Distribution Agreement

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

Signature:

_____ _____

Abby D. Mutic Date

PBDE Exposure, Thyroid Disruption, and Antenatal Depression in African American Women

By Abby D. Mutic

Doctor of Philosophy

Nursing

_____

Linda McCauley, Ph.D, RN, FAAOHN, FAAN
Advisor

_____

Dana Barr, Ph.D.
Committee Member

_____

Patricia Brennan, Ph.D.
Committee Member

_____

Anne Dunlop, M.D., M.P.H.
Committee Member

_____

Vicki Hertzberg, Ph.D.
Committee Member

Accepted:

_____

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

_____

Date

PBDE Exposure, Thyroid Disruption, and Antenatal Depression in African American Women

By

Abby D. Mutic
B.A., Drury University, 2003
B.S.N., Saint Louis University, 2004
M.S.N., Vanderbilt University, 2009

Advisor: Linda A. McCauley, Ph.D., RN, FAAOHN, FAAN

An abstract of
A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in Nursing
2019

Abstract

PBDE Exposure, Thyroid Disruption, and Antenatal Depression in African American Women
By Abby D. Mutic

Background: Polybrominated diphenyl ethers (PBDEs) are environmental chemicals once used to prevent or reduce flammability. Phased out of production nearly 10 years ago, PBDEs remain ubiquitous in the environment. These persistent endocrine disrupting chemicals have potential to disrupt normal neuroendocrine pathways such as thyroid hormone synthesis resulting in depression. Pregnant African American (AA) women living in urban areas may be at higher risk of depression due to high PBDE exposure. This study examined complex interactions between PBDE serum concentrations, tyrosine metabolites within thyroid hormone synthesis, and the risk of antenatal depression in pregnant AA women.

Methods: Nested in a larger study, data was collected from 193 pregnant AA women between 8-14 weeks gestation. Serum PBDEs were determined by GC/MS. Levels of tyrosine-associated metabolites in plasma were determined using apLCMS and xMSanalyzer to improve high-resolution LC/MS. Socio-demographic variables were collected. The Edinburgh Depression Scale (EDS) was used to identify depressive symptoms experienced in the last seven days with a score ≥10 indicating high risk. A weighted quantile sum (WQS) index was constructed for PBDE mixture effect. Linear and logistic regression models investigated associations with PBDE concentrations, WQS, and depression. Seven metabolites within the tyrosine pathway were considered as potential mediators between PBDEs and depression symptoms.

Results: 52 women (26.9%) were at a high risk of depression. BDE-47 was positively associated with depressive symptoms with a 4.52 increased risk of depression (CI 1.50, 13.60) and 1.58 for BDE-99 (CI 1.08, 2.29) for each unit increase in PBDE congener. The WQS index was also positively associated with a higher risk of depression (OR=2.93; CI 1.18, 7.82). Tyrosine metabolite suspects did not mediate the effect of PBDEs on depression scores.

Conclusion: BDE-47 and -99 exposures were associated with an increased risk of depression and Tyrosine metabolites were identified possibly indicating thyroid disruption. However, the metabolites did not mediate the PBDE-depression relationship. Interventions should focus on PBDE exposure mitigation to reduce the risk of depression on vulnerable pregnant women.

PBDE Exposure, Thyroid Disruption, and Antenatal Depression in African American Women

By

Abby D. Mutic
B.A., Drury University, 2003
B.S.N., Saint Louis University, 2004
M.S.N., Vanderbilt University, 2009

Advisor: Linda A. McCauley, Ph.D., RN, FAAOHN, FAAN

A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in Nursing
2019

Acknowledgments

I would like to take this opportunity to thank my trusted PhD committee members for their guidance and support of my dissertation throughout the process. My advisor, Dean Linda McCauley, for her creative influence, encouraging me to dabble in many different professional opportunities, and for modeling the kind of nurse leader and researcher I can aspire to be. Dr. Vicki Hertzberg for your regular input in my analyses, helping to build my self-esteem as a researcher, and for our few "come to Jesus" moments. Dr. Anne Dunlop for guiding me through research details while helping me balance my other roles as a clinician and a mother. Dr. Dana Barr for always being available for my pressing EH questions and for your interest and excitement about my ideas. And to Dr. Patty Brennan for your calm, down-to-earth perspective as well as your critical feedback and editing.

Many thanks to my friends and colleagues in the School of Nursing and around the globe. Whether work related or not, you were available at just the right times to pick me up, cheer me up, or give me a good laugh.

I will be forever grateful to my family and to my husband, Nathan. Without your unconditional love, support, and encouragement, this dissertation and degree would not be possible. Thank you for pouring into me over the last 4-5 years and filling in the gaps at home and with my girls. To God be the glory. His grace, strength, and blessings have given me the courage and ambition. May I use these skills to honor him and help others.

Table of Contents

List of Tables

List of Figures

INTRODUCTION

Statement of the problem

Environmental toxicant exposures and subsequent adverse health outcomes disproportionately affect minority individuals living in poor urban environments. According to NHANES population data, non-Hispanic blacks have higher amounts of polybrominated diphenyl ether (PBDE) body burden when compared to non-Hispanic whites[1]. Pregnant women are especially vulnerable due to increased risk of toxicity related to normal physiological changes in pregnancy[2-4]. PBDEs are flame-retardant chemicals found in many household products and thus, exposure to humans is ubiquitous occurring through ingestion of contaminated dust, air, or food[5,6]. They are known endocrine disrupting chemicals that can alter thyroid homeostasis by modifying essential thyroid hormone production or function[7,8]. Thyroid homeostasis is critical for human growth and development as well as for proper cognition and mood stability[4]. PBDE exposure in utero and thyroid dysfunction have independently been associated with adverse psychological functions such as attention deficit[9], impaired neurodevelopment[10-12], and poor mental and psychomotor development[11] but mechanistic pathways and linkages have yet to be established. Specifically, no studies have examined whether PBDE exposure in pregnant women can be linked to perinatal depression by thyroid pathway disruption. Therefore, the **purpose of this dissertation** was to characterize the complex interactions between PBDE serum concentrations, tyrosine metabolites responsible for thyroid hormone synthesis, and the risk of antenatal depression in urban African American (AA) pregnant women.

Background

Environmental health disparities

Communities of color represent approximately 60% of metro-Atlanta where the median household income is $51,000. Researchers have repeatedly documented that African Americans (AAs), also referred to as non-Hispanic blacks, consistently exhibit higher levels of PBDEs in serum compared to their White counterparts[13-16] and are nine times more likely to have high concentrations of persistent organic pollutants, which includes PBDEs, after adjusting for income[17]. Similarly, NHANES population data collected between years 2003-2008 consistently reported higher serum concentrations of polybrominated diphenyl ethers (PBDEs) among non-Hispanic black females when compared to non-Hispanic white females[18]. This type of inequality among minority populations to heavy toxicant exposures contributes to disparities in health status. Environmental injustice is highly correlated with poverty, poor living conditions, lack of healthy food, crime, and poor access to health care[19]. Environmental injustice research to date has largely focused on issues related to infants and children. Phil Landrigan, an expert in children's environmental health, urges human studies to assess exposures and health effects into adulthood and to include biomarkers of exposure and neuropsychological and behavioral endpoints[20].

Vulnerability of pregnant women

Pregnant women are especially vulnerable to environmental-related illnesses and are at an increased risk of chemical toxicity related to normal physiological changes in pregnancy[2-4]. Normal anatomical, hormonal, and metabolic shifts begin at conception and persist until return to pre-pregnancy function in the postpartum period. Especially of value to the pregnant woman, the thyroid gland undergoes significant demands

required of the growing fetus. An increase in thyroid hormone is found in circulation after maternal estrogen and human chorionic gonadotropin spikes trigger a hypothalamic-pituitary response[21]. Additionally, normal hemodilution and increased plasma volume contributes to a decrease in available iodine essential for thyroid hormone binding. The body compensates again by increasing thyroid hormone. The increased metabolic and growth demands on the mother as a result of pregnancy, makes the body more vulnerable to disruption by exogenous hazards such as environmental toxicants. In a vulnerable state, the likelihood of environmental-related illnesses as a result is amplified.

PBDEs as persistent organic pollutants

PBDEs are lipophilic chemicals found in the environment used to prevent or reduce the ability of materials to burn. They are found in many electronic products, carpets, furniture, baby products, and automobile interiors. They have been widely added to consumer products since the 1970's[22]. PBDEs are not covalently bound and when mixed with plastics and foam, they readily make their way into the environment during use or in the manufacturing process[23]. Once the chemicals breach the soil, sediment and water, they enter the food chain and accumulate. Fish, marine mammals, farm animals, birds, and wildlife become exposed. Subsequently, exposure is ubiquitous in the environment and, for humans, occurs mainly through indoor dust and diet[5,6]. These ubiquitous persistent organic pollutants (POPs) are present in humans[24], animals[25], and biota[26]. Human concentrations of PBDEs have steadily increased since inception[27,28] peaking in 2006 with congeners BDE -47, -99, and -100 having the highest reported levels in a minority cohort[29]. In the past three decades, concentrations in serum and breastmilk have increased exponentially and are estimated to be detected in

the tissues of virtually every North American. As data emerged on the health effects of PBDEs, new production and import of penta and octa PBDEs started to phase out in the US and Europe[30]. However, the phase out is slow and the toxicants are anticipated to remain present in our environment for years. Once-produced mass quantities of PBDE-containing products such as sofas and electronics are still being used in homes, businesses, and schools[23] across the US.

PBDE metabolic pathways and health effects

PBDEs are part of a large group of chemicals with endocrine disrupting properties capable of altering essential functions of the endocrine, reproductive, and nervous systems. Other well-studied endocrine disrupting chemicals are associated with adverse health effects such as genital malformations, preterm birth, low birth weight, endocrine-related cancers, early onset breast development, developmental neurotoxicity, obesity, and diabetes[31]. A growing body of evidence in human and animal studies links PBDE exposures to serious adverse health outcomes such as thyroid disease[32-34], reproductive changes[35], neurodevelopmental deficits[9,36-40], and gestational diabetes[41]. PBDEs have a chemical structure that is similar to thyroid hormones enabling the chemical to mimic hormone activities. PBDEs can alter thyroid homeostasis by disrupting thyroid hormone production and essential hormone function[7,8]. Disruption of thyroid hormone synthesis and activities have been linked to crippling health conditions such as cardiovascular disease[42], systemic lupus erythematosus[43], liver disease[44], and cancer[45]. Thyroid hormone signaling and disruption in homeostasis has a long history of association with mood disorders such as depression with a prevalence rate of 60% affecting women of childbearing age more often[46-48]. Classic hypothyroid disease, a condition hallmarked by decreased thyroid

hormone in the blood, typically exhibits significant psychiatric and cognitive symptoms such as forgetfulness, fogginess, fatigue, depressed mood, and sleep disturbance. While the relationships between thyroid disruption, mood, and cognition have been well-established, researchers have only recently begun to investigate potential mechanisms which may explain the relationships between endocrine disrupting chemicals and neuropsychological dysfunction.

PBDE exposure and thyroid dysfunction have independently been associated with adverse psychological functions such as attention deficit[9], impaired neurodevelopment[10-12], and poor mental and psychomotor development[11]. Neurotoxic effects occur when a toxic agent produces structural or functional changes within the nervous system. The changes can lead to permanent or reversible brain damage exemplified by learning impairment, memory loss, motor control or behavior changes such as mood instability. Many studies have elucidated the effects of prenatal PBDE exposure on the developing fetus and children later in life, but to date, only two studies have investigated the effects of PBDE exposure outside of pregnancy *and* subsequent neuropsychological effects[49,50]. It is unknown if the PBDE-associated neuropsychological and behavioral dysfunction observed in children of cohort studies are also present in other populations.

Preliminary studies have begun to implicate specific metabolites in the association between environmental chemicals and depression[51]. Metabolites are intermediate products produced from metabolic reactions that naturally occur within cells. An upregulation or downregulation of metabolic processes can occur in response to environmental toxicants such as PBDEs causing more or less receptors and thus, sensitivity to a molecule. Thyroid hormone synthesis occurs within the tyrosine

metabolic pathway, produced in the thyroid gland from tyrosine and iodine. One way to identify whether metabolic processes have been up or downregulated is by utilizing high-resolution metabolomics where researchers are able to detect tens of thousands of metabolites present in human plasma. Assessing individual metabolic profiles can provide useful information about disease states, toxicity, and mechanisms of action at a given point in time. No studies to date have examined whether PBDE exposure can be linked to antenatal depression by tyrosine pathway disruption.

Specific Aims of Dissertation

In order to better describe the PBDE exposures to urban African American pregnant women and the relationship with neuropsychological outcomes, we designed 3 specific aims to guide the research and hypothesis testing.

Aim 1. Characterize serum PBDE concentrations of urban AA pregnant women and evaluate their association with the risk of antenatal depressive symptoms.

H_1: Higher concentrations of PBDE toxicants (measured in serum) are associated with a higher risk of depression (Edinburgh Depression Scale) among urban AA pregnant women.

Aim 2. Examine the association between serum PBDE concentrations and the tyrosine metabolic pathway.

H_1: Higher concentrations of PBDE toxicants (measured in serum) are associated with downregulation of metabolites within the tyrosine pathway.

Aim 3. Examine the tyrosine metabolic pathway as a mediator between PBDE concentrations and the risk of antenatal depressive symptoms in the AA cohort.

H_1: The tyrosine metabolic pathway acts as a mediator between PBDE concentrations and the risk of antenatal depression (strength of the relationship

between PBDE levels and antenatal depressive symptoms goes down after accounting for tyrosine metabolites).

Conceptual Model

To guide the dissertation and illustrate the interrelationships between the main concepts, we constructed a conceptual model (Figure 1), presented below.

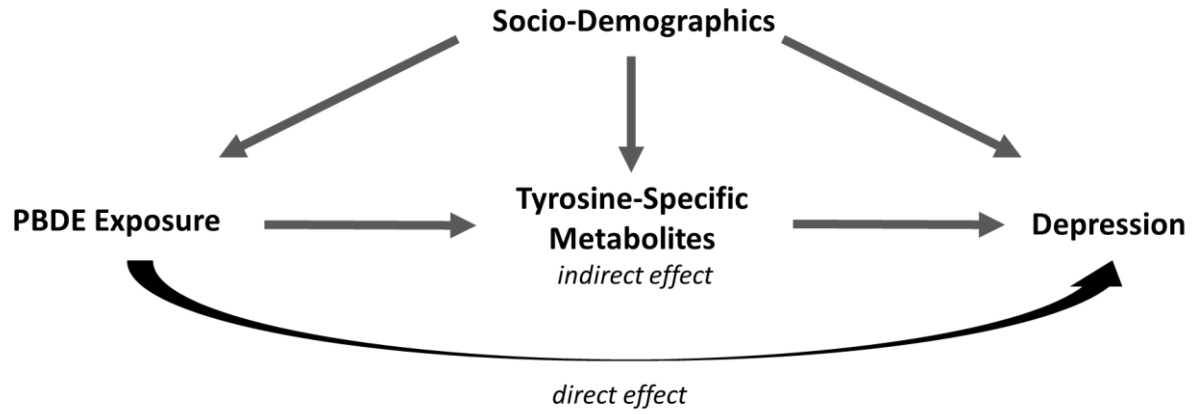


Figure 1. Conceptual model depicting two mechanisms between PBDE exposure and depression. *Adapted from Eldridge et al. (2017)*

The proposed causal framework depicts two mechanisms between PBDE exposure and depression. We propose that 1) tyrosine-specific metabolites mediate the relationship between PBDE exposures and depression (indirect effect) and 2) PBDEs may have alternative mechanisms which lead to depression that does not include the tyrosine pathway (direct effect).

Introduction to manuscripts

In manuscript 1 entitled, *"Thyroid hormone disruption as a mediator between polybrominated diphenyl ether and antenatal depression"*, the relationships between the dissertation concepts are highly scrutinized and evaluated in the literature. We review the literature on the known health effects of PBDE exposures, the role of thyroid

hormone disruption in neuropsychological outcomes, and increased vulnerability in pregnant women.

Manuscript 2 entitled, *"Polybrominated diphenyl ether serum concentrations and depressive symptomatology in pregnant African American women"*, describes the PBDE serum concentrations and examines whether PBDE exposure in pregnant women is associated with depressive symptomatology in the African American cohort. The toxicant and depression data were collected between 8-14 weeks gestation. We used statistical modeling to estimate associations between detected PBDE congeners, a mixture of congeners, and depressive symptoms (direct effect in the causal framework).

Finally, in manuscript 3 entitled, "*Using tyrosine metabolomics to describe the relationship between serum polybrominated diphenyl ether and depression"*, the causal framework is further explored. Tyrosine-associated metabolic pathways were detected using a metabolomics suspect screening approach to explore causal effects of PBDE exposure and antepartum depression in African American women. The screening approach resulted in 278 compounds having human metabolomic database matches with our custom metabolite database based on the mass to charge ratio (*m/z*). The evaluation results identified 7 of those metabolic compounds named in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and were further used in the analysis. The models partition the effects into indirect and direct mechanisms where an indirect causal effect implicates the metabolite-mediated mechanism and a direct effect represents all other potential mechanisms that may lead to depression.

Relevance to Nursing and Public Health

Health conditions resulting from environmental hazards are preventable. The ability to modify environments and change human activities that result in pollution is

achievable. Detectable levels of PBDEs and other endocrine disrupting chemicals in human matrices cannot be overlooked. Environmental injustice resulting from poor living conditions affects the most vulnerable and heavily contributes to the health disparities that currently exist in the United States. This intersection between the environment and human health is where nurses and public health professionals excel. Whether it is through education, research, advocacy, or providing actual nursing care, nurses can influence healthy interventions and change among individuals, communities, and whole populations. Prevention is always better than dealing with disease and illness after it occurs. Not only is it beneficial for cost, it reduces the human suffering accrued by unknowing but needless exposures. Nurses have the unique capacity to mobilize systems, large and small, to make the changes required to end disproportionate exposures and ultimately halt the short and long-term health consequences.

Summary

In summary, this dissertation reviews toxicological information about PBDE chemicals commonly found in the environment and in the homes of most Americans. Once thought to be important in reducing the frequency and duration of fire, PBDEs are known endocrine disrupting chemicals that can alter thyroid homeostasis in humans. Thyroid homeostasis is essential for many system processes including proper cognition and mood but major gaps in research have been identified. This compilation of research attempts to fill some of those gaps by shifting the focus toward understudied African American pregnant women, characterizing the relationships between PBDEs and neuropsychiatric outcomes, and testing whether the thyroid acts as a mediator between the endocrine disruptors and depression.

Thyroid Hormone Disruption as a Mediator Between PBDEs and Perinatal Depression

Abstract

Polybrominated diphenyl ethers (PBDEs) are lipophilic chemicals used to prevent or reduce the ability of materials to burn. They are found in many electronic products, carpets, furniture, baby products, and automobile interiors. PBDEs are ubiquitous and persistent in our environment. As an endocrine disrupting chemical, a clear connection exists between PBDE exposure and thyroid disruption in both animal and human studies. Pregnancy is a time of rapid alterations in thyroid hormone signaling and production possibly making the woman more susceptible to PBDE-induced thyroid dysfunction. Disruption in thyroid homeostasis can lead to psychiatric symptoms and mood disorders such as depression. As many as 19.2% of women suffer from prenatal depression, and up to 18.7% including postpartum women which is detrimental to not only the mother but also the developing fetus and infant. While many studies have elucidated the effects of prenatal PBDE exposure on the developing fetus and children later in life, few have investigated the effects of PBDE exposure outside of pregnancy *and* subsequent neuropsychological effects. Additionally, researchers have begun to investigate potential mechanisms, which may explain the relationships between endocrine disrupting chemicals and neuropsychological dysfunction. We review the literature on the known health effects of PBDE exposures, the role of thyroid hormone disruption in neuropsychological outcomes, and increased vulnerability in pregnant women.

Introduction

Polybrominated diphenyl ethers (PBDEs) are lipophilic chemicals used to prevent or reduce the ability of materials to burn. They are found in many electronic products, carpets, furniture, baby products, and automobile interiors. They have been widely added to consumer products since the 1970's[22]. As PBDEs are mixed, not covalently bound, with plastics and foam, they readily make their way into the environment during their use or manufacturing process[23]. These ubiquitous persistent organic pollutants (POPs) are present in humans, animals, and biota[24-26]. Human concentrations of PBDEs have been increasing since they were initially manufactured[1,28] and peaked in 2006 with congeners BDE -47, -99, and -100 having the highest reported levels in a minority cohort[29]. As data emerged on the health effects of PBDEs, the production and import of penta and octa PBDEs started to be phased out in 2004 in the US and Europe[30]. However, they are anticipated to remain present in our environment for years due to once-produced mass quantities of PBDE-containing products such as sofas and electronics. These are still being used in homes, businesses, and schools across the U.S.[23]. In the past three decades, concentrations in serum and breastmilk have increased exponentially and are estimated to be detected in the tissues of virtually every North American.

