EPIGENETIC EFFECTS OF POLYBROMINATED DIPHENYL ETHER EXPOSURE ON AIRWAY HYPERRESPONSIVENESS IN OFFSPRING

by

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A thesis submitted to Johns Hopkins University in conformity with the requirements for the degree of Master of Science

Baltimore, Maryland

April 2020

Abstract

Asthma is a chronic lung disease with staggering disease burden in youth. Current literature suggests that maternal exposure to certain xenobiotic chemicals can affect developing offspring epigenetics, leading to a predisposition for asthma. Our previous study links polybrominated diphenyl ether congener 47 (PBDE-47), a chemical used as a flame-retardant within household products, with increased airway hyperresponsiveness (AHR). Of further concern is the potential influence of PBDE-47 in mice lung on the epigenetic alterations regulating asthma related genes that may play a role in pathogenesis, such as Tumor Necrosis Factor Alpha (*TNFA*) and Brain Derived Neurotrophic Factor (*BDNF*). This thesis tested the hypothesis that altered DNA methylation of key asthma related genes due to PBDE-47 exposure leads to modulation of gene transcription.

In Chapter 1, I begin by introducing PBDEs, and discuss the role of altered DNA methylation in disease pathogenesis. The major genes studied in the experiment, *Bdnf* and *Tnfa*, are also detailed, and I discuss the effects that dysregulation of these key genes has on health. In Chapter 2, I observed there is no difference in gene expression and DNA methylation of *Bdnf* and *Tnfa* in mouse exposed to a single postnatal PBDE-47. In Chapter 3, I investigated the epigenetic effect of maternal PBDE-47 exposure on offspring's airway reactivity and asthma phenotypic genes. This multi-dose experiment found increased gene expression and promoter demethylation for *Bdnf* and *Tnfa* in the mouse model.

Overall, the completion of this research illuminates the epigenetic effects of exposure to PBDE-47 on asthma genes, and their connection with previously observed increased AHR. This study presents an innovative approach to observing the effect of PBDE-47 exposure on asthma risk through utilizing methylation profiling. While many previous studies look at direct adverse health effects, my approach demonstrates how PBDE-47 exposures have epigenetic effects in offspring of impacted mothers. Looking at in utero effects demonstrates that epigenetics underpins a mechanism of toxic action with PBDEs that would not be commonly seen in traditional animal experiments by establish a heritable

epigenetic model of toxic action of PBDEs on key genes related to the development of asthma in mammals.

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CHAPTER 1: Background

1.1 Background on Polybrominated Diphenyl Ethers

Polybrominated Diethyl Ethers, or PBDEs, are chemicals with a wide variety of industrial uses and have a large range of human health impacts. They are classified as persistent organic pollutants (POPs) due to their ability to bioaccumulate in the environment, and have been extensively studied in both experimental and epidemiologic settings. PBDEs can be chemically identified through their bromination pattern; they are organic hydrocarbon molecules with bromines attached to a minimum of 2 and maximum of 10 ether carbons (1). PBDEs in industry are most commonly used as flame retardants, and as a result, are ubiquitously present in many household products, particularly in the U.S. These products include, but are not limited to: plastics, furniture, upholstery, electrical equipment, electronic devices, and textiles (1). Commercial PBDEs are typically congers of penta-, octa-, or deca-PBDEs; the prefix referring to the number of attached bromines (1). However, the commercially used PBDEs contain mixtures of several lower brominated BDEs, which pose a greater health risk than pure versions of the higher brominated PBDEs (2). This increased hazard is because PBDEs with lower bromination have a greater tendency to bioaccumulate, due to their higher degree of fat solubility and hydrophobicity (2).

While penta- and octa-PBDEs have been prohibited for commercial use since 2009 in the United States, deca-BDE usage remains prominent throughout the world (3). Additionally, many older products purchased before the ban of certain PBDE species still possess potentially harmful levels of penta- and octa-PBDEs, as it does not quickly degrade in the environment due to its long half-life (4).

1.2 Exposure Assessment and Epidemiology Studies of PBDEs

Human exposure commonly occurs from inhalation and ingestion of dust containing PBDE (1, 41). Another common pathway of PBDEs entering the human body is via ingestion of foods; particularly

in areas where PBDE contaminated products are disposed (3, 4). Disposal of PBDE contaminated products leads to contamination of potentially ingested plants, animals, or soil as the waste products degrade, leaving the PBDEs behind. Dermal absorption is also possible through from products that still contain PBDEs (1).

While traditionally, other persistent organic pollutants attribute their exposure almost exclusively to ingestion through food exposure due to their tendency to bioaccumulate, PBDEs enter the human body primarily through dust residue from consumer products and furniture, giving them a reputation as a "indoor POP" (41). As a result of this near ubiquitous indoor exposure, over 98% of the U.S. population has some level of PBDE burden, with the 95th percentile of the population having a concentration of ~291ng/g of PBDE, over 7 times the geometric mean PBDE-47 burden (41, 42). The burden is even more stark in the top 1% of exposures, where these subjects have ~3680ng/g, over 12 times the burden of those at the 95th percent (41). It is also difficult to predict who may develop high blood serum levels of PBDE, due to only weak associations between normal predictors of exposures, such as race and socioeconomic status (43). However, it is known that PBDE burden is pronounced in children, as they are a subpopulation that spends the most time having their hands in their mouth, giving a direct pathway from PBDE contaminated products to digestion.

1.3 Known Health Effects of PBDEs Exposure

The most well known, albeit not fully understood, health effects attributable to exposure by PBDEs include endocrine disruption, developmental neuropathologies, and immune system disruption (5). However, there are several other less explored health implications of these chemicals, and their toxicodynamic mechanisms are just beginning to be understood. My research aimed to obtain a better understanding of the ways in which PBDEs contribute to the development of human disease by looking at ways in which PBDEs may lead to an asthmatic phenotype in mammals.

