be rated for methodological bias by two individual reviewers using the Newcastle-Ottawa Scale. Disagreements will be resolved via consultation

with a third reviewer. The Newcastle-Ottawa Scale (NOS) is an ongoing collaboration between the Universities of Newcastle, Australia and Ottawa, Canada. The NOS assigns up to a maximum of nine points for the least risk of bias in three domains: 1) selection of study groups (four points); 2) comparability of groups (two points); and 3) ascertainment of exposure and outcomes (three points) for case–control and cohort studies, respectively[46].

Aim 2

Objective

Identify associations between prenatal exposure to OPFRs with gestational age and birth weight in the PROTECT cohort

Hypothesis

We hypothesize that women who have higher exposure levels to OPFRs will have decreased gestational age and lower birth weight

Methods

Exposure

The main exposures for this aim are 8 OPFR metabolites measured in the PROTECT cohort listed in **Table 3.1**

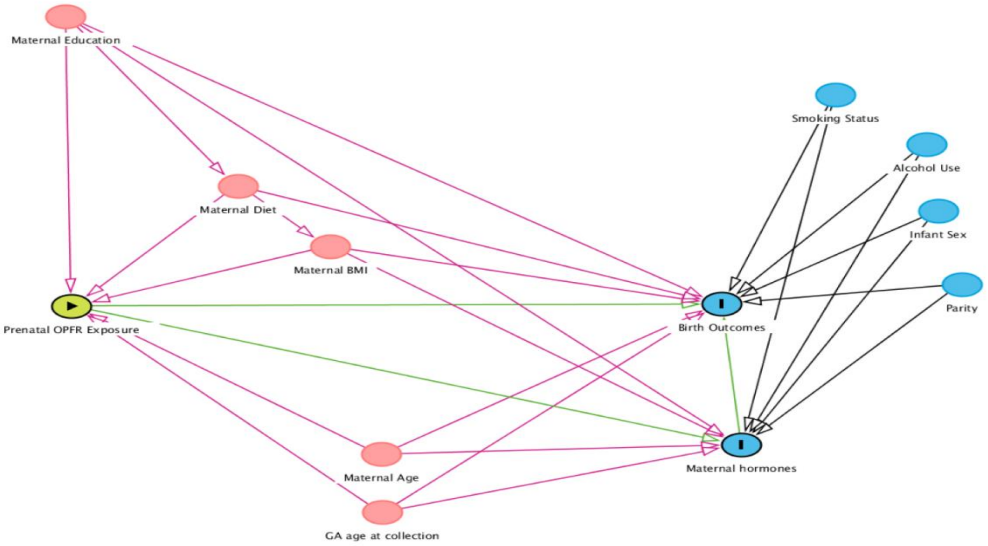
Outcome

Our outcomes of interest are gestational age and birthweight z-scores.

Table 3.1: Flame retardants measured in PROTECT

Abbreviation	Metabolite Name
BCEtP	bis(2-chloroethyl) phosphate
BCPP	bis(1-chloro-2-propyl) phosphate
BDCPP	bis(1,3-dichloro-2-propyl) phosphate
DBuP	di-n-butyl phosphate
DBzP	di-benzyl phosphate
DCP	di-cresyl phosphate
DPhP	Diphenyl phosphate
TBBA	2,3,4,5-tetrabromobenzoic acid

Figure 3.2: Directed Acyclic Graph for Aims 2 & 3



Covariates

Maternal education, maternal age, marital status, smoking status, alcohol use, employment status, parity, infant sex, and pre-pregnancy weight were included as potential covariates in our analyses. We used a directed a directed acyclic graph (DAG) to select confounders that were not causal intermediates and associated with both OPFR concentrations and birth outcomes (Figure 3.2). Covariates included in the final adjusted model were determined by both a priori knowledge and bivariate association with any of the outcomes.

Table 3.2: Coding of covariates for Aims 2 & 3

Covariate	Variable Type	Coding
Maternal education	Categorical	< high school diploma or GED Some college or a two-year degree Bachelor's degree Some graduate school or
Maternal age, years	Categorical	18-24 25-29 30-34 35+
Marital Status	Categorical	Married Living Together Single
Smoking Status	Categorical	Yes No
Alcohol Use	Categorical	Before Pregnancy Currently Drinks Does not drink
Employment Status	Categorical	Unemployed Currently employed
Parity	Categorical	Yes No
Infant Sex	Categorical	Male Female
Pre-pregnancy Weight (Lbs.)	Continuous	Number

Statistical Analysis

The distributions of covariates were described using frequencies and percentages for categorical variables, or median and IQR for continuous variables.

The distributions of all concentration measurements were described using the minimum, median, maximum, IQR, geometric mean, and 95% confidence intervals for the geometric mean. All concentrations were reported with limits of detection (LODs) and the percentage of concentrations for each LOD which were below the limit of detection was calculated.

Additionally, flame retardant concentrations were adjusted for specific gravity using the formula

$$C_{adj} = C\left(\frac{\text{median(SG)} - 1}{\text{SG}_i - 1}\right),$$

where C_{adj} is the specific gravity adjusted biomarker concentration, C is the unadjusted biomarker concentration, median (SG) is the median of all specific gravity samples, and SG_i is an individual's specific gravity measurement.[47] All specific gravity adjusted flame retardant concentrations were assumed to be drawn from a log-normal population and were thus log-transformed to correct for skewness in their distributions. Only OPFRs with 70% of measurements above the limit of detection were used for analysis. We fit linear models for gestational age and birthweight by covariates and average OPFR concentrations. The response variables are only measured at one time point, so a repeated measures model cannot be used.

Aim 3

Objective

Investigate associations between OPFRs and maternal hormones in the PROTECT Cohort

Hypothesis

We hypothesized that exposure to OPFR metabolites will result in significant changes in maternal hormone concentrations. Due to different windows of susceptibility and influence by fetal physiology, there will be unique associations at each study visit and between fetal sexes.

Methods

Exposure

The main exposures for this aim, OPFRs, have been previously described within the methods section for aim 2

Outcome

Our main outcomes of interest are the following maternal hormones: Corticotrophin-releasing hormone (CRH), estriol, sex hormone binding globulin (SHBG), progesterone, thyroid-stimulating hormone (TSH), total triiodothyronine(T3), free thyroxine(T4), total thyroxine(T4), and testosterone

Covariates

Covariates included in Aim 3 were the same as those included in Aim 2. We used a directed a directed acyclic graph (DAG) (Figure 3.2) to select confounders that were not causal intermediates and associated with both OPFR concentrations and maternal hormones. Education status, age range, smoking status, alcohol use, current employment status, parity, marital status, infant sex, and pre-pregnancy weight were included as potential covariates in our analyses. Covariates included in the final adjusted model were determined by both a priori knowledge and bivariate association with any of the outcomes. A detailed description of all included covariates is available on Table 3.2 within the methods section of Aim 2.

Statistical Analysis

The distributions of covariates were described using counts and frequencies for categorical variables, or median and IQR for continuous variables. The distributions of all concentration measurements were described using the minimum, median, maximum, IQR, geometric mean, and 95% confidence intervals for the geometric mean. Several of the maternal hormone concentrations appeared to follow a log-normal distribution. To maintain consistency with the treatment of each outcome, all maternal hormone concentrations were log-transformed. All concentrations were reported with limits of detection (LODs) and the percentage of concentrations for each LOD which were below the limit of detection was calculated.

Additionally, flame retardant concentrations were adjusted for specific gravity using the formula

$$C_{adj} = C\left(\frac{\text{median(SG)} - 1}{\text{SG}_i - 1}\right),$$

where C_{adj} is the specific gravity adjusted biomarker concentration, C is the unadjusted biomarker concentration, median (SG) is the median of all specific gravity samples, and SG_i is an individual's specific gravity measurement.[47] All specific gravity adjusted flame retardant concentrations were assumed to be drawn from a log-normal population and were thus log-transformed to correct for skewness in their distributions. Only OPFRs with 70% of measurements above the limit of detection were used for analysis. To assess whether hormone or OPFR concentrations varied across the two visit dates, Welch's paired 2-sample t-test was used to compare the log-transformed concentrations at Visit 1 for each individual with the log-transformed concentrations at Visit 3 for each interval. The p-values were adjusted using the method of Benjamini and Hochberg[48] to maintain a false discovery rate of 5%. We fit linear mixed effects models to estimate fixed effects for independent variables while accounting for between-subject variability with random intercepts for each subject.

CHAPTER 4

FLAME RETARDANTS AND ADVERSE PREGNANCY OUTCOMES: A REVIEW OF EPIDEMIOLOGICAL LITERATURE[1]

[1]Velasquez, SG., Claridy, MD., Roberson, C., Goldring, A., Bouchi, Y., Pak, A., Hammerton, SM., Knight, JH., Rathbun, SL., Meeker, JD., Cordero, JF.

To be submitted to Environment International.

Abstract

Purpose of Review- Flame retardants are chemicals that are applied to materials to prevent the start or slow the growth of fire. They have been used in many consumer and industrial products since the 1970s to decrease the ability of materials to ignite.[9] Since PBDEs have been phased out, some organophosphate flame retardants (OPFRs) and other alternatives have been identified as replacements. The increased use of OPFRs has caused concerns regarding their adverse effects on the environment, animal, and human health. Pregnant women and children are especially vulnerable to these exposures due to prenatal exposure having lasting detrimental impacts on children and infants engaging in frequent hand-to-mouth contact as well as spending more time indoors.

Objectives: The objective of this systematic review was to summarize the epidemiologic current literature on OPFRs and adverse pregnancy outcomes, with a focus on preterm birth and low birth weight. A secondary objective of this review was to summarize these same associations with PBDEs, a predecessor of OPFRs.

Methods: A systematic review was conducted using PubMed, Web of Science, EMBASE, and COCHRANE databases up to September 1, 2021. Published cohort, cross-sectional, and case-control studies exploring the relationship between OPFRs, and adverse pregnancy outcomes were included.

Results: In total, 40 epidemiological studies meeting the pre-determined inclusion criteria were included. In addition to preterm birth and low birth weight, other

plausible adverse pregnancy outcomes associated with OPFR exposure include spontaneous abortion, pregnancy loss, adverse IVF outcomes, and fetal chromosome abnormalities. These studies conducted in adult cohorts suggest that OPFRs may be endocrine disruptors, but the results are mixed. Continuing studies on PBDEs still reveal adverse associations on pregnancy outcomes and show that PBDEs remain an important public health problem even though it has been over a decade after its removal from the market.

Summary- A growing body of evidence demonstrates that OPFRs are associated with preterm birth and low birth weight, as well as other adverse pregnancy outcomes, but the results are inconsistent. PBDEs continue to reveal adverse associations on pregnancy outcomes. Still, additional research is urgently needed to elucidate the full impact of OPFRs on birth outcomes.

Introduction

Flame retardants are chemicals that are applied to materials to prevent the start or slow the growth of fire. They have been used in many consumer and industrial products since the 1970s to decrease the ability of materials to ignite.[9] There are hundreds of different flame retardants. They are often broken down into categories based on chemical structure and properties. In general, flame retardants are grouped based on whether they contain bromine, chlorine, phosphorus, nitrogen, metals, or boron. The National Institute of Environmental Health Sciences (NIEHS) breaks them down into the following categories[9]:

Table 4.1: Flame retardant categories adapted from NIEHS[9]

Category	Description
Brominated flame retardants	• Contain bromine and are the most abundantly used flame retardants • Used in consumer goods such as electronics, furniture, building materials etc.
Tetrabromobisphenol A	• Widely used to make computer circuit boards and electronics • Also used in some textiles and paper • Used as an additive in other flame retardants
Hexabromocyclododecane (HBCD)	• Additive primarily used in polystyrene foam building materials • Primary risk to humans is from leaching out of products and getting into indoor dust • Low levels of HBCD have also been found in some food products
Polybrominated diphenyl ethers (PBDE's)	• Do not chemically bind with the products to which they are added so they easily release and enter air and dust • PBDEs have been found to have associations with lower birth weight/length of children, and impair neurological development
Organophosphate flame retardants (OPFRs)	• Emerging flame retardants due to the phasing out of PBDEs

Widespread exposure to flame retardants

People can be exposed to flame retardants through a variety of ways, including diet, consumer products in the home, car, airplane, workplace, and house dust. These chemicals can also get into the air, water, and soil during manufacture. They can also leak from products into dust and into air. If the contaminated dust gets on the hands, it can then get onto food and into the mouth when food is eaten. Additionally, exposure can happen from uncontrolled burning and dismantling of electronic and electric waste. Though most people are exposed to flame retardants, there are some groups that are more vulnerable to these exposures. Two of these

groups are pregnant women and children. Pregnant women are vulnerable due to prenatal exposure having lasting detrimental impacts on children and can cause diseases that show up in childhood. Infants and young children are believed to have a higher exposure to flame retardants when compared with adults because they spend more time indoors, in close proximity to contaminant sources, and engage in frequent hand-to-mouth contact.[28]

Organophosphate Flame retardants (OPFRs)

Due to the environmental and health concerns of brominated flame retardants, such as polybrominated diphenyl ethers (PBDEs), organophosphate flame retardants (OPFRs) emerged. They are used as flame retardant additives in plasticizers, foams, hydraulic fluids, anti-foam agents, coatings for electronic components/devices, furniture, textile, electronics, construction, vehicle, and petroleum industries. The increased use of OPFRs has caused concerns regarding their adverse effects on the environment, animal, and human health. In addition, studies have shown that indoor air and dust had concentrations of OPFRs higher than that of PBDEs.[29] Because OPFRs are frequently present as additives rather than chemically bonded to materials, they are easily released to different environmental compartments via volatilization, leaching and/or abrasion.[28] A major concern is the potential carcinogenic, neurological effects, and endocrine disruption of these compounds.[29] Another issue of concern is that many of these OPFRs were rapidly marketed due to the need of rapid PBDE substitution, however, their environmental behavior and toxicological effects were not properly assessed.[28]

Polybrominated Diphenyl Ethers (PBDEs)

Polybrominated diphenyl ethers (PBDEs) are a class of recalcitrant and bio accumulative halogenated compounds that have emerged as a major environmental pollutant used since the 1960s.[49] PBDEs are synthetic compounds used in additives to retard fire and flames in a variety of commercial and household products and resist degradation in the environment. They were added to consumer products including furniture, children's products, and electronics.[29] After extensive research showed that PBDEs were persistent, bio accumulative, and toxic, in 2004 the European Commission and California banned the use of Penta- and OctaBDE, two mixtures primarily used in North America.[29] Additionally, in 2004, the U.S Environmental Protection Agency (US EPA) negotiated a phase-out of new production of these two PBDE commercial mixtures with US manufacturers. Penta- and OctaBDE were added to the Stockholm Convention in 2009, prompting more than 150 signatures to legislate their phase out. DecaBDE was added to the Stockholm Convention in 2017 and similarly phased out of use in most countries. Unfortunately, old furniture, electronics, vehicles, and other products containing PBDEs continue to be used and reused. As the use of PBDEs is declining due to regulatory action, the use of OPFRs is increasing. OPFRs production has increased. It is estimated that by 2019 the annual production of global FRs was six billion pounds, and phosphorus-based flame retardants accounted for 16% of the global market share. In 1992, the total consumption of OPFRs worldwide was only 100,000 tons, while the consumption in

2011 was 500,000 tons and in 2015 it reached 680,000 tons.[50] During this time frame, the U.S production volume of various OPFRs has remained constant or increased.[29]

OPFRs and PBDEs as endocrine disruptors

The endocrine system regulates key interrelated functions in the body, including reproduction, early development, as well as metabolic and neurologic processes. Some chemicals or chemical mixtures that can cause adverse health effects by perturbing any aspect of hormone action are defined as endocrine disrupting chemicals (EDCs).[30] The endocrine system is composed of glands of an organism that secrete hormones directly into the circulatory system which are carried to a distant target organ. The major endocrine glands include the pineal gland, pituitary gland, pancreas, ovaries, testes, thyroid gland, parathyroid gland, gastrointestinal tract, adrenal glands, and hypothalamus. EDCs cause endocrine diseases that are characterized by dysregulated hormone releases of the endocrine system.[31] UNEP has established a list of 45 EDCs or potential EDCs by reviewing evidence from multiple sources, including some flame retardants. However, it is likely that this list is incomplete due to many more chemicals showing endocrine disrupting activities in humans and experimental animals.