PBDEs are part of a large group of chemicals with endocrine disrupting properties, including the capacity to disrupt thyroid hormone production and function. In pregnancy, normal physiologic changes alter the amount of thyroid production and hormone utilization as demands from the fetus increases[3]. Thyroid hormone disruption has been linked to a myriad of health conditions[43-45,52] but growing scientific interest has been given to thyroid hormone disruption and the potential contribution to perinatal

depression- including both pre- and postnatal time periods[53-55]. Perinatal depression includes major or minor episodes of depression during pregnancy or up to 1 year after delivery[56]. It is estimated that as many as 19.2% of women suffer from prenatal depression, and up to 18.7% including postpartum women[57], which is detrimental to not only the mother but also the developing fetus leading to poor birth outcomes, infant or child morbidity[58-60]. While many studies have elucidated the effects of prenatal PBDE exposure on the developing fetus and children later in life, to date only two studies have investigated the effects of PBDE exposure outside of pregnancy *and* subsequent neuropsychological effects[49,50]. It is unknown if the PBDE-associated neuropsychological and behavioral dysfunction observed in children of cohort studies are also present in other populations. Psychological assessments were measured in a small cross-sectional sample of children aged 9-11 and compared to PBDE congeners. Measures of hostility and anger were significantly associated with BDE -28, -47, and -99 as well as parent-reported conduct problems[49]. Similar neuropsychological tests were conducted among older adults living near previous industrial sites known for heavy polychlorinated biphenyl (PCB) usage, a group of compounds structurally similar to PBDEs. The researchers hypothesized PCBs may biologically interact with PBDEs enhancing neurological outcomes. While findings did not support associations between PBDEs and neuropsychological functions, they did support the hypothesis that PCBs may potentiate the effects of PBDEs, especially regarding learning and memory[50]. Neither study had associations with depressive symptoms. This review describes the common ways that humans are exposed to PBDEs and the populations most vulnerable to ill-effects. The evidence is summarized regarding the effects of PBDEs on thyroid function, correlations between thyroid hormones and perinatal depression, and the

effects of endocrine disrupting chemicals on neuropsychological functioning. Finally, we propose that disruption of normal thyroid homeostasis may be a plausible mechanism by which PBDEs can cause neuropsychological effects such as depression.

Routes of PBDE Exposure

With the exception of breastfed infants, the primary routes of PBDE exposures are in contaminated air[61], house dust, water, soil, and direct exposure during manufacturing[23]. Once in the body, the half-life of PBDEs congeners with fewer bromine atoms can range from two to 12 years[62]. The primary source of PBDE exposure to children is through diet and dust, however the estimated contributions of various exposure sources varies by age[28]. In breastfeed infants, 91% of this comes from diet[63] and these infants may have higher exposures than their formula-fed peers[23]. The amount of PBDE in breastmilk is positively associated with maternal consumption of dairy and meat[64] and air levels[65]. As infants become more independent, ingestion of dust becomes more common likely due to their characteristic hand-to-mouth behaviors and frequent time spent near the floor and other dust-collecting surfaces[18]. Regardless of the route or source of exposure, children are thought to be the most vulnerable to PBDE exposure because they eat, drink, and breathe more per kilogram of body mass than adults and have a much larger surface area to body volume proportion than adults[23]. PBDEs also have the capacity to cross the placenta during pregnancy, potentially affecting the development of the fetus. Data on prenatal exposures shows significant correlations between pentaBDE congeners (specifically PBDE 153) found in house dust and levels measured in the placenta[66]. In a longitudinal study of PBDE levels in placental tissue and fetal liver samples, it was found that levels of PBDEs in the fetus steadily increased over time. The findings suggested PBDEs pass from the placenta to

the fetus and accumulate[66]. PBDE concentrations have been well documented in newborn cord blood to further support the concept of maternal-fetal exposure[33,67,68]. In a large cohort study investigating the presence of several persistent organic pollutants (POPs) including PBDEs, seven congeners were detectable in maternal plasma and four present in cord blood[67] demonstrating the placenta is clearly filtering some but not all of the toxins. It has also been suggested that PBDEs could have an additional transplacental transfer mechanism than other POP chemicals due to the different amount of fetal accumulation when POPs were compared within maternal serum and cord blood[69]. In comparing feto-maternal samples, detectable PBDEs were not of the highest POP concentrations in maternal samples but, specifically BDE -99 and -153, were the highest in the cord blood. Additionally, the molecular size of PBDEs in cord serum, which is larger than other prominent POPs, should theoretically restrict the transfer across the placenta but this theory was not validated in the feto-maternal ratios[69].

Variability of PBDE Exposure within Populations

PBDE body burden is dependent on the duration, route, and intensity of the exposure. PBDEs are ubiquitous in our environment yet their presence varies by geography, occupation, income, and possibly race[70,71]. Regulation differences for the use of flame retardants in consumer products is associated with marked geographic differences in PBDE body burden[72]. Levels of PBDEs measured in serum and adipose tissue in the United States are 5 times higher than those in Europe[24,66,68,73], with US levels as high as 35-38 ng/g lipid compared to European levels of 2 ng/g lipid[74]. This is reflected in PBDE concentration differences in human breast milk[64,75] and dust[76] with North America asserting breast milk concentrations 6 times higher than Europe and

Asia[75]. Within the United States, California leads with the highest documented levels of PBDEs[40,73]. Proximity to urban areas has been associated with higher PBDE concentrations in air and soil samples according to a study by Harrad and Hunter (2006) ultimately raising questions about whether geography and population density play a role in total PBDE body burden[77]. Geographical differences in PBDE levels were also found in other studies determined to be congener dependent[78-80]. A study of e-waste recycling facilities showed PBDE levels in dust were highest inside the recycling facility and declined with increased distance away from the facility[79]. This finding was replicated in a recycling plant located in Australia using more controlled recycling methods but did not improve the exposure levels[81].

It is well established that individuals working in specific occupations have higher exposures to some flame retardants. Gymnasts and gymnastic coaches have elevated exposures compared to the general population due to frequent contact with foam blocks and foam mats[82]. Other groups with elevated exposures of various PBDEs include flame retardant rubber manufacturers[83], e-waste recycling[84], and aircraft maintenance workers[85].

PBDE exposures and body burden estimates appear to differ across ages and by human matrices. Some report infants <1 year of age to have the highest estimated daily exposure of 86.4 ng/kg/day[63] while others document increasing concentrations from birth peaking in children 4-6 years old[18]. Compared to adolescents aged 12-19 from the 2003–2004 National Health and Nutrition Examination Survey (NHANES), a representative sample in the United States, children aged 4-8 years old in Texas had statistically higher medians of BDE -47, -99, -99, and 153[18]. Unfortunately, NHANES does not collect biomonitoring from children less than 12 years for direct comparison.

Estimated exposures appear to decline with age until age 31[26,63,86]. In a systematic review looking at relationships between maternal age and PBDE concentrations globally, the sum of PBDE levels and individual congeners BDE -47 and -153 in breast milk were positively correlated with the age of the mother but these congeners in placentas were negatively associated with age[68]. Since we know PBDEs have a high affinity for adipose tissue, this difference could be explained by chronic exposures that are naturally sequestered by the body later being mobilized due to breastfeeding demands.

Racial disparities in PBDE exposure have also been observed. White pre-adolescent[13], adolescent girls[14], pregnant women[15], and post-menopausal women[61] consistently exhibit lower levels of PBDEs compared to other races, implying that the elevated PBDE burden may be disproportionally affecting a subset of the population. Pumarega et al. (2016) conferred that non-Hispanic blacks were nine times more likely to have high concentrations of persistent organic pollutants, including PBDEs, compared to Whites after adjusting for income[17]. However, data from 2003-2004 NHANES do not reflect these racial disparities when all incomes are combined. In serum from women aged 16-49 years, the median blood concentrations of PBDEs were not statistically different among races without accounting for cofounding variables. After accounting for income and age, White non-Hispanic women had statistically higher levels than Black non-Hispanics who had statically higher levels than Mexican American's[71]. This discrepancy in PBDE body burdens states that income does not influence a person's risk of PBDE exposure and White women are have more body burden than Black women.

PBDEs as Endocrine Disrupting Chemicals

Endocrine disrupting chemicals (EDCs) interfere with the development and homeostasis of the endocrine system by altering normal physiology within the negative feedback loop and can disrupt multiple organ systems leading to increased morbidity and mortality[87]. The Endocrine Society states EDCs cause long-term permanent physiologic changes that directly influence one's susceptibility to chronic disease[88]. Approximately 800 chemicals are currently known to potentially interrupt normal endocrine function and include toxicants such as phthalates, PBDE flame retardants, bisphenol A (BPA), pesticides and fungicides[89]. A growing body of evidence in human and animal studies links PBDE exposures to serious adverse health outcomes[70] such as thyroid disease[32,34,90], reproductive changes[14,35], neurodevelopmental deficits[9,36-40], and gestational diabetes[41]. PBDEs have a chemical structure that is similar to thyroid hormones enabling the chemical to mimic hormone activities (Figure 2). Decades ago the capacity of PBDEs to disrupt thyroid homeostasis disruption were described[91]. The specific mechanistic relationship between PBDE exposure and thyroid disruption is still not clearly defined in animal or human studies. In part, this is due to the complex normal regulation of thyroid chemicals in pregnancy, variability in effects of specific congeners, and a lack of large-scale epidemiologic studies.

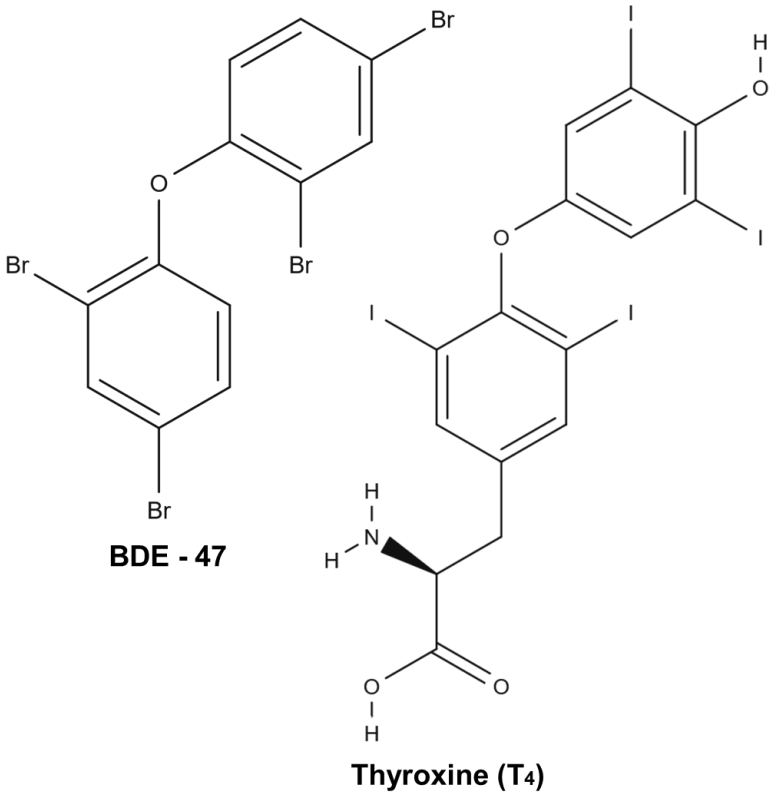


Figure 2. Chemical Structures of BDE -47 and Thyroxine (T_4).

Thyroid Function During Development

The thyroid gland, a component of the hypothalamic-pituitary adrenal (HPA) axis, mediates many normal physiological changes during pregnancy. The hypothalamus is considered the command center of the endocrine system and regulates signaling within the HPA. The thyroid gland is considered profoundly important in fetal development and is one of the largest endocrine glands, first developing around three to four weeks gestation[92]. The fetus is solely dependent on the mother's thyroid for hormone production and utilization until 18-20 weeks gestation. Total thyroxine (T_4) concentrations, one of the primary thyroid hormones, have been measured as early as four weeks gestation in amniotic fluid and as many as six transporters have been identified in placental tissue[93]. Animal models demonstrate that individuals with little

maternal thyroid hormone transfer experienced severe growth restriction in the fetus and increases in fetal malformations[94]. Maternal thyroid hormone regulation is essential for normal fetal neurodevelopment and metabolism and is protective against maternal hypothyroid effects during early development[94]. The amount of transferred maternal T_4 decreases as the fetus becomes self-sufficient but remains a crucial component to brain and organ maturation for the duration of development[92].

Thyroid Hormone Changes during Pregnancy

The demands of the growing fetus for thyroid hormone results in several successive events ensuing in the mother during pregnancy[95]. Initially, rising maternal estrogen stimulates a two to three-fold increase in thyroxine-binding globulin (TBG)[96]. TBG has a high affinity for thyroid hormones and binding of TGB to T_4 results in a direct increase in circulating T_4 and triiodothyronine (T_3) in the bloodstream[3]. The increased levels are due to a higher distribution of bound hormone (also known as total T_4) while diminished free T_4 and T_3 levels are unable to enter the cell for metabolism. Elevated total and free thyroid hormone levels are subsequently found circulating throughout pregnancy and have been calculated at 50% above normal pre-pregnancy levels[3].

Additionally, the rise in maternal human chorionic gonadotropin (hCG), a hormone responsible for establishing and maintaining early pregnancy, inhibits the pituitary release of thyrotropin[3]. Transiently, the decreased TSH levels contribute to the production and bioavailability of T_3 and T_4. By the end of the first trimester, hCG levels decrease and TSH concentrations rise to maintain homeostasis for the remainder of the pregnancy. Increasing thyrotrophic activity is also thought to stimulate thyroid cellular growth but only in areas of iodine deficiency[94,97]. A review of the literature by Berghout and Wiersinga (1998) highlighted an increase in thyroid volume and conferred it can be

reversed or completely eliminated by supplementing iodine at a dose of 100 μg per day[97].

Interestingly, normal serum thyroid parameters vary by ethnic groups and it has been suggested that these differences could account for 18% of misdiagnosed thyroid diseases in a sample of almost 4,000 women[98]. African Americans have been found to have lower overall levels of TSH than Whites and lower upper limits of TSH compared to whites, Hispanics, and Asians during pregnancy[99,100]. Similarly, a comparison of Sub-Saharan and North African pregnancy women to Caucasian pregnant women found mean TSH levels in the Black African women to be lower and suggested the differences may to some extent be due to genetic predisposition[101].

Human Studies Linking PBDE Exposure to Thyroid Disruption

The capacity of PBDEs to disrupt endocrine function and the critical physiological changes during pregnancy raises the question of whether PBDEs-related thyroid disruption is different among pregnant women versus nonpregnant women. A meta-analysis conducted in 2015 found disjointed results within 16 published studies on PBDE serum levels and thyroid function. Specifically, low levels of PBDEs (less than 30 ng/g lipids) were *negatively* correlated with TSH serum levels. *No* correlations were found at intermediate levels (30ng/g and 100 ng/g lipids). And *positive* correlations resulted at levels greater than 100 ng/g lipids[102]. This U-shaped correlation curve indicates complex mechanisms underlie the physiological thyroid response to PBDE exposures[102]. Longitudinal studies have begun to emerge evaluating prenatal PBDE exposures and child outcomes. Table 1 includes the findings of 11 articles published between 2015 and 2018. The inclusion and exclusion criteria followed by Zhao et al (2015) were used for this literature review[102]. In addition, study data was only included

if the exposure and the outcome were within the same person: no findings involving maternal exposures and infant/child health outcomes were reviewed. The direction of significant findings in regards to thyroid hormones is presented.

Table 1. Evidence Table linking PBDE Exposure and Thyroid Dysfunction

Author (year)	Study Design/ Population	Proposed Risk Factor(s)	Outcome	Direction of Significant Findings	Limitations
Vuong (2018)	N= 162 Repeated measures/ prenatal and children in U.S.	<u>Prenatal</u> ∑ PBDEs, BDE -47 <u>Child</u> ∑ PBDEs, BDE -28, -47, -99, -100, -153 BDE -47, -99	<u>Child</u> TSH TT_4 TSH	Neg Pos Pos *14-28% decrease in TSH levels assoc w 10-fold increase in PBDEs *examined PBDEs on volume basis (pg/g serum) and adjusted for lipid levels and compared to standardizing PBDEs (ng/g lipid) while including total lipids in model and found similar findings.	1st to present data on assoc. between prenatal PBDEs and childhood thyroid disruption (no comparison abilities) Low sample size ages 1-3, imputed measures of PBDEs to increase power No iodine levels in children- may be an effect modifier Selection bias (diff between maternal characteristics of missing vs sample) Unable to include OH-PBDEs which have higher

					affinity TH binding proteins
Guo (2018)	Cross-sectional/ geographically exposed children in China	∑ PBDEs, BDE -47, -99 BDE -209	FT_4 T_3	Neg Pos *median ∑PBDEs significantly > gen pop levels of Asia, Europe, and North Am	Inconsistent relationships between PBDEs and THs (may be due to influences of PBDEs and transport, metabolism and function of THs Not generalizable since highly exposed
Byrne (2018)	N= 85 Cross sectional/ Alaska Natives with diet high in PBDE exposure	BDE -153 BDE -28/33, -47, -99, -100 BDE -28/33, -47, -100	T_3 TSH FT_3	Neg Pos Pos *lipids adjusted in models * modification effect by sex	Cannot examine temporal relationship between exposure and outcome. Small sample size may complicate interpretation of results Did not measure OH-PBDE metabolites (more closely resemble T3 and T4) Also highly exposed to other POPs that may

					confound results
Chen (2018)	N= 72 Cross sectional/ BDE-209 manufacture workers	BDE -209	TT_4	Pos *suggesting hyperthyroidism	Cannot examine temporal relationship between exposure and outcome. Highly exposed subpopulation difficult to generalize. Did not measure OH-PBDE metabolites (more closely resemble T3 and T4)
Ding (2017)	N= 123 Prospective cohort/ cord serum in China	∑ PBDEs, BDE -99	TT_4	Pos	Results may be confounded by other POPs or pesticides Did not measure OH-PBDE metabolites Did not measure higher-brominated congeners widely used Small sample size
Zheng (2017)	N= 79 Cross sectional/e-waste recycling	BDE -47, -66, -85 BDE -66, -85	TT_3 TT_4 FT_3 FT_4	Pos Pos Pos Pos	Did not measure OH-PBDE metabolites

	workers in China	BDE -66, -85 BDE -66, -85		*PBDE concentrations were 5 times higher than reported in other countries *no gender differences except decaBDEs	Did not measure higher-brominated congeners widely used Small sample size
Jacobson (2016)	N= 80 Cross sectional/children in U.S.	∑ PBDEs, BDE -47, -99, -100	TSH	Pos *results suggest hypothyroid *tendency toward lower total T4 and >FT3 *47 and 99 were big players in sum *results unchanged with lipid adjustment	Cannot examine temporal relationship between exposure and outcome. Small sample size Low detection of BDE -153 d/t high LOD
Makey (2016)	N=51 Repeated measures/healthy office workers in U.S.	BDE -28, -47, -99, -100, -153	TT_4	Neg *for every 1 unit increase in 47, 2.6-ug/dL decrease in TT4 *may mean BDEs might decrease binding of T4 to binding proteins	Did not measure OH-PBDE metabolites Did not have three samples from all participants Questionable generalizability
Xu (2015)	N=55 Cross sectional/e-waste workers and controls in China	BDE -47	FT_4	Neg *PCBs had more influence on THs	Small sample size Did not examine BDE-209
Kim (2015)	N=148 Prospective cohort / cord	BDE -47, 99	TSH	pos *among the newborns	Assays of free hormones can be affected by

	serum and newborn bloodspot in Korea			*sum PBDEs higher in cord serum than maternal *measured 3 groups of POPs	other proteins that increase in pregnancy THs fluctuate during pregnancy so timing of collection makes a difference
Vuong (2015)	N=165 maternal N=226 cord Prospective cohort/ prenatal and cord serum in U.S.	<u>Maternal</u> BDE -28, 47 BDE 28, 47 BDE -28, 47 BDE -47 BDE -28	<u>Maternal</u> TT_4 FT_4 FT_3 TT_3 <u>Cord</u> FT_3	Pos Pos Pos Pos Neg *one of the only studies to observe THs in second trimester	Did not examine OH-PBDEs Assays of free hormones can be affected by other proteins that increase in pregnancy Competitive binding

Since Zhao's meta-analysis in 2015, findings have consistently reported positive associations between PBDE concentrations and TSH levels. In a study that compared childhood PBDE exposures and TSH levels at repeated time points, a 39-50% increase in TSH levels were associated with a 10-fold increase in all prominent PBDEs as well as the sum measure[32]. This supports other studies of associations between specific congeners and TSH[34,103,104].

Human studies on the relationships between PBDE exposures and T_3 or T_4 levels have been conflicting. This could be due to the complex regulation of thyroid hormones, differences in study protocols, variability in measurement timing, the extent and type of PBDE exposure, or concomitant exposures to other POPs. A small study of 36 individuals residing near PBDE production sites plus other studies have found negative relationships between PBDE concentrations, and T_4 levels[7,105,106]. Conversely, the opposite effect was seen with PBDE concentrations and T_3[34,105] or T_4[32,107]. Likewise, positive correlations were found between rarely-identified BDE -209, a common byproduct during manufacturing, with T_3 and T_4[7,90]. While there are strong studies on the influence of PBDE exposure on T_3 and T_4 hormone levels, little agreement has been made on the direction of the hormones as a result of the exposures or why some populations or genders have higher congener burden than others[34].

Additional studies which did not adhere to the inclusion criteria followed by Zhao et al. (2015) provide additional insight to the relationship between PBDE exposure and thyroid disease in humans[102]. An analysis of 2003-2004 NHANES data found an association between exposure to BDE -47, -99, and -100 with thyroid disease in a sample of 2613 women. This association was strongest in post-menopausal women which the authors speculated could be due to the reduced estrogen reserves of post-menopausal women[61]. During pregnancy estrogen levels are generally elevated beyond pre-pregnant levels. Therefore, the associations between PBDEs and thyroid hormones may be difficult to measure. PBDE concentrations have been associated with hypothyroidism in women aged 30-50[108] and with an elevated risk of developing papillary thyroid cancer[109]. Thyroid disease, including thyroid cancer is a significant health concern and is expected to continue to be a public health burden. Aschebrook-

Kilfory et al. predicts that by 2019, papillary thyroid cancer will be the third most common cancer affecting US women[110]. Few quantitative studies have examined how the exposures disrupt the thyroid and lead to concomitant health effects. To our knowledge, none have looked at thyroid dysregulation as a result of PBDE exposure and maternal health outcomes during pregnancy.