The endocrine effects of PBDEs are amongst the most well studied outcomes of the chemical

exposure. Martin et al. found that PBDEs primarily cause reproductive toxicity by delaying onset of testicular descent and decreasing sperm count, along with an association with cryptorchidism (5). Exposure to endocrine disrupting chemicals (EDCs) has a medium to weak trend with sperm damage, spontaneous abortion, male reproductive tract issues, prostatitis, endometriosis, polycystic ovaries syndrome, and shortened lactation (5). PBDEs may also lead to elevated levels of T4 hormone produced by the thyroid, which can have notable effects on the metabolic activity in those exposed (6). Outside of the reproductive system, there has been evidence for PBDE induced neurobehavioral changes, neurodegenerative effects, and brain damage. While the mechanism, until recently, has been relatively unknown, the effect of inflammation and altered growth factors from PBDE exposure may provide an explanation for observed neurological phenomena associated with chemical exposure. This may have important implications in many neurodegenerative diseases, including Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis (8).

PBDEs may also influence immune health. The production of several inflammatory cytokines species, which promote the inflammatory immune response, has been linked to PBDE exposure (9, 46). The inflammatory response increases blood flow to the exposed area, leading to greater white blood cell phagocytosis of pathogens and xenobiotics, but in excess may cause damage to tissue and hyper-reactivity in many diseases. This modulated immune response has high variability between individuals, with unclear mechanism as to what stimulates its effect (47). Finally, PBDEs also show an association to many cancers through indirect mechanisms. TRKB is a receptor of BDNF, one of our genes of interest that is affected by PBDEs, and is highly expressed in many cancers. A 2012 study by Kimura et al. saw that having higher TRKB and BDNF lead to poor prognosis of patients with small cell lung cancer due to promotion of migration of TRKB overexpressing SC lung cancer cells (15).

1.4 Effect of PBDEs on Asthma Pathogenesis

The role of PBDEs in asthma remains unclear, with plenty of epidemiological evidence to

support a potential connection between PBDE exposure and asthma, but with no clear mechanism for asthma development. Over 300 million people worldwide experience asthma, and the rates of asthma development in children and young adults is increasing by approximately 5% every year (30). Asthma morbidity is a particular problem in the U.S., with over 22 million Americans having the disease (29). It is estimated that every year in the United States, asthma causes at least 15 million outpatient visits, 2 million emergency room visits, and 500,000 full hospitalizations (29). Asthma patients experience a wide level of phenotypic expression, with many risk factors that may differentiate moderate vs. severe asthma symptoms. Dougherty and Fehy identified many factors that may lead to exacerbation of the respiratory symptoms of asthma, which include host response to viral infection, location of infections, airborne pollutants, cigarette smoke, medication non-compliance, psychosocial factors, co-morbidities, and deficiencies in epithelial cell production of anti-inflammatory products (29). They also mention that exacerbation events are more common amongst post-pubertal women, leading more adult women to experience acute asthma hospitalization than men (29). Additionally, there are greater amounts of acute asthma events amongst African Americans and Hispanic Americans unexplained by socioeconomic factors and asthma therapy options (29).

Several other early-life xenobiotic exposures have a definitive link to childhood asthma. One of the better-characterized risk factors for developing childhood asthma is air pollution, with a variety of indoor and outdoor pollutants being known causes of asthma development in children. In the Kaiser air pollution (KAPPA) study in Atlanta, Georgia, they measured pollution levels of fine particulate matter 2.5 (PM 2.5), NO_x compounds, and CO levels at several residential locations within Atlanta. The KAPPA study found that there was a significant difference in asthma risk in children for every quintile increase for each of the studied air pollutants, with the risk in asthma development being most pronounced going from the first to the second quintile, and having a leveling off of risk in the further quintiles (33). Rumachev et al. looked at several volatile organic compounds that were hypothesized to have

association with asthma. While benzene alone was not a significant risk factor in asthma, combinations of benzene along with ethylbenzene and toluene had the highest odds ratio for asthma (38). Choi et al. found that the natural-log of summed propylene glycol and glycol ethers in bedroom air was associated with a 1.5 fold increase in asthma rates (37).

Many other exposures are well known triggers of asthmatic response, such as several dust mite, bacterial, and fungal species; however, most of these species are not considered risk factors for developing asthma in the first place (35). A notable exception to this is the exposure from certain bacterial species that release endotoxin upon the death and disintegration of the bacterial cell (35). In this instance, high endotoxin concentrations was a significant risk factor for the development of wheezing and asthma amongst newborns (35).

Factors underlying development and exacerbation of asthma are not fully understood, and my proposal investigates the role of persistent organic pollutants like PBDEs on asthma. While PBDEs have many undetermined effects on asthma pathogenesis, previous research has elucidated the role of other POPs, such as polychlorinated biphenyls (PCBs). High levels of PCB exposure has been epidemiologically linked to a high degree of respiratory symptoms, including asthmatic airway phenotype (36). Phosphorus flame retardants, which have replaced PBDEs in countries where PBDEs were banned, have been found to enhance the odds ratio for asthma development when the chemical TNBP was found on the floor or household surfaces (40).

Many studies have attempted to correlate early childhood PBDE exposure to development of asthma, but have been mostly unsuccessful. For example, one study looked at the levels of PBDE found in dust samples collected from the mattresses of children in Stockholm, Sweden. While PBDEs were found in the mattresses of asthmatic subjects, the concentrations of PBDE congeners did not vary significantly between these subjects and control subjects with no asthma (32). Another case control study in Shanghai observed children ages 3 to 6 years old attempting to link their diagnosed asthma to