EDCs may act on nuclear receptors by mimicking the endogenous hormone and activating the receptor(agonists), or by inhibiting the effect of the hormone (antagonists).[32] Nuclear receptors are ligand-inducible transcription factors that specifically regulate the expression of target genes involved in metabolism, development, and reproduction.[32] Their primary function is to mediate the

transcriptional response in target cells to hormones, such as the sex steroids, adrenal steroids, vitamin D3, and thyroid and retinoid hormones, in addition to a variety of other metabolic ligands. Forty-eight nuclear receptors are known to exist in humans, and these proteins comprise the single largest family of metazoan transcription factors, the nuclear receptor superfamily. Particularly, experimental studies suggest that PBDEs affect transactivation via nuclear receptors such as estrogen receptors (ERs), androgen receptors (AR), and pregnane X receptor (PXR). For OPFRS, it remains unclear whether this toxin has any potential nuclear receptor activity. However, studies show that OPFRs are endocrine-disrupting compounds that may interfere with these receptors. In vitro, OPFRs interfere with the estrogen receptors (ERα and ERβ), androgen receptor (AR), glucocorticoid receptor (GR), pregnane X receptor (PXR), peroxisome proliferator-activated receptor gamma (PPARγ), and mineralocorticoid receptor (MR), which, in turn, could potentially affects steroidogenesis, growth, development and metabolic homeostasis.[12]

Methods

A research protocol was developed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework. A search strategy was developed with the advice of a professional librarian. We conducted a systematic literature search in PUBMED, Web of Science, Cochrane Central Register of Controlled Trials (CENTRAL), and Excerpta Medica Database (EMBASE) on September 1, 2021. Search strings were developed that would address our population

of interest (pregnant women and child dyads), exposures of interest (OPFRs and PBDEs) and outcomes of interest (preterm birth, low birth weight) and secondary outcomes (spontaneous abortion, pregnancy loss, and fetal growth restriction). A combination of medical subject headings and free text words were used with no exclusion on publication dates. The search strategy was limited to studies conducted in humans, written in the English language, and empirical or epidemiological studies only. The complete search term for each database is detailed in Appendix A. In addition, we searched the reference lists of all identified relevant publications and relevant reviews to screen for any additional studies that were not retrieved by the initial literature search.

Study selection process

Endnote software was used for the management of the articles and to de-duplicate before the review was started. Two investigators (S.M. and A.G.) independently screened the titles and abstracts of potential articles. Discrepancies were resolved by two investigators (S.V. and M.C.). Three authors (A.G., Y.B., and A.P.,) independently examined the full text articles and extracted the necessary information using a pre-specified form that includes information such as type of flame retardant and concentration, level of detection, date of publication, study design, outcomes and types of measurement, and participant demographics. Studies that did not meet the defined eligibility criteria were excluded and the reason for exclusion was recorded.

Results

Summary of Studies

The search strategy retrieved 1878 studies across the four databases, but 471 of these were identified as duplicates and excluded. One-thousand three hundred and forty-four (1344) articles were excluded during the title and abstract review. No studies were added via hand searching, which led to 63 studies undergoing full text review. Forty of the 63 studies selected for full text review met the inclusion criteria. The full study selection process is described in Figure 4.1.

On the associations between OPFRs and PTB/LBW, there were 6 studies that assessed this relationship. Of these studies, 5 were prospective cohort design and 1 was a nested case-control study design. These studies were performed either in the United States or China, used prenatal urine as the exposure metric, and the earliest year of sampling was 2001. For the associations between OPFRs and other adverse pregnancy outcomes, there were 4 studies that assessed spontaneous abortion, pregnancy loss, IVF outcomes, and fetal chromosome abnormalities. Among these 4 studies, 2 were case-control design and 2 were prospective cohorts. The earliest year of sampling for these studies was 2004.

For the association between PBDEs and PTB/LBW, there were 24 studies that assessed this relationship and 5 studies that assessed other adverse pregnancy outcomes. Of these, 18 were cohort studies, 5 case-control, and 1 cross-sectional study. These studies took place in the United States, China, Singapore, Spain, Canada, Taiwan, Greece, Greenland, and Australia. The most common exposure

metric for these studies was maternal blood and urine, followed by colostrum, meconium, and breast milk. The earliest year of sampling for these studies was 1994.

OPFRs and preterm birth and low birth weight

Preterm birth and low birth weight, as well as other adverse pregnancy outcomes, represent the leading causes of neonatal mortality in developed countries, and while most babies survive, those born too early or too small are at increased risk of chronic health conditions throughout their lifetimes.[51]

Epidemiologic evidence for the impact of OPFRs on birth outcomes is limited to six studies and the findings are mixed. Four studies found significant associations between OPFRs and birth outcomes, while two found no significant associations. Hoffman et al. identified an inverse trend between urinary isopropyl-phenyl phosphate(ip-PPP) levels and birth weight among female infants.[52] Luo et al(2020) found that maternal urinary diphenyl phosphate (DPHP) levels were positively associated with the risk of low birth weight.[53] Luo et al (2021). found that bis(1, 3-dichloro-2-propyl) phosphate(BDCIPP) and bis(2-butoxyethyl) phosphate(BBOEP) in the third trimester, 4-hydroxyphenyl-diphenyl phosphate (4-HO-DPHP) in the second trimester, and diphenyl phosphate(DPHP) in the first trimester were negatively associated with birth weight, among which a significant difference in exposure-effect relationships across the three trimesters was observed for BDCIPP.[54] Crawford et al., found that BDCPP was associated with increased weight in males and DPHP was inversely associated with female weight.[12]However, Feng et al., and

Kuiper et al., found no statistically significant associations of OPFR exposure during pregnancy with birth weight and gestational age.[55,56]

OPFRs and other adverse pregnancy outcomes

Five studies assessed the relationship between exposure to OPFRs and other adverse pregnancy outcomes such as spontaneous abortion, pregnancy loss, fetal abnormalities, small and large for gestational age, and outcomes among women who underwent In vitro fertilization (IVF). In a case-control study in Shanghai, China, BCIPP was significantly different among spontaneous abortion (SAB) cases and controls.[57] Similarly, Zhao et al, assessed SAB and fetal chromosome abnormalities in a case control and found that BCIPP was associated with increased odds SAB.[58] One study of 155 women who were enrolled in the EARTH study found that DPHP was associated with an increased risk of biochemical pregnancy loss for women in the 4^{th} vs 1^{st} quartile.[59] There was also an elevated risk of loss among women in the highest quartile of the molar sum of urinary OPFR metabolites compared to the lowest. In a study that was assessing small- or -large for gestational age, DPHP was associated with lower odds of LGA in individual models. Using quantile g-computation, higher OPFR metabolite concentrations were also associated with lower odds of LGA.[60]

PBDEs and preterm birth and low birth weight

Global levels of PBDEs have been detected in air, soil, water, and biota ranging from invertebrates to humans, and the levels have increased in the past 30

years.[61] 2,2′4,4′-tetrabromodiphenyl ether (BDE-47) is the dominant PBDE congener in humans, wildlife, and the environment. BDE-47 has been assessed in several studies. In animals, BDE47 has been identified as a developmental, reproductive, and neurological toxicant, and a disrupter of multiple endocrine systems.[61] In a case-cohort study sampled from the Flame Retardants and Adverse Pregnancy Outcomes (FRAPO) study, high concentrations of PBDE-47 in the first trimester significantly increased the odds of both indicated and spontaneous preterm birth.[62] Peltier et al. also found that higher levels of PBDE-47 were associated with preterm birth compared to lower levels among 140 pregnant women enrolled in the Healthy Pregnancy, Healthy Baby cohort study.[63] Similarly, Eick et al. found that the highest tertile of BDE-47 compared to the lowest tertile was associated with shorter gestational age. In the middle tertile, BDE-47 and BDE-99 were associated with a reduction in birth weight z-scores.[10]

Some studies reported small or null associations with gestational age and birth weight, but they diminished when controlling for other potential confounders. For example, investigators reported that BDE-47, BDE-99, and BDE-100 were negatively associated with birth weight, but these associations diminished when controlling for maternal weight gain.[64] In the HOME study, investigators reported that PBDEs had null or small associations with birth weight.[65] Seven studies reported no associations.[66-72]

PBDEs and other adverse pregnancy outcomes

BDE-47, BDE-153, and BDE-99 were associated with a decrease in head circumference, but the association was attenuated after controlling for maternal risk factors.[73] There were 2 studies that examined pregnancy loss. Choi et al. found that BDE-17, 28, 66, and homolog triBDE were positively associated with incident pregnancy loss.[74] Similarly, in the LIFE study, PBDE-28 was associated with hCG pregnancy loss.[75] One study that focused on IVF outcomes found that higher levels of BDE-153 in follicular fluid had elevated odds of failed implantation.[76] Another adverse pregnancy outcome assessed was fetal growth restriction. Fetal growth restriction refers to the fetus that does not grow to its expected biological potential in utero, and is a relatively common complication of pregnancy.[77] True FGR, as compared to constitutional smallness, is a pathological condition wherein the placental fails to deliver an adequate supply of oxygen and nutrients to the developing fetus, termed placental insufficiency. As a consequence, fetal growth becomes stunted.[77] In a nested case control sampled from the Wenzhou Birth Cohort Study in China, investigators found that elevated BDE-206, BDE-17-190, BDE-196-209 and Σ_{19} PBDE concentrations were associated with increased risk of FGR in newborns.[78] In a similar study that measured the exposure in maternal serum and colostrum, investigators found increased concentration of higher brominated BDEs in maternal serum and low-to-moderately brominated BDEs in colostrum were associated with increased risk of FGR.[79]

Conclusions

In summary, continuing studies on historically used FRs still reveal long-term adverse impacts of PBDE exposures decades after the ban. Epidemiological evidence of the associations of OPFRs on preterm birth and low birth weight are very limited. There were only 6 studies that examined the effect of OPFRs on gestational age and birth weight. There were only 5 studies that assessed the relationship of OPFRs with other adverse pregnancy outcomes such as pregnancy loss, spontaneous abortion, IVF outcomes, and small- or large-for-gestational age births. Evidence from epidemiological studies show that prenatal exposure to PBDEs may impact birth weight and gestational age as well as pregnancy loss, fetal growth restriction, and IVF outcomes. However, these findings are inconsistent. Of the 29 studies that examined the relationship between PBDEs and PTB/LBW and other adverse pregnancy outcomes, 6 of these studies found null or no associations. Two other studies that had significant associations stated that these diminished after controlling for maternal risk factors. The studies for OPFRs and adverse birth outcomes are very limited, and therefore more research on this class of flame retardants and birth outcomes is urgently needed.

Regardless of the inconsistent evidence, there is enough to justify that a coordinated global effort be taken to reduce flame retardant exposure in humans, especially during sensitive times in development where there can be lasting detrimental impacts in children that may progress into adulthood. Also, their use is driven by flammability standards, usually based on small-scale fire testing, which may not accurately predict real life fire behavior.[29] In addition, it is important to note

that OPFRs were rapidly marketed due to the need of rapid PBDE substitution, however, their environmental behavior and toxicological effects were not properly assessed.[28] The idea that OPFRs are less harmful than PBDEs was largely based on the presumption that OPFRs are less environmentally persistent and hence have a lower potential for widespread environmental distribution and exposure. However, as an increasing number of studies suggest ubiquitous detection in human samples and possible health impacts, the safety of OPFRs should be rigorously investigated to not delay mitigating actions for several decades - as was done with PBDEs - and prevent OPFRs from being another regrettable substitution.

Figure 4.1: Final Study Selection Process

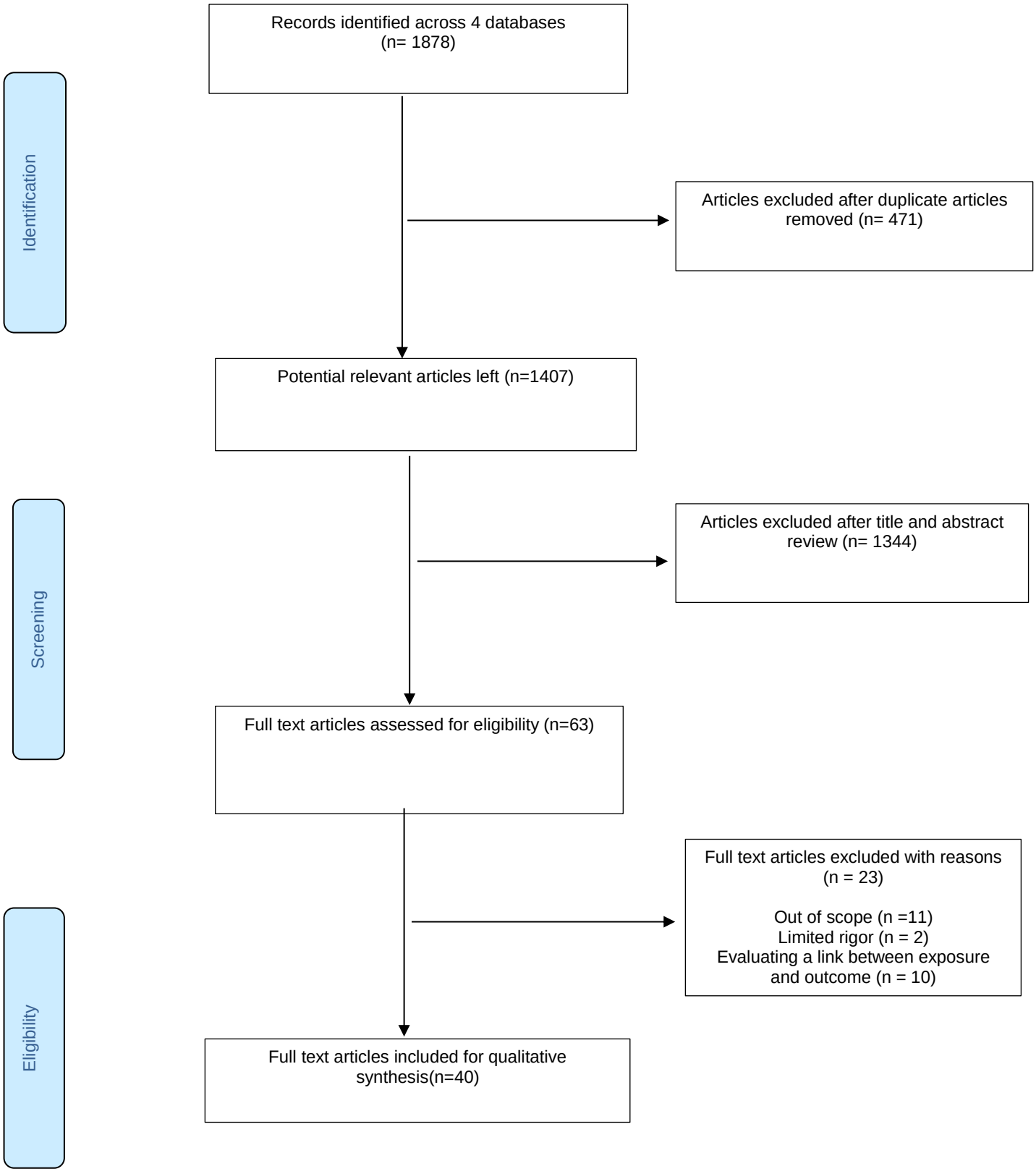


Table 4.2: Epidemiologic studies investigating OPFR exposures and impacts on adverse pregnancy outcomes