Thyroid Dysfunction and Perinatal Depression

As many as 20-22% of women suffer from perinatal depression with higher rates reported in low- and middle-income countries[58,111]. Perinatal depression is a major or minor depressive disorder affecting mothers during pregnancy or within the first year after birth. Perinatal depression can take a toll on a woman's ability to perform daily tasks and care for herself or her child. Symptoms include feelings of shame and inadequacy, withdrawal from family and friends, as well as thoughts of harming oneself or the baby. Approximately 1:10-12 women in the United States experience depression during or following pregnancy, according to a recent report from the American Academy of Pediatrics[112]. AA women, particularly those of lower socioeconomic status, may also be at elevated risk for perinatal depression. Melville et al. (2010) estimated nearly 19% of the AA women met diagnostic criteria for either minor or major depression during pregnancy, considerably higher than the national average of 8.5%[113].

In pregnancy, thyroid dysfunction has a prevalence of 2-4%[114,115]. Thyroid dysregulation in pregnancy is associated with a number of adverse health outcomes such as miscarriage, intrauterine growth retardation, preterm delivery, hypertensive disorders, and decreased child IQ[95]. Some of these outcomes can even be seen in subclinical thyroid dysfunction[95].

Thyroid hormone signaling and disruption in homeostasis has a long history of association with mood disorders such as depression[46,47]. Women of childbearing age are affected more often with approximately a 60% prevalence rate of depression among those with a known thyroid disease[48]. Classic hypothyroidism disease, a condition hallmarked by decreased thyroid hormone in the blood, typically exhibits significant psychiatric and cognitive symptoms such as forgetfulness, fogginess, fatigue, depressed mood, and sleep disturbance. A wide range of symptom severity may exist from low mood to psychosis or mania[116]. In cases where major depressive disorder and hypothyroid states were observed, patients were also more likely to have anxiety and agitation[117]. Interestingly, thyroid hormones coupled with short term antipsychotics have been used to effectively treat both mania and depressive symptoms[118,119]. A series of newer studies conducted by Pedersen et. al. (2007 & 2016), draw clear connections between thyroid hormone levels and perinatal depression[54,120]. They document a strong, statistically significant inverse relationship between total or free T_4 and perinatal depression measured by two independent depression scales: the Edinburgh Postnatal Depression Scale (EPDS) and Beck Depression Inventory (BDI). When comparing participants stratified by low versus high antenatal thyroid levels, EPDS scores were consistently higher among participants with low thyroid hormones across five time points measured pre- and postnatally[120]. This inverse relationship was later confirmed with other perinatal populations[121,122] while no association was seen by Albacar et al. (2010) (Table 2)[123]. In a follow up study by Pedersen et al. (2016), free T_4 and TBG, measured at 31-33 weeks pregnant, were both significantly predictive of perinatal depression[54]. Lower TBG was found to be a stronger predictor of perinatal syndromal depression (otherwise known as to symptomatic depression) in a combined model

accounting for trauma and a history of major depression. Because TBG is regulated by estrogen, perinatal depression may be linked to low estrogen levels[54].

Table 2. Evidence Table linking Thyroid Dysfunction and Perinatal Depression

Author (year)	Study Design/ Population	Proposed Risk Factor(s)	Outcome	Direction of Significant Findings	Limitations
Pedersen (2016)	N=199 Prospective cohort/ perinatal women in U.S.	FT_4, TBG FT_4, TBG	Risk of depression Diagnosis of depression	Neg Neg *18.8% of the total sample met DSM-IV criteria during the perinatal period for major or minor depression *African Americans more likely to be depressed on the EPDS over time compared to all other races. * "The odds of having clinical depression during the study period were 1.60 times higher for a one standard deviation decrease in FT4"	After accounting for confounders - trauma and lifetime depression, clinical diagnosis becomes more significantly associated with TBG but not FT4. Measures were not collected simultaneously.

				* findings indicate that lower pregnancy week 31–33 TBG concentrations robustly predict subsequent perinatal syndromal depression.	
Wesseloo (2018)	N=1075 Prospective cohort/ perinatal women in Netherlands	TPO-ab(+)	Risk of depression	Pos *Looked at first-onset self reported depression at 4 mo pp when TPO-ab titers are thought to be at their lowest (Jansson 1984) *121 women (11.3%) had positive TPO-ab status (>20 IU/mL) at 12 weeks gestation. Women with positive TPO-ab had an almost 4 times higher risk of depression at 4 months PP than those with a	If woman developed depression during pregnancy, she was excluded. Thyroid function was not assessed during PP period, only antenatal. Not generalizable to women with a psychiatric history or antenatal depression. Not able to adjust for sexual child abuse (a potential confounder)

				negative status.	
Groer (2013)	N=135 Longitudinal correlation/ perinatal women with TPO-ab (+) and (-) in U.S.	<u>Pre&Postnatal</u> TPO-ab(+)	<u>Pre&Postnatal</u> Dysphoria symptoms	Higher scores * suggests that the risk for perinatal depressive symptoms is increased by TPO positivity.	Instrument used screens for dysphoria symptoms rather than clinical depression Minimal thyroid measures included
Sylvén (2013)	N=347 Prospective cohort/ postpartum women in Sweden	TSH FT_4	Risk of depression	Pos Neg *15% self-reported depression (EDS score of >=12) at 5 days PP, 8% at 6wk PP *high TSH and dep only at 6 mo PP not early	Thyroid measures taken only at 1 time point. Wide confidence intervals
Albacar (2010)	N=1053 Repeated measures/ postpartum women in Spain	FT_4 TSH TPO-ab	Risk of depression	No association *8% classified as depressed *in subgroup of TPO-ab+, 5.55 times more likely to have hypothyroidism	Thyroid measures taken only at 1 time point.
Lambrinoudaki (2010)	N=57 Cross-sectional/peri	FT_3 FT_4	Risk of depression	Neg *prepartum FT3 and	Small sample size

	natal women in Greece	FT4 neg corr with baby blues in first week PP	Unable to collect PP data further out than 1 week High rate of C-section delivery in population

Thyroid peroxidase antibodies (TPO-ab) have also been named as a significant predictor of perinatal depression. Women with positive TPO-ab during pregnancy are at an increased risk for developing auto-immune thyroid dysfunction postpartum[53,124] that may be induced by the postpartum TPO-ab rebound phenomena[125]. To further delineate this relationship, Wesseloo et al. (2018) isolated the timing of the first-onset postpartum depression to be congruent with the timing of typical postpartum TPO-ab titer changes, peaking at 4 months[126].

Perinatal depression not only affects mothers but infants as well. More than 400,000 children are born each year to depressed mothers, making perinatal depression the top undiagnosed obstetric complication in America[112]. One of the main focuses of the AAP is the effect of postpartum depression on the social and cognitive development of infants. MRI-based research indicates that infants living in home environments where depression is present are likely to show impairments in their social interactions, maternal infant bonding, and develop mentally at a slower pace[112]. These potential long-term effects underscore the need for research focused on how the environment impacts perinatal women and the role it plays in perinatal depression. Strong correlations exist between thyroid status and depression. Research is beginning to uncover the role pregnancy plays on thyroid hormone demands, TBG sensitivities,

and perinatal depression risk. However, there is a dearth of evidence on the possibility that PBDE exposures contribute to perturbations in thyroid hormone concentrations thereby leading to prenatal depression. Additionally, to date, no one has attempted to draw connections between these interrelated concepts or inquired about possible mechanisms that may explain them.

PBDE-Associated Depression

The mechanism of action and toxic effects of PBDEs is similar to other structurally similar chemicals such as PCBs[11,35,127,128]. Potential corresponding health effects of PCBs and PBDEs include endocrine disruption of the hypothalamic-pituitary-gonadal (HPG) axis, which regulates neurodevelopment, behavior, and reproductive functioning[32,129-131]. In adults, PCB exposure has been associated with depressive symptoms[50,132] from neurotransmitter disruption. Gaum et al. (2017) followed workers exposed to PCBs at their workplace over two years and found homovanillic acid, a dopamine metabolite, mediated the relationship between many PCB congeners and depressive symptoms[133]. A follow up study in the same population, elucidated a significant negative effect of exposure on homovanillic acid for dopamine and vanillylmandelic acid for norepinephrine[51]. Reductions in tyrosine, a naturally occurring amino acid and precursor for dopamine and norepinephrine, have also been associated with decreased production and function of the monoamines in the brain. Monoamine depletion has been shown to cause depressed mood in individuals with personal or familial history of major depressive disorder providing insight into a possible mechanistic pathway[134]. Additionally, thyroid hormones are produced in the thyroid gland from tyrosine and iodine precursors. Given the strong interconnections between PBDEs and thyroid disruption, and thyroid disruption and mood disorders, it has been

postulated that neurotransmitters, such as dopamine and norepinephrine, may be key players[50,132].

The potential for environmental toxicants such as PBDEs to disrupt normal neuroendocrine pathways resulting in depression and other neurological symptoms has been largely unstudied. Only 2 studies have investigated the relationships between[135] PBDE and depression and neither found supportive evidence for PBDEs alone though limitations were of concern (Table 3). A significant negative relationship was found in older adults between the interaction of ∑PBDEs and ∑PCBs and learning and memory tests[49,136]. While reasonable connections between PBDE levels and thyroid disruption have been documented, only preliminary studies have begun to consider linkages between PBDE exposures and depression-like behaviors[49,136]. High resolution metabolomics (HRM) is a promising tool which has the capacity to elucidate potential mechanisms that are clinically relevant with detrimental health outcomes in response to EDC exposures such as PBDEs[135,137]. Accordingly, metabolomics could provide insights into the mechanisms by which PBDEs can affect mood disorders. Assessing individual profiles during an exposure event can provide useful information about disease states, toxicity, and mechanisms of action at a given point in time.

Table 3. Evidence Table linking PBDEs and Depression

Author (year)	Study Design/ Population	Proposed Risk Factor(s)	Outcome	Direction of Significant Findings	Limitations
Gump (2014)	N=43 Cross sectional/ children in U.S.	PBDE	Risk of depression	No Association	Small sample size
			Hostility	Pos	Low exposure group
			Aggression	Pos	
			Temperament	Pos	Whole blood

					measured versus usual serum
Fitzgerald (2012)	N=144 Cross sectional/Older adults age 55-74yrs in U.S.	∑PBDEs	Risk of depression	No association	Small sample size may limit effect detection
		∑PBDEs * ∑PCBs	Risk of depression	No association	
		∑PBDEs * ∑PCBs	Learning and Memory	Neg *results not completely reported	Measured in whole blood

Conclusion

To date, literature regarding PBDE exposure and subsequent short- and long-term health effects has focused on endocrine and neurological effects primarily among vulnerable infants and children. However, pregnant women are also vulnerable to the effects of toxicants[2-4] due to unstable immune and endocrine states throughout pregnancy. Endocrine disrupting chemicals such as PBDEs have the ability to modulate normal thyroid signaling through various mechanisms. Scientific literature has historically made clear associations between thyroid hormone dysfunction and mood disorders however, further research must continue to investigate relationships between PBDE concentrations and depressive symptomatology. We posit that PBDE exposure can lead to thyroid hormone disruption and subsequent depression in vulnerable pregnant women. High resolution metabolomics is a promising tool capable of elucidating specific mechanisms by which environmental toxicants can affect health. The need for additional research is compelling, especially in the context for African American pregnant women, to address gaps in the current literature. Given the known

detrimental effects of perinatal depression to the mother, the fetus, and the children, understanding the basic biology of EDCs and their role in perinatal depression protects the health of entire families.

Polybrominated diphenyl ether serum concentrations and depressive symptomatology in pregnant African American women.

Abstract

Background Polybrominated diphenyl ethers (PBDEs) are lipophilic, persistent endocrine disrupting chemicals often used as flame retardants in products that were widely produced in the United States until 2004. The potential for environmental toxicants such as PBDEs to disrupt normal neuroendocrine pathways resulting in

depression and other neurological symptoms has been largely understudied. This study examined whether PBDE exposure in pregnant women was associated with antenatal depressive symptomatology.

Methods This study is part of a larger longitudinal pregnancy and birth cohort study. Data were collected from 193 African American pregnant women at 8-14 weeks gestation. Serum PBDEs were analyzed using gas chromatography-tandem mass spectrometry. The Edinburgh Depression Scale (EDS) was used to identify depressive symptoms experienced in the last seven days prior to biosampling. The dichotomous depression variable was used to explore varying high-risk EDS cutoffs and illustrated with receiver operating characteristic curves. Logistic regression models were constructed to investigate associations with antenatal depression and a weighted quantile sum (WQS) index was calculated to account for the mixture of PBDE congeners.

Results Of the total sample, 52 women (26.9%) were categorized as having a high risk of depression. PBDE congeners -47, -99, and -100 were detected in 50% or more of the samples tested. BDE-47 was positively associated with depressive symptoms (β =2.36, p=0.05). The risk of being mild to moderately depressed increased by a factor of 4.52 for BDE-47 (CI 1.50, 13.60) and 1.58 for BDE-99 (CI 1.08, 2.29). The WQS index, a weighted estimate of the body burden of the congener mixture was positively associated with a higher risk of mild to moderate depression using an EDS cutoff ≥10 (OR=2.93; CI 1.18, 7.82).

Conclusion BDE-47 and -99 exposures are significantly associated with depressive symptomatology in a pregnant cohort. These exposures will likely continue for years due

to slow chemical degradation. Interventions should focus on PBDE mitigation to reduce toxic neuroendocrine effects on vulnerable pregnant women.

Background

Polybrominated diphenyl ethers (PBDEs) are a family of chemicals with a common structure of a diphenyl ether molecule attached to one to ten bromine atoms. PBDEs were once added to consumer products and materials to attenuate the risk of fire and to increase the escape time in the event of a fire. A voluntary phase-out of pentaBDEs and octaBDEs began in the United States in 2004 and later, decaBDEs,

however, they remain prevalent in the environment over 10 years later. PBDE chemicals are not covalently bound to materials and leach from consumer products into the environment. Additionally, they are not easily biodegraded and thus remain persistent and ubiquitous in the environment. Levels of PBDEs in humans and the environment are higher in North America than in other regions of the world, likely because of the widespread commercial use of PBDE mixtures in the United States: decaBDE and pentaBDE (widely used in textiles, plastics, electronics, and polyurethane foam)[71]. The chemicals are highly detectable in air, dust, sediment, soil, and ground water and, with their hydrophobic properties, PBDEs can bioaccumulate in lipid-rich human tissue[23]. Exposure to PBDEs has been associated with thyroid disease[32,34,90], reproductive changes[14,35], neurodevelopmental deficits[9,36-40], and gestational diabetes[41]. The potential for environmental toxicants such as PBDEs to disrupt normal neuroendocrine pathways resulting in depression and other neurological symptoms has been largely unstudied. Literature regarding PBDE exposure and subsequent short- and long-term health effects has focused on neurological effects among infants and children. To date, only two studies have investigated the effects of PBDE exposure outside of pregnancy *and* subsequent neuropsychological effects[49,136]. In this study, we focus on PBDE exposure in pregnant adults and their relationship with depressive symptoms.

Clear connections between PBDE levels and thyroid disruption have been made[32,34,90,105,138,139]. PBDEs have similar shape and structure to thyroid hormones and are thought to mimic thyroid activities as a result. Further, the essentiality of thyroid homeostasis for cognition and mood stability has been long recognized[46-48]. Of the two available studies investigating PBDE exposure and risk of depression within the same individual, no associations were found. However, measures of hostility, aggression, and

temperament among children were significantly correlated with PBDE concentrations[49]. In another study of older adults with high occupational polychlorinated biphenyl (PCB) exposure, significant associations were found between the mixture of PCBs and PBDEs and learning and memory[136]. PCBs and PBDEs have been postulated to share mechanistic actions and toxic effects[11,35,127,128] and others endocrine disrupting chemicals and have been correlated with depression-like symptoms as well[50,136,140,141].

Pregnant women may be an overlooked vulnerable population to the effects of toxicants due to normal physiological changes in pregnancy such as higher lipid profiles and thyroid strain[2-4]. African Americans (AAs) specifically may also be at elevated risk of toxicant exposure and depression. According to the 2003-2008 National Health and Nutrition Examination Survey (NHANES) population data, non-Hispanic blacks have elevated PBDE body burden when compared to non-Hispanic whites[1,18]. Cross-sectional studies have found similar findings predicting non-Hispanic blacks are nine times more likely to have high concentrations of persistent organic pollutants (which include PBDEs) than non-Hispanic whites after adjusting for income[17]. AA women may also be at increased risk for perinatal depression- a mood disorder present in the antenatal or postpartum period for up to one year. Melville et al. (2010) estimated nearly 19% of AA women met diagnostic criteria for either minor or major depression during pregnancy[113], considerably higher than the national average of 8.5%[142]. African American women of low income have the highest reported prevalence of perinatal depression (28%) in the U.S.[143]. Major depressive disorder (MDD) is associated with adverse outcomes in many aspects of role performance[144]. Specifically, MDD is associated with poor outcomes in marital functioning[145], parental functioning[146], absenteeism and low work performance[147,148], and personal income or household

earnings[149]. Unfortunately, some populations of African Americans are less likely to be diagnosed and treated for depressive symptoms compared to white patients[150], and across subgroups, African Americans are more likely to have persistent disorders after becoming ill[151]. Additionally, mental health concerns are often overlooked, misdiagnosed, mistreated, or not treated and, historically, both patients and providers have poor follow-up of mental health concerns.

It is also widely accepted that not all populations conform to the standard EDS scoring criteria. The EDS has been validated in multiple race and ethnic groups[152,153] with varied cut-off scores recommended to accurately predict a diagnosis of mild to moderate depression resulting from socioeconomic status, race, and gender differences[142,154]. Additionally, researchers have argued that cultural differences and timing of screenings influence clinical cut-off scores[142,155,156].

Few quantitative studies have examined health effects of PBDE exposures in pregnant women and, to our knowledge, none have studied AA women. For these reasons, we designed our study to investigate whether PBDE exposure in pregnant AA women is associated with depressive symptomatology during pregnancy. Due to uncertainty in applicability of standard EDS cutoff scores to our vulnerable population of pregnant AA women, we took additional measures to ensure appropriate cutoff scores were utilized in our analysis. We hypothesized a positive relationship would be found between PBDE serum concentration and depression among pregnant AA women.

Methods

This study used data drawn from a larger longitudinal pregnancy and birth cohort aimed to investigate the maternal prenatal microbiome as a predictor of birth outcomes[157]. Women were enrolled in the study between 2014 and 2017 and were

included if they self-identified as AA, had a singleton pregnancy, were 18-35 years of age, resided in the Atlanta Georgia area, and could comprehend and speak English. Women were recruited during their first trimester from prenatal clinics serving two local hospitals. Members of this SES-diverse cohort were individually informed of the study protocol and written consent was obtained. Participants were followed through delivery. For this analysis, a subset of 193 women were included if they had serum PBDE concentrations and complete EDS scores. All study procedures were approved and reviewed by the Institutional Review Board at Emory University.

Measures

Pregnancy dating was based on the participant's known last menstrual period or first trimester ultrasound if a discrepancy of ± 5-7 days exists per standard dating protocol[157]. Measures (questionnaire, clinical, and biological data) were collected in person at the initial visit or by electronic medical record.

Demographic data

Socio-demographic variables included age, marital status, years of education, and insurance status. The poverty/income ratio was determined from family size and household income data.

Clinical and Edinburgh Depression Scale Data

Health data extracted from EMR included height, weight, parity, past and current tobacco use, and alcohol or drug use in the first trimester of pregnancy. Depression data was collected by trained research coordinators at 8-14 weeks gestation. Depression was defined using the Edinburgh Depression Scale (EDS); a 10-item scale of depressive symptoms experienced in the last seven days[153]. The EDS is a reliable tool to predict antenatal combined major or minor depression with psychometric properties of 64-87%

sensitivity, 73-96% specificity, and 73% positive predictive value[152]. Individual question scores were summed and total scores ranged from 0-30 with higher scores associated with a higher risk of depression. Four cut-off scores were explored (≥7, ≥8, ≥9, and the universal standard ≥10) to determine the optimal cutoff value to detect depressive symptomatology in our AA study sample. Receiver operating characteristic curves were constructed to visualize and compare the specificity of the cutoff scores. An optimal EDS cutoff of ≥10, area under the curve 0.6867, was determined to differentiate the groups of high versus low or no risk of depression (Figure 3).