exposure to PBDE and PCB exposure. In this study, 100% of the 124 pooled serum samples contained PBDE-47, the chemical of focus for our study, along with many other congeners of the chemical (31). These studies together suggest that PBDEs may not cause direct asthma development upon exposure as a child, but may lead to sensitization triggering asthma symptoms in children (31). As a result, our study focuses on “in-utero” exposures and their impact on early embryonic lung development to determine if exposure to PBDE-47 may lead to a priming of asthma. In addition, another proposed mechanism of asthma development in response to exposure to PBDEs involves immune system modulation. One clear indicator of the involvement of the immune system is the IgE count, a predictor of immune involvement, increases in response to PBDE-47 exposure (37). Additionally, in adult PBMCs, it was found that PBDE71 stimulated high levels of cytokine production, which are increasingly expressed in all types of asthmatic lung, particularly allergic asthma (40). A study by Zota et al. found that doubling the concentration PBDEs was associated with a 15.26% and 3.74% increase in IL6 and *TNFA* expression in the blood, indicating moderate increase in inflammation response proteins following PBDE exposure (49). The *TNFA* gene produces the inflammatory cytokine TNFA, a protein used in immune inflammation response by macrophages and monocytes. TNFA is responsible for a diverse range of signaling events within cells, which may lead to necrosis or apoptosis (14). The protein is also important for resistance to infection and is involved in many cancer pathways (14). Elevated TNFA levels are highly associated with asthmatic phenotype, showing a clear connection between inflammation and asthma. TNFA is involved in lung airway smooth muscle cell hyperreactivity; TNFA induces the release of LT_{C4} and LT_{D4} mediators along with histamine from mast cells, leading to AHR (27). TNFA also increases the cytotoxic effect of eosinophils, which are involved in signaling, activation, and migration of T cells to stimulate AHR (27). As a result of this altered expression of TNFA in human cells, I wish to investigate further the role of *TNFA* in lung tissue to determine whether it may modulate asthma response in our model.

1.5. New direction: Epigenetic Changes Associated with PBDE Exposures

The effect of PBDEs on gene transcription is strongly associated with an epigenetic mechanism of change for gene regulation (9, 12, 17). Epigenetic changes in genes are changes outside of the direct DNA sequence that effect expression levels of the gene that may be influenced by both one's internal and external environment. Epigenetic changes include non-coding RNAs, which reduce functionality of mRNAs leading to reduced protein production, histone modifications, which are covalent modifications to the histone structure that alter transcription of a gene, or DNA methylation, the method of epigenetic regulation that my experiment will be investigating (22). DNA methylation is involved in silencing of gene transcription, and while there is still debate on the exact mechanism of this silencing, methylation may lead to either interference with transcription factor binding, creation of inactive chromatin structure, or lead to transcriptional repression complexes (23). DNA methylation can also be heritable, leading to epigenetic changes in the progeny of mothers exposed to PBDEs and other environmental hazards (24).

In addition, it has been postulated that changes to regulation of asthma related genes through PBDEs exposure may be caused by epigenetic reprogramming. Epigenetic reprogramming relates to the concept of a fetal origin of disease, also known as the "Barker Hypothesis", which is used to describe how the pathogenesis of many chronic illnesses can be traced back to rapid growth periods that occur in utero, and can be affected by environmental factors (62, 63). These rapid growth periods correspond to periods of methylation following embryonic genome activation, and alterations in specific genes occurring during fetal development. Aberrant epigenetic reprogramming established in utero ultimately contributes to disease risk in later life. We previously reported that, using the Boston Birth Cohort, maternal exposure to PBDE-47 showed cord blood demethylation of the *TNFA* gene (9), which would lead to cell damage/death by increased inflammation cytokine production. It also speculates that in-utero exposure to PBDEs may cause overall epigenetic reprogramming in order to alter immune

response, leading to hyperreactivity (9). PBDE-47 has also been shown to elicit alterations in global DNA methylation patterns within several tissue types, including placenta and brain tissue (28, 65). Other mechanisms, such as histone modifications and microRNAs also play a part in the epigenetic regulation of genes, and can similarly be affected by environmental toxicants, such as PBDE-47.

In addition to *TNFA*, epigenetic regulation of *BDNF* is associated with PBDE exposure. The BDNF protein is primarily used as a growth factor in neurons, influencing activity, function, and survival of neurons. BDNF is viewed as a neuroprotective protein, associated with improved prognosis and function of neurons in a variety of neurological disorders (8). *BDNF* is expressed in the lung; during embryonic development, neurons in the developing brain create axons within the lung. These neurons exist within the dorsal and ventral respiratory nuclei in the medulla oblongata, and provide parasympathetic efferent information and much of the sensory afferent information to the lungs (13). This complex neural network helps control breathing, smooth muscle tone, mucus secretion, and trigger coughing reflexes (13). BDNF serves as a target-derived neurotrophic factor for airway smooth muscle (ASM) innervation by extrinsic neurons during embryogenesis. BDNF is coordinated with ASM differentiation for proper innervation. BDNF is often regulated through miRNAs (particularly miR-206), and is downregulated with ASM differentiation (13). Strikingly, TNFA is positively correlated with that of BDNF, particularly within ASM cells, demonstrating the direct link that both have in inflammation effects in asthma (7). In the presence of inflammatory cytokines, ASM response to BDNF increases, and causes a positive feedback loop for BDNF production. This pathway of feed forward BDNF production begins with transient receptor potential channels TRPC3/6 receiving elevated levels of [Ca^{2+}] and inducing airway smooth muscle BDNF secretion (26). From here, BDNF binds to its receptor, TRKB, which activates a variety of signaling events; pathways that elevate calcium levels further, leading to the positive feedback (26).

Changes in *Bdnf* expression have been observed in hippocampal tissues of mice exposed to various PBDE congeners (8). Arita et al. also found that basal production of BDNF significantly reduced in human placenta exposed to PBDE-47 and 99 (50). However, epigenetic alterations in *Bdnf* and *Tnfa* expression in mouse lung as a result of PBDE exposure has not been explored, providing an opportunity for us to observe the changes in their expression that may alter asthma risk .

CHAPTER 2: Effect of Single Dose Exposure to PBDE-47 on Offspring Asthma Associated Phenotypes

2.1 Abstract

The regulation of inflammation related genes has been shown to be affected exposure to PBDE congeners, including the most environmentally persistent congener, PBDE-47. In order to ascertain the effect of PBDE-47 on lung inflammation, we investigated the effects of a single dose of PBDE-47 on expression of inflammatory genes in mouse lung. Previous studies have suggested there is an association between PBDE-47 exposure and the expression of TNFA and BDNF. In the present study, no trend was observed between exposure group and gene regulation or promoter methylation for these genes in this single-dose model. Perhaps, changes in epigenetic regulation of these genes requires a longer period of chemical stressor, which ultimately alter the lung functions as well as airway inflammation.