	Study Sample	Location	Study Design	Exposure Metric	Year of Sampling	Outcomes of interest	Summary Findings
OPFRs							
Birth Outcomes							
Feng et al. 2016	23 pregnant women (14 delivering term infants	Shanghai, China	Prospective cohort	Prenatal urine	2015	neonatal birthweight, gestational diabetes, miscarriages	No reported associations
Hoffman et al. 2018	349 women enrolled in the PIN study	North Carolina, USA	Prospective cohort	Prenatal urine	2001-2006	Gestational age in days and birth weight for gestational age	Females: ip-PPP and BDCIPP inversely related to gestational age and odds of preterm birth Males: ip-PPP associated with decreased odds of preterm birth and DPHP suggestively associated with longer gestational age
Crawford et al. 2020	56 women from Women &Infants Hospital of Rhode Island (WIHRI)	Rhode Island, USA	Prospective cohort	Prenatal urine	2014	Gestational age at delivery, gestational weight gain, infant anthropometry	Males: BDCPP was associated with increased infant length and weight Females: DPHP was inversely associated with female weight
Kuiper et al. 2020	76 offspring of women enrolled in the ORigins of Child Health and Resilience in Development (ORCHARD) pregnancy cohort	Baltimore, MD	Prospective cohort	Prenatal urine	2017-2019	Birth weight, gestational age at delivery, birth length, ponderal index	No reported associations
Luo et al. 2020	113 cases and 226 matched controls	Wuhan, China	Nested case-control	Prenatal urine	2014-2016	Birth weight	DPHP was associated with increased risk for giving birth to LBW infants, with evidence of sex as an effect modifier
Luo et al. 2021	213 pregnant women enrolled in birth cohort project in the Wuhan Maternal and Child Healthcare Hospital	Wuhan, China	Prospective cohort	Prenatal urine	2014-2016	Birth size, birth weight	BDCIPP and BBOEP in the third trimester, and 4-HO-DPHP in the second trimester, and DPHP in the first trimester were negatively associated with birth weight
Other adverse pregnancy outcomes							
Carignan, C et al 2017	211 from the EARTH study	Boston, MA	Prospective cohort	Prenatal Urine	2005-2015	IVF outcomes	Decreased success for several IVF outcomes across increasing quartiles of both summed and individual DPHP and ip-PPP metabolites
Messerlian, C et al 2020	155 women enrolled in the EARTH Study	Boston, USA	Prospective cohort	Prenatal urine	2004-2015	Pregnancy loss among women who underwent assisted reproductive technology (ART)	DPHP was associated with an increased risk of biochemical loss for women in the 4^{th} vs 1^{st} quartile. There was also an elevated risk of loss among women in the highest quartile of the molar sum of urinary OPFR metabolites compared to the lowest
Li. L et al 2021	55 cases and 55 controls that was conducted in the	Shanghai, China	Case-control	Prenatal Urine	2019-2020	Spontaneous abortion (SAB)	BCIPP was significantly different among the SAB cases and controls

	Shanghai First Maternity and Infant Hospital						
Zhao, Y. 2021	136 cases and 136 controls that was conducted in the Shanghai First Maternity and Infant Hospital	Shanghai, China	Case-control	Prenatal Urine	2019-2020	Spontaneous abortion (SAB) & fetal chromosome abnormalities	BCIPP was associated with increased odds of SAB
Bommarito, P et al 2021	90 participants from the LIFECODES study	Boston, MA	Nested case-control	Prenatal Urine	2006	Small- or large-for-gestational age births	Diphenyl Phosphate was associated with lower odds of LGA Quantile g-computation: higher OPE metabolite concentrations were associated with lower odds of LGA

Table 4.3: Epidemiologic studies investigating PBDE exposures and impacts on adverse pregnancy outcomes

	Study Sample	Location	Study Design	Exposure Metric	Year of Sampling	Outcomes	Summary Findings
PBDEs							
Chao, H et al. 2007	20 women recruited from a medical center in Taichung	Taichung, Taiwan	Prospective Cohort Study	Maternal breast milk	2000-2001	Birth weight, birth length, Quetelets index, menstrual cycle length	Higher levels of PBDEs were associated with decreased birth weight, birth length, and Quetelets index
Tan, J et al. 2009	41 mothers admitted to the National Hospital of Singapore for a c-section	Singapore city, Singapore	Cohort study	Maternal Blood	2006	Birth weight, length, head circumference, ponderal index, Apgar scores	PBDE-47 and PBDE-99 was associated with a higher Apgar score
Wu, K et al. 2009	153 women recruited from hospitals in Guiyu and Chaonan	Guiyu & Chaonan, China	Prospective Cohort Study	Maternal Blood	2007	Premature birth, low birth weight, and stillbirth	PBDE levels significantly differed in neonates by normal birth and low birth weight, premature birth, and stillbirth
Foster, W et al. 2011	Women enrolled in the FAMILY cohort study	Ontario, Canada	Prospective Cohort Study	Maternal Blood & cord serum	2004-2005	Birth weight	Only the umbilical cord serum was negatively associated with birth weight
Harley, K et al. 2011	286 pregnant women enrolled in the CHAMACOS cohort	California, United States	Prospective cohort study	Maternal Blood	1999-2000	Birth weight, birth length, head circumference, length of gestation	BDE-47, BDE-99, and BDE-100 were negatively associated with birth weight, but these associations diminished when controlling for maternal weight gain
Stasinska, A et al. 2014	173 women enrolled in the AMETS study	Western Australia	Prospective Cohort Study	Maternal Blood	2008-2011	Birth weight	No reported associations
Vafeiadi, M et al. 2014	1117 mothers and infants enrolled in the Rhea Study	Crete, Greece	Prospective Cohort Study	Maternal Blood	2007-2008	Birth weight, gestational age, and head circumference	No reported associations
Chen, L et al. 2015	215 mothers enrolled in the LWBC Cohort Study	Laizhou Wan of the Bohai Sea, Shandong province, China	Prospective Cohort Study	Maternal Blood	2010-2012	Birth weight, length, head circumference, and gestational age	There was a negative association between BDE-28, -100 and birth length. BDE-28 showed a negative association with birth weight among males
Lopez-Espinosa, MJ et al. 2015	670 women enrolled in the INMA Project	Different sites around Spain	Prospective Cohort Study	Maternal and umbilical cord serum	2003-2008	Gestational age, birth weight, infant anthropometric measures	BDE-99 was inversely associated with birth weight. For maternal serum, there was inverse associations between PBDEs with birth weight
Miranda, M et al. 2015	140 pregnant women enrolled in the Healthy Pregnancy, Healthy Baby cohort study	North Carolina, United States	Prospective cohort study	Maternal Blood	2008-2010	Birth weight, head circumference, birth length, birth weight percentile	BDE-153, BDE-47, BDE-99 were associated with a decrease in head circumference, but the association was attenuated after controlling for maternal risk factors
Peltier, MR et al. 2015	82 cases and 197 controls women enrolled from Centennial Women's Hospital	Tennessee, USA	Case-control study	Maternal Blood	2008-2011	Gestational Age	Higher levels of PBDE-47 were associated with preterm birth compared to low levels of the same

Serme-Gbedo, Y et al. 2016	349 women recruited from the GESTE cohort	Quebec, Canada	Prospective Cohort Study	Maternal Blood	2007-2008	Birth weight	No reported associations
Gao, Y et al 2016	207 pregnant women enrolled in the LWBC cohort study	Laizhou Wan of the Bohai Sea, Shandong province, China	Prospective cohort study	Maternal blood	2010-2012	Premature birth, spontaneous abortion	BDE-85, -153, and -183 were associated with an increased risk of threatened abortion. BDE-153 was associated with an increased risk of PTB. BDE-28 was associated with longer time to pregnancy
Woods, M et al. 2017	272 pregnant women enrolled in the HOME Study	Ohio, USA	Prospective Cohort Study	Maternal blood and urine	2003-2006	Birth weight	PBDEs had null or small associations with birth weight
Buck Louis, BM et al. 2018	2106 Women who were enrolled in the NICHD Fetal Growth Studies	12 clinical sites, USA	Prospective Cohort study	Maternal Blood	2009-2012	Gestational age, birth weight, infant anthropometry measurements	No reported associations
Bell, GA et al. 2019	2065 singleton infants enrolled in the Upstate KIDS Study	New York, USA	Prospective Cohort Study	Maternal Blood	2008-2010	Birth weight, gestational age, birth length, ponderal index	No reported associations
Kallo, G et al. 2019	380 enrolled in the HOME Study	Ohio, USA	Prospective Cohort Study	Maternal urine or blood	2003-2006	Gestational-age-specific birth weight z-scores, birth length, head circumference, and gestational age	No reported associations
Yin, S et al. 2019	60 mothers recruited from municipal hospitals across 3 cities in China	Mianyang, Wuhan, and Hangzhou China	Prospective Cohort Study	Maternal Colostrum	2016-2017	Birth weight, head circumference, birth length	BDE-28 was positively associated with birth weight, while BDE-99 was positively associated with head circumference
Eick, SM et al. 2020	506 women enrolled in the CIOB cohort study	California, United States	Prospective cohort study	Maternal Blood	2014-2018	Gestational age, birth weight z-scores, infant length, head circumference	The highest tertile of BDE-47 compared to the lowest tertile was associated with shorter gestational age. BDE-47 and BDE-99 in the middle tertile were associated with a reduction in birth weight z-scores
Gross R et al. 2020	333 women and child pairs enrolled in the StEP randomized controlled trial	New York, USA	Case-control study	Maternal and infant blood	2012-2014	Birth weight, prenatal diet quality and overweight status at 18 months old	No reported associations
Hjermitslev, M et al. 2020	504 mothers enrolled in a mother-child cohort	16 different towns across Greenland	Cross-sectional study	Maternal Blood	2010-2011, 2013-2015	Birth weight, length, head circumference and gestational age	PBDEs were positively associated with low birth weight
Jin, Y.T et al. 2020	101 cases and 101 controls sampled from the Wenzhou Birth Cohort study	Wenzhou, China	Nested case-control study	Maternal Blood	N/A	Birth weight, birth length, gestational age, Quetelet index, FGR	Increased BDE-207, -208, -209, and Σ_{19} PBDEs were associated with birth weight, birth length, gestational age, and Quetelet index. BDE-207 and Σ_{19} PBDEs were significantly associated with an increased risk of FGR
Alvarez-Silvares, E et al. 2021	50 pregnant women from prospective and retrospective data collection from medical records	University Hospital of Ourense, Spain	Case-control	Meconium	2017	Birth Weight	PBDEs were detected with the highest levels in meconium for small for gestational age newborns
Peltier, MR et al. 2021	368 Women enrolled in the FRAPO study	California, USA	Case-cohort study	Maternal Blood	2014-2017	Preterm birth and its subtypes	High concentrations of PBDE-47 in the first trimester significantly increased the odds of both indicated and spontaneous preterm birth

Other Adverse Pregnancy Outcomes							
Johnson, PI et al. 2012	65 women enrolled in Boston IVF study	Boston, USA	Prospective Cohort Study	Maternal Blood	1994-1998, 1999-2003	Failed embryo implantation/ IVF outcomes	Higher levels of BDE 153 in follicular fluid had elevated odds of failed implantation
Choi, G et al. 2019	344 women enrolled in the LIFE study	Michigan & Texas, USA	Prospective Cohort Study	Maternal Blood	2005-2009	Incident pregnancy loss	BDE-17, 28, 66, and homolog triBDE were positively associated with incident pregnancy loss
Zhao Y et al. 2019	130 fetal growth retardation (FGR) cases and 130 controls enrolled in the Wenzhou Birth Cohort study	Wenzhou, China	Nested case-control study	Maternal Blood	2016-2017	Fetal growth retardation, differential methylation region (DMR)	Elevated BDE-206, BDE-17-190, BDE-196-209, and Σ_{19} PBDE concentrations were associated with increased risk of FGR in newborns
Jin, Y et al. 2020	98 cases and 195 controls enrolled in the Wenzhou Birth Cohort Study	Wenzhou, China	Nested case-control	Maternal serum and colostrum	2016-2017	Fetal Growth Restriction	Increased BDE-207, BDE-209, ΣBDE196-209 and ΣPBDEs levels in maternal serum and BDE—99, ΣBDE17-154 and ΣPBDEs levels in colostrum were correlated with decreased birth weight z-scores. Increased concentration of higher brominated BDEs in maternal serum and low-to-moderately brominated BDEs in colostrum were associated with increased risk of FGR
Smarr, M et al. 2021	344 from the LIFE study	Michigan & Texas, USA	Prospective Cohort Study	Maternal Blood	2005-2009	Pregnancy Loss	PBDE-28 was associated with hCG pregnancy loss

CHAPTER 5

ASSOCIATIONS OF PRENATAL EXPOSURE TO OPFRS AND BIRTH OUTCOMES IN THE PROTECT COHORT STUDY (2011-2017)[1]

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Abstract

Background- Organophosphate flame retardants (OPFRs) as used as flame retardants and plasticizers. OPFR exposure is widespread. Prior research suggests that prenatal OPFR exposure may influence gestational duration and birth weight, but published results have been inconsistent.

Methods- In a cohort of 146 pregnant women from the PROTECT study, based in Northern Puerto Rico, we measured eight OPFR metabolites in urine measured twice during pregnancy. Gestational age and birth weight were extracted from medical records. We used multivariable linear regression to estimate covariate adjusted associations between urinary OPFR metabolite concentrations and birth outcomes.

Results – In unadjusted models, the findings are suggestive of an increase in gestational age and a decrease in birth weight z-score for all OPFRs. Gestational age increased by 0.12 weeks for BCEtP and BDCPP, however, these associations were not statistically significant. These associations remained the same after covariate adjustment.

Conclusions: In this subset of the PROTECT cohort, OPFR concentrations during pregnancy were not associated with gestational age and birth weight z-scores. Additional research is needed to determine if exposures during different periods of fetal development are associated with birth outcomes.