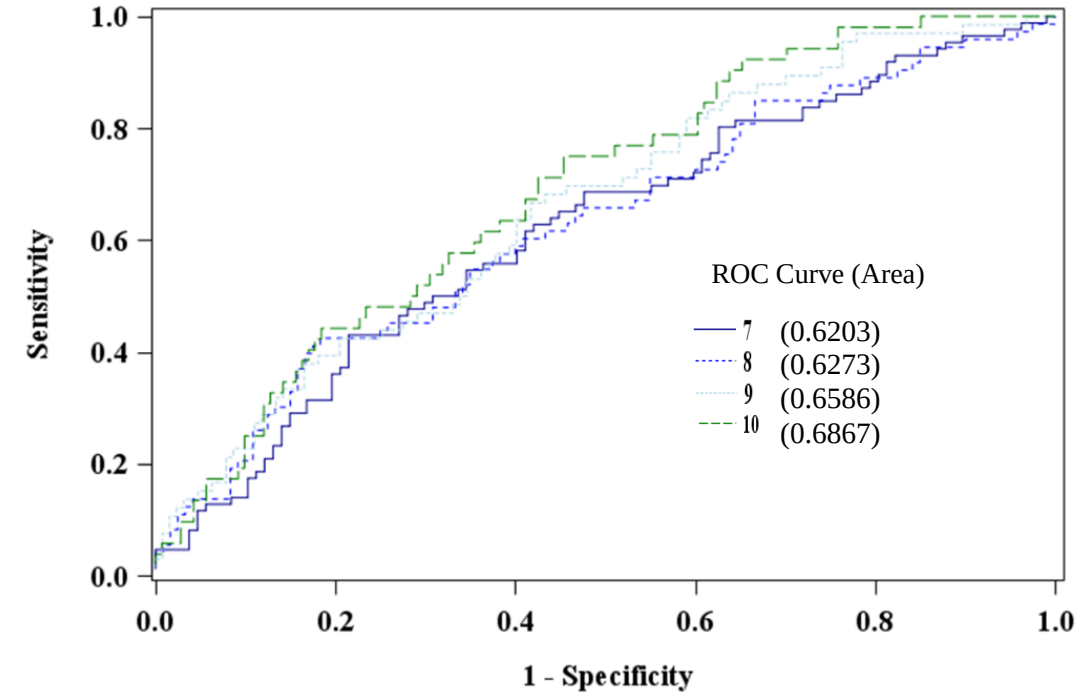


Figure 3. Comparison of Edinburgh Depression Scale Cut Points using PBDE 47.

Serum PBDE Concentrations

Concentrations of PBDEs congeners 47, 85, 99, 100, 153, and 154 were measured in maternal serum using a modification of a previous method[103,158]. Samples were fortified with isotopically labeled analogues of the target chemicals, homogenized and

deprotonated. Supernatants were extracted twice with hexane and dichloromethane and passed through an activated silica gel column to remove residual biogenic material. Sample extracts were concentrated and analyzed using gas chromatography-tandem mass spectrometry with isotope dilution calibration. The limits of detection (LODs) (see supplemental Table 1) were in the low pg/mL range. Values below the LOD were replaced with a value equal to the LOD, divided by the square root of two[159]. Maternal serum total cholesterol and free triglycerides were not collected, therefore lipid adjustment was not done.

Covariates and Potential Confounders

In order to test the relationship between PBDE congeners and depression, covariates and/or potential confounders were considered *a priori* and reassessed after bivariate analyses. The confounders identified *a priori* were age, gravidity, marital status, education, BMI, and income based on associations noted in previous literature[160-163]. Income had >20% missing values (n = 39) so insurance type was used as a proxy. Parity, indicating the number of pregnancies reaching viable gestational age, was tested as a covariate. During bivariate analysis, education and parity were dropped because no significant associations with the outcome of depression were observed.

Statistical Analysis

PBDE congeners were log transformed (base 10) to reduce the influence of outliers and to conform to normality. For congeners with a 50% or greater presence (-47, -99, and -100), geometric means were used to describe PBDE data and reduce the right skewed data distribution resulting from imputed data. Congeners -85 and -154 were not detected in any samples (above instrumental LOD) and were therefore not

reported and excluded from analysis. Congener -153 was detected in only 14% of the samples and was subsequently not used in further analyses.

We examined univariate associations within demographic characteristics, PBDE concentrations, and EDS scores. Geometric means were used to describe PBDE concentrations and to compare to 2013-2014 NHANES data restricted to non-Hispanic black women aged 18-35 years. Wilcoxon nonparametric tests were used to compare PBDE congeners between low and high depression groups. Parametric tests of association were completed for covariates and depression variables. Spearman rank-order and Kruskal-Wallis tests were conducted to compare means among skewed data. Bivariate analysis between PBDE 47 and depression groups yielded unequal variances so Satterthwaite test statistic was reported. Missing data were explored within each covariate and accounted for <10% of the sample and were not further manipulated. Each PBDE congener was regressed with confounding variables in linear and logistic regression models to investigate associations with depression. Exposure to a PBDE mixture (congeners -47, -99, and -100) was evaluated using a weighted quantile sum (WQS) approach in conjunction with multiple linear and nonlinear logistic regression[164,165]. The WQS method estimates a weighted linear index corresponding to chosen quantiles of PBDEs. Bootstrap sampling is used to empirically determine the weights, constrained between 0 and 1 and summed to 1. Since environmental exposures co-occur, interact, and are highly correlated, the WQS method reduces dimensionality and addresses multicollinearity[166]. The WQS index was placed in the best-fit model where $\exp(\beta 1)$ is the odds ratio associated with a unit (quartile) increase in the weighted quartile sum of PBDE exposures. The WQS index was regressed using an EDS cutoff ≥10. The significance of the test represents a test for a mixture effect.

Statistical significance was set at 0.05 and all analyses were performed using SAS v9.4 and R v3.4.

Results

Basic descriptive statistics for the total sample of 193 women are presented in Table 4. Of the total sample, 52 women (26.9%) were categorized as having a higher number of depressive symptoms (EDS cutoff ≥10) further referred to as having a high risk of depression and 141 (73.1%) had a lower number of depressive symptoms further referred to as low or no risk of depression. In this sample, the alpha coefficient was 0.85. The majority of participants were unmarried (86.5%), had at least some college education (48.2%), and qualified for Medicaid health insurance (77.8%) (Table 4). Both high and low or no risk of depression groups had an approximately normal distribution of educational attainment. Approximately half were overweight or obese (54.4%) and nulliparous (48.2%). A small proportion reported consuming alcohol (4.2%) or marijuana (17.1%) in the last month, or ever smoked tobacco (10.9%) (Table 5). The women's mean BMI scores were significantly different between the depression groups. Substance use in the last month and ever used tobacco were also associated with having an EDS score of ≥10. When comparing the 2014-2014 NHANES data reflecting the pooled samples of non-Hispanic Black women aged 20-39, our study population had similar levels of BDE -47 and -99 but lower levels of BDE -100 (Table 6). Consistent with previous literature, PBDE congeners were significantly intercorrelated ($r= 0.6\text{-}0.7$, $p<0.001$). Although not strong, serum PBDE 47 concentrations and marijuana use were positively associated ($r=0.12$, $p=0.03$). Neither tobacco nor alcohol use were associated with any congener. Levels of BDE -47 and -99 were significantly different between low and high depression groups ($p=0.04$ and $p=0.02$, respectively) (Table 7). As

concentrations of PBDE -47 in the serum increase, the probability of having depressive symptoms also increases (β =2.36, p=0.05) (Figure 4). However, this association accounted for only 6% of the variability.

Table 4. Demographic characteristics of AA cohort 2014-2015 associated with high depressive symptoms using an EDS cutoff ≥10.

	Total sample (n=193)	**High depressive symptoms (n=52)**	**Low or no depressive symptoms (n=141)**	***P* value**
Characteristics	**n (%)**	**n (%)**	**n (%)**	
Age (mean, SD)	24.0 ± 4.4	23.8 ± 3.9	24.4 ± 4.6	.46
Married	26 (13.5)	3 (5.8)	23 (16.3)	.06
Education				.30
Some high school	35 (18.1)	12 (23.1)	23 (16.3)	
Graduated high school or GED	65 (33.7)	18 (34.6)	47 (33.3)	
Some college or technical school	63 (32.6)	16 (30.8)	47 (33.3)	
Graduated college	20 (10.4)	6 (11.5)	14 (9.9)	
Some graduate work or degree	10 (5.2)	0 (0.0)	10 (7.1)	
Insurance				
Low Income	53 (27.5)	10 (19.2)	43 (30.5)	.08
Medicaid<100%	97 (50.3)	33 (63.5)	64 (45.4)	
Right from the Start				
Medicaid≤ 200%	43 (22.2)	9 (17.3)	34 (24.1)	
Private				

Table 5. Health-related characteristics of AA cohort 2014-2015 associated with high depressive symptoms using an EDS cutoff of ≥10.

	Total sample (n=193)	**High depressive symptoms (n=52)**	**Low or no depressive symptoms (n=141)**	***P* value**
Characteristics	**n (%)**	**n (%)**	**n (%)**	
Body mass index				.03*
Underweight (<18.5)	8 (4.2)	4 (7.7)	4 (2.8)	
Normal (18.5 – < 25)	80 (41.5)	24 (46.2)	56 (39.7)	
Overweight (25 – < 30)	39 (20.2)	14 (26.9)	25 (17.7)	
Obese (≥ 30)	66 (34.2)	10 (19.2)	56 (39.7)	

Number of children				.63
0	93 (48.2)	28 (53.9)	65 (46.1)	
1	58 (30.1)	14 (26.9)	44 (31.2)	
2+	42 (21.8)	10 (19.2)	32 (22.7)	
Drinks alcohol in last month	8 (4.2)	4 (50.0)	4 (50.0)	.14
Ever smoked tobacco	21 (10.9)	14 (26.9)	7 (5.0)	<.0001*
Smoked marijuana in last month	33 (17.1)	14 (26.9)	19 (13.5)	.03*

*Statistical significance at p<0.05

Table 6. Wet weight PBDE congeners present in the study population compared to 2013-2014 NHANES data reported in pg/mL serum (N=193).

Metabolite	mean ± SD	GM	NHANES mean	% detected
PBDE 47	124.9 ± 137.6	86.8	87.3	100%
PBDE 99	35.0 ± 45.3	20.8	18.3	81%
PBDE 100	22.3 ± 21.1	13.4	18.2	79%

* Reported NHANES data reflects arithmetic mean from pooled samples of non-Hispanic Black women aged 20-39 years.

Table 7. PBDE concentrations (pg/mL) in serum and risk of antenatal depression among African American cohort at 8-14 weeks gestation (N=193).

	High depressive symptoms (n=52)	Low or no depressive symptoms (n=141)	
Characteristic	**mean ± SD**	**mean ± SD**	***P* value**
PBDE 47	155.3 ± 198.3	113.7 ± 105.8	.04*
PBDE 99	112.1 ± 342.9	91.4 ± 342.9	.02*
PBDE 100	104.7 ± 343.0	94.1 ± 343.0	.24

*Statistical significance at p<0.05

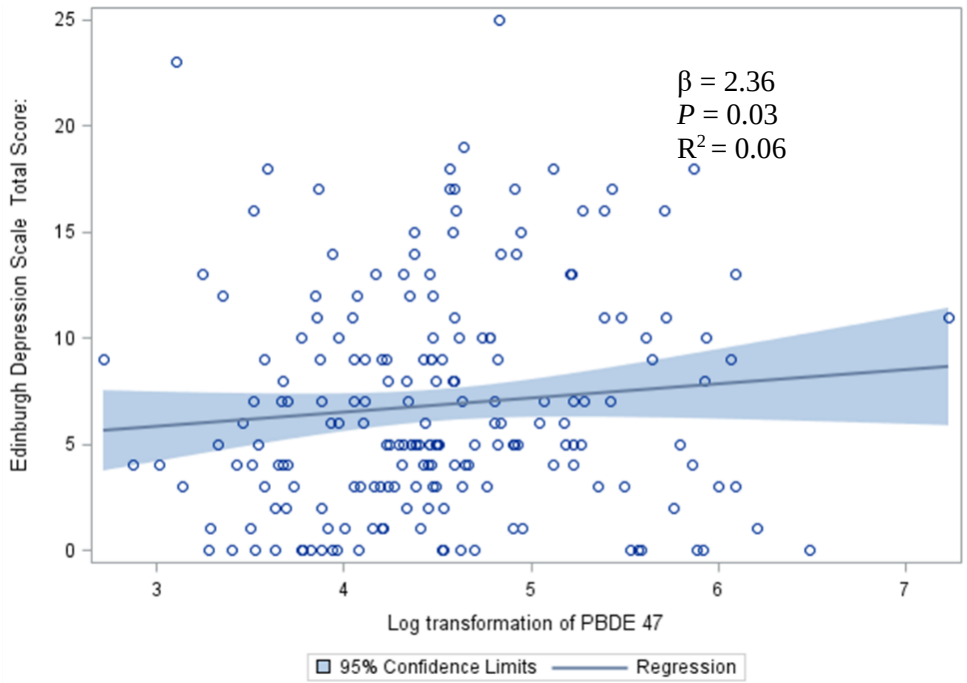


Figure 4. Relationship between PBDE47 and Depressive Symptoms.

All covariates and confounding variables identified *a priori* were entered into the multivariate model. The best-fit model controlled for age, marital status, and BMI to test for associations between PBDEs and depression. In the adjusted regression analysis, models were used to examine the associations between PBDEs (Table 8), and depressive symptoms. Statistical significance was detected with BDEs -47 and 99, but not BDE -100. For every one unit increase in concentration, the risk of being mild to moderately depressed increased by a factor of 4.52 for BDE-47, 1.58 for BDE-99, and 1.22 for BDE-100 adjusting for age, marital status, and BMI. No issues of multicollinearity existed since all various inflation factors values were low (<2). The Hosmer-Lemeshow goodness of fit (0.461-0.594) suggests good fit for each model.

Table 8. Multiple logistic regressions of high antenatal depressive symptoms among African American women using an EDS cutoff ≥10.

	OR [95% CI]	AOR [95% CI]

PBDE 47	2.62 (1.00, 6.87)	4.52 (1.50, 13.60)*
PBDE 99	1.39 (0.99, 1.93)	1.58 (1.08, 2.29)*
PBDE 100	1.17 (0.88, 1.58)	1.22 (0.90, 1.67)

*Statistical significance at p<0.05
*Adjusted for age, BMI, and marital status

In order to show which PBDE congener had the most robust association with depressive symptoms, a PBDE WQS index was regressed with the dichotomous depression variable and presented in Table 9. The distribution of weights comprising the index was led by BDE-47 (w= 0.71) followed by BDE-100 (w= 0.29), then BDE-99 (w= 0. 00000000817). The fitted coefficients exp(β) provided an estimated odds ratio for risk of depression resulting from the PBDE mixture. Specifically, increases in the weighted PBDE index were significantly associated with higher depressive symptoms after adjusting for age, marital status, and BMI (OR=2.93; CI 1.18, 7.82).

Table 9. Associations between Weighted Quantile Sum Regression Index and high antenatal depression symptoms using an EDS cutoff ≥10 in the study population.

Chemical	**Weights**
PBDE 47	0.71
PBDE 99	.00000000817
PBDE 100	0.29
	AOR [95% CI]
WQS	2.93 (1.18, 7.82)*

*Statistical significance at p=0.02
*Adjusted for age, BMI, and marital status

Discussion

The purpose of this study was to examine the relationship between concentrations of PBDEs in serum and depressive symptoms in pregnant AA women, a vulnerable and traditionally understudied population in the context of both PBDE

exposure and prenatal mental health outcomes. The bulk of PBDE and health outcome research has centered on maternal exposures and fetal development but, as concentrations continue to be detect remote from the production phase-out, health concerns beyond early childhood should be explored. Cowell et al. (2018) found no significant differences in PBDE cord blood concentrations collected before and after the phase out supporting the persistent properties and ongoing release if the chemicals from products[29]. Exposures and their body burdens are not well-characterized in pregnant women versus nonpregnant. In our sample of 193 pregnant women, 100% had detectable concentrations of BDE -47. Body burden estimates are similar to serum concentrations from the most recent 2013-2014 NHANES national sample of non-Hispanic black women of reproductive ages. Findings indicate positive associations between PBDE congeners, the PBDE mixture, and high depressive symptoms among our study population. The weighted PBDE index identified BDE -47 to be the "bad actor" within the mixture. We anticipated this since it is highly prevalent across multiple matrices[81,167,168]. Due to the small amount of variability in the final regression models, further studies should focus on potential covariates that could better explain the relationship between serum PBDE concentration and prevalence of depressive symptoms. We understand humans are exposed to many environmental chemicals at once and the preliminary findings in this study using the WQS method broadens our perspectives on analyzing highly correlated variables and provides better estimates of the PBDE mixture effect. For more comprehensive mixture estimates, additional endocrine disrupting chemicals should be considered. Theoretically, endocrine disruptors have similar health effects and may be working through similar pathways[88,169] to affect mood. Assessing for exposure to other endocrine disruptors and

neuropsychological outcomes in this population could provide insight into shared metabolic pathways.

Consistent with previous studies of tobacco use and depression in pregnancy, tobacco use and substance abuse correlated with EDS scores[170]. However, our data collection protocol did not allow us to account for the onset of depressive symptoms to further analyze the correlations. It is plausible that depressed or stressed women are likely to smoke tobacco or use marijuana as a coping mechanism[26,171]. It is also plausible there is no causal relationship between substance use or smoking and depression, but rather the behavior is an expression of one's attitude or emotion[172]. Lazarus and Folkman (1987) described stress as being physiologic and psychologic, both having the opportunity to lead to problematic health outcomes[173]. In the context of this study, we believe stressors lead to physiologic and psychologic depressive responses without regard to co-occurring behaviors such as smoking or marijuana use.

Based on this study, there is a high risk of perinatal depression among African American (27%) compared to the national average of all perinatal women regardless of race (8.5%). In our study of only AA women, those with lower income, using insurance as a proxy, had disproportionate rates of depression (29%) compared to those with higher income or private insurance (21%). This is consistent with other studies who have been reported elevated perinatal major depression rates of 19-28% also in low income AA women[113,142,174,175].

Our outcome measure, EDS score, is a measure of depression *risk*, not a diagnostic measure. It is primarily utilized by health professionals and researchers to objectively identify mothers suffering from stress that may be interfering with normal activities or enjoyment of life. Clinically, the EDS cutoff used for referral or to warrant

repeat testing remains variable[176-178]. In light of this uncertainty, we took a careful analytic approach to identify an appropriate value specific for our sample. Surprisingly, our analysis demonstrates that using a standard cutoff of ≥10 is optimal for decision making in regards to depression risk for urban AA pregnant women. Using a cutoff of ≥10 is likely to accurately predict depression in most women but some suggest repeat testing as frequent as two weeks later regardless of the cutoff score[179], especially among populations where cultural variations may exist in the expression of depressive symptoms[180]. Tandon et al. (2012) collected depression measures and performed subsequent sensitivities on the EDS[142]. Similar to this study, they recommended an optimal cutoff score for major and minor depression of ≥10 in this population. In general, AAs are less likely to be diagnosed with depression or treated for depressive symptoms compared to white patients[150], and across subgroups, African Americans are more likely to have persistent mental disorders after becoming ill[151]. At a minimum, providers should be alerted when EDS scores fall between 7 and 10 because patients could be developing depression and a trustworthy rapport can positively impact patient compliance and follow-up[181,182].

Limitations

There are notable limitations to this study. Since our study relied on maternal-self report to document tobacco use, substance abuse, and depressive symptoms, it is possible that recall bias interfered with our results. The study sample is based on convenience sampling from two metropolitan prenatal clinics. While it may represent most AA pregnant women living in urban environments, attempts were not made to include hard-to-reach groups. Especially during pregnancy, study participants may have

been reluctant to disclose the presence or absence of depressive symptoms or current use of tobacco or illegal drugs. Women report difficulty disclosing perinatal mental illness because of stigmas of inadequacy as a mother or stigmas of treatment in pregnancy[183]. Others refuse to seek help because of poor mental health knowledge or literacy[184]. Self-report of substance abuse is underrepresented resulting from maternal guilt or remorse[185]. A cross-sectional study design comprised of one time point, limits the ability to cultivate causal relationships. We attempted to compensate for this lack of temporality in part by excluding anyone with a personal or family history of depression. Further longitudinal data is needed to substantiate the findings and more confidently predict conclusions. Another limitation of our analytic approach was the lack of serum lipid data which hindered our ability to lipid adjust serum PBDE concentrations. Variation of PBDE concentrations in humans is common due to multiple exposure routes and elimination differences among each congener. A final key limitation was the inability to determine the major sources of environmental exposures to PBDEs in this population which impacts future development of preventive interventions for pregnant AA populations.