2.2 Introduction

Environmental exposure to PBDE species has been speculated to affect gene regulation in several genes related to asthma and airway hyperresponsiveness. In particular, the PBDE species, PBDE-47, has been cited to alter epigenetic regulation of the genes *Bdnf* and *Tnfa*. PBDE-47 is the chosen PBDE species due to it being the most persistent of the species and having the highest body burden in humans due to its ability to bioaccumulate. *Bdnf* was found to have reduced nuclear 5-mC methylation after a low dose exposure in Rattus norvegicus (11). Similarly, *TNFA* had decreased promoter methylation in cord blood associated with high maternal PBDE-47 exposure in the human Boston Birth Cohort study (9). Strikingly, *TNFA* promoter methylation showed significantly hypomethylation in young girls whose mothers had a high blood PBDE-47 level.

These findings are of particular concern when considering its effects on asthma pathogenesis and severity. BDNF is associated with innervation of the lungs, and demethylation observed in the rat

study could lead to increased production of BDNF in the developing lung, which could cause increased airway hyperresponsiveness (AHR). With TNFA, there is concern the cytokines could lead to increased inflammation, exacerbating and increasing the severity of asthma symptoms.

As a result of these findings, we hypothesized that a single dose of PBDE-47 could upregulate the gene expression of *Tnfa* and *Bdnf* measured in mouse lung tissue. We further speculated that decreased promoter methylation would lead to the increased gene expression. To test this hypothesis, we exposed the mice to a 10mg/kg/BW dose in order to ascertain if the exposure can have a direct effect on gene promoter. A dose of 10mg/kg/BW was used for this study to replicate the effective dose that modulated gene expression in a PBDE study designed by the European Food Safety Commission (70)

2.3 Materials and Methods

Exposure to PBDE-47

PBDE-47 was purchased from AccuStandard (New Haven, CT). A single dose of 10mg/kg/bw of PBDE-47 in corn oil was administrated via oral gavage to 8 weeks old B6S129 mice. Two weeks after the exposure, lung tissue was then collected and snap-frozen for gene expression analysis.

RNA and DNA Isolation

DNA and RNA were extracted from mouse lung tissue utilizing the AllPrep Universal Kit (Qiagen). Lysate from the lung samples are passed through a DNA spin column to selectively isolate DNA then on a RNeasy spin column to selectively isolate RNA. Quality of DNA and RNA was accessed by the analysis of the two wavelengths (260nm and 280nm) used for UV-Vis absorbance measurements via the Epoch Take3 Microplate Spectrophotometer (BioTek). Only the RNA samples showing a ratio of 1-8-2.0 and the DNA samples showing a ratio of 1.5-1.7 were utilized in the gene expression and methylation studies.

Quantity was also determined using the UV absorbance reading. These isolated samples were then stored at 4°C (DNA) and -80°C (RNA) until further use.

Gene Expression via qPCR

cDNA used for measurement of gene expression was made from one thousand ng of RNA extracted from mouse lung using the iScript cDNA synthesis kit (Biorad). Primer sequences are as follows: mBdnf-2: Forward sequence: CAT AAG GAC GCG GAC TTG TAC A, Reverse sequence: AGA CAT GTT TGC GGC ATC CA. mTnfa-m1: Forward sequence: AGC CGA TGG GTT GTA CCT TG, Reverse sequence: ATA GCA AAT CGG CTG ACG GT. mRNA levels were measured by the SYBR-Green based real time PCR performed at AB7500 Fast qPCR machine (Applied Biosystems). The $2^{-\Delta\Delta Ct}$ method was used to calculate relative expression levels through a comparison to an *Rpl19* loading control normalization.

Methylation-specific PCR (MS-PCR).

Two hundred ng of genomic DNA was used for bisulfite conversion using EZ DNA Methylation kit (Zymo Research, Irvine, CA). Bisulfite treated samples were compared to a 100% methylated control group to confirm if there was changes in methylation patterns seen by band intensity in the region of interest, as determined by the primers used. Primer sequences specific for the methylated DNA sequence of the *Bdnf* promoter used for this study are as follows: mMS-Bdnf-c1: Forward sequence: CGG GAA GTA TTT AAA ATA GGG TAG C, Reverse sequence: CTA ATA ACT AAA CGA AAA ACA CCC G.

Statistical Analysis.

Data are expressed as mean ± SEM using a t-test or one way ANOVA with Tukey's multiple comparisons test. Statistically significant was defined as $p < 0.05$ using GraphPad Prism v7 software (La Jolla, CA).

2.4 Results and Discussion

In order to determine the epigenetic effect of PBDE-47 on lung inflammation, I followed up with a measurement of promoter methylation and mRNA levels of the inflammatory genes, *Bdnf* and *Tnfa*, to see if there was any correlation between observed gene expression and promoter methylation.

The relative expression data acquired via qPCR showed no difference between the control samples and PBDE exposed mice (Table 1). Despite the reduction in gene expression (Bdnf reduction of ~19% and Tnfa reduction of ~18%) that was measured from the samples, the p values were high suggesting that PBDE-47 did not affect both gene expression of *Bdnf* and *Tnfa*. On the other hand, the promoter demethylation, usually correlates with an increased gene expression measured if DNA methylation contributes to regulation of gene transcription. Take Bdnf as an example, the methylation data obtained following bisulfite treatment and MSPCR did not show any difference in promoter methylation. Also, there was no correlation between DNA methylation and gene expression.

The results displayed (Tables 1 and 2) currently contradict the findings seen in the rat nuclear gene study conducted by Byun et al, where they found significant changes in the regulation of *Bdnf*. Additionally, Zota et al found a dose dependency, where doubling the concentration of PBDE would lead to a 3.74% increase in *Tnfa* expression, and this trend of a dose response is not tested here. Most likely, it suggests that one single dose of PBDE does not necessarily result in a statistically significant phenotypic difference in asthma related genes. Our previous study demonstrated that the maternal exposure to PBDE-47 study was associated with the demethylation in the promoter of *TNFA* in cord blood in humans (9, 11). Similarly, increasing *Bdnf* expression was shown in other study in which the animals were administrated PBDE-47 during post-natal lung development, it is possible that *Bdnf*, which is most active as a growth factor for ASM during embryogenesis (13). Taken together, it suggests that epigenetic impact on gene regulation through PBDE-47 may require more specific dosing during critical windows of exposure. Early life exposures to chemical stressors impacts the later life asthma risk, at

least through epigenetic modulation of the genome. In order to fully ascertain the effects of PBDE-47 on gene expression and methylation, I followed up the single dose experiment by exposing a new cohort of mice to repeated doses of PBDE-47, as described in Chapter 3.