Introduction

Organophosphate flame retardants are a class of synthetic chemicals primarily used as additive flame retardants that are found in a variety of products including clothing, furniture, electronics, and baby products.[56] Human exposure to OPFRs is understood to be ubiquitous and multiple studies have detected OPFR metabolites in the United States and around the world.[12] Though most people are exposed to flame retardants, pregnant women and children are more vulnerable to these exposures. Pregnant women are vulnerable due to prenatal exposure having lasting detrimental impacts on children and can cause diseases that show up in childhood and later in life. Infants and young children are believed to have a higher exposure to flame retardants when compared with adults because they spend more time indoors, in close proximity to contaminant sources, and engage in frequent hand-to-mouth contact.[28]

Preterm birth (PTB), defined as birth before 37 completed weeks of gestation, is one of the leading causes of neonatal mortality in the world.[2] Babies born too early have higher rates of infant death and disability. Low birth weight (LBW) is defined as a baby born weighing less than 5 pounds, 8 ounces.[4] LBW is an established risk factor for numerous adverse health outcomes, including increased risk of neonatal and post neonatal morbidity and mortality in adulthood. Although the preterm birth rate declined, from 10.2% in 2019 to 10.1% in 2020[15], the United States is among the top 10 countries with the highest numbers of preterm births.[16] In 2021, the rate of preterm birth in Puerto Rico was 11.6%, higher than the national rate of 10.1%.[3] Gestational age is more predictive of risk of neonatal and childhood mortality than low birth weight, but preterm birth is more difficult to ascertain accurately than birthweight.

Although preterm birth is a major reason for a baby being born LBW, LBW is an imperfect surrogate for preterm birth. Mechanisms and risk factors for preterm and for LBW babies may differ despite a substantial proportion of LBW being contributed by preterm births as LBW infants are also a result of intrauterine growth restriction.[6] For this reason, as well as the higher than normal PTB rates in Puerto Rico, the outcomes of interest for this study are PTB and LBW.

It is hypothesized that flame retardant exposure contributes to adverse pregnancy outcomes, such as PTB and LBW, through endocrine disruption that may interfere with growth and metabolism. In vitro, OPEs interfere with the estrogen receptors (ERα and ERβ), androgen receptors (AR), glucocorticoid receptor (GR), pregnane X receptor (PXR), peroxisome proliferator-activated receptor gamma (PPARγ), and mineralocorticoid receptor (MR), which, in turn, could potentially affect steroidogenesis, growth, development, and metabolic homeostasis.[12] Our knowledge and understanding of what causes adverse pregnancy outcomes has greatly increased over the past decades. However, there is still much that is not known. In particular, the role of environmental exposures in reproductive and infant health is complex and largely not understood.

Despite the omnipresence of OPFRs, studies identifying predictors and health effects of OPFR exposures are very limited. To date, there are six epidemiological studies from the United States and China investigating the relationship of prenatal OPFR exposure with birth outcomes However, their findings are not consistent. Four studies found significant associations between OPFRs and birth outcomes, while two found no significant associations.[12,52-56] Hoffman et al. identified an inverse trend

between urinary isopropyl-phenyl phosphate(ip-PPP) levels and birth weight among female infants.[52] Luo et al (2020) found that maternal urinary diphenyl phosphate (DPHP) levels were positively associated with the risk of low birth weight.[53] Luo et al (2021) found that bis(1, 3-dichloro-2-propyl) phosphate(BDCIPP) and bis(2-butoxyethyl) phosphate(BBOEP) in the third trimester, 4-hydroxyphenyl-diphenyl phosphate (4-HO-DPHP) in the second trimester, and diphenyl phosphate(DPHP) in the first trimester were negatively associated with birth weight, among which a significant difference in exposure-effect relationships across the three trimesters was observed for BDCIPP.[54] Crawford et al., found that BDCPP was associated with increased weight in males and DPHP was inversely associated with female weight.[12] However, Feng et al., and Kuiper et al., found no significant associations of OPFR exposure during pregnancy with birth weight and length.[55,56]

Due to the growing body of evidence suggesting adverse effects of OPFR exposures on adverse pregnancy outcomes and scarce information on OPFRs and their effects on human health, the objective of this study is to examine whether exposure to gestational OPFR metabolites were associated with gestational age and birth weight in the PROTECT cohort.

Methods

Study Population

The pregnant women included in the present study were enrolled in the PROTECT cohort, an ongoing prospective birth cohort in Northern Puerto Rico. PROTECT has been previously described in detail.[80] Briefly, study participants were

recruited at approximately 14+/-2 weeks gestation at seven prenatal clinics and hospitals throughout Northern Puerto Rico. Women were eligible if they intended to deliver at one of the two collaborating hospitals, had an age of 18 - 40 years old, reside in a municipality in the northern Karst region of the island, did not use oral contraceptives for at least 3 months prior to becoming pregnant, did not use in vitro fertilization to become pregnant, and were free of known medical or obstetrical complications, including diabetes. Women were invited to participate in three study visits that were targeted for 18 ± 2 weeks, 22 ± 2 weeks, and 26 ± 2 weeks gestation. Demographic information was collected during the first visit. For the present analyses, participating women provided blood and spot urine samples for analysis for at least two time points during pregnancy. We included a subset of 146 women who had complete OPFR concentration for two visits (18 and 26 weeks) and had complete birth outcome data available. All women provided informed consent and the Institutional Review Board at the University of Puerto Rico, University of Georgia, Northeastern University, and University of Michigan approved the PROTECT study.

Urinary collection and quantification of OPFR biomarkers

Measurement of urinary concentrations has been previously described.[81] Urine samples were collected at the two visits, and the specific gravity of the sample (SG) was measured at the University of Puerto Rico Medical Sciences campus using a hand-held digital refractometer (Atago Co., Ltd., Tokyo, Japan). Aliquots were stored at -80 °C before being shipped overnight to the National Center for Environmental Health, CDC, Atlanta, GA. Metabolites were extracted via automated

off-line solid phase extraction, isolated using reversed phase high-performance liquid chromatography, and concentrations were quantified using isotope dilution-electrospray ionization tandem mass spectrometry. Participating women provided two spot urine samples at approximately 18- and 26-weeks' gestation. Eight FR metabolites: BCEtP, BCPP, BDCPP, DNBP, DBzP, DCP, DPHP, and TBBA were considered as the main exposures. The limits of detection (LOD) were 0.05 μg/L (DBzP and TBBA), 0.50 μg/L (DCP), and 0.10 μg/L (all other metabolites). FR concentrations below LOD were imputed a value equal to the LOD divided by the square root of 2.

Birth Outcome measurements

All birth outcome data were extracted from medical records. The American Congress of Gynecologists (ACOG) recommendations for best obstetrical estimate to calculate the gestational age for complete pregnancies were used in our study as previously described.[82] Birthweight z-scores (defined as the number of standard deviations by which a birthweight is above or below the mean) are commonly used to compare individual birthweights with the cohort. Birth weight z-scores for gestational z-scores were calculated using a U.S population reference.[83] Birth weight z-scores are preferred over birth weight, as they account for gestational age at delivery and disentangle the effects of gestational age versus fetal growth.[10]

Statistical Analyses

Frequencies, percentages, medians, and IQR were used to describe the demographic characteristics. These characteristics include education status, maternal age, household annual income, smoking status, alcohol use, employment status, parity, marital status, infant sex, and pre-pregnancy weight.

All concentrations were reported with limits of detection (LODs) and the percentage of concentrations for each LOD which were below the limit of detection was calculated. Additionally, flame retardant concentrations were adjusted for specific gravity using the expression

$$C_{adj} = C\left(\frac{\text{median(SG)} - 1}{\text{SG}_i - 1}\right),$$

where C_{adj} is the specific gravity adjusted biomarker concentration, C is the unadjusted biomarker concentration, median (SG) is the median of all specific gravity samples, and SG_i is an individual's specific gravity measurement.[47] Distributions of all OPFR metabolites were right-skewed and so were log transformed for all analyses. Descriptive statistics were calculated for all OPFR metabolites and specific gravity-adjusted values for all urinary biomarkers among the total study sample and for each study visit. The distributions of all concentration measurements were described using the minimum, median, maximum, IQR, geometric mean, and 95% confidence intervals. OPFR concentrations below the limit of detection (LOD) were replaced by LOD/square root of 2. For statistical analysis, we included OPFRs with at least 70% of samples having concentrations above the LOD (BCEtP, BCPP, BDCPP, DPhP). OPFRs with low detection rate (<30%), DNPB, DBZP, DCP, and TBBA were excluded from analyses.

Covariates retained in the final adjusted models were associated with birth weight or gestational age in bivariate analyses and had empirical evidence in the literature supporting an association with both the exposure and outcome. We fit linear regression models to calculate crude and adjusted β estimates and CIs for the association between for gestational age and birthweight by covariates and average OPFR concentrations. Final models were adjusted for maternal age, maternal education, and pre-pregnancy weight.

Results

Demographic characteristics of the study population are summarized in Table 1. Pluralities of participants had some college/2-year degree (34%) and were 18-24 years old (36%). The majority of women had never smoked (81%) and nearly half did not currently drink (47%). Forty-three percent (43%) used to drink before pregnancy. For over half of the women in this population, this was not their first pregnancy (60%), they were currently employed (58%), and they reported being married (60%). Most infants born during this study were male (58%). The median pre-pregnancy weight of the women was 140 lbs., with an IQR of 120 to 160 lbs.

Summary statistics of the concentrations of each OPFR are shown in table 2. BCEtP, BCPP, BDCPP, and DPHP were detected frequently (78%-99% of the samples had concentrations above the LOD). OPFRs with low detection rate (<30%) including DNPB, DBZP, DCP, and TBBA were excluded from the analyses. While the concentration of BCEtP appeared to increase on average at visit 3, there appears to be no significant difference in concentrations of all OPFRs between visits.

Correlations between different mean urinary metabolite concentrations ranged between 0.08 (BCEtP and BDCPP) and 0.24 (BCEtP and DPhP) (Table 5.3).

In adjusted models, the findings are suggestive for an increase in gestational age for all OPFRs and a decrease in birth weight z-scores with increasing concentrations of all OPFRs. Gestational age increased by 0.12 weeks for BCEtP and BDCPP, however, these associations were not significant. For birth weight z-scores, the results are suggestive of a decrease in birth weight z-scores with increasing BCEtP, BCPP, BDCPP, and DPhP concentrations. Similar relationships are observed in unadjusted models.

Conclusions & Discussion

In this study, we investigated the associations between prenatal exposure to four OPFRs (BCEtP, BCPP, BDCPP, and DPhP) and gestational age as well as birth weight z-scores. There was a suggestive negative effect of prenatal exposure of OPFRs on birth weight z-scores, however, these results were not statistically significant. When examining birth weight z-scores, we observed a suggestive negative effect of all OPFRs. For gestational age, we observed an increase in gestational age per week for all OPFRs, but the associations were not significant. Although increased gestational duration is generally considered beneficial, post-term birth (occurring after 42 weeks gestation) carries health risks for both the infants and the mother.[52] Post term pregnancy is associated with an increased risk of fetal and neonatal mortality and morbidity as well as an increased maternal morbidity. Fetal, neonatal, and maternal complications associated with this condition have always been

underestimated. It is not well understood why some women become post term although obesity, hormonal, and genetic factors have been implicated.[84]

Our study was subject to several limitations. This study is limited by the small sample size (n = 146). Sample size may be one of the explanations of the observed non-statistically significant association of levels of urinary OPFR pregnancy outcomes. Follow up findings should include a larger sample size to have higher statistical power. Studies with larger sample sizes are also needed to address potential improvement of models by including interactions between OPFRs and covariates that were not accounted for in the current analysis. Another limitation of this study is that the subjects' occupation and lifestyle information was not detailed enough to indicate sources of exposure. The women included in our current analyses are not representative of the general population, suggesting that the generalizability of our results to other populations could be limited. However, we do not anticipate that this would limit the validity of our findings. To our knowledge, this is the first study to assess the association of OPFRs and gestational age and birth weight in a cohort of Hispanic, Puerto Rican women. Another limitation is that we studied a population that was limited to pregnant women who did not have comorbidities, such as diabetes, which have been associated with poor pregnancy outcomes. While this may limit the generalizability of our study to a certain extent, it also enables us to better examine the association between urinary metabolites and PTB/LBW without confounding by other health conditions.

The results of this study suggest that maternal urinary OPFR metabolite concentrations are not associated with gestational age and birth weight in our cohort

of women in Puerto Rico after covariate adjustment. Additional research is needed in larger studies assessing OPFR exposure in pregnancy to confirm these findings.

Table 5.1: Demographic characteristics of PROTECT study population (2011-2017)

	N = 146[1]
Education	
< HS Diploma	32 (22%)
Some college/2-year degree	50(34%)
Bachelor ‘s Degree	42(29%)
Graduate or Doctoral Degree	22(15%)
Age	
18-24	52 (36%)
25-29	48 (33%)
30-34	33 (23%)
35+	13 (8.9%)
Has ever smoked	
Yes	28(19%)
No	118(81%)
Alcohol Use	
Before pregnancy	62(43%)
Currently drink	14(9.7%)
Does not drink	68(47%)
Missing	2
Currently Employed	
Yes	84(58%)
No	62(42%)
First Pregnancy	
Yes	60(41%)
No	85(58%)
Missing	1(0.68)
Marital Status	
Single	36(25%)
Married	88(60%)
Living Together	22(15%)
Infant Sex	
Female	59(42%)
Male	83(58%)
Missing	4
Pre-pregnancy weight (lbs.)	140(120,160)
Birth Weight (lbs)	7.06(6.31, 7.62)
Missing	5
Gestational Age at Delivery(weeks)	39.14(38.14, 40.00_
Missing	1

[1] n (%); Median (IQR)

Table 5.2: Summary statistics for the distribution of OPFR (ng/mL) concentrations in the Overall Study Population and at Visits 1 and 3

		N	% > LOD	min	median	max	IQR	GM[1]	95% CI[2]	p[3]
BCEtP	Overall	292	97.3	0.05	0.82	61.94	1.32	0.93	(0.82, 1.05)	0.75
	Visit 1	146		0.05	0.82	11.21	1.10	0.90	(0.76, 1.07)	
	Visit 3	146		0.07	0.84	61.94	1.33	0.96	(0.81, 1.17)	
BCPP	Overall	292	78.8	0.04	0.27	5.51	0.37	0.27	(0.24, 0.30)	0.83
	Visit 1	146		0.04	0.27	5.51	0.38	0.27	(0.23, 0.31)	
	Visit 3	146		0.04	0.27	2.48	0.35	0.26	(0.23, 0.30)	
BDCPP	Overall	292	97.3	0.07	1.31	20.54	1.86	1.24	(1.09, 1.39)	0.73
	Visit 1	146		0.07	1.42	20.54	1.83	1.28	(1.07, 1.52)	
	Visit 3	146		0.07	1.24	12.46	1.89	1.19	(1.00, 1.40)	
DPhP	Overall	292	99	0.05	1.48	85.69	1.92	1.64	(1.46, 1.85)	0.94
	Visit 1	146		0.05	1.51	85.69	1.86	1.65	(1.39, 1.94)	
	Visit 3	146		0.06	1.43	81.32	1.99	1.64	(1.36, 1.97)	

[1]Geometric mean.

[2]95% confidence interval, calculated using the empirical percentiles of 1000 bootstrap resamples.

[3]The p-value is from a paired t-test between concentration at visit 1 and concentration at visit 3 for each OPFR. SG-adjusted concentrations were log-transformed before the test was conducted. Adjusted to maintain a false discovery rate of 5% using the method of Benjamini and Hochberg.