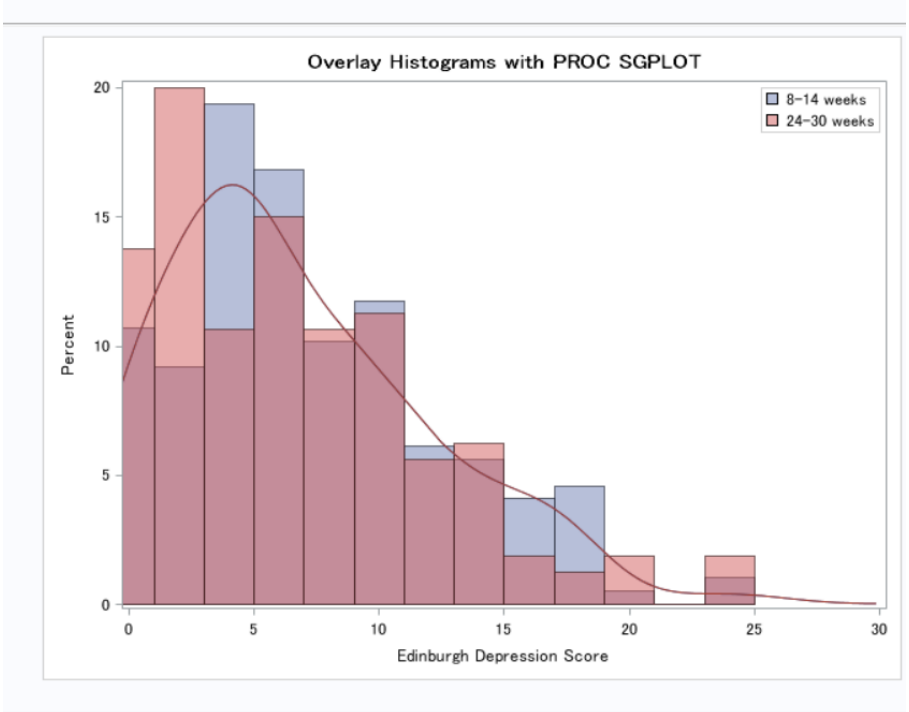
Conclusion

Health concerns related to PBDE exposure are becoming apparent and will likely continue for years due to slow chemical degradation leading to persistence and accumulation in our environment. Products with PBDEs are common and continue to be used in homes, schools, and businesses across the country. Even those removed from homes continue to decompose in landfills and recycling centers releasing harmful toxicants into the air, water, and soil. Our analysis revealed BDE-47, -99, and -100 are present in a cohort of pregnant AA women and the weighted mixture is associated with

high depressive symptoms. The novel associations were found in a relatively small sample size and among a non-occupationally exposed group. Given the strong association between antepartum depression and subsequent postpartum depression, serum PBDE concentrations could be used as a biomarker with predicative validity to identify women at high risk for depression. The need for replication and consistent results is suggested. Because of limited research and slow PBDE degradation, it is unclear whether PBDE accumulation has other long-term implications on pregnant women. Additional studies are needed to address population health impacts resulting from PBDE bioaccumulation since the chemicals are likely to be present in the environment for many years ahead. The cross-sectional methodology of this study can be particularly useful in informing researchers and public health professionals about depressive symptomatology among AA pregnant women and changes in PBDE concentrations over time. Assessing environmental burdens and human toxicant effects, especially mixtures, can inform planning and allocation of health resources for mitigation and prevention. Endocrine disrupting chemicals such as PBDEs are completely modifiable and prevention efforts should be given high priority.

Supplemental Table 1. Level of detection for Each PBDE Congener

Compound	**LOD (pg/mL)**
PBDE 47	3.13
PBDE 85	78.13
PBDE 99	7.81
PBDE 100	3.13
PBDE 153	78.13
PBDE 154	78.13



Supplemental Figure 1. Comparison of EDS scores between 8-14 weeks and 24-30 weeks gestation.

Supplemental Table 2. Comparison of high depressive symptoms using an EDS cutoff ≥10 at 8-14 weeks and 24-30 weeks gestation (N=193).

Time point	N	EDS Score Mean (SD)	EDS Score ≥ 10 N (%)	Difference in Mean P Value
8-14 weeks	193	6.82 (5.24)	52 (27)	0.28
24-30 weeks	160	6.20 (5.52)	39 (20)	

Using tyrosine metabolomics to describe the relationship between serum polybrominated diphenyl ether and depression

Abstract

Background Polybrominated diphenyl ethers (PBDEs) are persistent organic pollutants capable of disrupting thyroid hormone synthesis that leads to multi-organ systems effects. PBDE exposure and thyroid dysfunction have independently been associated with adverse neuropsychological functions but their mechanistic actions are not well understood. High-resolution metabolomics (HRM) is a promising method that detects subtle alterations in biological pathways to provide insight into mechanisms associated with depression.

Methods HRM profiling, PBDE concentrations, and Edinburgh Depression Scale (EDS) data were collected on 193 pregnant African American (AA) women between 8-14

weeks gestation. A suspect screening approach was used to re-analyze plasma HRM data to enable better sensitivity of tyrosine-specific metabolic pathway identification. Seven metabolites within the tyrosine pathway were evaluated for relationships between PBDEs and depression symptoms then considered as potential mediators in a causal mediation analysis.

Results The sample was grouped into high and low or no risk of depression based on EDS score ≥10. Women with high depressive symptoms had statistically higher PBDE concentrations (pg/ml) for BDE -47, and -99. Metabolite with m/z 746.0871 annotated as Nicotinamide adenine dinucleotide phosphate was positively associated with BDE -47 and -100. The tyrosine metabolite suspects did not mediate the effect of PBDE exposures on EDS scores to comprise an indirect effect. However, the direct effect of PBDE 47 remained significantly associated with EDS scores. EDS scores increased by 2 points for each unit increase in PBDE 47 serum concentration.

Conclusion Tyrosine metabolite suspects were identified in the plasma of pregnant AA women exposed to PBDE toxicants. PBDE congeners were associated with a high risk of depression but tyrosine metabolites did not indirectly mediate the relationship. Limitations of HRM may prohibit its use in cases where metabolic pathways are highly regulated by multiple endocrine feedback loops. A case control study design may elicit a clearer depiction of the metabolic differences between depressed and nondepressed women with similar environmental exposure.

Keywords:

PBDE; Depression; Thyroid hormones; Tyrosine; Metabolomics; Metabolite

Background

Polybrominated diphenyl ethers (PBDEs) are persistent organic pollutants, commonly found in the air, soil, and dust (EPA). Although PBDEs are no longer commercially produced in the United States, the chemical compounds continue to pollute outdoor and indoor environments. Health effects resulting from PBDE exposure are well-documented including disruptions of the endocrine system, reproductive system, liver, nervous system, cognitive function, and behavior[70,106,186-189]. PBDEs are classified as endocrine disrupting chemicals due to their interference with the synthesis, secretion, transport, binding, operation, or elimination of hormones critical for homeostasis of many organ systems[12]. Rodent studies have consistently documented reduced thyroid hormone production associated with PBDE exposures, even at low doses[12,190]. In humans, however, PBDE exposures have been linked to thyroid hormone disturbance consistent with a hyperthyroid state demonstrated by decreased thyroid

stimulating hormone (TSH) and elevated thyroxine (T4) levels[12] or a hypothyroid state mirroring the opposite laboratory diagnostics[102]. Either way, disturbed thyroid homeostasis can lead to life-altering consequences.

PBDE exposure and thyroid dysfunction have independently been associated with adverse psychological functions such as attention deficit[191], impaired neurodevelopment[11,12], and poor mental and psychomotor development[11]. Neurotoxic effects occur when a toxic agent produces structural or functional changes within the nervous system. The changes can lead to permanent or reversible brain damage exemplified by learning impairment, memory loss, motor control or behavior changes such as mood instability. Many studies have elucidated the effects of prenatal PBDE exposure on the developing fetus and children later in life but only a few have investigated the effects of PBDE exposure outside of pregnancy and neuropsychological effects[49,136]. It is unknown if the PBDE-associated neuropsychological and behavioral dysfunction observed in children of cohort studies are also present in other populations.

The mechanisms of action by which PBDE effects cognition or behavior is understudied and not well characterized. Given the strong interconnections between PBDEs and thyroid disruption, and thyroid disruption and mood disorders, some have suggested neurotransmitters may be key players[50,132]. PBDEs share similar mechanistic actions and health effects with polychlorinated biphenyls (PCBs), another persistent organic pollutant[11,35,127-131]. It has been reported that PCBs, much like PBDEs, disrupt thyroid homeostasis and can lead to neurotoxic effects[128]. Specific metabolites have been implicated in the association between PCBs and depression such as homovanillic acid and vanillylmandelic acid but the findings are preliminary[51]. These metabolites are central to metabolic pathways within dopamine and norepinephrine synthesis of the

tyrosine metabolic pathway. Thyroid hormone synthesis also occurs within the greater tyrosine metabolic pathway, produced in the thyroid gland from tyrosine and iodine. With new methods such as high-resolution metabolomics (HRM), researchers are able to detect tens of thousands of metabolites present in human plasma. The HRM approach includes liquid chromatography and ultra-high resolution mass spectrometry (LC-MS) to produce a large network of cellular processes representing intermediate metabolites and end products. Diseases, infections, mutations, and environmental exposures can alter the metabolic profile. Assessing individual profiles during such an event can provide useful information about disease states, toxicity, and mechanisms of action at a given point in time[192]. In this study, we explore tyrosine-associated metabolic pathways by using a suspect screening approach to explore causal effects of PBDE exposure and antepartum depression in African American (AA) women.

Methods

This cross-sectional analysis was based on a subset of 193 women drawn from a larger longitudinal pregnancy and birth cohort study investigating the prenatal microbiome as a predictor of birth outcomes[157]. Women were included if they self-identified as AA, had a singleton pregnancy, were 18-35 years of age, resided in the study area, could comprehend and speak English, and were in the first trimester of pregnancy. None of the participants had a history or current report of thyroid/parathyroid disease, depression, or diabetes. Recruitment sites included prenatal clinics serving two local hospitals. Participants were informed of the study protocol and written consent obtained. Study procedures were approved and reviewed annually by the Emory University Institutional Review Board (Atlanta, GA).

Demographic questionnaires and peripheral blood samples were obtained for the PBDE and metabolomics analysis between 8-14 weeks gestation.

Measures

Edinburgh Depression Scale

The Edinburgh Depression Scale (EDS), a 10-item scale of depressive symptoms experienced in the last seven days, was used to measure depression among the participants[153]. Scores can range from 0-30 with higher scores associated with a higher risk of depression[153]. The EDS is a reliable tool to predict antenatal major or minor depression with psychometric properties of 64-87% sensitivity, 73-96% specificity, and 73% positive predictive value[152]. It has been validated in multiple race and ethnic groups and our work supports using of a cut-off score of ≥10 to predict depression among our AA cohort[152,153].

PBDE Analysis

Concentrations of PBDEs congeners 47, 85, 99, 100, 153, and 154 were measured in maternal serum using a modification of a previous method[103,158]. Samples were fortified, concentrated, and analyzed using gas chromatography-tandem mass spectrometry and calibrated with isotope dilution. Values less than the limit of detection were imputed with values divided by the square root of two[159]. Maternal serum total cholesterol and free triglycerides were not collected, therefore we could not perform lipid adjustment.

Metabolomics Analysis and Metabolite Identification

Maternal plasma was processed for metabolomics analysis using apLCMS and xMSanalyzer per the method described by Soltow et al.[193]. Data were filtered using quality control methods and batch-corrected yielding 18,675 untargeted metabolic

features in HILIC (positive ion mode) and C18 (negative ion mode) plasma columns. The median coefficient of variation was 27% for C18 and 21% for HILIC. A suspect screening approach was used to re-analyze plasma HRM data for better sensitivity of tyrosine-specific metabolic pathway identification. The custom database was created based on known Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and included all metabolites, isotopes, and adducts in the tyrosine synthesis pathway. xMSannotator, a free R package, uses KEGG, HMDB, Toxin and Toxin Target Database (T3DB) and ChemSpider to build a multi-criteria algorithm for compound annotation. The algorithm utilizes the HRM data and known metabolic pathways to confirm identities based on physicochemical properties, reduces incorrect matches, and assigns confidence levels to the results[194]. For this study, 278 metabolic compounds had human database matches with our custom metabolite database of tyrosine-specific metabolites based on the mass to charge ratio (*m/z*). The evaluation results identified seven metabolic compounds assigned with high confidence and 23 with medium confidence for which the *m/z* values were correctly matched to the metabolites named in the KEGG database (Figure 5).

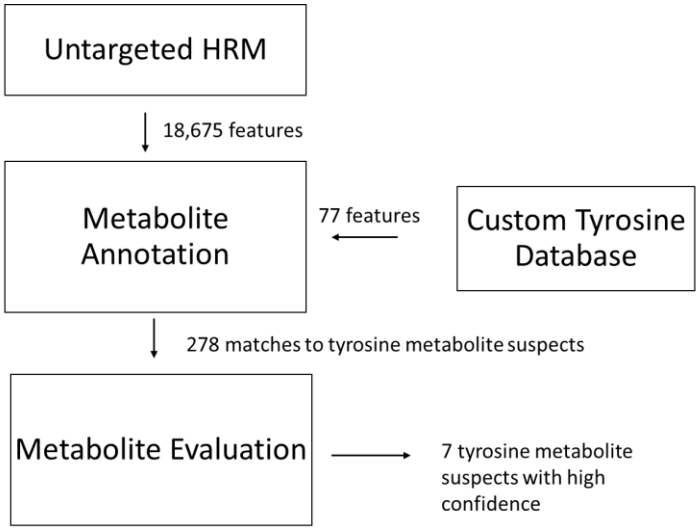


Figure 5. Tyrosine-specific metabolite identification using high-resolution metabolomics and suspect screen analysis (N=193).

Statis6tical analysis

Descriptive analyses was performed on demographic and clinical characteristics and bivariate associations with high depressive symptoms using an EDS cutoff score of ≥10. Log transformed PBDE congeners were included in analyses if they were present in >50% of the samples. Less than 10% of missing data existed. Wilcoxon signed-rank test was used for all PBDE associations and correlated with the chosen metabolites that yielded a high confidence (level 3) of correctly matching the metabolites to the well-established KEGG database. We conducted formal mediation analyses to further understand the effect of PBDEs on depressive symptoms. We considered each metabolite a potential mediator. The metabolite intensity profiles were log transformed and each were modeled as continuous mediators in the R mediation package. The models partition the effects into indirect and direct mechanisms. An indirect causal effect implicates the metabolite-mediated mechanism and a direct effect represents all other potential mechanisms that may lead to depression (Figure 1).

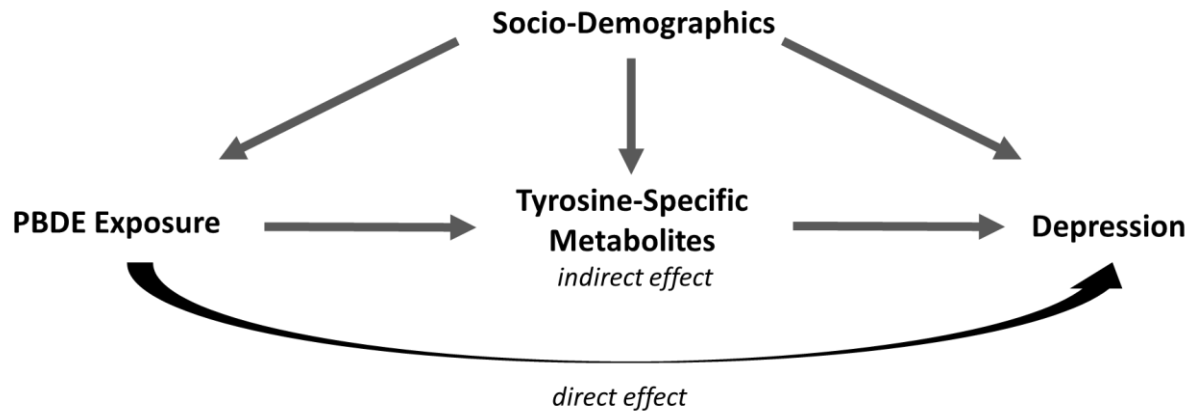


Figure 1. The proposed causal framework depicted two mechanisms between PBDE exposure and depression. We propose that 1) tyrosine-specific metabolites mediate the relationship between PBDE exposures and depression (indirect effect) and 2) PBDEs may have alternative mechanisms which lead to depression that does not include the tyrosine pathway (direct effect). *Adapted from Eldridge et al. (2017)*

The causal model shown in Figure 1 represents the modeling of two equations by the R mediator package. First, the package models the mediator with exposure and covariates. The mediator consisted of seven metabolites, each modeled with the three PBDE congeners and the covariates. A total of 21 models were constructed, controlling for age, BMI, and marital status. Second, the package models the 21 outcomes with exposures, covariates, and each mediator. The final analysis step of the mediation package evaluates another 21 models to determine if the effect of PBDE on depressive symptoms occurs through the metabolite, or indirectly. Once these relationships were identified, we evaluated the mediation effect of the tyrosine metabolites by investigating the effect of PBDEs on EDS scores while accounting for the multiplicative term between each PBDE times each metabolite, controlling for age, BMI, and marital status. The final aspect of the mediate function in the R mediation package takes the model objects and inputs them as independent variables with mediators. Beta coefficients and corresponding confidence intervals were estimated for each PBDE congener and each

metabolite using nonparametric bootstrapping of 1000 simulations. The average causal mediation effect results are compared to zero. All analyses were performed in R version 3.5.1.

Results

Age did not differ significantly between the high depressive symptom group (N=52) and those with low or no depressive symptoms (N=141), mean of 23.8 and 24.4, respectively (Table 10). Married women had less depressive symptoms than unmarried though the difference was not statistically significant (p=0.6). Most women qualified for Medicaid at or below poverty (77.8%). Approximately half of the total sample reported having some college education (48.2%), were overweight or obese (54.4), and nulliparous (48.2%). Women with high depressive symptoms were more likely to ever smoke tobacco (26.9% vs 5%, p<.0001). Thirty-three women reported using marijuana in the last month and were also more likely to have high depressive symptoms (26.9% vs 13.5%, p=0.3). Women with high depressive symptoms had higher PBDE concentrations (pg/ml) for BDE -47, -99, and -100 with statistical significance noted for BDE -47 and -99 (p= 0.04 and 0.02).

Of the 278 metabolic compounds annotated based on the tyrosine-specific custom database, seven matched with high confidence to the KEGG database based on the *m/z* ratios and correct annotation. Those seven were used in the statistical analysis and mapped to three distinct pathways, all within the custom tyrosine database (Table 11). Metabolites were regressed with each PBDE congener separately with and without the covariates age, BMI, and marital status. No differences were seen therefore, the unadjusted results are reported (Table 12). Metabolite with *m/z* value of 746.0871 pertaining to pathway three was positively associated with BDE- 47 and -100 at p<0.05.

Pathway 3 was annotated as nicotinamide adenine dinucleotide phosphate (NADP), b-NADP, its metabolic precursor, and Triphosphopyridine nucleotide (TPN).

To evaluate the tyrosine metabolite suspects as mediators between each PBDE congener and the EDS scores, a 1,000 simulation bootstrap regression analysis was conducted. The analysis revealed that, in the case of each congener, the tyrosine metabolites did not appear to mediate the effect of PBDE exposures on EDS scores, when controlling for age, BMI, and marital status (Table 13). The indirect effect on EDS scores through tyrosine metabolites, using PBDE 47 as the exposure, ranged from point estimates equal to -0.56 to 0.42. However, the direct effect of PBDE 47 remained significantly associated with EDS scores. For each unit increase in PBDE 47 serum concentration, we observed greater than 2 point increase in EDS scores. Full mediation results can be found in supplemental Table 3.

Table 10. Demographic characteristics of AA cohort 2014-2015 associated with high depressive symptoms (N=193).

	High depressive symptoms (n=52)	**Low or no depressive symptoms (n=141)**
Characteristics	**n (%)**	**n (%)**
Age (mean, SD)	23.8 ± 3.9	24.4 ± 4.6
Married	3 (5.8)	23 (16.3)
Insurance		
Low Income Medicaid<100%	10 (19.2)	43 (30.5)
Right from the Start Medicaid≤ 200%	33 (63.5)	64 (45.4)
Private	9 (17.3)	34 (24.1)
Education		
Some high school	12 (23.1)	23 (16.3)
Graduated high school or GED	18 (34.6)	47 (33.3)

Some college or technical school	16 (30.8)	47 (33.3)
Graduated college	6 (11.5)	14 (9.9)
Some graduate work or degree	0 (0.0)	10 (7.1)
Body mass index*		
Underweight (<18.5)	4 (7.7)	4 (2.8)
Normal (18.5 – < 25)	24 (46.2)	56 (39.7)
Overweight (25 – < 30)	14 (26.9)	25 (17.7)
Obese (≥ 30)	10 (19.2)	56 (39.7)
Substance use		
Tobacco, ever*	14 (26.9)	7 (5.0)
Marijuana, in last month*	14 (26.9)	19 (13.5)
Alcohol, in last month	4 (7.7)	4 (2.8)
PBDE 47 (pg/ml) * (GM, GSD)	107.7 ± 2.2	84.3 ± 2.1
PBDE 99 (pg/ml) * (GM, GSD)	26.5 ± 2.4	19.6 ± 2.7
PBDE 100 (pg/ml) (GM, GSD)	15.5 ± 3.1	12.7 ± 3.0

*Denotes statistical significance at $p<0.05$

*GM= geometric mean, GSD= geometric standard deviation

Table 11. Tyrosine-specific metabolic pathways identified by high-resolution metabolomics methods among AA pregnant women (N=193).

Pathway 1	m/z 166.058 m/z 165.0546	4-Coumarate; p-Coumaric acid; trans-4-Hydroxycinnamate; trans-p-Hydroxycinnamate; 4-Hydroxycinnamate
Pathway 2	m/z 184.0877 m/z 183.0837 m/z 182.0812	L-Tyrosine; (S)-3-(p-Hydroxyphenyl) alanine; (S)-2-Amino-3-(p-hydroxyphenyl) propionic acid; Tyrosine
Pathway 3	m/z 746.0871 m/z 745.0851	Nicotinamide adenine dinucleotide phosphate (NADP); beta-NADP; Triphosphopyridine nucleotide (TPN)

Table 12. Simple linear regression between PBDE exposures and identified tyrosine-specific metabolites. (N=193).