Table 1: Effect of PBDE-47 on gene expression in *Bdnf* and *Tnfa* in mouse lung

Gene	Oil (RER, SEM)	PBDE (RER, SEM)	*P* value vs. Oil control
Bdnf	0.78, 0.22	0.63, 0.12	0.55
Tnfa	0.63, 0.09	0.52, 0.09	0.40

Note: Gene expression was examined by real-time PCR and normalized to the housekeeping gene, *Rpl19*. Relative Expression Ratio (RER) values were calculated by a 2-ddCt method, in which RER was assigned a value of 1.00 for mouse universal RNA. Data were shown as mean values ± SEM (n=3-12). An unpaired parametric t-test was used to achieve these values.

Table 2: Effect of PBDE-47 on promoter methylation of *Bdnf*

Gene	Oil (Met%, SEM)	PBDE (Met%, SEM)	*P* value vs. Oil control
Bdnf	10.50, 6.33	9.96, 4.94	0.94

Note: Percent of methylation (Met%) was determined by MS-PCR as the ratio of the sample's methylation percentage relative to a fully methylated DNA control. Data were shown as mean values ± SEM (n=3-12). An unpaired parametric t-test was used to achieve these values.

CHAPTER 3: Effect of Multi Dose Exposures on Offspring Asthma Associated Phenotypes

3.1 Abstract

Following completion of the single dose PBDE experiment and observing no change in experimental vs control animals, a model in which mice exposed to multiple PBDE exposures in utero was developed to see the epigenetic effect of PBDE-47 on mouse lung genome when administered at several timepoints such as pre-conception, fetal lung development, and postnatal lung development. Following dosing, the epigenetic effect of PBDE on gene transcription of *Tnfa*, *Bdnf*, the asthma related genes, was tested in lung of F1 offspring when they reached adulthood. In addition, we determined if the epigenetic effect of early life exposures to PBDE on offspring's lung genome was further enhanced in mice received an additional administration of house allergens, which is known to induce allergen-induced airway hyperresponsiveness. Overall, the data suggests that maternal PBDE exposure impact the methylation of genes related to inflammatory pathways, which supports the hypothesis that epigenetic regulation of asthma pathogenesis in response to PBDE in an experimental asthma model.

3.2 Introduction

Environmental exposure to PBDE species has been speculated to affect gene regulation in several genes related to immune responses and asthma. As mentioned previously in chapter 2, the PBDE species, PBDE-47, has been cited to alter epigenetic regulation of the genes *BDNF* and *TNFA* (9, 11). These findings are of particular concern when considering its effects on asthma pathogenesis and severity. The BDNF protein is associated with innervation of the lungs, and demethylation observed in the rat study could lead to increased production of BDNF in the developing lung, which could cause increased hyperresponsiveness. With the cytokine TNFA, there is concern the cytokines could lead to increased inflammation, exacerbating and increasing the severity of asthma symptoms.

As a result of these findings and following the result of the single dose experiment where no

change was found after a one time dose was administered, we hypothesized that multiple doses of PBDE-47 through various points of development and epigenetic reprogramming would modulate the gene expression of *Tnfa* and *Bdnf* measured in lung tissue. We expected that this upregulated gene expression would be associated with decreased promoter methylation, which would ultimately contribute to the increased airway inflammation seen in asthmatics. To test this hypothesis, mice were exposed to PBDE during pre-conception, and the F1 mice exposed to 0.1 mg/kg/bw PBDE in utero and postnatally to ascertain if exposures at these specific timepoints could have epigenetic effect on the asthma related genes. These dose amounts were also chosen to replicate values similar to the median concentration of PBDE-47 found in human cord blood (11ng/g lipid BW) (9) and the benchmark dose of 0.47mg/kg seen in the rodent neurotoxicity study (66).

3.3 Materials and methods

Exposure to PBDE-47 and House Dust Mite (HDM)

0.1 mg/kg/bw of PBDE-47 or corn oil (as control) was administered to 7-week old adult mice at five critical windows (2 weeks before conception to prime the immunity setup, 1st and 2nd week of gestation for *in utero* epigenetic reprogramming, and 4th and 16th day of lactation for postnatal lung development) via oral gavage. Breeding pairs were established as 2 females with 1 male (B6S129SF1/J). The offspring were separated from dams after weaning. Among them, half of the eight-week-old offspring from each dam (F0-Saline, F0-PBDE)were subjected for HDM challenge to induce airway hyperresponsiveness. The mice were first sensitized with an intraperitoneal injection of 100 µg HDM containing 0.53EU endotoxin in sterile phosphate buffered saline (PBS) or PBS only (as a control). Two weeks after, the offspring were challenged by 100µg of HDM with 0.53EU endotoxin or PBS 3 times a week intratracheally. Two days after the last challenge, the mice were anesthetized with 1:1

ketamine:xylazine, paralyzed with succinylcholine, and maintained on a ventilator to assess AHR followed by tissues harvesting.

Reduced Representation Bisulfite Sequencing

Reduced representation bisulfite sequencing (RRBS) is a method of determining genome wide methylation profiles. This method of sequencing uses highly methylation specific restriction enzymes to hone in on specific regions of the genome with methylation, particularly within gene promoters. This has the advantage of pinpointing genome locations of specific interest, those with higher methylation, at a cheaper price and faster rate than comparable techniques such as whole genome bisulfite sequencing (WGBS). This method allows us to see if epigenetic gene regulation can alter disease susceptibility through altering gene expression, but at the cost of overlooking non-promoter regions that can be analyzed using WGBS.