Table 5.3: Correlation between average prenatal OPFR metabolites concentrations and corresponding p-values with >70% detection in maternal urine (N = 146)[a]

OPFR Metabolites	BCEtP	BCPP	BDCPP	DPhP
BCEtP	1.00			
BCPP	0.24*	1.00		
BDCPP	0.08	0.12	1.00	
DPhP	0.24*	0.11	-0.02	1.00

[a] Average of natural log transformed at 18 and 26 weeks
*indicates $p < 0.05$

Table 5.4: Adjusted linear regression coefficients and 95% confidence intervals for the associations between the average log OPFR (ng/mL) concentrations > 70% detection in maternal urine and gestational age in weeks and birth weight z-scores

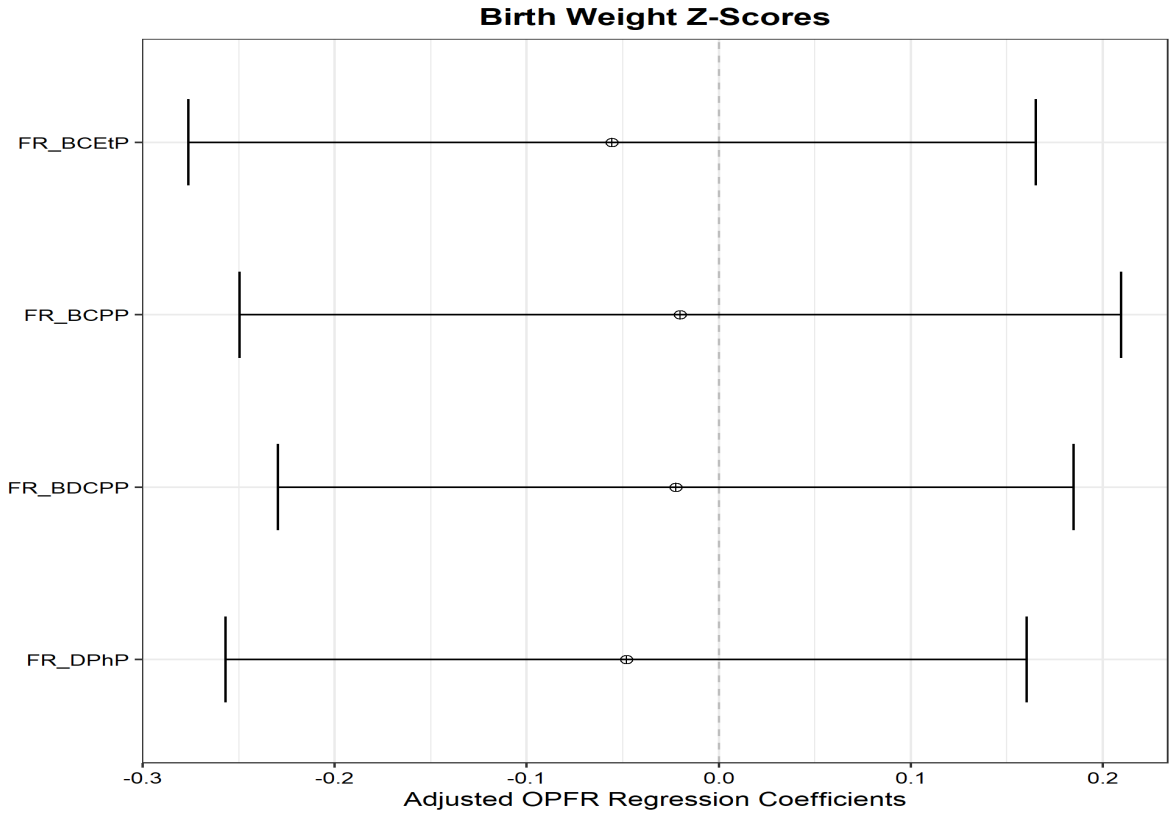
	Gestational Age		**Birth Weight Z-Score**	
	β	**95% CI**	**β**	**95% CI**
OPFRs				
BCEtP	0.12	[-0.24, 0.47]	-0.06	[-0.28, 0.17]
BCPP	0.10	[-0.27, 0.47]	-0.02	[-0.25, 0.21]
BDCPP	0.12	[-0.21, 0.45]	-0.02	[-0.23, 0.18]
DPhP	0.22	[-0.12, 0.55]	-0.05	[-0.26, 0.16]

Models adjusted for maternal education, maternal age, and maternal pre-pregnancy weight

Table 5.5. Crude linear regression coefficients and 95% confidence intervals for the associations between the average OPFR (ng/mL) concentrations > 70% detection in maternal urine and gestational age in weeks and birth weight z-scores

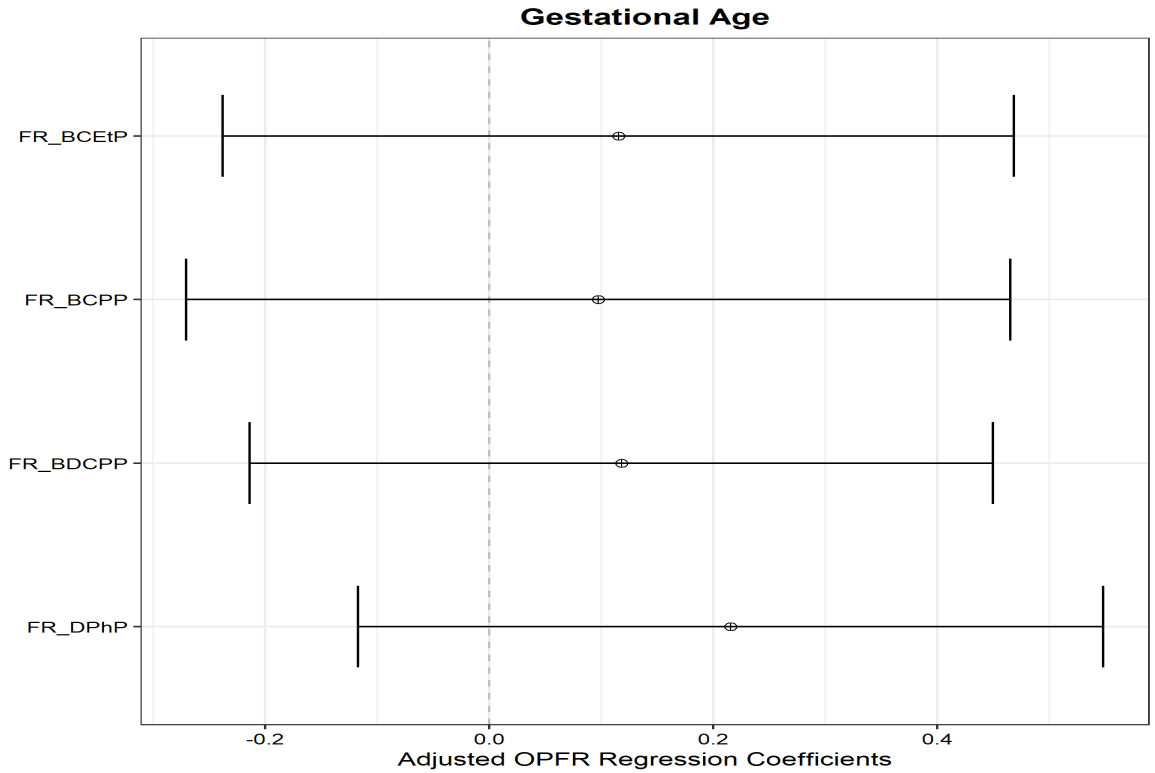
	Gestational Age		**Birth Weight Z-Score**	
	β	**95% CI**	**β**	**95% CI**
OPFRs				
BCEtP	0.14	[-0.21, 0.49]	-0.07	[-0.28, 0.14]
BCPP	0.05	[-0.30, 0.41]	-0.03	[-0.25, 0.18]
BDCPP	0.13	[-0.19, 0.45]	-0.02	[-0.22, 0.17]
DPhP	0.29	[-0.04, 0.62]	-0.05	[-0.25, 0.15]

Figure 5.1: Plot of regression coefficients and 95% confidence intervals for average OPFR concentrations and birth weight z-scores



Models adjusted for maternal education, maternal age, and maternal pre-pregnancy weight

Figure 5.2: Plot of regression coefficients and 95% confidence intervals for average OPFR concentrations and gestational age



Models adjusted for maternal education, maternal age, and maternal pre-pregnancy weight

CHAPTER 6

ASSOCIATIONS OF PRENATAL OPFR EXPOSURE WITH MATERNAL REPRODUCTIVE AND THYROID HORMONES AMONG PREGNANT WOMEN IN PUERTO RICO (2011-2017)[1]

[1]Velasquez, SG., Billings, WZ., Knight, JH., Rathbun, SL., Meeker, JD., Cordero, JF. To be submitted to Environmental Health Perspectives.

Abstract

Background: Organophosphate Flame Retardants (OPFRs) are widely used as flame retardants and plasticizers in consumer and industrial products. Human exposure to OPFRs raises concerns due to their endocrine disruptive potentials. Dysregulation of maternal endocrine homeostasis could be a possible biological pathway between OPFRs and birth outcomes

Methods: Pregnant women were recruited through the Puerto Rico Testsite for Exploring Contamination Threats (PROTECT). Urine, blood, demographic, and pregnancy-related data were collected at recruitment and subsequent visits. Eight OPFRs were analyzed in maternal urine, while nine maternal hormones (corticotropin-releasing hormone (CRH), sex-hormone binding globulin (SHBG), estriol(E3), progesterone, testosterone, thyroid-stimulating hormone (TSH), total triiodothyronine (T3), total thyroxine (T4), and free thyroxine (FT4)) were measured in serum samples twice during pregnancy. Linear mixed models with random intercepts were used to examine associations between OPFRs and maternal hormones concentrations.

Results: Increased levels of FT4 were associated with BCPP (%Δ:0.25, 95% CI: 0.05, 0.45) and BDCPP (%Δ:0.24, 95% CI: 0.07, 0.41) A decrease in FT4 was associated with DPhP (%Δ: -0.21, 95% CI: -0.37, -0.06). DPhP was associated with a decrease in T4(%Δ: -0.16, 95% CI: -0.31, -0.01). BCPP was also associated with an increase in T3(%Δ:1.23, 95% CI: 0.35, 2.12). BDCPP was associated with an increase in E3(%Δ:0.73, 95% CI: 0.16, 1.31).

Conclusions: Our analysis suggests that some OPFRs may act as endocrine disruptors by altering prenatal hormone levels. Prenatal exposures to OPFRs may be particularly important to consider in future human health studies.

Introduction

Pregnancy, childbirth, and postpartum are periods of dramatic hormonal and physiologic changes that heighten susceptibility to external factors like environmental chemicals.[85] Particularly, environmental toxins that are endocrine disrupting chemicals can impact endogenous hormone levels in animals and humans. An endocrine disrupting chemical (EDC) is a substance that is either an agonist or antagonist of any nuclear hormone receptor.[86] The incidence and prevalence of endocrine disruption related health problems have recently increased.[85] Because pregnancy is a sensitive window for toxicant exposure, identifying EDCs and analyzing disruption mechanisms are critical issues.[86,87]

Environmental toxicants, which include EDCs, are globally ubiquitous and represent an area of major public health concern.[88] Alterations in the delicate hormonal balance occurring during pregnancy can disrupt processes affecting both mother and baby. One EDC of interest is organophosphate flame retardants. Beginning in the 1970s, polybrominated diphenyl ethers (PBDEs), one kind of flame retardant, were added to consumer products, including furniture, children's products, and electronics. After extensive research showed that PBDEs were persistent, bio accumulative, and toxic, in 2004 the European Commission and California banned the use of Penta- and OctaBDE, two commercial mixtures primarily used in North

America. After the banning of PBDEs, there has been an increase of replacement flame retardants to assure compliance with flammability standards. Replacement flame retardants, such as OPFRs, are a good example of chemicals of emerging concern. OPFRs can be found in a variety of consumer products, such as furniture, electronics, food packaging, etc. OPFRs are not physically bound to products, so they readily leach out and contaminate home and office environments as well as foods.[36] OPFRs are quickly metabolized in the body and excreted into the urine, where the chemical metabolites are readily available.[36] As a result, the replacement flame retardants are now detected in indoor air and dust, in the environment, biota, the food chain, as well as human samples.[31]

Although there is clear evidence of increasing population exposure, there is little or no information regarding the effects on human health for many replacement flame retardants including OPFRs, but the few available studies suggest that some of them can induce adverse outcomes.[31] Percy et al., found that some associations in maternal and neonatal sera with exposure to OPFRs. They found that increased maternal urinary BDCIPP, DPHT, and DNBP concentrations were associated with decreased infant free T3, free T4, total T3, total T4, and increased log-TSH concentrations. They also found evidence that maternal urinary DPHP and DNBP concentrations were linearly associated with increased maternal TSH concentration.[36] They, however, did not observe evidence of effect modification by infant sex.[36] Another study also found that OPFR metabolites were positively associated with maternal and neonatal TSH, however, they did observe effect modification by newborn sex.[39] Women with polycystic ovarian syndrome or other hyperandrogenic

conditions that are characterized by higher levels of testosterone have been shown to be associated with preterm birth.[41] Elevated testosterone levels are associated with in utero growth restriction, development of gestational diabetes, and preeclampsia.[41] Animal studies in zebrafish, chickens, and rodents also add to the body of evidence that OPFRs can affect hormones.[36] Because of the growing body of evidence suggesting adverse effects of OPFR exposures on maternal homeostasis and scarce information on OPFRs and their effects on human health, the objective of this study is to examine whether exposure to gestational OPFR metabolites is associated with alterations in maternal thyroid and reproductive hormones measured at two time points during pregnancy in the PROTECT(Puerto Rico Testsite for Exploring Contamination Threats) cohort.

Methods

Study Population

Pregnant women included in the present study were enrolled in the PROTECT cohort, an ongoing prospective birth cohort in Northern Puerto Rico. PROTECT has been previously described in detail.[80] Briefly, study participants were recruited at approximately 14+/-2 weeks gestation at seven prenatal clinics and hospitals throughout Northern Puerto Rico. Women were eligible if they intended to deliver at one of the two collaborating hospitals, had an age of 18-40 years old, reside in a municipality in the northern Karst region of the island, did not use oral contraceptives for at least 3 months prior to becoming pregnant, did not use in vitro fertilization to become pregnant, and were free of known medical or obstetrical complications,

including diabetes. Women were invited to participate in three study visits that were targeted for 18 ± 2 weeks, 22 ± 2 weeks, and 26 ± 2 weeks gestation. Demographic information was collected during the first visit. For the present analyses, participating women provided blood and spot urine samples for analysis for at least two time points during pregnancy. We included a subset of 148 women who had complete OPFR concentrations for two visits (18 and 26 weeks) and at least one hormone measurement available for at least one of the two study visits. All women provided full informed consent prior to participation. The Institutional Review Board at the University of Puerto Rico, University of Georgia, Northeastern University, and University of Michigan approved the PROTECT study.

Urinary collection and quantification of OPFR biomarkers

Measurement of urinary concentrations of OPFRs has been previously described.[81] Urine samples were collected at the two visits, and the specific gravity of the sample (SG) was measured at the University of Puerto Rico Medical Sciences campus suing a hand-held digital refractometer (Atago Co., Ltd., Tokyo, Japan). Aliquots were stored at -80 °C before being shipped overnight to the National Center for Environmental Health, CDC, Atlanta, GA. Metabolites were extracted via automated off-line solid phase extraction, isolated using reversed phase high-performance liquid chromatography, and concentrations were quantified using isotope dilution-electrospray ionization tandem mass spectrometry. Participating women provided two spot urine samples at approximately 18- and 26-weeks' gestation. Eight flame retardant (FR) metabolites: BCEtP, BCPP, BDCPP, DNBP,

DBzP, DCP, DPHP, and TBBA were considered as the main exposures. The limits of detection (LOD) were 0.05 μg/L (DBzP and TBBA), 0.50 μg/L (DCP), and 0.10 μg/L (all other metabolites). FR concentrations below LOD were imputed a value equal to the LOD divided by the square root of 2.