Metabolite	**m/z 166.058**	**m/z 165.0546**	**m/z 184.0877**	**m/z 183.0837**	**m/z 182.0812**	**m/z 746.0871**	**m/z 745.0851**
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
BDE- 47	-0.04 (-0.14, 0.05)	-0.04 (-0.13, 0.05)	0.00 (-0.11, 0.15)	-0.03 (-0.24, 0.19)	-0.02 (-0.13, 0.09)	0.30 (0.10, 0.50)*	0.08 (-0.05, 0.22)
BDE- 99	-0.02 (-0.05, 0.02)	-0.02 (-0.05, 0.01)	0.00 (-0.04, 0.05)	0.00 (-0.08, 0.07)	-0.02 (-0.05, 0.02)	0.04 (-0.03, 0.11)	0.00 (-0.05, 0.05)
BDE- 100	-0.01 (-0.04, 0.00)	-0.01 (-0.04, 0.02)	-0.02 (-0.06, 0.02)	-0.03 (-0.09, 0.04)	-0.02 (-0.05, 0.01)	-0.02 (-0.05, 0.01)*	0.02 (-0.02, 0.06)

*Denotes statistical significance at <0.05 (unadjusted)

Table 13. Results of mediation model of PBDE 47 exposure on tyrosine metabolites and EDS scores (N=193).

Effect	Pathway 1		Pathway 2			Pathway 3	
	m/z 166.058 β (95% CI)	m/z 165.0546 β (95% CI)	m/z 184.0877 β (95% CI)	m/z 183.0837 β (95% CI)	m/z 182.0812 β (95% CI)	m/z 746.0871 β (95% CI)	m/z 745.0851 β (95% CI)
Indirect	0.30 (- 0.58, 1.40)	0.37 (-.059, 1.68)	-0.02 (-1.06, 0.86)	0.16 (-0.91, 1.46)	0.42 (-0.40, 1.74)	-0.56 (-2.72, 0.9)	-0.18 (-1.32, 0.62)
Direct	2.79 (0.32, 5.44)	2.64 (0.29, 5.50)	2.38 (-0.20, 5.10)	2.36 (-0.00, 4.83)	2.77 (0.30, 5.51)	2.29 (-0.98, 5.16)	2.36 (-0.15, 4.95)

*Adjusted for BMI, age, and marital status

Supplemental Table 3. Causal mediation effects of PBDE exposure on depressive symptoms.

Metabolite Group 1: 4-Coumarate; p-Coumaric acid; trans-4-Hydroxycinnamate; trans-p-Hydroxycinnamate; 4-Hydroxycinnamate

	m/z 166.058 β (95% CI)	m/z 165.0546 β (95% CI)
PBDE 47		
Indirect effect	0.30 (- 0.58, 1.40)	0.37 (-.059, 1.68)
Direct effect	2.79 (0.32, 5.44)	2.64 (0.29, 5.50)
PBDE 99		
Indirect effect	0.02 (-0.21, 0.22)	0.02 (-0.26, 0.24)
Direct effect	0.53 (-0.34, 1.29)	0.46 (-0.38, 1.27)
PBDE 100		
Indirect effect	-0.00 (-0.16, 0.06)	-0.00 (-0.16, 0.09)
Direct effect	0.47 (-0.11, 1.12)	0.45 (-0.23, 1.06)

Metabolite Group 2: L-Tyrosine; (S)-3-(p-Hydroxyphenyl) alanine; (S)-2-Amino-3-(p-hydroxyphenyl) propionic acid; Tyrosine

	m/z 184.0877 β (95% CI)	m/z 183.0837 β (95% CI)	m/z 182.0812 β (95% CI)
PBDE 47			
Indirect effect	-0.02 (-1.06, 0.86)	0.16 (-0.91, 1.46)	0.42 (-0.40, 1.74)
Direct effect	2.38 (-0.20, 5.10)	2.36 (-0.00, 4.83)	2.77 (0.30, 5.51)
PBDE 99			
Indirect effect	0.02 (-0.22, 0.24)	0.01 (-0.17, 0.24)	0.10 (-0.12, 0.41)
Direct effect	0.47 (-0.28, 1.20)	0.43 (-0.30, 1.15)	0.57 (-0.21, 1.38)
PBDE 100			
Indirect effect	-0.01 (-0.17, 0.10)	0.01 (-0.12, 0.14)	0.01 (-0.18, 0.15)
Direct effect	0.46 (-0.21, 1.10)	0.44 (-0.15, 1.13)	0.45 (-0.17, 1.12)

Metabolite Group 3: Nicotinamide adenine dinucleotide phosphate (NADP); beta-NADP; Triphosphopyridine nucleotide (TPN)

	m/z 746.0871 β (95% CI)	m/z 745.0851 β (95% CI)
PBDE 47		
Indirect effect	-0.56 (-2.72, 0.99)	-0.18 (-1.32, 0.62)
Direct effect	2.29 (-0.98, 5.16)	2.36 (-0.15, 4.95)
PBDE 99		
Indirect effect	0.00 (-0.22, 0.19)	0.00 (-0.14, 0.17)
Direct effect	0.47 (-0.40, 1.26)	0.40 (-0.32, 1.21)
PBDE 100		
Indirect effect	0.01 (-0.20, 0.18)	-0.01 (-0.15, 0.07)
Direct effect	0.51 (-0.16, 1.17)	0.45 (-0.14, 1.06)

Discussion

We took a suspect screening approach to identify metabolites specific to the tyrosine pathway potentially involved in the mediation of depressive symptoms resulting from PBDE exposure in African American pregnant women. We observed a significant association between PBDE exposure level and depressive symptoms. Our mediation analysis, however, suggests that PBDE toxicants are not indirectly influencing depressive symptoms through tyrosine metabolism in our sample. Metabolomics is a highly sensitive and specific tool for identifying chemicals present in the blood. Yet, a key limitation of the technique is that it provides only a snapshot in time of the dynamic catabolic and anabolic biochemical reactions taking place within the body. Therefore, it is possible that in the case of metabolic pathways that are highly regulated by multiple feedback loops, metabolomics may not be able to elucidate changes in metabolism due to the static nature of the data it produces. A few studies that have identified tyrosine metabolites as part of an inflammatory response implicate nitric oxide (NO)-tyrosine and other NO-adducts among depressed patients[195,196]. Grounded in the principals of oxidative stress, inflammation generates free radicals and decreases antioxidant balance. Under these conditions, tyrosine nitration, a key biomarker of nitro-oxidative stress, is found in multiple pathological conditions[197]. However, protein tyrosine nitration is a low yield process, thus the levels of breakdown products, or metabolites in our case, may be too low for detection in some cases[198].

Pregnancy-related changes in thyroid hormone signaling could lead to excessive variability further impeding a tyrosine metabolic effect of PBDE exposure on depression. For example, participants in our study provided blood samples and took the EDS survey between 8-14 weeks gestation. It is possible that there were as many as six

week's difference in data collection among participants which could inaccurately reflect the expected thyroid demand in early pregnancy. The anticipated physiologic thyroid demands (distinguished by free T_3 and T_4 levels) of the mother increase by as much as 50% and last until the end of the first trimester while TSH levels decrease[199,200]. When levels of T3 and T4 increase, TSH decreases through midgestation before plateauing for the remainder of pregnancy[200]. Like tyrosine metabolites, thyroid hormones could test the mediation hypothesis between PBDE exposure and depressive symptomatology. Thyroid hormone levels were not measured in our study and thus, could not be included as covariates in our modeling.

In addition to normal physiologic shifts in pregnancy, environmental exposures, lifestyles, and genetic predispositions can contribute to changes in thyroid hormone levels and the metabolic profile of an individual[201,202]. Diet and exercise in particular plays a major role in thyroid hormone metabolism but we were unable to identify participant's last meal or type of food recently ingested[203]. Additionally, we lacked serum lipid data which hindered our ability to lipid adjust serum PBDE concentrations and account for the role of diet, PBDE sequestering, and thyroid hormone metabolism. A broader assessment of psychosocial stressors, trauma, and diet could greatly improve our ability to assess the environmental effect and reduce the unexplained variation found in this study.

Conclusion

PBDEs are persistent in the environment and present in human serum as evidenced in biomonitoring studies. Normal physiologic changes in pregnancy can potentially complicate the metabolism of xenobiotics such as PBDEs. It is well-known that a variety of environmental factors, medical conditions, and diseases may trigger

depression. The relationship between environmental chemicals and mental health is an understudied area of research but incredibly important to adjudicate. Our findings indicate a significant direct causal relationship between PBDE exposure and the risk of depression that does not appear to be mediated by the tyrosine metabolic pathway. This study contributes to the environmental exposure literature that evaluates mechanistic actions associated with the endocrine disrupting chemical, PBDE, and neuropsychological effects. Isolating particular pathways that may be mediating these relationships is challenging as we have uncovered. In addition to a larger sample size, future studies should use case control designs that may elicit a clearer depiction of the metabolic differences between depressed and nondepressed women with similar environmental exposures. Further research should incorporate potential confounders such as psychosocial stressors, health behaviors, and diet that may be mediating this association.

Dissertation Conclusion

PBDEs are ubiquitous in our environment and commonly found in homes of most Americans. These chemicals were originally developed to reduce the ignition time of the products they were added to. In the past two decades, the negative health effects of these chemicals have been well characterized in the literature. Long term health concerns related to PBDE exposure are becoming apparent and will likely continue for years due to slow chemical degradation leading to persistent accumulation in our environment. Relevant to this dissertation, they have been characterized as endocrine disrupting chemicals capable of altering thyroid homeostasis in humans. Thyroid signaling is responsible for maintaining normal physiological processes of numerous body systems including respiratory, cardiac, and central and peripheral nervous systems. Thyroid homeostasis is essential for many system processes including proper cognition and mood but major gaps in available research exist specific to the mechanisms by which thyroid hormone disruption leads to adverse health outcomes. Additionally, the majority of existing studies focus on prenatal exposures and their effects on the infant or fetus. Prior to this dissertation, no studies existed that evaluate the effects of PBDE exposures on a socioeconomically diverse cohort of African American women living in the urban Southeast. The ultimate purpose of this dissertation work was to address the aforementioned gaps by shifting the focus to a population of understudied African American pregnant women and elucidate health effects to them with deliberate attention to their physiologic changes associated with pregnancy. We sought to characterize the relationships between PBDEs and neuropsychiatric outcomes, and test whether the thyroid performs as an intermediary between PBDEs and depression.

"Thyroid hormone disruption as a mediator between polybrominated diphenyl ether and antenatal depression"

In the first manuscript of this dissertation, we review the literature on the known health effects of PBDE exposures, the role of thyroid hormone disruption in neuropsychological outcomes, and increased vulnerability in pregnant women. Existing literature on PBDE exposure and subsequent health effects has focused on endocrine and neurological effects primarily among infants and children. Pregnancy is also a time of vulnerability to the effects of toxicants due to natural variability immune and endocrine states[2-4]. PBDEs and other endocrine disrupting chemicals can modulate thyroid signaling through various mechanisms. A clear associations between thyroid hormone dysfunction and mood disorders exists in the scientific literature however, additional research is needed to elucidate relationships between PBDE exposures and depressive symptomatology. We posit PBDE exposure leads to disruption of thyroid hormone signaling and subsequently leads to depression in pregnant women. High-resolution metabolomics (HRM) shows promise as a tool for illuminating the mechanisms by which environmental toxicants alter normal physiology. A need exists for research on African American pregnant women in the Southeast to address gaps in the current literature. In light of the well-established effects of perinatal depression to the mother, fetus, and the children at multiple life stages, understanding the basic biology of EDCs and their role in perinatal depression would substantially add to our ability to design interventions to protect the health of pregnant mothers and children.

"Polybrominated diphenyl ether serum concentrations and depressive symptomatology in pregnant African American women"

In the second manuscript of this dissertation, we describe the PBDE serum concentration in our study sample and examine whether exposure in pregnant women is associated with depressive symptomatology. Toxicant and depression data were collected between 8-14 weeks gestation. Statistical modeling estimated associations between detected PBDE congeners, a mixture of congeners, and depressive symptoms (direct effect in the causal framework). Our analysis revealed BDE-47, -99, and -100 are present in a cohort of socioeconomically diverse pregnant AA women and the weighted mixture is associated with high depressive symptoms. The novel associations were found in a relatively small sample size and among a non-occupationally exposed group. Because of limited research and slow PBDE degradation, it is unclear whether PBDE accumulation has other long-term implications on pregnant women. Additional studies are needed to address population health impacts resulting from PBDE bioaccumulation since the chemicals are likely to be present in the environment for the foreseeable future. The cross-sectional methodology of this study can be useful in informing researchers and public health professionals about depressive symptomatology among AA pregnant women and changes in PBDE concentrations over time. Assessing environmental burdens and human toxicant effects, especially mixtures, can inform planning and allocation of health resources for mitigation and prevention.

"*Using tyrosine metabolomics to describe the relationship between serum polybrominated diphenyl ether and depression"*

In the final manuscript of this dissertation, the causal framework was further explored. Tyrosine-associated metabolic pathways were detected using a metabolomics suspect screening approach to explore causal effects of PBDE exposure and antepartum depression in AA women. The approach resulted in 278 compounds with human metabolomic database matches with our custom metabolite database based on the mass to charge ratio (*m/z*). The evaluation results identified 7 of those metabolic compounds named in the KEGG database and were further used in the analysis. The models partition the effects into indirect and direct mechanisms where an indirect causal effect implicates the metabolite-mediated mechanism and a direct effect represents all other potential mechanisms that may lead to depression. Normal physiologic changes in pregnancy can potentially complicate the metabolism of xenobiotics such as PBDEs. It is well established that a variety of environmental factors, medical conditions, and diseases may trigger depression. Our findings indicate a significant direct causal relationship between PBDE exposure and the risk of depression but does not appear to be mediated by the tyrosine metabolic pathway. This study contributes to the literature evaluating mechanistic actions associated with PBDE its and neuropsychological effects. Isolating particular pathways that may be mediating these relationships is challenging as we have uncovered. In addition to a larger sample size, future studies should design case controls which may elicit a clearer depiction of the metabolic differences between depressed and nondepressed women with similar environmental exposure. Further research should incorporate potential confounders such as psychosocial stressors, health behaviors, and diet that may be mediating this association.

Strengths and limitations

A key strength of this study is the focus on vulnerable pregnant AA women which may be at higher risk of PBDE exposure and perinatal depression. It addresses a major gap in research by examining PBDE exposure in a non-occupational setting and in pregnant women. To date, existing studies have focused on prenatal exposures and their effects on the infant or fetus. To our knowledge, this is the first study to examine PBDE exposures and depression during pregnancy. Innovative methods such as the weighted quantile sum and the metabolomics suspect screen analyses were utilized to explain the PBDE mixture effect and to determine the tyrosine-specific metabolites involved in thyroid hormone synthesis. These methods add depth and description to the analysis and interpretation which significantly contribute to the nursing and public health literature.

There are notable limitations to this study. Since our study relied on maternal-self report to document tobacco use, substance abuse, and depressive symptoms, it is possible that recall bias interfered with our results. Especially during pregnancy, study participants may have been reluctant to disclose the presence or absence of depressive symptoms or current use of tobacco or illegal drugs. A broader assessment of psychosocial stressors, trauma, and diet could greatly improve the environmental effect and reduce the unexplained variation found in this study.

The study sample is based on a convenience sampling from two metropolitan prenatal clinics. Attempts were made to represent most AA pregnant women living in urban environments but results may not be generalizable to suburban or rural areas. Additionally, the cross-sectional study design comprised of one time point limits the ability to cultivate causal relationships. Also, because the of the design collected blood

anywhere within the 6 weeks period (8-14 weeks), thyroid metabolites may not reflect the first trimester demands of pregnancy similarly between individuals. Longitudinal data or a repeated measures study would improve the findings and more confidently predict the conclusions.

Metabolomics is a highly sensitive and specific tool for identifying chemicals and metabolite byproducts present in the blood. Yet, a key limitation of the technique is that it provides only a snapshot in time of the multiple biochemical synthesis and degradation pathways that take place in the body. Therefore, the temporally regulated pathways may not be able to discern pregnancy-related changes in metabolism from those induced by environmental exposures. Pregnancy-related changes in thyroid hormone signaling could also lead to excessive variability impeding a tyrosine metabolic effect of PBDE exposure on depression.

Challenges encountered during study

The main challenges encountered during this dissertation was the inability to measure additional confounders that were identified a priori. As previously mentioned, thyroid hormone levels, liver and kidney function tests, and total lipid data would greatly enhance the interpretations of the study findings. Inherent difficulties with human research were also present such as participant follow-through and timeliness of appointments. It is difficult to organize participants to obtain biospecimen and questionnaire data at the same exact time point in pregnancy for equal comparison between individuals.

Another challenge for this dissertation was managing large omics data and three software programs with multiple algorithms for estimating and annotating tyrosine-specific metabolites. The behind-the-scene computing offered by the software packages

were complex and cumbersome. Nonetheless, the research aims and necessary analysis methods enhanced the research skills of the team and proved to be informative for health sciences research and practice.

Implications for clinical practice

The standard set forth for all clinicians is currently to *not* screen universally for thyroid hormones or autoantibodies during pregnancy. The American College of Obstetricians and Gynecologists (ACOG) supports screening only those with a personal history or those exhibiting symptoms of thyroid disease[204]. Those in opposition argue the recommendations are unequivocally flawed due to stark similarities in normal symptoms of pregnancy and symptoms related to thyroid disease. Those that oppose argue that universal screening is the only way to identify subclinical or covert cases that would be otherwise masked by early pregnancy. Additionally, early thyroid screening and detection of an abnormality provides the largest impact for the prevention of poor maternal and fetal outcomes. The literature review for this dissertation supports broadening the standard purview to universally screen all pregnant women exhibiting depressive symptoms for thyroid dysfunction.

Universal screening standards are in place for depression and environmental hazards in pregnancy. For depression screening, ACOG recommends at least 1 screening in the perinatal period using a validated screening tool in addition to a full emotional well-being exam during the postpartum follow-up visit[205]. At this time, no recommendations are made for specific screening during the antenatal period. Our work revealed a high antenatal depression risk of 27% in AA women. At a minimum, this supports additional screening in pregnancy. In terms of identifying environmental risk factors in pregnancy, ACOG has provided a committee opinion outlining the effects of

environmental exposures on reproductive health and populations that may be more susceptible to toxicity[206]. In conjunction with the International Federation of Gynecology and Obstetrics, four main exposure prevention recommendations have been made for women's health and childbirth providers[207]. The gaps between clinical recommendations and implementation into practice could involve misunderstandings in the translation process as well as poor competency in environmental health literacy among medical practitioners. Clinical researchers well-versed in environmental health and reproduction are invaluable in translating the risks of common environmental exposures such as PBDEs and most, importantly, avoidance and mitigation practices.

Recommendations for policy

Advocacy and political activism may be the most impactful and efficient way to reduce harmful environmental exposures to pregnant women and children. For example, recent nationwide involvement in prohibiting the use of tobacco smoke in public spaces has significantly reduced exposure and improved health outcomes across multiple populations[208]. Individual changes are important but improving policy and advocating for stricter environmental health and safety guidelines will have larger bearings on local, state national, and international policies. Consumers can also have a powerful political voice by being knowledgeable and savvy shoppers. When consumers do not purchase products containing toxicants, manufacturers do not profit and are forced to understand public concern and create healthier products.

Recommendations for future research

This research is a first step in identifying environmental exposures in a pregnant AA cohort. The methods utilized in this dissertation can be easily applied to incorporate additional endocrine disrupting chemicals with similar toxic effects on thyroid hormone

synthesis. Limited information is available about how endocrine disrupting chemical concentrations differ by race and characterization pf racial differences could greatly improve our knowledge of exposures specific to the southeast United States. Further, a case control design may elicit a clearer depiction of the metabolic differences between depressed and nondepressed women with similar environmental exposures. Additional metabolic pathways should be incorporated that are also known to be associated with depressive symptoms and are closely involved with thyroid hormone synthesis such as dopamine, norepinephrine, and epinephrine. Further research should also incorporate potential confounders such as psychosocial stressors, health behaviors, and diet that may affect PBDE concentrations, thyroid function, and/or depressive symptoms. Finally, identification of major sources of environmental exposures to PBDEs in this population may shed light on future findings and provide insights for exposure mitigation or intervention research studies.

Summary

PBDEs are persistent in the environment and present in human serum as evidenced in this biomonitoring study. Normal physiologic changes in pregnancy can potentially complicate the metabolism of xenobiotics such as PBDEs. It is well-known that a variety of environmental factors, medical conditions, and diseases may trigger depression. The relationship between environmental chemicals and mental health is an understudied area of research but incredibly important to adjudicate. Our findings indicate a significant direct causal relationship between PBDE exposure and the risk of depression that does not appear to be mediated by the tyrosine metabolic pathway. This study contributes to the environmental exposure literature that evaluates mechanistic

actions associated with the endocrine disrupting chemical, PBDE, and neuropsychological effects.

References

1. Sjodin A, Papke O, McGahee E, et al. Concentration of polybrominated diphenyl ethers (PBDEs) in household dust from various countries. *Chemosphere.* 2008;73(1 Suppl):S131-136.
2. Lignell S, Aune M, Darnerud PO, et al. Maternal body burdens of PCDD/Fs and PBDEs are associated with maternal serum levels of thyroid hormones in early pregnancy: a cross-sectional study. *Environ Health.* 2016;15:55.
3. Tan EK, Tan EL. Alterations in physiology and anatomy during pregnancy. *Best Pract Res Clin Obstet Gynaecol.* 2013;27(6):791-802.
4. Budenhofer BK, Ditsch N, Jeschke U, Gartner R, Toth B. Thyroid (dys-)function in normal and disturbed pregnancy. *Archives of gynecology and obstetrics.* 2013;287(1):1-7.
5. Fraser AJ, Webster TF, McClean MD. Diet contributes significantly to the body burden of PBDEs in the general U.S. population. *Environ Health Perspect.* 2009;117(10):1520-1525.
6. Watkins DJ, McClean MD, Fraser AJ, et al. Impact of dust from multiple microenvironments and diet on PentaBDE body burden. *Environ Sci Technol.* 2012;46(2):1192-1200.
7. Makey CM, McClean MD, Braverman LE, et al. Polybrominated Diphenyl Ether Exposure and Thyroid Function Tests in North American Adults. *Environ Health Perspect.* 2016;124(4):420-425.
8. Dishaw LV, Macaulay LJ, Roberts SC, Stapleton HM. Exposures, mechanisms, and impacts of endocrine-active flame retardants. *Current opinion in pharmacology.* 2014;19:125-133.