Our RRBS-Seq study used the following protocol: The Genomic DNA extracted from lung tissues using the protocol described in 2.3.2 underwent MspI digestion (NEB) and bisulfite treatment (Zymo) before undergoing DNA library synthesis and then pair ended at Hi-Seq4000 (at 50-70M read pairs per sample). FASTQ files obtained from the RRBS-Seq study were imported into CLC BioMedical Workbench version 4.0 with the default import settings. Pair-ended reads were counted, trimmed and quality checked prior next step. After basic data preparation (Prepare Raw Data), quality reads were mapped to the Mus musculus Ensembl v80 Reference Sequence with bisulfite sequencing specific algorithm. Reads mapped to the mice genome were further “soft-clipped 5 bases” before the comparison analysis to remove methylation detection bias presumably due to end repair process in library preparation. A total of 403 peak reads showing differentially methylated ($p<0.05$, fold difference in methylation level >2) were identified. We next annotated these peak reads to genes and mapped the differential methylated genes to biological networks by panther pathway analysis. The top three pathways were enriched in

WNT signaling pathway (mapped genes: 11); Gonadotropin-releasing hormone receptor pathway (mapped genes: 10) and Inflammation mediated by chemokine and cytokine signaling pathway (mapped genes: 8). Among these 29 genes, some of them are mapped for more than one pathway. *In silico* analysis was done to examine if there are CpG islands at the 5'regulatory region (-2000 to +2000 from TSS) of these methylated gene candidates. A list of seven genes with 5' CpG island(s) was chosen to test the hypothesis that the gene expression altered by maternal PBDE-47 exposure could contribute to lung inflammation and associate with the increased HDM-induced AHR in offspring.

Other Methods

Gene expression analysis, methylation specific PCR, and statistical analyses were performed as outlined in chapter 2.3. Sequences of primers used for gene expression and methylation studies listed in Table 1 and 2. This analysis was carried out on the male mice only.

Table 3: MSPCR primers for *Tnfa* and *Bdnf*

Primer List	(Sequence 5' to 3'')
mMS-Bdnf-C1F	CGG GAA GTA TTT AAA ATA GGG TAG C
mMS-Bdnf-C1R	CTA ATA ACT AAA CGA AAA ACA CCC G
mMS-Tnfa-MF1	AAT TTT TTA AAT TTT TTG TTT TCG C
mMS-Tnfa-MR1	TAA CTA ATC CCT TAC TAT CCT CGC T

Table 4: qPCR primer list for RRBS genes

Primer Name	Gene Name	Sequence (5' to 3')
mAdcy2-F1	Adenylate cyclase 2	CGG ATG TGC CAA TGG GTC AA
mAdcy2-R1	Adenylate cyclase 2	GCC ATT GCT TTT GTG CGT TG
mItpr3-F1	Inositol 1,4,5-triphosphate receptor 3	GCT GTC AAC ATG AGC GAC CT
mItpr3-R1	Inositol 1,4,5-triphosphate receptor 3	GAC ACG CGG CCT TTG GT
mPcdh1-F1	Protocadherin 1	GAG AAG GTG AGG ACT AGG GC
mPcdh1-R1	Protocadherin 1	AAT CAG GAG AAC TTC TGG GTT GTC
mPparg-F1	Peroxisome proliferator-activated receptor gamma	CTG ACG GGG TCT CGG TTG
mPparg-R1	Peroxisome proliferator-activated receptor gamma	AAC CAT GGT AAT TTC AGT AAA GGG T
mPtk2-F1	Protein tyrosine kinase 2	TGC CCT CGA CCA GGG ATT AT
mPtk2-R1	Protein tyrosine kinase 2	AAG CTG GAT TCT CTG GGC TC
mSfrp1-F1	Secreted frizzled-related protein 1	GAA GCC TCT AAG CCC CAA GG
mSfrp1-R1	Secreted frizzled-related protein 1	ATC CTC AGT GCA AAC TCG CT

mSiah1b-F1	Siah E3 ubiquitin protein ligase 1B	AGG GCG GGT CAG GAG TCA A
mSiah1b-R1	Siah E3 ubiquitin protein ligase 1B	ACC TGG TCT TCC CAA GTT TCC A
mSocs5-F1	Suppressor of cytokine 5 signaling	AGG CTC TCG AAG GCT CAG G
mSocs5-R1	Suppressor of cytokine 5 signaling	TGA TTA GAA GAG CTC CAC GGC

3.4 Results and Discussion

To demonstrate if maternal PBDE exposures contributes to asthma pathogenesis, we determined if the effects of PBDE exposure at multiple timepoints during mouse lung development affected the gene expression of select genes related to asthma pathogenesis. When looking at the two focal genes of the study, *Bdnf* and *Tnfa*, both genes show a strong upwards trend in gene expression between the maternal oil control (F0-Oil_F1-Saline) and PBDE exposed (F0-PBDE47_F1-Saline) groups without the adult exposure to HDM (Figure 1 and Table 3). Over a two fold increase in average gene expression of *Bdnf* between the F0-Oil_F1 PBS and the F0-PBDE_F1-PBS was seen, although the changes is not statistically significant. We additionally observed over a two fold increase in average *Tnfa* mRNA levels between F0-Oil_F1-Saline and the F0-PBDE_F1 Saline, but this observed difference is also not statistically significant. Upon the adult exposure to HDM, the group with PBDE exposed F0 mothers showed a trend of further increase in *Bdnf* and *Tnfa* expression (F0-Oil_F1-HDM vs the F0-PBDE_F1-HDM).

While the observed difference in gene expression of *Bdnf* and *Tnfa* was not found to be significant, we did find a clear trend in increased expression of these pro-inflammatory genes. Both of these gene expression upregulations correlated well with the observed decreased promoter methylation

(Figure 2). Results indicates that maternal exposure to PBDE with or without adult HDM exposure caused demethylation of *Bdnf* and *Tnfa* promoters that resulted in the increased gene expression. A reduction in CpG on a promoter could organize the chromatin to unwind and make these pro-inflammatory genes become transcriptionally active. We speculate the increase in *Bdnf* and *Tnfa* could contribute to the asthma-related phenotypes, ASM innervation and inflammation respectively, which gives them plausibility as driving genes for asthma that PBDE-47 can act on.