Serum Hormone Measurement

The primary outcome variables to be used in this study are serum concentrations of several maternal hormones, namely: T4, SHBG, progesterone, FT4, E3, testosterone, T3, CRH, and TSH. Measurement protocols were described previously.[41] All serum samples were analyzed at the Central Ligand Assay Satellite Services (CLASS) lab at the School of Public Health, University of Michigan. Progesterone, SHBG, testosterone, T3, T4, FT4, and TSH were measured using chemiluminescence immunoassay, while E3 and CRH were measured using enzyme immunoassay. Due to low sample volume for some serum samples, hormone measurements were not completed for all subjects at Visit 3.

Statistical Analyses

Demographic characteristics were described using frequencies and percentages for categorical variables or median and IQR for continuous variables. These characteristics include education status, maternal age, household annual income, smoking status, alcohol use, employment status, parity, marital status, infant sex, and pre-pregnancy weight.

Distribution of all OPFR metabolites were right skewed and were log transformed for all analyses. Several of the maternal hormone concentrations appeared to follow a log-normal distribution. To maintain consistency, all maternal hormone concentrations were log-transformed. Descriptive statistics for all OPFR metabolites were calculated using specific gravity-adjusted values for all urinary biomarkers among the total study sample and for each study visit. The distributions of all concentration measurements were described using the minimum, median, maximum, IQR, geometric mean, and 95% confidence intervals. For statistical analysis, we included OPFRs with at least 70% of samples having concentrations above the LOD as continuous variables (BCEtP, BCPP, BDCPP, DPhP). OPFRs with low detection rates <30% (DNPB, DBZP, DCP, and TBBA) were excluded from analyses.

Relationships between exposure and outcome variables and potential confounders were assessed using ANOVA to test for differences between categories of covariates. Covariates to be included in the final adjusted model were determined by both a priori knowledge and bivariate association with any of the outcomes. Linear mixed models with repeated measures were fit to regress hormones on OPFR metabolites with random intercepts to account for intra-individual correlation of exposure and outcome measures. Final models were adjusted for maternal age, maternal education, and pre-pregnancy weight.

Results

Demographic characteristics of the study population are summarized in Table 1. A plurality of participants had some college/2-year degree (34%) and were 18-24 years old (36%). The majority of women had never smoked (81%) and did not currently drink (47%). Forty three percent (43%) drank before pregnancy. For over half of the women in this population, this was not their first pregnancy (60%), they were currently employed (58%), and they reported being married (60%). Most infants born during this study were male (58%). The median pre-pregnancy weight of the women was 140 lbs. with an IQR of 120 to 160 lbs.

Summary statistics of the concentrations of each OPFR are shown in table 2. BCEtP, BCPP, BDCPP, and DPHP were detected frequently (78%-99% of the samples had concentrations above the LOD). OPFRs with low detection rates (<30%) including, DNPB, DBZP, DCP, and TBBA were excluded from analyses. While the concentration of BCEtP appeared to increase on average at visit 3, there appears to be no significant difference between concentrations of all OPFRs between visits.

Similarly, the summary measurements of concentrations for each maternal hormone are shown in Table 3. All maternal hormones have more than 70% of values above the limit of detection. In contrast to the OPFR concentrations, several of the hormones change across visits. Concentrations of E3, Progesterone, SHBG, and Testosterone increased while FT4 decreased significantly from visit 1 to visit 3 ($p < 0.01$).

Results from linear mixed models indicating associations between OPFR metabolite biomarkers and serum hormones over the study period are shown in table

6.4. We estimated significant increases in FT4 for a 10% increase in BCPP (%Δ:0.25, 95% CI: 0.05, 0.45) and BDCPP (%Δ:0.24, 95% CI: 0.07, 0.41). DPhP was associated with a decrease in T4(%Δ: -0.16, 95% CI: -0.31, -0.01). BCPP was also associated with an increase in T3(%Δ:1.23, 95% CI: 0.35, 2.12). There were no significant associations with TSH.

For reproductive hormones, BDCPP was associated with an increase in E3(%Δ:0.73, 95% CI: 0.16, 1.31). There were no significant associations for testosterone, progesterone, and SHBG. There were also no significant associations for CRH.

Conclusions & Discussion

In this study, we examined the association between biomarkers of OPFR exposure and maternal serum hormones measured at two time points during pregnancy. Three of the four OPFRs assessed were significantly associated with thyroid hormones, while only BDCPP was significantly associated with reproductive hormones. BDCPP and BCPP were associated with an increase in FT4, while DPhP was associated with a decrease in T4. BCPP was also associated with an increase in T3. BDCPP was associated with a significant increase in E3. There were no significant associations among any of the assessed OPFRs and TSH, testosterone, progesterone, and SHBG. There were also no significant associations for CRH.

Thyroid Hormones:

Thyroid hormone balance is important for maintaining normal physiological processes in humans and its disruption may bring adverse effects on human health. During pregnancy, maternal thyroid hormone balance is an important factor for normal fetal development especially during early pregnancy when the fetal thyroid gland is not mature.[35] Wang et al. showed that treating zebrafish with tris(1,3-dichloro-2-propyl) phosphate, the parent compound of BDCIPP, caused decreased transcription of genes related to the hypothalamic-pituitary-thyroid axis.[38] In contrast, limited epidemiological data are available in human studies. Urinary DPHP was found associated with an increase of total T4, which was less significant in males.[36] Another study in China investigated these associations in pregnant women and newborns. They observed associations between maternal urinary DNBP levels during pregnancy and increased TSH concentrations in newborns and also between cross-sectional maternal urinary DPHP levels during pregnancy and maternal TSH levels.[39] Choi et al, investigated the influence of pregnancy exposure to OPEs on maternal thyroid hormones, and found that urinary diphenyl phosphate (DPHP) was positively associated with the ratio of total triiodothyronine(T3) and total thyroxine (T4). Yao et al, reported that maternal urinary dibutyl phosphate (DBP) and DPHP concentrations were associated with increased maternal or neonatal thyroid-stimulating hormone (TSH) levels, and such positive associations were particularly observed in females as stratified by infant sex, indicating a sex-dependent effect of OPFRs.[89]

During early brain development, the fetus is entirely dependent on maternal thyroid hormones during the first trimester. The baby begins to produce a higher

proportion of thyroid hormones throughout the second and third trimesters, becoming successively less dependent on maternal contributions. Small changes in maternal T4 concentration cause large changes in fetal thyroid hormones, due to differences in the binding of carrier proteins between adults and fetuses. Therefore, our observation of DPhP being associated with a decrease in T4 may have important implications in the disruption of hormonal homeostasis. Increased activation of thyroid hormone nuclear receptors and enhanced T4 binding to transporter proteins could cause decreased pressure on the negative feedback loop that regulates thyroid hormone nuclear receptor and enhanced T4 binding to transporter proteins could cause decreased pressure on the negative feedback loop that regulates thyroid hormone homeostasis.

Although there are no epidemiological studies assessing the impact of OPFRs on sex steroid hormones, some studies have examined prenatal sex-steroid hormones in relation to other endocrine disruptors including phthalates, parabens, BPA, and some pesticides.[90,91] In the TIDES study, a large pregnancy cohort including women from four U.S cities (n = 591), reported that first trimester phthalate exposure was positively associated with estrone and estradiol measure during the first half of pregnancy (<20 weeks). Similar work in PROTECT observed some positive associations between E3 and various metals (Co, Mn, Ni, Pb).[87] Windows of rapid estrogen and testosterone production are particularly important points at which endocrine disruptors may interact with normal production and function of sex steroid hormones and alter fetal development. Alterations in estrogen and testosterone during pregnancy can lead to a wide range of adverse birth outcomes including abnormalities

of the reproductive system, miscarriage, intrauterine growth restriction, and preterm birth.[92]

Our study has several limitations. We did not have data on maternal serum concentrations of iodine or thyroid peroxidase antibodies, both of which can impact measured concentrations of serum thyroid hormones.[41] Measuring OPFRs and hormones at two time points during pregnancy that align with periods of rapid fetal growth rather than trimesters is an improvement on most published research on this topic. However, two time points may not be sufficient to detect different effects of OPFRs on hormones at different times through gestation. Our study is limited by a small sample size (n = 146) of women who had complete OPFR measurements at the two time points. Sample size may be one of the explanations of the observed non-statistically significant associations. However, this is one of the first studies to examine prenatal OPFR exposures and maternal serum hormones in Puerto Rico. Finally, we carried out many comparisons and thus some of our significant results may have been found by chance. Future studies utilizing more frequent measurement through pregnancy and larger sample sizes for OPFR metabolites are needed to support our findings. People are rarely exposed to individual OPFRs and thus studying exposures to phthalates will be an important future step to gain a potentially fuller understanding of associations between environmental exposures and hormone levels.

Table 6.1: Demographic characteristics of women with urinary OPFR data from the PROTECT cohort (2011-2017)

	N = 146[1]
Education	
< HS Diploma	32 (22%)
Some college/2-year degree	50(34%)
Bachelor ‘s Degree	42(29%)
Graduate or Doctoral Degree	22(15%)
Age	
18-24	52 (36%)
25-29	48 (33%)
30-34	33 (23%)
35+	13 (8.9%)
Has ever smoked	
Yes	28(19%)
No	118(81%)
Alcohol Use	
Before pregnancy	62(43%)
Currently drink	14(9.7%)
Does not drink	68(47%)
Missing	2
Currently Employed	
Yes	84(58%)
No	62(42%)
First Pregnancy	
Yes	60(41%)
No	86(59%)
Marital Status	
Single	36(25%)
Married	88(60%)
Living Together	22(15%)
Infant Sex	
Female	59(42%)
Male	83(58%)
Missing	4
Pre-pregnancy weight (lbs.)	140(120,160)

[1] n (%); Median (IQR)

Table 6.2: Summary statistics for the distribution of OPFR (ng/mL) concentrations in the Overall Study Population and at Visits 1 and 3

		N	% > LOD	min	median	max	IQR	GM[1]	95% CI[2]	p[3]
BCEtP	Overall	292	97.3	0.05	0.82	61.94	1.32	0.93	(0.82, 1.05)	0.75
	Visit 1	146		0.05	0.82	11.21	1.10	0.90	(0.76, 1.07)	
	Visit 3	146		0.07	0.84	61.94	1.33	0.96	(0.81, 1.17)	
BCPP	Overall	292	78.8	0.04	0.27	5.51	0.37	0.27	(0.24, 0.30)	0.83
	Visit 1	146		0.04	0.27	5.51	0.38	0.27	(0.23, 0.31)	
	Visit 3	146		0.04	0.27	2.48	0.35	0.26	(0.23, 0.30)	
BDCPP	Overall	292	97.3	0.07	1.31	20.54	1.86	1.24	(1.09, 1.39)	0.73
	Visit 1	146		0.07	1.42	20.54	1.83	1.28	(1.07, 1.52)	
	Visit 3	146		0.07	1.24	12.46	1.89	1.19	(1.00, 1.40)	
DPhP	Overall	292	99	0.05	1.48	85.69	1.92	1.64	(1.46, 1.85)	0.94
	Visit 1	146		0.05	1.51	85.69	1.86	1.65	(1.39, 1.94)	
	Visit 3	146		0.06	1.43	81.32	1.99	1.64	(1.36, 1.97)	

[1]Geometric mean.

[2]95% confidence interval, calculated using the empirical percentiles of 1000 bootstrap resamples.

[3]The p-value is from a paired t-test between concentration at visit 1 and concentration at visit 3 for each OPFR. SG-adjusted concentrations were log-transformed before the test was conducted. Adjusted to maintain a false discovery rate of 5% using the method of Benjamini and Hochberg.

Table 6.3: Summary statistics for the distribution of maternal serum hormones in the overall study population and at visits 1 and 3

		N	min	median	max	IQR	GM[1]	95% CI[2]	p[3]
CRH	Overall	269	3.47	32.00	254.10	60.10	29.76	(26.18, 33.76)	0.78
	Visit 1	139	3.47	33.00	254.10	63.60	30.81	(25.61, 36.58)	
	Visit 3	130	3.47	31.80	207.70	51.93	28.67	(23.70, 34.18)	
E3	Overall	269	5.60	25.80	97.30	28.10	25.32	(23.46, 27.46)	< 0.01
	Visit 1	139	5.60	15.70	91.90	11.20	16.99	(15.56, 18.62)	
	Visit 3	130	6.90	41.65	97.30	23.72	38.80	(35.92, 41.91)	
FT4	Overall	268	0.44	0.92	1.40	0.26	0.91	(0.88, 0.93)	< 0.01
	Visit 1	138	0.54	0.96	1.28	0.26	0.94	(0.91, 0.97)	
	Visit 3	130	0.44	0.90	1.40	0.28	0.88	(0.84, 0.91)	
Prog	Overall	269	13.80	51.30	282.50	41.20	52.54	(49.14, 56.15)	< 0.01
	Visit 1	139	13.80	38.00	282.50	21.95	39.13	(36.46, 42.27)	
	Visit 3	130	27.60	71.50	222.80	46.88	71.99	(66.43, 78.48)	
SHBG	Overall	269	117.00	502.70	1460.70	273.60	498.30	(473.90, 521.22)	< 0.01
	Visit 1	139	117.00	485.20	1460.70	277.65	475.66	(443.06, 509.79)	
	Visit 3	130	142.30	512.30	1192.30	268.30	523.70	(489.31, 556.27)	
T	Overall	269	3.68	158.00	1910.00	533.40	170.53	(145.10, 198.40)	< 0.01
	Visit 1	139	3.68	146.70	1325.00	482.80	160.80	(128.08, 199.78)	
	Visit 3	130	3.68	216.50	1910.00	565.60	181.59	(145.70, 225.18)	
T3	Overall	267	0.11	1.47	3.16	1.08	1.17	(1.07, 1.28)	0.73

		N	min	median	max	IQR	GM[1]	95% CI[2]	p[3]
	Visit 1	137	0.11	1.49	3.16	1.00	1.16	(1.01, 1.31)	
	Visit 3	130	0.11	1.46	3.07	1.11	1.19	(1.06, 1.32)	
T4	Overall	268	5.30	11.50	16.80	2.20	11.30	(11.09, 11.52)	0.12
	Visit 1	138	7.10	11.50	15.60	2.00	11.42	(11.16, 11.70)	
	Visit 3	130	5.30	11.50	16.80	2.35	11.18	(10.85, 11.49)	
TSH	Overall	265	0.11	1.05	4.93	1.02	1.01	(0.94, 1.10)	0.85
	Visit 1	136	0.21	1.06	3.93	1.02	1.01	(0.91, 1.12)	
	Visit 3	129	0.11	1.03	4.93	0.97	1.02	(0.90, 1.14)	

[1]Geometric mean.