9. Eskenazi B, Chevrier J, Rauch SA, et al. In utero and childhood polybrominated diphenyl ether (PBDE) exposures and neurodevelopment in the CHAMACOS study. *Environmental health perspectives.* 2012;121(2):257-262.

10. Herbstman JB, Mall JK. Developmental Exposure to Polybrominated Diphenyl Ethers and Neurodevelopment. *Current environmental health reports.* 2014;1(2):101-112.

11. Herbstman JB, Sjodin A, Kurzon M, et al. Prenatal exposure to PBDEs and neurodevelopment. *Environ Health Perspect.* 2010;118(5):712-719.

12. Czerska M, Zieliński M, Kamińska J, Ligocka D. Effects of polybrominated diphenyl ethers on thyroid hormone, neurodevelopment and fertility in rodents and humans. *International journal of occupational medicine and environmental health.* 2013;26(4):498-510.

13. Windham GC, Pinney SM, Sjodin A, et al. Body burdens of brominated flame retardants and other persistent organo-halogenated compounds and their descriptors in US girls. *Environ Res.* 2010;110(3):251-257.

14. Chen A, Chung E, DeFranco EA, Pinney SM, Dietrich KN. Serum PBDEs and age at menarche in adolescent girls: analysis of the National Health and Nutrition Examination Survey 2003–2004. *Environmental research.* 2011;111(6):831-837.

15. Vuong AM, Webster GM, Romano ME, et al. Maternal Polybrominated Diphenyl Ether (PBDE) Exposure and Thyroid Hormones in Maternal and Cord Sera: The HOME Study, Cincinnati, USA. *Environ Health Perspect.* 2015;123(10):1079-1085.

16. Parry E, Zota AR, Park JS, Woodruff TJ. Polybrominated diphenyl ethers (PBDEs) and hydroxylated PBDE metabolites (OH-PBDEs): A six-year temporal trend in Northern California pregnant women. *Chemosphere.* 2018;195:777-783.

17. Pumarega J, Gasull M, Lee DH, Lopez T, Porta M. Number of Persistent Organic Pollutants Detected at High Concentrations in Blood Samples of the United States Population. *PLoS One.* 2016;11(8):e0160432.

18. Sjodin A, Schecter A, Jones R, et al. Polybrominated diphenyl ethers, 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153), and p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE) concentrations in sera collected in 2009 from Texas children. *Environ Sci Technol.* 2014;48(14):8196-8202.

19. Brulle RJ, Pellow DN. Environmental justice: human health and environmental inequalities. *Annu Rev Public Health.* 2006;27:103-124.

20. Landrigan PJ, Rauh VA, Galvez MP. Environmental justice and the health of children. *The Mount Sinai journal of medicine, New York.* 2010;77(2):178-187.

21. Mutic AD, Baker BJ, McCauley LA. Deleterious Effects From Occupational Exposure to Ethylene Thiourea in Pregnant Women. *Workplace health & safety.* 2017;65(12):595-602.

22. Zota AR, Adamkiewicz G, Morello-Frosch RA. Are PBDEs an environmental equity concern? Exposure disparities by socioeconomic status. *Environ Sci Technol.* 2010;44(15):5691-5692.

23. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Polybrominated Diphenyl Ethers (PBDEs) 2017;

https://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=901&tid=183. Accessed January 10, 2018.

24. Johnson-Restrepo B, Kannan K, Rapaport DP, Rodan BD. Polybrominated diphenyl ethers and polychlorinated biphenyls in human adipose tissue from New York. *Environ Sci Technol.* 2005;39(14):5177-5182.

25. Johnson-Restrepo B, Kannan K, Addink R, Adams DH. Polybrominated diphenyl ethers and polychlorinated biphenyls in a marine foodweb of coastal Florida. *Environ Sci Technol.* 2005;39(21):8243-8250.

26. Lorber M. Exposure of Americans to polybrominated diphenyl ethers. *J Expo Sci Environ Epidemiol.* 2008;18(1):2-19.

27. Hurley S, Goldberg D, Nelson DO, et al. Temporal Evaluation of Polybrominated Diphenyl Ether (PBDE) Serum Levels in Middle-Aged and Older California Women, 2011-2015. *Environ Sci Technol.* 2017;51(8):4697-4704.

28. Sjodin A, Wong LY, Jones RS, et al. Serum concentrations of polybrominated diphenyl ethers (PBDEs) and polybrominated biphenyl (PBB) in the United States population: 2003-2004. *Environ Sci Technol.* 2008;42(4):1377-1384.

29. Cowell WJ, Sjödin A, Jones R, Wang Y, Wang S, Herbstman JB. Determinants of prenatal exposure to polybrominated diphenyl ethers (PBDEs) among urban, minority infants born between 1998 and 2006. *Environmental Pollution.* 2018;233:774-781.

30. Frederiksen M, Vorkamp K, Thomsen M, Knudsen LE. Human internal and external exposure to PBDEs–a review of levels and sources. *International journal of hygiene and environmental health.* 2009;212(2):109-134.

31. Bergman Å, Heindel JJ, Kasten T, et al. The impact of endocrine disruption: a consensus statement on the state of the science. National Institute of Environmental Health Sciences; 2013.

32. Vuong AM, Yolton K, Poston KL, et al. Childhood polybrominated diphenyl ether (PBDE) exposure and executive function in children in the HOME Study. *Int J Hyg Environ Health.* 2018;221(1):87-94.

33. Chen L, Wang C, Zhang Y, et al. Polybrominated diphenyl ethers in cord blood and perinatal outcomes from Laizhou Wan Birth Cohort, China. *Environmental Science and Pollution Research.* 2018;25(21):20802-20808.

34. Byrne SC, Miller P, Seguinot-Medina S, et al. Associations between serum polybrominated diphenyl ethers and thyroid hormones in a cross sectional study of a remote Alaska Native population. *Scientific reports.* 2018;8(1):2198.

35. Harley KG, Marks AR, Chevrier J, Bradman A, Sjodin A, Eskenazi B. PBDE concentrations in women's serum and fecundability. *Environ Health Perspect.* 2010;118(5):699-704.

36. Kuriyama SN, Talsness CE, Grote K, Chahoud I. Developmental exposure to low dose PBDE 99: effects on male fertility and neurobehavior in rat offspring. *Environ Health Perspect.* 2005;113(2):149-154.

37. Shy CG, Huang HL, Chang-Chien GP, Chao HR, Tsou TC. Neurodevelopment of infants with prenatal exposure to polybrominated diphenyl ethers. *Bulletin of environmental contamination and toxicology.* 2011;87(6):643-648.

38. Hoffman K, Adgent M, Goldman BD, Sjodin A, Daniels JL. Lactational exposure to polybrominated diphenyl ethers and its relation to social and emotional development among toddlers. *Environ Health Perspect.* 2012;120(10):1438-1442.

39. Bradman A, Castorina R, Sjödin A, et al. Factors associated with serum polybrominated diphenyl ether (PBDE) levels among school-age children in the CHAMACOS cohort. *Environmental science & technology.* 2012;46(13):7373-7381.

40. Eskenazi B, Fenster L, Castorina R, et al. A comparison of PBDE serum concentrations in Mexican and Mexican-American children living in California. *Environmental health perspectives.* 2011;119(10):1442-1448.

41. Liu X, Zhang L, Li J, et al. A nested case-control study of the association between exposure to polybrominated diphenyl ethers and the risk of gestational diabetes mellitus. *Environ Int.* 2018;119:232-238.

42. Ochs N, Auer R, Bauer DC, et al. Meta-analysis: subclinical thyroid dysfunction and the risk for coronary heart disease and mortality. *Annals of internal medicine.* 2008;148(11):832-845.

43. Luo W, Mao P, Zhang L, Yang Z. Association between systemic lupus erythematosus and thyroid dysfunction: a meta-analysis. *Lupus.* 2018;27(13):2120-2128.

44. Guo Z, Li M, Han B, Qi X. Association of non-alcoholic fatty liver disease with thyroid function: A systematic review and meta-analysis. *Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver.* 2018;50(11):1153-1162.

45. Shi XZ, Jin X, Xu P, Shen HM. Relationship between breast cancer and levels of serum thyroid hormones and antibodies: a meta-analysis. *Asian Pacific journal of cancer prevention : APJCP.* 2014;15(16):6643-6647.

46. Hendrick V, Altshuler L, Whybrow P. Psychoneuroendocrinology of mood disorders. The hypothalamic-pituitary-thyroid axis. *The Psychiatric clinics of North America.* 1998;21(2):277-292.

47. Joffe RT, Sokolov ST. Thyroid hormones, the brain, and affective disorders. *Critical reviews in neurobiology.* 1994;8(1-2):45-63.

48. Bathla M, Singh M, Relan P. Prevalence of anxiety and depressive symptoms among patients with hypothyroidism. *Indian journal of endocrinology and metabolism.* 2016;20(4):468.

49. Gump BB, Yun S, Kannan K. Polybrominated diphenyl ether (PBDE) exposure in children: possible associations with cardiovascular and psychological functions. *Environ Res.* 2014;132:244-250.

50. Fitzgerald EF, Belanger EE, Gomez MI, et al. Polychlorinated biphenyl exposure and neuropsychological status among older residents of upper Hudson River communities. *Environmental health perspectives.* 2007;116(2):209-215.

51. Putschogl FM, Gaum PM, Schettgen T, Kraus T, Gube M, Lang J. Effects of occupational exposure to polychlorinated biphenyls on urinary metabolites of neurotransmitters: A cross-sectional and longitudinal perspective. *Int J Hyg Environ Health.* 2015;218(5):452-460.

52. Wang B, Liu S, Li L, et al. Non-thyroidal illness syndrome in patients with cardiovascular diseases: A systematic review and meta-analysis. *International journal of cardiology.* 2017;226:1-10.

53. Dama M, Steiner M, Van Lieshout R. Thyroid peroxidase autoantibodies and perinatal depression risk: a systematic review. *Journal of Affective Disorders.* 2016;198:108-121.

54. Pedersen C, Leserman J, Garcia N, Stansbury M, Meltzer-Brody S, Johnson J. Late pregnancy thyroid-binding globulin predicts perinatal depression. *Psychoneuroendocrinology.* 2016;65:84-93.

55. Szpunar MJ, Parry BL. A systematic review of cortisol, thyroid-stimulating hormone, and prolactin in peripartum women with major depression. *Archives of women's mental health.* 2018;21(2):149-161.

56. The American College of Obstetricians and Gynecologists. Screening for Perinatal Depression. Committee Opinion No. 757. *Obstetrics and gynecology.* 2018;132(5):e208-e212.

57. Woody CA, Ferrari AJ, Siskind DJ, Whiteford HA, Harris MG. A systematic review and meta-regression of the prevalence and incidence of perinatal depression. *J Affect Disord.* 2017;219:86-92.

58. Leung BM, Kaplan BJ. Perinatal depression: prevalence, risks, and the nutrition link--a review of the literature. *Journal of the American Dietetic Association.* 2009;109(9):1566-1575.

59. Liu Y, Kaaya S, Chai J, et al. Maternal depressive symptoms and early childhood cognitive development: a meta-analysis. *Psychological medicine.* 2017;47(4):680-689.

60. Surkan PJ, Patel SA, Rahman A. Preventing infant and child morbidity and mortality due to maternal depression. *Best practice & research Clinical obstetrics & gynaecology.* 2016;36:156-168.

61. Allen JG, Gale S, Zoeller RT, Spengler JD, Birnbaum L, McNeely E. PBDE flame retardants, thyroid disease, and menopausal status in U.S. women. *Environ Health.* 2016;15(1):60.

62. Geyer HJ, Schramm K-W, Darnerud PO, et al. Terminal elimination half-lives of the brominated flame retardants TBBPA, HBCD, and lower brominated PBDEs in humans. *ORGANOHALOGEN COMPOUNDS.* 2004;66:3820.

63. Johnson-Restrepo B, Kannan K. An assessment of sources and pathways of human exposure to polybrominated diphenyl ethers in the United States. *Chemosphere.* 2009;76(4):542-548.

64. Wu N, Herrmann T, Paepke O, et al. Human exposure to PBDEs: associations of PBDE body burdens with food consumption and house dust concentrations. *Environ Sci Technol.* 2007;41(5):1584-1589.

65. Toms LM, Hearn L, Kennedy K, et al. Concentrations of polybrominated diphenyl ethers (PBDEs) in matched samples of human milk, dust and indoor air. *Environ Int.* 2009;35(6):864-869.

66. Frederiksen M, Thomsen C, Froshaug M, et al. Polybrominated diphenyl ethers in paired samples of maternal and umbilical cord blood plasma and associations with house dust in a Danish cohort. *Int J Hyg Environ Health.* 2010;213(4):233-242.

67. Fisher M, Arbuckle TE, Liang CL, et al. Concentrations of persistent organic pollutants in maternal and cord blood from the maternal-infant research on environmental chemicals (MIREC) cohort study. *Environ Health.* 2016;15(1):59.

68. Tang J, Zhai JX. Distribution of polybrominated diphenyl ethers in breast milk, cord blood and placentas: a systematic review. *Environmental science and pollution research international.* 2017;24(27):21548-21573.

69. Kim JT, Son MH, Lee DH, Seong WJ, Han S, Chang YS. Partitioning behavior of heavy metals and persistent organic pollutants among feto-maternal bloods and tissues. *Environ Sci Technol.* 2015;49(12):7411-7422.

70. Linares V, Belles M, Domingo JL. Human exposure to PBDE and critical evaluation of health hazards. *Archives of toxicology.* 2015;89(3):335-356.

71. United States Environmental Protection Agency. America's Children and the Environment: Biomonitoring- Polybrominated Diphenyl Ethers (PBDEs). 2018; https://www.epa.gov/ace/ace-biomonitoring-polybrominated-diphenyl-ethers-pbdes. Accessed January 10, 2018.

72. Jinhui L, Yuan C, Wenjing X. Polybrominated diphenyl ethers in articles: a review of its applications and legislation. *Environmental science and pollution research international.* 2017;24(5):4312-4321.

73. Zota AR, Rudel RA, Morello-Frosch RA, Brody JG. Elevated house dust and serum concentrations of PBDEs in California: unintended consequences of furniture flammability standards? *Environ Sci Technol.* 2008;42(21):8158-8164.

74. Hites RA, Foran JA, Schwager SJ, Knuth BA, Hamilton MC, Carpenter DO. Global assessment of polybrominated diphenyl ethers in farmed and wild salmon. *Environ Sci Technol.* 2004;38(19):4945-4949.

75. Zhang J, Chen L, Xiao L, Ouyang F, Zhang QY, Luo ZC. Polybrominated Diphenyl Ether Concentrations in Human Breast Milk Specimens Worldwide. *Epidemiology (Cambridge, Mass).* 2017;28 Suppl 1:S89-s97.

76. Fromme H, Hilger B, Kopp E, Miserok M, Völkel W. Polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane (HBCD) and “novel” brominated flame retardants in house dust in Germany. *Environment international.* 2014;64:61-68.

77. Harrad S, Hunter S. Concentrations of polybrominated diphenyl ethers in air and soil on a rural-urban transect across a major UK conurbation. *Environ Sci Technol.* 2006;40(15):4548-4553.

78. Malliari E, Kalantzi OI. Children's exposure to brominated flame retardants in indoor environments - A review. *Environ Int.* 2017;108:146-169.

79. Wu Z, Han W, Xie M, Han M, Li Y, Wang Y. Occurrence and distribution of polybrominated diphenyl ethers in soils from an e-waste recycling area in northern China. *Ecotoxicology and environmental safety.* 2019;167:467-475.

80. Li H, La Guardia MJ, Liu H, et al. Brominated and organophosphate flame retardants along a sediment transect encompassing the Guiyu, China e-waste recycling zone. *The Science of the total environment.* 2019;646:58-67.

81. McGrath TJ, Morrison PD, Ball AS, Clarke BO. Spatial Distribution of Novel and Legacy Brominated Flame Retardants in Soils Surrounding Two Australian Electronic Waste Recycling Facilities. *Environ Sci Technol.* 2018;52(15):8194-8204.

82. Ceballos DM, Broadwater K, Page E, Croteau G, La Guardia MJ. Occupational exposure to polybrominated diphenyl ethers (PBDEs) and other flame retardant foam additives at gymnastics studios: Before, during and after the replacement of pit foam with PBDE-free foams. *Environ Int.* 2018;116:1-9.

83. Thuresson K, Bergman A, Jakobsson K. Occupational exposure to commercial decabromodiphenyl ether in workers manufacturing or handling flame-retarded rubber. *Environ Sci Technol.* 2005;39(7):1980-1986.

84. Leung AO, Chan JK, Xing GH, et al. Body burdens of polybrominated diphenyl ethers in childbearing-aged women at an intensive electronic-waste recycling site in China. *Environmental science and pollution research international.* 2010;17(7):1300-1313.

85. Strid A, Smedje G, Athanassiadis I, et al. Brominated flame retardant exposure of aircraft personnel. *Chemosphere.* 2014;116:83-90.

86. Toms LM, Sjodin A, Harden F, et al. Serum polybrominated diphenyl ether (PBDE) levels are higher in children (2-5 years of age) than in infants and adults. *Environ Health Perspect.* 2009;117(9):1461-1465.

87. Mantovani A. Risk assessment of endocrine disrupters: the role of toxicological studies. *Annals of the New York Academy of Sciences.* 2006;1076:239-252.

88. Gore AC, Chappell VA, Fenton SE, et al. Executive Summary to EDC-2: The Endocrine Society's Second Scientific Statement on Endocrine-Disrupting Chemicals. *Endocrine reviews.* 2015;36(6):593-602.

89. Bergman A, Heindel J, Jobling S, Kidd K, Zoeller RT. State of the Science of Endocrine Disrupting Chemicals- 2012. In: Organization WH, ed2013:258.

90. Chen T, Niu P, Kong F, et al. Disruption of thyroid hormone levels by decabrominated diphenyl ethers (BDE-209) in occupational workers from a deca-BDE manufacturing plant. *Environment international.* 2018;120:505-515.

91. International Research and Development Corporation. *Decabromodiphenyl ether and octabromodiphenyl ether. A twenty-eight day toxicity study in rats. .* U.S. Environmental Protection Agency under TSCA Section 8D. 1976. OTS0523322.

92. Kay D. Embryology of the Thyroid and Parathyroids. 2014.

93. Landers K, Richard K. Traversing barriers - How thyroid hormones pass placental, blood-brain and blood-cerebrospinal fluid barriers. *Mol Cell Endocrinol.* 2017;458:22-28.

94. Forhead AJ, Fowden AL. Thyroid hormones in fetal growth and prepartum maturation. *The Journal of endocrinology.* 2014;221(3):R87-r103.

95. Medici M, Korevaar TI, Visser WE, Visser TJ, Peeters RP. Thyroid function in pregnancy: what is normal? *Clinical chemistry.* 2015;61(5):704-713.

96. Zhang J, Hisada A, Yoshinaga J, et al. Exposure to pyrethroids insecticides and serum levels of thyroid-related measures in pregnant women. *Environ Res.* 2013;127:16-21.

97. Berghout A, Wiersinga W. Thyroid size and thyroid function during pregnancy: an analysis. *European journal of endocrinology / European Federation of Endocrine Societies.* 1998;138(5):536-542.

98. Korevaar TI, Medici M, de Rijke YB, et al. Ethnic differences in maternal thyroid parameters during pregnancy: the Generation R study. *The Journal of clinical endocrinology and metabolism.* 2013;98(9):3678-3686.

99. Walker JA, Illions EH, Huddleston JF, Smallridge RC. Racial comparisons of thyroid function and autoimmunity during pregnancy and the postpartum period. *Obstetrics and gynecology.* 2005;106(6):1365-1371.

100. La'ulu SL, Roberts WL. Ethnic differences in first-trimester thyroid reference intervals. *Clinical chemistry.* 2011;57(6):913-915.

101. Veltri F, Belhomme J, Kleynen P, et al. Maternal thyroid parameters in pregnant women with different ethnic backgrounds: Do ethnicity-specific reference ranges improve the diagnosis of subclinical hypothyroidism? *Clin Endocrinol (Oxf).* 2017;86(6):830-836.

102. Zhao X, Wang H, Li J, Shan Z, Teng W, Teng X. The Correlation between Polybrominated Diphenyl Ethers (PBDEs) and Thyroid Hormones in the General Population: A Meta-Analysis. *PLoS One.* 2015;10(5):e0126989.

103. Jacobson MH, Barr DB, Marcus M, et al. Serum polybrominated diphenyl ether concentrations and thyroid function in young children. *Environmental research.* 2016;149:222-230.

104. Kim S, Park J, Kim HJ, et al. Association between several persistent organic pollutants and thyroid hormone levels in serum among the pregnant women of Korea. *Environ Int.* 2013;59:442-448.