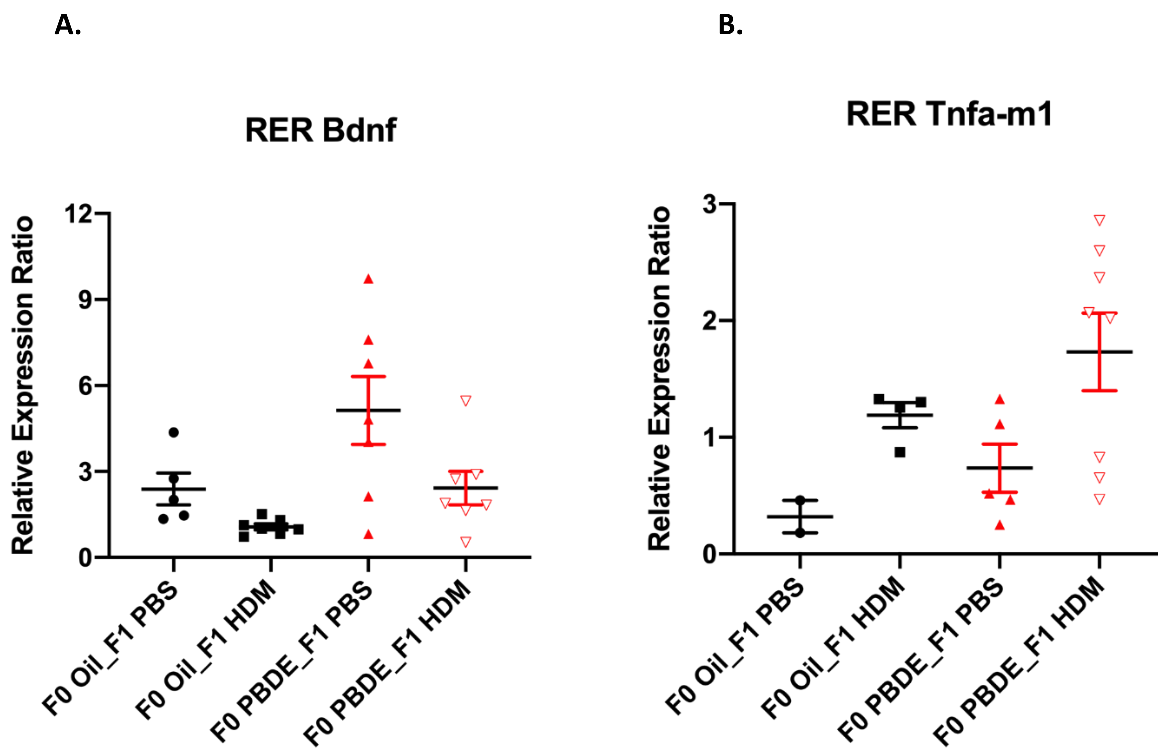


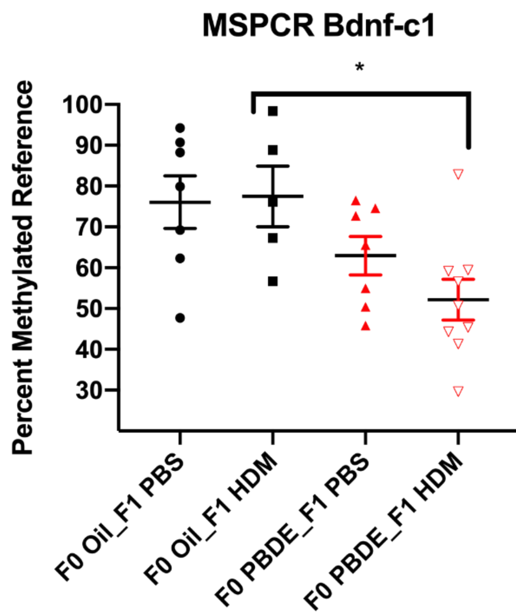
Figure 1: Effect of maternal PBDE-47 exposure and adult HDM exposure on gene expression of *Bdnf* and *Tnfa*. Relative gene expression (RER) ratios for *Bdnf* (A) and *Tnfa* (B) were observed in F1 offspring. The transcription levels of *Tnfa* and *Bdnf* were examined by real-time PCR and normalized to the housekeeping gene, *Rpl19*. Relative Expression Ratio values were calculated by a 2-ddCt method, in which RER was assigned a value of 1.00 for mouse universal RNA. Data were shown as mean values ± SEM.

Table 3: Statistical analysis of the effect of PBDE and HDM on gene expression of Bdnf and Tnfa

Gene	F0 Oil_F1 PBS (A) Mean, SEM	F0 Oil_F1 HDM (B) Mean, SEM, **P value vs (A)**	F0 PBDE_F1 PBS (C) Mean, SEM, **P value vs (A)**	F0 PBDE_F1 HDM (D) Mean, SEM, **P value vs (B)**, **P value vs (C)**
Bdnf	2.39 0.55	1.07 0.10 **0.64**	5.13 1.18 **0.09**	2.42 0.59 **0.56** **0.06**
Tnfa	0.32 0.14	1.19 0.11 **0.49**	0.74 0.21 **0.89**	1.73 0.33 **0.59** **0.10**

Note: Relative gene expression ratios for *Bdnf* (A) and *Tnfa* (B) observed in F1 offspring. The transcription levels of *Tnfa* and *Bdnf* were examined by real-time PCR and normalized to the housekeeping gene, *Rpl19*. Relative Expression Ratio values were calculated by a 2-ddCt method, in which RER was assigned a value of 1.00 for mouse universal RNA. Data were shown as mean values ± SEM. Statistical analyses conducted were ordinary one-way ANOVAs with Tukey's multiple comparisons.

A.



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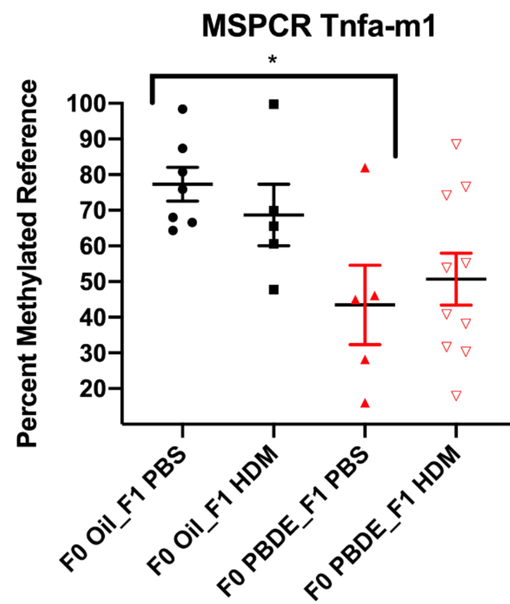

Figure 2: Effect of maternal PBDE-47 exposure and adult HDM exposure on promoter methyation of *Bdnf* and *Tnfa*. Methylation percentages for *Bdnf* (A) and *Tnfa* (B) were observed in F1 offspring. Percent of methylation reference was determined by MS-PCR as the ratio of the sample's methylation percentage relative to a fully methylated DNA control. An ordinary one-way ANOVA with Tukey's multiple comparisons. * = P<0.05.