2Units are reported in pg/mL for CRH, FT4, T, and T3; ng/mL for E3 and progesterone; nmol/L for SHBG; µg/dL for T4; and uIU/mL for TSH

3The p-value is from a paired t-test between concentration at visit 1 and concentration at visit 3 for each biomarker. Adjusted using the method of Benjamini and Hochberg

Table 6.4: Results from Linear Mixed Models showing the Percent Change in Serum Hormone Levels Corresponding with a 10% increase in OPFR concentration

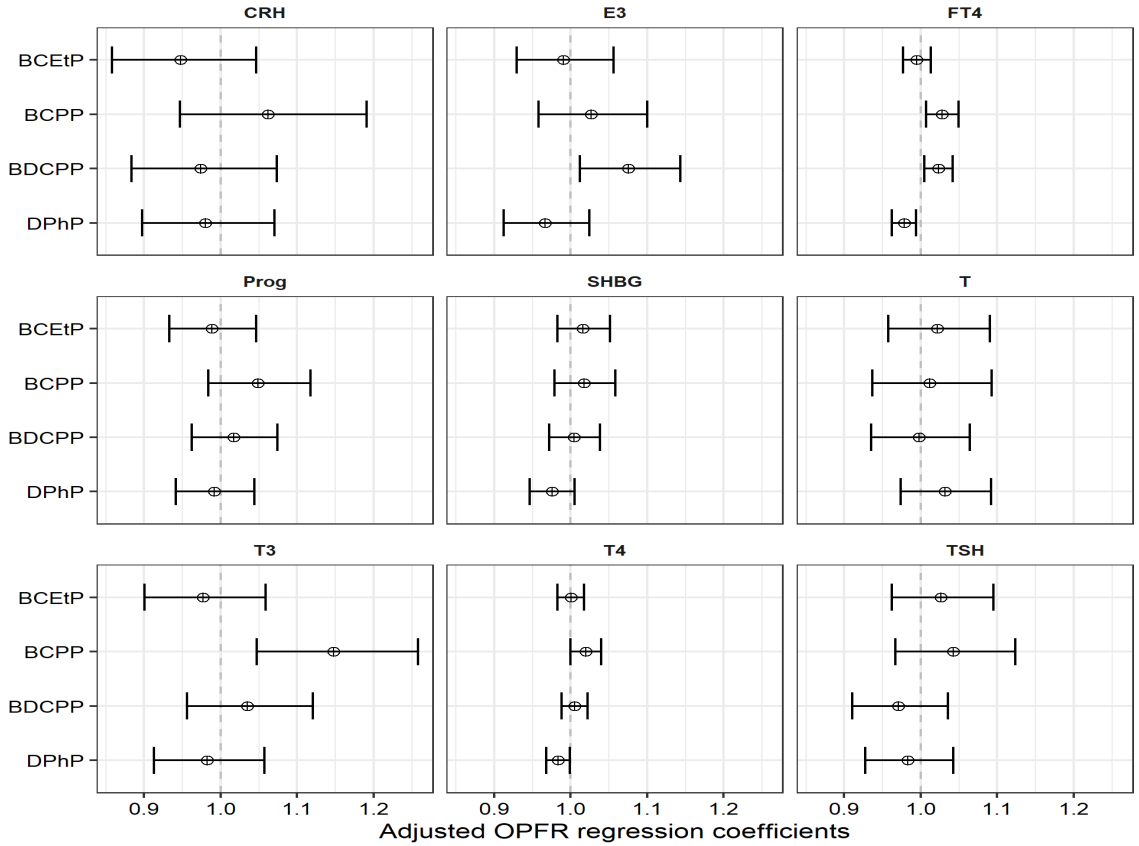
hormone	term	% change
FT4	BCEtP	-0.06 (-0.23, 0.12)
	BCPP	0.25 (0.05, 0.45)
	BDCPP	0.24 (0.07, 0.41)
	DPhP	-0.21 (-0.37, -0.06)
T3	BCEtP	-0.27 (-1.04, 0.51)
	BCPP	1.23 (0.35, 2.12)
	BDCPP	0.45 (-0.30, 1.21)
	DPhP	-0.17 (-0.86, 0.53)
TSH	BCEtP	0.25 (-0.37, 0.87)
	BCPP	0.40 (-0.32, 1.12)
	BDCPP	-0.30 (-0.91, 0.31)
	DPhP	-0.15 (-0.70, 0.41)

hormone	term	% change
T4	BCEtP	-0.00 (-0.16, 0.16)
	BCPP	0.18 (-0.00, 0.37)
	BDCPP	0.05 (-0.11, 0.21)
	DPhP	-0.16 (-0.31, -0.01)
E3	BCEtP	-0.11 (-0.71, 0.50)
	BCPP	0.23 (-0.42, 0.89)
	BDCPP	0.73 (0.16, 1.31)
	DPhP	-0.32 (-0.86, 0.23)
T	BCEtP	0.21 (-0.40, 0.84)
	BCPP	0.14 (-0.59, 0.88)
	BDCPP	-0.06 (-0.67, 0.55)
	DPhP	0.30 (-0.25, 0.85)

hormone	term	% change
CRH	BCEtP	-0.55 (-1.48, 0.40)
	BCPP	0.49 (-0.61, 1.59)
	BDCPP	-0.14 (-1.06, 0.78)
	DPhP	-0.20 (-1.04, 0.64)
Prog	BCEtP	-0.11 (-0.65, 0.44)
	BCPP	0.46 (-0.14, 1.07)
	BDCPP	0.15 (-0.37, 0.67)
	DPhP	-0.09 (-0.58, 0.40)
SHBG	BCEtP	0.14 (-0.18, 0.47)
	BCPP	0.12 (-0.26, 0.49)
	BDCPP	0.09 (-0.23, 0.40)
	DPhP	-0.23 (-0.52, 0.06)

Models adjusted for maternal education, maternal age, and maternal pre-pregnancy weight

Figure 6.1: Adjusted coefficient plots for percent change in maternal hormone levels corresponding to a 10% increase in OPFR concentration



Models adjusted for maternal education, maternal age, and maternal pre-pregnancy weight

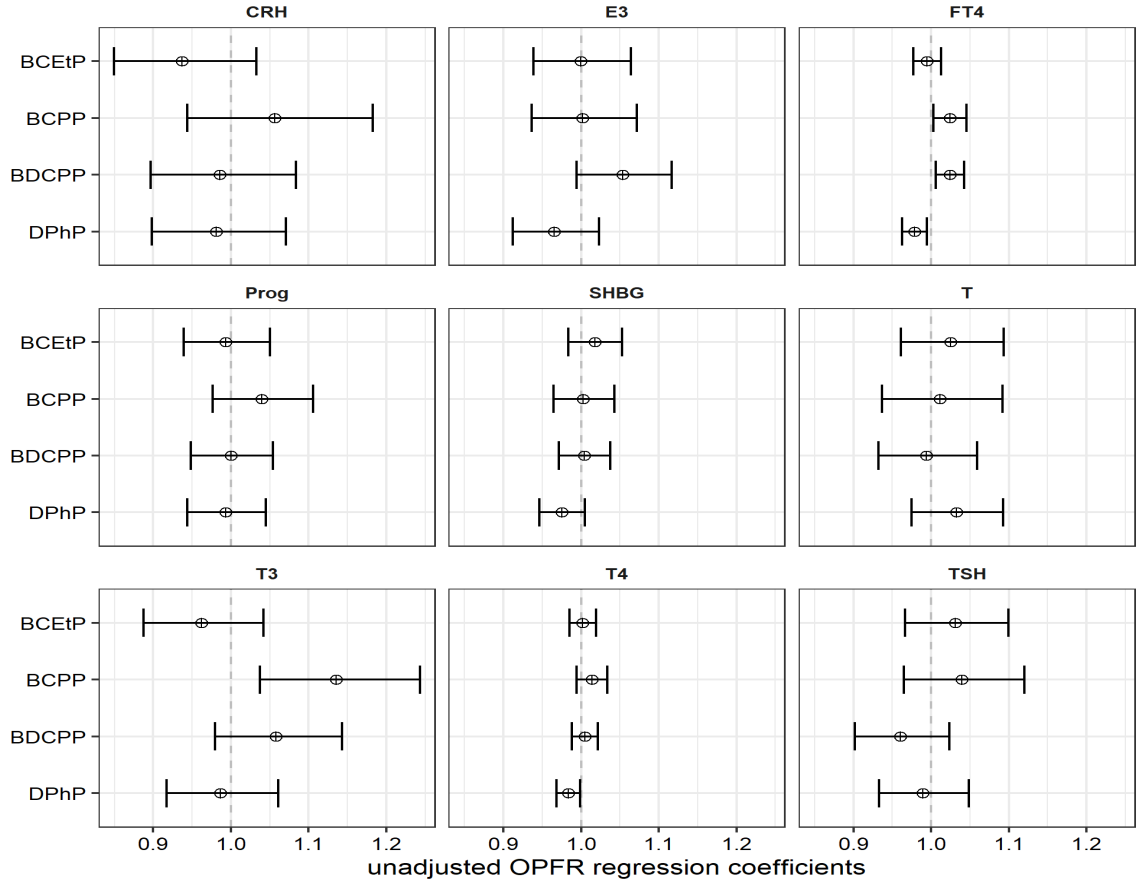
Table 6.5: Unadjusted coefficient plots for percent change in maternal hormone levels corresponding to a 10% change in OPFR concentration

hormone	term	% change
FT4	BCEtP	-0.05 (-0.22, 0.12)
	BCPP	0.23 (0.03, 0.42)
	BDCPP	0.23 (0.06, 0.39)
	DPhP	-0.21 (-0.36, -0.05)
T3	BCEtP	-0.37 (-1.13, 0.39)
	BCPP	1.22 (0.35, 2.10)
	BDCPP	0.54 (-0.20, 1.28)
	DPhP	-0.13 (-0.82, 0.57)
TSH	BCEtP	0.29 (-0.32, 0.91)
	BCPP	0.37 (-0.34, 1.09)
	BDCPP	-0.38 (-0.98, 0.22)
	DPhP	-0.10 (-0.66, 0.45)

hormone	term	% change
T4	BCEtP	0.02 (-0.14, 0.18)
	BCPP	0.13 (-0.06, 0.32)
	BDCPP	0.04 (-0.11, 0.20)
	DPhP	-0.16 (-0.31, -0.01)
E3	BCEtP	-0.00 (-0.60, 0.60)
	BCPP	0.02 (-0.63, 0.66)
	BDCPP	0.50 (-0.06, 1.06)
	DPhP	-0.33 (-0.88, 0.22)
T	BCEtP	0.24 (-0.38, 0.86)
	BCPP	0.11 (-0.62, 0.85)
	BDCPP	-0.06 (-0.67, 0.55)
	DPhP	0.30 (-0.24, 0.85)

hormone	term	% change
CRH	BCEtP	-0.62 (-1.54, 0.31)
	BCPP	0.52 (-0.55, 1.61)
	BDCPP	-0.14 (-1.04, 0.77)
	DPhP	-0.18 (-1.02, 0.65)
Prog	BCEtP	-0.06 (-0.59, 0.47)
	BCPP	0.37 (-0.23, 0.96)
	BDCPP	-0.00 (-0.50, 0.50)
	DPhP	-0.06 (-0.55, 0.42)
SHBG	BCEtP	0.17 (-0.15, 0.49)
	BCPP	0.03 (-0.34, 0.40)
	BDCPP	0.04 (-0.28, 0.35)
	DPhP	-0.24 (-0.53, 0.05)

Figure 6.2: Unadjusted coefficient plots for percent change in maternal hormone level corresponding to a 10% increase in OPFR concentration



CHAPTER 7

CONCLUSIONS

Summary

This dissertation addressed the public health issue of environmental exposures and its possible role on adverse pregnancy outcomes in Puerto Rico. The rates of preterm birth in Puerto Rico are some of the highest both globally and in the United States. There is an increasing public health concern that exposure to persistent organic pollutants, even at low-levels, may have adverse health impacts, particularly during fetal, neonatal, and childhood development. Many of these pollutants are considered endocrine disrupting chemicals (EDCs) that interfere with the body's endocrine system. Due to its abundance in the environment and concerns about its' impact on human health, the focus of this study was on one POP of interest: flame retardants, particularly organophosphate flame retardants. Our study used data from a subset of women enrolled in an ongoing, prospective cohort study, Puerto Rico Testsite for Exploring Contamination Threats (PROTECT), from 2011 to 2017. The PROTECT cohort was established as part of the Superfund Research Program and women are recruited from Puerto Rico's Northern Karst region. The Northern Karst Region of Puerto Rico is home to the majority of the superfund sites on the island. Here, the superfund sites over the Karst aquifers, and water sampling in this region has consistently shown the presence of many environmental contaminants. To the

best of our knowledge, this is one of the first studies that looks at exposure of OPFRs in pregnant women and its effect on adverse pregnancy outcomes in Puerto Rico.

This dissertation discussed three major areas of interest. In the first aim, we sought to identify knowledge gaps on the role of flame retardants on adverse pregnancy outcomes and assess the current knowledge of the effects of flame retardants as endocrine disruptors on these outcomes. Second, aim 2 investigated the associations between prenatal exposure to 8 OPFRs with gestational age and birth weight, hypothesizing that some of these OPFRs would decrease gestational age and birth weight. Lastly, aim 3 examined hormones as a possible mechanism of OPFRs and adverse birth outcomes by assessing the effect of prenatal exposure to OPFRs and its effects on maternal thyroid and reproductive hormones. We hypothesized that OPFRs would disrupt maternal homeostasis. Our results for aim 3 supported our hypotheses and provided information on the effect of OPFRs on maternal hormones as a potential mechanism linking OPFRs and adverse pregnancy outcomes. Aim 1 identified current knowledge in epidemiological literature of the effects of OPFRs and PBDEs as endocrine disruptors on adverse pregnancy outcomes. Although our results for aim 2 did not support our hypotheses, our results provide important information regarding OPFR exposure during pregnancy in Puerto Rico prior to the arrival of Hurricane Maria in September 2017.

Strengths and Limitations

Our results should be interpreted in light of its strengths and limitations. One strength is that our study is sampled from a prospective study design with rich

covariate information including information on co-exposures of interest. Another strength is that we collected two urine samples from our subset of women, enabling us to better characterize exposure and reduce exposure misclassification. However, the degree of exposure misclassification likely varies according to within-person variability of OPFR exposure sources. Our study is limited by a small sample size (n = 146) of women who had complete OPFR measurements at two time points. Sample size may be one of the explanations of the observed non-statistically significant associations in aims 2 and 3, but particularly aim 2. However, this is one of the first studies to examine prenatal OPFR exposures and adverse pregnancy outcomes in Puerto Rico. Another limitation of this study is that the participants' occupation and life style information was not detailed enough to indicate other important sources of exposure. The women included in our current analyses are not representative of the general population, suggesting that the generalizability of our results to other populations could be limited. However, we do not anticipate that this would limit the validity of our findings. Another limitation is that we studied a population that was limited to pregnant women who did not have comorbidities, such as diabetes, which have been associated with poor pregnancy outcomes. While this may limit the generalizability of our study to a certain extent, it also enables us to better examine the association between urinary metabolites and PTB/LBW without confounding by other health conditions. To our knowledge, this is the first study to assess the association of OPFRs and gestational age, birth weight, and maternal thyroid and reproductive hormones

Suggestions for Future Research

The findings from this dissertation have many implications for future research. The results of the systematic review (Aim 1) indicate that although there are many studies still revealing adverse associations on pregnancy outcomes of historical flame retardants such as PBDEs, there is still scarce information on emerging flame retardants such as OPFRs. OPFRs were rapidly marked due to the need of rapid PBDE substitution, however, their environmental behavior and toxicological effects were not properly assessed. This is the case for many other chemical toxins today. Although there was no date restriction for the search strategy, only 6 studies across four databases assessed OPFRs and their effects on gestational age and birth weight, as well as other adverse pregnancy outcomes. Therefore, additional research is urgently needed to elucidate the full impact of OPFRs on maternal and child health. Follow up findings on the association of OPFRs and birth outcomes assessed in aim 2 should include a larger sample size in order to have higher statistical power. Studies with larger sample sizes are also needed to address potential improvement of models by including interactions between OPFRs and covariates that were not accounted for in the current analysis. Additionally, this relationship should be explored in other populations, such as Puerto Ricans in the mainland United States who may be different from women enrolled in PROTECT and other Hispanic groups. In regards to the association of OPFRs and maternal hormone homeostasis, future studies utilizing more frequent measurements through pregnancy and larger sample sizes for OPFRs are needed to support out findings. Because people are rarely exposed to

individual flame-retardant chemicals, studying exposures to mixtures of flame retardants is an important future step to gain a better understanding of associations between environmental exposures and hormone levels. Future studies should also aim to assess how the impact of OPFR exposure on maternal hormones may mediate birth outcomes and child development.