105. Guo LC, Xiao J, Zhang Y, et al. Association between serum polybrominated diphenyl ethers, new flame retardants and thyroid hormone levels for school students near a petrochemical complex, South China. *Chemosphere.* 2018;202:476-482.

106. Xu X, Liu J, Zeng X, Lu F, Chen A, Huo X. Elevated serum polybrominated diphenyl ethers and alteration of thyroid hormones in children from Guiyu, China. *PLoS One.* 2014;9(11):e113699.

107. Chen L, Wang C, Zhang Y, et al. Polybrominated diphenyl ethers in cord blood and perinatal outcomes from Laizhou Wan Birth Cohort, China. *Environmental science and pollution research international.* 2018;25(21):20802-20808.

108. Oulhote Y, Chevrier J, Bouchard MF. Exposure to Polybrominated Diphenyl Ethers (PBDEs) and Hypothyroidism in Canadian Women. *The Journal of clinical endocrinology and metabolism.* 2016;101(2):590-598.

109. Hoffman K, Lorenzo A, Butt CM, et al. Exposure to flame retardant chemicals and occurrence and severity of papillary thyroid cancer: A case-control study. *Environ Int.* 2017;107:235-242.

110. Aschebrook-Kilfoy B, Schechter RB, Shih YC, et al. The clinical and economic burden of a sustained increase in thyroid cancer incidence. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology.* 2013;22(7):1252-1259.

111. Fisher J, Mello MCd, Patel V, et al. Prevalence and determinants of common perinatal mental disorders in women in low-and lower-middle-income countries: a systematic review. *Bulletin of the World Health Organization.* 2012;90:139-149.

112. Earls MF, Yogman MW, Mattson G, Rafferty J. Incorporating Recognition and Management of Perinatal Depression Into Pediatric Practice. *Pediatrics.* 2019;143(1).

113. Melville JL, Gavin A, Guo Y, Fan MY, Katon WJ. Depressive disorders during pregnancy: prevalence and risk factors in a large urban sample. *Obstetrics and gynecology.* 2010;116(5):1064-1070.

114. Glinoer D. The regulation of thyroid function in pregnancy: pathways of endocrine adaptation from physiology to pathology. *Endocrine reviews.* 1997;18(3):404-433.

115. Krassas GE, Poppe K, Glinoer D. Thyroid function and human reproductive health. *Endocrine reviews.* 2010;31(5):702-755.

116. Ritchie M, Yeap BB. Thyroid hormone: Influences on mood and cognition in adults. *Maturitas.* 2015;81(2):266-275.

117. Mowla A, Kalantarhormozi MR, Khazraee S. Clinical characteristics of patients with major depressive disorder with and without hypothyroidism: a comparative study. *Journal of psychiatric practice.* 2011;17(1):67-71.

118. Lin CL, Yang SN, Shiah IS. Acute mania in a patient with hypothyroidism resulting from Hashimoto's Thyroiditis. *General hospital psychiatry.* 2013;35(6):683.e681-682.

119. Heinrich TW, Grahm G. Hypothyroidism Presenting as Psychosis: Myxedema Madness Revisited. *Primary care companion to the Journal of clinical psychiatry.* 2003;5(6):260-266.

120. Pedersen CA, Johnson JL, Silva S, et al. Antenatal thyroid correlates of postpartum depression. *Psychoneuroendocrinology.* 2007;32(3):235-245.

121. Sylven SM, Elenis E, Michelakos T, et al. Thyroid function tests at delivery and risk for postpartum depressive symptoms. *Psychoneuroendocrinology.* 2013;38(7):1007-1013.

122. Lambrinoudaki I, Rizos D, Armeni E, et al. Thyroid function and postpartum mood disturbances in Greek women. *J Affect Disord.* 2010;121(3):278-282.

123. Albacar G, Sans T, Martin-Santos R, et al. Thyroid function 48h after delivery as a marker for subsequent postpartum depression. *Psychoneuroendocrinology.* 2010;35(5):738-742.

124. Groer MW, Vaughan JH. Positive thyroid peroxidase antibody titer is associated with dysphoric moods during pregnancy and postpartum. *Journal of obstetric, gynecologic, and neonatal nursing : JOGNN.* 2013;42(1):E26-32.

125. Balucan FS, Morshed SA, Davies TF. Thyroid autoantibodies in pregnancy: their role, regulation and clinical relevance. *Journal of thyroid research.* 2013;2013.

126. Wesseloo R, Kamperman AM, Bergink V, Pop VJM. Thyroid peroxidase antibodies during early gestation and the subsequent risk of first-onset postpartum depression: A prospective cohort study. *J Affect Disord.* 2018;225:399-403.

127. Sethi S, Chen X, Kass PH, Puschner B. Polychlorinated biphenyl and polybrominated diphenyl ether profiles in serum from cattle, sheep, and goats across California. *Chemosphere.* 2017;181:63-73.

128. Dingemans MM, Kock M, van den Berg M. Mechanisms of Action Point Towards Combined PBDE/NDL-PCB Risk Assessment. *Toxicological sciences : an official journal of the Society of Toxicology.* 2016;153(2):215-224.

129. Wang BL, Pang ST, Sun JP, et al. Levels of polychlorinated biphenyls in settled house dust from urban dwellings in China and their neurodevelopmental effects on preschool-aged children. *The Science of the total environment.* 2015;505:402-408.

130. Vuong AM, Yolton K, Xie C, et al. Childhood polybrominated diphenyl ether (PBDE) exposure and neurobehavior in children at 8 years. *Environmental research.* 2017;158:677-684.

131. Rattan S, Zhou C, Chiang C, Mahalingam S, Brehm E, Flaws JA. Exposure to endocrine disruptors during adulthood: consequences for female fertility. *The Journal of endocrinology.* 2017;233(3):R109-r129.

132. Gaum PM, Esser A, Schettgen T, Gube M, Kraus T, Lang J. Prevalence and incidence rates of mental syndromes after occupational exposure to polychlorinated biphenyls. *Int J Hyg Environ Health.* 2014;217(7):765-774.

133. Gaum PM, Gube M, Schettgen T, et al. Polychlorinated biphenyls and depression: cross-sectional and longitudinal investigation of a dopamine-related Neurochemical path in the German HELPcB surveillance program. *Environ Health.* 2017;16(1):106.

134. Ruhe HG, Mason NS, Schene AH. Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. *Molecular psychiatry.* 2007;12(4):331-359.

135. Zhou M, Ford B, Lee D, et al. Metabolomic Markers of Phthalate Exposure in Plasma and Urine of Pregnant Women. *Frontiers in public health.* 2018;6:298.

136. Fitzgerald EF, Shrestha S, Gomez MI, et al. Polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs) and neuropsychological status among older adults in New York. *Neurotoxicology.* 2012;33(1):8-15.

137. Xia B, Zhu Q, Zhao Y, et al. Phthalate exposure and childhood overweight and obesity: Urinary metabolomic evidence. *Environ Int.* 2018;121(Pt 1):159-168.

138. Ding G, Yu J, Chen L, et al. Polybrominated diphenyl ethers (PBDEs) and thyroid hormones in cord blood. *Environmental pollution (Barking, Essex : 1987).* 2017;229:489-495.

139. Zheng J, He CT, Chen SJ, et al. Disruption of thyroid hormone (TH) levels and TH-regulated gene expression by polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and hydroxylated PCBs in e-waste recycling workers. *Environ Int.* 2017;102:138-144.

140. Xu X, Dong F, Yang Y, Wang Y, Wang R, Shen X. Sex-specific effects of long-term exposure to bisphenol-A on anxiety- and depression-like behaviors in adult mice. *Chemosphere.* 2015;120:258-266.

141. Braun JM, Kalkbrenner AE, Calafat AM, et al. Impact of early-life bisphenol A exposure on behavior and executive function in children. *Pediatrics.* 2011;128(5):873-882.

142. Tandon SD, Cluxton-Keller F, Leis J, Le HN, Perry DF. A comparison of three screening tools to identify perinatal depression among low-income African American women. *J Affect Disord.* 2012;136(1-2):155-162.

143. Centers for Disease Control and Prevention (CDC). National Center for Health Statistics. Depression Among Women. 2017.

144. Kessler RC. The costs of depression. *The Psychiatric clinics of North America.* 2012;35(1):1-14.

145. Zlotnick C, Kohn R, Keitner G, Della Grotta SA. The relationship between quality of interpersonal relationships and major depressive disorder: findings from the National Comorbidity Survey. *J Affect Disord.* 2000;59(3):205-215.

146. Field T. Postpartum depression effects on early interactions, parenting, and safety practices: a review. *Infant Behavior and Development.* 2010;33(1):1-6.

147. Ivandic I, Kamenov K, Rojas D, Ceron G, Nowak D, Sabariego C. Determinants of Work Performance in Workers with Depression and Anxiety: A Cross-Sectional Study. *International journal of environmental research and public health.* 2017;14(5).

148. Plaisier I, Beekman AT, de Graaf R, Smit JH, van Dyck R, Penninx BW. Work functioning in persons with depressive and anxiety disorders: the role of specific psychopathological characteristics. *J Affect Disord.* 2010;125(1-3):198-206.

149. Hakulinen C, Elovainio M, Pulkki-Raback L, et al. Depressive symptoms and long-term income: The Young Finns Study. *J Affect Disord.* 2016;204:120-123.

150. Gallo JJ, Bogner HR, Morales KH, Ford DE. Patient ethnicity and the identification and active management of depression in late life. *Archives of internal medicine.* 2005;165(17):1962-1968.

151. Breslau J, Kendler KS, Su M, Gaxiola-Aguilar S, Kessler RC. Lifetime risk and persistence of psychiatric disorders across ethnic groups in the United States. *Psychological medicine.* 2005;35(3):317-327.

152. Kozinszky Z, Dudas RB. Validation studies of the Edinburgh Postnatal Depression Scale for the antenatal period. *J Affect Disord.* 2015;176:95-105.

153. Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression: development of the 10-item Edinburgh Postnatal Depression Scale. *The British journal of psychiatry.* 1987;150(6):782-786.

154. Matthey S, Barnett B, Kavanagh DJ, Howie P. Validation of the Edinburgh Postnatal Depression Scale for men, and comparison of item endorsement with their partners. *J Affect Disord.* 2001;64(2-3):175-184.

155. Chaudron LH, Szilagyi PG, Tang W, et al. Accuracy of depression screening tools for identifying postpartum depression among urban mothers. *Pediatrics.* 2010;125(3):e609-617.

156. Gjerdingen D, Crow S, McGovern P, Miner M, Center B. Postpartum depression screening at well-child visits: validity of a 2-question screen and the PHQ-9. *Annals of family medicine.* 2009;7(1):63-70.

157. Corwin EJ, Hogue CJ, Pearce B, et al. Protocol for the emory university african american vaginal, oral, and gut microbiome in pregnancy cohort study. *BMC pregnancy and childbirth.* 2017;17(1):161.

158. Darrow LA, Jacobson MH, Preston EV, et al. Predictors of serum polybrominated diphenyl ether (PBDE) concentrations among children aged 1–5 years. *Environmental science & technology.* 2016;51(1):645-654.

159. Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. *Applied occupational and environmental hygiene.* 1990;5(1):46-51.

160. Faravelli C, Alessandra Scarpato M, Castellini G, Lo Sauro C. Gender differences in depression and anxiety: the role of age. *Psychiatry research.* 2013;210(3):1301-1303.

161. Feder KA, Heatherington L, Mojtabai R, Eaton WW. Perceived Marital Support and Incident Mental Illness: Evidence from the National Comorbidity Survey. *Journal of marital and family therapy.* 2018.

162. Bracke P, van de Straat V, Missinne S. Education, mental health, and education-labor market misfit. *Journal of health and social behavior.* 2014;55(4):442-459.

163. Wiltink J, Michal M, Wild PS, et al. Associations between depression and different measures of obesity (BMI, WC, WHtR, WHR). *BMC psychiatry.* 2013;13:223.

164. Czarnota J, Gennings C, Colt JS, et al. Analysis of Environmental Chemical Mixtures and Non-Hodgkin Lymphoma Risk in the NCI-SEER NHL Study. *Environ Health Perspect.* 2015;123(10):965-970.

165. Gennings C, Carrico C, Factor-Litvak P, Krigbaum N, Cirillo PM, Cohn BA. A cohort study evaluation of maternal PCB exposure related to time to pregnancy in daughters. *Environ Health.* 2013;12(1):66.

166. Carrico C, Gennings C, Wheeler DC, Factor-Litvak P. Characterization of Weighted Quantile Sum Regression for Highly Correlated Data in a Risk Analysis Setting. *Journal of agricultural, biological, and environmental statistics.* 2015;20(1):100-120.

167. Karpeta A, Gregoraszczuk E. Mixture of dominant PBDE congeners (BDE-47, -99, -100 and -209) at levels noted in human blood dramatically enhances progesterone secretion by ovarian follicles. *Endocrine regulations.* 2010;44(2):49-55.

168. Holmes AK, Koller KR, Kieszak SM, et al. Case-control study of breast cancer and exposure to synthetic environmental chemicals among Alaska Native women. *International journal of circumpolar health.* 2014;73:25760.

169. Boas M, Feldt-Rasmussen U, Main KM. Thyroid effects of endocrine disrupting chemicals. *Molecular and cellular endocrinology.* 2012;355(2):240-248.

170. Newport DJ, Ji S, Long Q, et al. Maternal depression and anxiety differentially impact fetal exposures during pregnancy. *The Journal of clinical psychiatry.* 2012;73(2):247-251.

171. Weaver K, Campbell R, Mermelstein R, Wakschlag L. Pregnancy smoking in context: the influence of multiple levels of stress. *Nicotine & tobacco research : official journal of the Society for Research on Nicotine and Tobacco.* 2008;10(6):1065-1073.

172. Rodell JB, Judge TA. Can "good" stressors spark "bad" behaviors? The mediating role of emotions in links of challenge and hindrance stressors with citizenship and counterproductive behaviors. *The Journal of applied psychology.* 2009;94(6):1438-1451.

173. Lazarus RS, Folkman S. Transactional theory and research on emotions and coping. *European Journal of personality.* 1987;1(3):141-169.

174. Grobman WA, Parker C, Wadhwa PD, et al. Racial/Ethnic Disparities in Measures of Self-reported Psychosocial States and Traits during Pregnancy. *American journal of perinatology.* 2016;33(14):1426-1432.

175. Hobfoll SE, Ritter C, Lavin J, Hulsizer MR, Cameron RP. Depression prevalence and incidence among inner-city pregnant and postpartum women. *Journal of consulting and clinical psychology.* 1995;63(3):445-453.

176. Bunevicius A, Kusminskas L, Pop VJ, Pedersen CA, Bunevicius R. Screening for antenatal depression with the Edinburgh Depression Scale. *Journal of Psychosomatic Obstetrics & Gynecology.* 2009;30(4):238-243.

177. Tsai AC, Scott JA, Hung KJ, et al. Reliability and validity of instruments for assessing perinatal depression in African settings: systematic review and meta-analysis. *PLoS One.* 2013;8(12):e82521.

178. Bergink V, Kooistra L, Lambregtse-van den Berg MP, et al. Validation of the Edinburgh Depression Scale during pregnancy. *Journal of psychosomatic research.* 2011;70(4):385-389.

179. Matthey S, Ross-Hamid C. Repeat testing on the Edinburgh Depression Scale and the HADS-A in pregnancy: differentiating between transient and enduring distress. *J Affect Disord.* 2012;141(2-3):213-221.

180. Smith-Nielsen J, Matthey S, Lange T, Vaever MS. Validation of the Edinburgh Postnatal Depression Scale against both DSM-5 and ICD-10 diagnostic criteria for depression. *BMC psychiatry.* 2018;18(1):393.

181. Gilbert DA, Hayes E. Communication and outcomes of visits between older patients and nurse practitioners. *Nursing research.* 2009;58(4):283-293.

182. Zink KL, Perry M, London K, et al. "Let Me Tell You About My..." Provider Self-Disclosure in the Emergency Department Builds Patient Rapport. *The western journal of emergency medicine.* 2017;18(1):43-49.

183. Moore D, Ayers S, Drey N. A Thematic Analysis of Stigma and Disclosure for Perinatal Depression on an Online Forum. *JMIR mental health.* 2016;3(2):e18.

184. Jorm AF, Christensen H, Griffiths KM. The public's ability to recognize mental disorders and their beliefs about treatment: changes in Australia over 8 years. *The Australian and New Zealand journal of psychiatry.* 2006;40(1):36-41.

185. Stengel C. The risk of being 'too honest': Drug use, stigma and pregnancy. *Health, risk & society.* 2014;16(1):36-50.

186. Chen L, Cao D, Li LX, Zhao Y, Xie CM, Zhang YH. Assessment of Exposure to Polybrominated Dipheny Ethers via Inhalation and Diet in China. *Biomedical and environmental sciences : BES.* 2014;27(11):872-882.

187. Cowens KR, Simpson S, Thomas WK, Carey GB. Polybrominated Diphenyl Ether (PBDE)-Induced Suppression of Phosphoenolpyruvate Carboxykinase (PEPCK) Decreases Hepatic Glyceroneogenesis and Disrupts Hepatic Lipid Homeostasis. *Journal of toxicology and environmental health Part A.* 2015;78(23-24):1437-1449.

188. Kim YR, Harden FA, Toms LM, Norman RE. Health consequences of exposure to brominated flame retardants: a systematic review. *Chemosphere.* 2014;106:1-19.

189. Lyche JL, Rosseland C, Berge G, Polder A. Human health risk associated with brominated flame-retardants (BFRs). *Environ Int.* 2015;74:170-180.

190. Andrade AJ, Kuriyama SN, Akkoc Z, Talsness CE, Chahoud I. Effects of developmental low dose PBDE 47 exposure on thyroid hormone status and serum concentrations of FSH and inhibin B in male rats. *Organohalogen compounds.* 2004;66:3907-3912.

191. Eskenazi B, Chevrier J, Rauch SA, et al. In utero and childhood polybrominated diphenyl ether (PBDE) exposures and neurodevelopment in the CHAMACOS study. *Environ Health Perspect.* 2013;121(2):257-262.

192. Uppal K, Walker DI, Liu K, Li S, Go YM, Jones DP. Computational Metabolomics: A Framework for the Million Metabolome. *Chemical research in toxicology.* 2016;29(12):1956-1975.

193. Soltow QA, Strobel FH, Mansfield KG, Wachtman L, Park Y, Jones DP. High-performance metabolic profiling with dual chromatography-Fourier-transform mass spectrometry (DC-FTMS) for study of the exposome. *Metabolomics : Official journal of the Metabolomic Society.* 2013;9(1 Suppl):S132-s143.

194. Uppal K, Walker DI, Jones DP. xMSannotator: An R Package for Network-Based Annotation of High-Resolution Metabolomics Data. *Analytical chemistry.* 2017;89(2):1063-1067.

195. Maes M, Galecki P, Chang YS, Berk M. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. *Progress in neuro-psychopharmacology & biological psychiatry.* 2011;35(3):676-692.

196. Maes M, Mihaylova I, Kubera M, Leunis JC, Geffard M. IgM-mediated autoimmune responses directed against multiple neoepitopes in depression: new pathways that

underpin the inflammatory and neuroprogressive pathophysiology. *J Affect Disord.* 2011;135(1-3):414-418.

197. Leonard B, Maes M. Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. *Neuroscience and biobehavioral reviews.* 2012;36(2):764-785.

198. Ríos N, Prolo C, Álvarez MN, Piacenza L, Radi R. Peroxynitrite Formation and Detection in Living Cells. *Nitric Oxide*: Elsevier; 2017:271-288.

199. Alexander EK, Pearce EN, Brent GA, et al. 2017 Guidelines of the American Thyroid Association for the Diagnosis and Management of Thyroid Disease During Pregnancy and the Postpartum. *Thyroid : official journal of the American Thyroid Association.* 2017;27(3):315-389.

200. Moleti M, Trimarchi F, Vermiglio F. Thyroid physiology in pregnancy. *Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists.* 2014;20(6):589-596.

201. Jourdan C, Linseisen J, Meisinger C, et al. Associations between thyroid hormones and serum metabolite profiles in an euthyroid population. *Metabolomics : Official journal of the Metabolomic Society.* 2014;10(1):152-164.

202. Suhre K, Meisinger C, Doring A, et al. Metabolic footprint of diabetes: a multiplatform metabolomics study in an epidemiological setting. *PLoS One.* 2010;5(11):e13953.

203. Mwafy S, Yassin M, Mousa R. Thyroid hormones, lipid profile and anthropometric changes after programmed weight loss in Palestinian obese adult females. *Diabetes & metabolic syndrome.* 2018;12(3):269-273.

204. The American College of Obstetricians and Gynecologists. Committee Opinion No. 148. Thyroid Disease in Pregnancy. *Obstetrics and gynecology.* 2015;125(4):996-1005.

205. ACOG Committee Opinion No. 757: Screening for Perinatal Depression. *Obstetrics and gynecology.* 2018;132(5):e208-e212.

206. The American College of Obstetricians and Gynecologists. Exposure to toxic environmental agents. Committee Opinion No. 575. *Obstetrics and gynecology.* 2013;122(4):931-935.

207. Di Renzo GC, Conry JA, Blake J, et al. International Federation of Gynecology and Obstetrics opinion on reproductive health impacts of exposure to toxic environmental chemicals. *International Journal of Gynecology & Obstetrics.* 2015;131(3):219-225.

208. Frazer K, Callinan JE, McHugh J, et al. Legislative smoking bans for reducing harms from secondhand smoke exposure, smoking prevalence and tobacco consumption. *Cochrane Database of Systematic Reviews.* 2016(2).