Conclusions

In the first aim, our systematic review of the literature on the effect of OPFRs on adverse pregnancy outcomes found that a growing body of evidence demonstrates that OPFRs are associated with preterm birth and low birth weight, as well as other adverse pregnancy outcomes, but the results are inconsistent. PBDEs continue to reveal adverse associations on pregnancy outcomes, even though they have been phased out of production. Additional research is urgently needed to elucidate the full impact of OPFRs on birth outcomes.

In our second aim, in unadjusted models, the findings are suggestive for an increase in gestational age and a decrease in birth weight z-score for all OPFRs, however, these associations were not significant. After covariate adjustment, these associations remained the same. Although we did not observe any statistically significant associations, this finding is similar to a study conducted in the PIN (Pregnancy Infection and Nutrition study). They found that among male infants, DPHP was associated with a modest increase in gestational duration, although not significant. For example, baby boys with the highest levels of prenatal exposure were born approximately 5 days later than those with the lowest levels of exposure (β=0.75

weeks; 95% CI:(0.01, 1.50); p=0.05).[52] There are also other studies that have non-significant associations of OPFR exposure and birth outcomes.[55,56] Our null results may also be explained by a small sample size (n = 146).

In our third aim, we assessed the association of four OPFRs on 9 maternal hormones. These hormones included both thyroid and reproductive hormones. We found some OPFRs were associated with thyroid hormones, suggesting that OPFRs may act as endocrine disruptors by altering prenatal hormone concentrations.

In conclusion, this dissertation adds to an existing body of literature of the effect of environmental pollutants and its implications for the health of human pregnancy. However, the focus of this dissertation is on a replacement flame retardant, organophosphate flame retardants, for which evidence of effects on human pregnancy is very limited. Our hope is that results from this dissertation further our efforts to understand increased rates of preterm birth observed on the island of Puerto Rico, and provide additional tools that can be used to predict at-risk pregnancies and better protect this highly vulnerable population.

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Appendix A: Search Strategies by Database

PubMed Search Strategy

(
(("organophosphates"[MeSH] OR "organophosph*"[tw] OR "diethyl dithiophosphate"[tw] OR "diethyl phosphate"[tw] OR "dimethyl dithiophosphate"[tw] OR "dimethyl phosphate"[tw] OR "dimethyl thiophosphate"[tw] OR "diethyl thiophosphate"[tw] OR "diphenyl phosphate"[tw] OR "di-cresyl phosphate"[tw] OR "di-benzyl phosphate"[tw] OR "di-n-butyl phosphate"[tw] OR "bis(2-chloroethyl) phosphate"[tw] OR "bis-(1-chloro-2propyl) phosphate"[tw] OR "bis(1-chloro-2-propyl) phosphate"[tw] OR "bis(1,3-dichloro-2-propyl) phosphate"[tw] OR "2,3,4,5-Tetrabromobenzoic acid"[tw] OR BCPP[tw] OR BCEtP[tw] OR BDCPP[tw])
OR
("Flame retardant*"[tw] OR "Flame retardants"[MESH] OR "fireproofing agent*"[tw] OR "fire retardant*"[tw] OR "PBDE*"[tw] OR "polybrominated diphenyl ether*"[tw] OR "OPEs"[tw] OR "endocrine disruptors"[MESH] OR "endocrine disrupt*"[tw] OR "endocrine-disrupt*"[tw] OR "Halogenated Diphenyl Ethers"[MeSH])
)

AND

(
("Infant, Low Birth Weight"[MeSH] OR "Infant, Premature"[MeSH] OR "Pregnancy Outcome"[MeSH])
OR
(
(
(prematur*[tw] OR preterm[tw] OR pre-term[tw] OR "low birth weight*"[tw] OR "low birthweight*"[tw])
AND
(neonat*[tw] OR newborn*[tw] OR infan*[tw] OR "Infant, Newborn"[MeSH])
)
OR
("small for gestational age"[tw] OR ("small"[tw] AND "gestational age"[tw]))
)
OR

("Fetal Death"[MeSH] OR "Abortion, Spontaneous"[MeSH] OR "early pregnancy loss*"[tw] OR "tubal abortion*"[tw] OR "miscarr*"[tw] OR "spontaneous abortion"[tw] OR ("spontaneous"[tw] AND "abortion"[tw]) OR "stillb*"[tw])

OR

(
("Premature Birth"[MeSH] OR "Obstetric Labor, Premature"[MeSH])
OR
(
(prematur*[tw] OR preterm[tw] OR pre-term[tw])
AND
("Parturition"[MeSH] OR parturition*[tw] OR birth*[tw] OR "Delivery, Obstetric"[MeSH]
OR
obstetric*[tw] OR deliver*[tw] OR "Labor, Obstetric"[MeSH] OR labor*[tw] OR
labour*[tw])
)
)
)

NOT

("Animals"[MeSH] NOT "Humans"[MeSH])

NOT

(“Letter”[PT] OR “Editorial”[PT] OR “Comment”[PT])

EMBASE Search Strategy

1 premature labor/ or prematurity/
2 exp low birth weight/
3 exp abortion/
4 exp induced abortion/
5 exp pregnancy termination/
6 pregnancy outcome/
7 pregnancy complication/
8 fetus mortality/ or infant mortality/ or maternal mortality/ or exp perinatal mortality/
9 ((prematur* or preterm or pre-term) adj2 (birth* or parturition* or delivery or labo?r)).mp.
10 ((prematur* or preterm or pre-term or low birth weight or low birthweight) adj2 (neonat* or newborn* or infant*)).mp.
11 "small for gestational age".mp.
12 (miscarr* or abort* or stillb* or ((f?etal or f?tus) adj (death* or mortality))).mp.
13 ((f?tal or f?tus* or f?ti or pregnan* or prenatal or pre-natal or antenatal or ante-natal or perinatal or peri-natal or birth* or obstetric* or parturition* or labo?r) adj2 (complicat* or outcome* or mortalit* or death*)).mp.
14 ((infant* or newborn* or neonat*) adj2 (complicat* or outcome* OR death* OR mortalit*)).mp.
15 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14
16 exp flame retardant/
17 exp endocrine disruptor/
18 exp organophosphate/
19 diphenyl ether/ or diphenyl ether derivative/
20 (organophosph* or diethyl dithiophosphate or diethyl phosphate or dimethyl dithiophosphate or dimethyl phosphate or dimethyl thiophosphate or diethyl thiophosphate or diphenyl phosphate).mp.
21 (di-cresyl phosphate or di-benzyl phosphate or di-n-butyl phosphate or BCPP or BCEtP or BDCPP or "bis(2-chloroethyl) phosphate" or "bis-(1-chloro-2-propyl) phosphate" or "bis(1-chloro-2propyl) phosphate" or "bis(1,3-dichloro-2-propyl) phosphate" or 2,3,4,5-Tetrabromobenzoic acid).mp.
22 (((fire* or flame*) adj6 retardant*) or fireproofing agent* or PBDE* or polybrominated diphenyl ether* or OPEs or endocrine-disrupt* or halogenated diphenyl ether* or (endocrine adj6 disrupt*)).mp.
23 16 or 17 or 18 or 19 or 20 or 21 or 22
24 15 and 23
25 exp animals/ not human/
26 24 not 25
27 limit 26 to (books or conference abstract or conference paper or "conference review" or editorial or letter or note)

28 26 not 27

Web of Science

1 TI=((prematur* OR preterm OR pre-term) NEAR/2 (birth* OR parturition* OR delivery
OR labo$r))
2 AB=((prematur* OR preterm OR pre-term) NEAR/2 (birth* OR parturition* OR delivery
OR labo$r))
3 AK=((prematur* OR preterm OR pre-term) NEAR/2 (birth* OR parturition* OR delivery
OR labo$r))
4 TI=((prematur* OR preterm OR pre-term OR "low birth weight" OR "low birthweight")
NEAR/2 (neonat* OR newborn* OR infant*))
5 AB=((prematur* OR preterm OR pre-term OR "low birth weight" OR "low birthweight")
NEAR/2 (neonat* OR newborn* OR infant*))
6 AK=((prematur* OR preterm OR pre-term OR "low birth weight" OR "low birthweight")
NEAR/2 (neonat* OR newborn* OR infant*))
7 TI=("small for gestational age")
8 AB=("small for gestational age")
9 AK=("small for gestational age")
10 TI=(miscarr* OR abort* OR stillb* OR ((f$etal OR f$tus) NEAR/0 (death* OR mortalit*)))
11 AB=(miscarr* OR abort* OR stillb* OR ((f$etal OR f$tus) NEAR/0 (death* OR mortalit*)))
12 AK=(miscarr* OR abort* OR stillb* OR ((f$etal OR f$tus) NEAR/0 (death* OR mortalit*)))
13 TI=((f$tal OR f$tus* OR f$ti OR pregnan* OR prenatal OR pre-natal OR antenatal OR ante-natal OR perinatal OR peri-natal OR birth OR obstetric* OR
parturition* OR labo?r) NEAR/2 (complicat* OR outcome* OR mortalit* OR death*))
14 AB=((f$tal OR f$tus* OR f$ti OR pregnan* OR prenatal OR pre-natal OR antenatal OR ante-natal OR perinatal OR peri-natal OR birth OR obstetric* OR
parturition* OR labo?r) NEAR/2 (complicat* OR outcome* OR mortalit* OR death*))
15 AK=((f$tal OR f$tus* OR f$ti OR pregnan* OR prenatal OR pre-natal OR antenatal OR ante-natal OR perinatal OR peri-natal OR birth OR obstetric* OR
parturition* OR labo?r) NEAR/2 (complicat* OR outcome* OR mortalit* OR death*))
16 TI=((infant* OR newborn* OR neonat*) NEAR/2 (complicat* OR outcome* OR death*
OR mortalit*))
17 AB=((infant* OR newborn* OR neonat*) NEAR/2 (complicat* OR outcome* OR death*
OR mortalit*))

18 AK=((infant* OR newborn* OR neonat*) NEAR/2 (complicat* OR outcome* OR death* OR mortalit*))
19 TI=(organophosph* OR "diethyl dithiophosphate" OR "diethyl phosphate" OR "dimethyl dithiophosphate" OR "dimethyl phosphate" OR "dimethyl thiophosphate" OR "diethyl thiophosphate" OR "diphenyl phosphate" OR "di-cresyl phosphate" OR "di-benzyl phosphate" OR "di-n-butyl phosphate" OR "bis(2-chloroethyl) phosphate" OR "bis-(1-chloro-2-propyl) phosphate" OR "bis(1-chloro-2propyl) phosphate" OR "bis(1,3-dichloro-2-propyl) phosphate" OR "2,3,4,5-Tetrabromobenzoic acid")
20 AB=(organophosph* OR "diethyl dithiophosphate" OR "diethyl phosphate" OR "dimethyl dithiophosphate" OR "dimethyl phosphate" OR "dimethyl thiophosphate" OR "diethyl thiophosphate" OR "diphenyl phosphate" OR "di-cresyl phosphate" OR "di-benzyl phosphate" OR "di-n-butyl phosphate" OR "bis(2-chloroethyl) phosphate" OR "bis-(1-chloro-2-propyl) phosphate" OR "bis(1-chloro-2propyl) phosphate" OR "bis(1,3-dichloro-2-propyl) phosphate" OR "2,3,4,5-Tetrabromobenzoic acid")
21 AK=(organophosph* OR "diethyl dithiophosphate" OR "diethyl phosphate" OR "dimethyl dithiophosphate" OR "dimethyl phosphate" OR "dimethyl thiophosphate" OR "diethyl thiophosphate" OR "diphenyl phosphate" OR "di-cresyl phosphate" OR "di-benzyl phosphate" OR "di-n-butyl phosphate" OR "bis(2-chloroethyl) phosphate" OR "bis-(1-chloro-2-propyl) phosphate" OR "bis(1-chloro-2propyl) phosphate" OR "bis(1,3-dichloro-2-propyl) phosphate" OR "2,3,4,5-Tetrabromobenzoic acid")
22 TI=(((fire* or flame*) NEAR/6 retardant*) or fireproofing agent* or PBDE* or polybrominated diphenyl ether* or OPEs or endocrine-disrupt* or halogenated diphenyl ether* or (endocrine NEAR/6 disrupt*))
23 AB=(((fire* or flame*) NEAR/6 retardant*) or fireproofing agent* or PBDE* or polybrominated diphenyl ether* or OPEs or endocrine-disrupt* or halogenated diphenyl ether* or (endocrine NEAR/6 disrupt*))
24 AK=(((fire* or flame*) NEAR/6 retardant*) or fireproofing agent* or PBDE* or polybrominated diphenyl ether* or OPEs or endocrine-disrupt* or halogenated diphenyl ether* or (endocrine NEAR/6 disrupt*))
25 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #12 OR #13 OR #14 OR #15 OR #16 OR #17 OR #18
26 #19 OR #20 OR #21 OR #22 OR #23 OR #24
27 #25 AND #26
28 SU=Veterinary Sciences
29 WC=Veterinary Sciences
30 #28 OR #29
31 #27 NOT #30

Then manually exclude these document types:

Proceedings Papers
Editorial Materials

Meeting Abstracts
Letters
Book Chapters
COCHRANE

1 (phosphate* OR organophosph* OR dithiophosphate* OR thiophosphate* OR tetrabromobenzoic OR ((flame OR fireproof*) NEAR/6 (retardant* OR agent*)) OR PBDE* OR ((polybrominated OR halogenated) NEAR/2 (“diphenyl ether” OR “diphenyl ethers”)) OR (endocrine NEAR/6 disrupt*)):ti,ab,kw

2 ((((prematur* OR preterm OR pre-term OR "low birth weight*" OR "low birthweight*")
NEAR/6 (neonat* OR newborn* OR infan*)) OR ("small for gestational age" OR ("small" NEAR/6 "gestational age"))) OR ("early pregnancy loss*" OR "tubal abortion*" OR "miscarr*" OR "spontaneous abortion" OR ("spontaneous" NEAR/6 "abortion") OR "stillb*") OR (((prematur* OR preterm OR pre-term) NEAR/6 (parturition* OR birth* OR obstetric* OR deliver* OR labor* OR labour*)))):ti,ab,kw

3 #1 AND #2

4 ([mh “animals”] NOT [mh “humans”])

5 #3 NOT #4