Table 4: Statistical analysis of the effect of PBDE and HDM on promoter methylation of Bdnf and Tnfa

Gene	**F0 Oil_F1 PBS (A)** Mean%, SEM	**F0 Oil_F1 HDM (B)** Mean%, SEM, P value vs (A)	**F0 PBDE_F1 PBS (C)** Mean%, SEM, P value vs (A)	**F0 PBDE_F1 HDM (D)** Mean%, SEM, P value vs (B), P value vs (C)
Bdnf	76.1 6.45	77.5 7.43 0.99	62.9 4.72 0.39	52.2 5.00 **0.03** 0.51
Tnfa	77.3 4.70	68.7 8.61 0.89	43.5 11.1 **0.05**	50.7 7.29 0.40 0.92

Note: Methylation percentages for *Bdnf* and *Tnfa* were observed in F1 offspring. Percent of methylation reference was determined by MS-PCR as the ratio of the sample's methylation percentage relative to a fully methylated DNA control. An ordinary one-way ANOVA with Tukey's multiple comparisons. **Bold** indicates statistical significance.

In addition to the analysis of genes related to asthma pathogenesis, we applied an unbiased genome-wide methylation profiling to identify other epigenetic changes induced by PBDE exposure. After completion of a reduced representation bisulfite sequencing (described above in 3.3) we identified differential methylation of genes outside of the primary focus of *Tnfa* and *Bdnf* : *Adcy2, Ascl1, Itpr3, Pparg, Ptk2, Sfrp3, Siah1,* and *Socs5*. Briefly, Adenylate cyclase 2 (*Adcy2*) encodes for an adenylate cyclase, which is a membrane associated enzyme involved in the catalyzing the formation of cyclic

adenosine monophosphate (cAMP). cAMP is important in the regulation of airway smooth muscle cells, cells which are inextricably linked to asthma, becoming contracted and inflamed during an asthmatic episode (55). *Ascl1* (Achaete-Scute Family BHLH Transcription Factor 1) is involved in the differentiation of pulmonary neuroendocrine cells and other autonomic neurons. As a result, it plays a key role in the innervation, and therefore sensitization, of the lung (56). *Itrp3* (Inositol 1,4,5-triphosphate receptor 3) encodes for a receptor on inositol 1,4,5-triphophate, which serves as a second messenger for calcium regulation. It was recently found to be dysregulated in asthma in a male-specific manner (57). *Pparg* (Peroxisome proliferator-activated receptor gamma) is a ligand activated transcription factor involved in the regulation of adipocyte differentiation and glucose homeostasis. It also upregulates PTEN, which can negatively modulate allergic inflammation in asthma (58). *Ptk2* (Protein tyrosine kinase 2) may be important in early cell growth and intracellular signal transduction pathways in response to neural peptides or interactions with extracellular matrix, meaning it also plays a role in early innervation of the lung (60). *Sfrp1* (Secreted frizzled-related protein 1) is a modulator of the Wnt signaling pathway, and epigenetic silencing of *Sfrp1* has been implicated in allergic asthma by altering airway inflammation and lung function (59). *Siah1* (Siah E3 ubiquitin protein ligase 1B) encodes for a protein involved in proteasome mediated degradation in the neuron, and is also highly expressed in the lung (60). Finally, *Socs5* (Suppressor of cytokine 5 signaling) is involved in cytokine signaling as negative regulators of the signaling pathway; where epigenetic silencing could lead to increased cytokine related inflammation in the lungs (61). Taken together, we demonstrated maternal exposure to PBDE could alter lung methylome, which may ultimate alter development of asthma.

To determine if the methylation patterns induced by PBDE exposure contribute to the gene expression, we applied qPCR to measure the mRNA level of the genes identified from RRBS (Figure 3 and Table 5). No trend was observed in *Adcy2* was seen between the F0-Oil_F1-PBS and the F0-PBDE47_F1-PBS, or between the F0-Oil _F1-HDM and the F0- PBDE47_F1-HDM. This result suggests that there is

little effect to Adcy2, which is responsible for increasing production of cAMP, which activates protein kinase A, leading to a relaxation of ASM (67). With the gene *Itrp3*, No trend was observed between the F0-Oil_F1-PBS and the F0-PBDE47_F1-PBS, and a weak trend was seen between the F0-Oil _F1-HDM and the F0- PBDE47_F1-HDM (p = 0.32). However, *Itrp3* did have a greater than eight-fold increase in expression F0-PBDE47_F1-PBS and the F0-PBDE47_F1-HDM. This somewhat corresponds with the findings seen in Gautam et al, where there was a sex specific increase in male *Itrp3* expression amongst asthmatics, suggesting it could have influence in asthma amongst men (57). However, the gene is far more strongly modulated by the HDM exposure than the PBDE47 exposure. *Pparg* also had no trend between the F0-Oil_F1-PBS and the F0-PBDE47_F1-PBS, or the F0-Oil_F1-HDM and the F0- PBDE47_F1-HDM. When comparing the differences between the F1-PBS and F1-HDM groups, the gene had around a seven-fold reduction in expression for both F0-Oil and F0-PBDE subgroups. *Pparg'*s knockdown has been cited by Nieto et al to cause a global upregulation of pro-inflammatory genes specifically involved with immune cell proliferation and migration, but is only significantly modified by HDM (68). *Ptk2* also saw no trend in between the F0-Oil_F1-PBS and the F0-PBDE47_F1-PBS, or between the F0-Oil _F1-HDM and the F0- PBDE47_F1-HDM. When looking at HDM exposure, *Ptk2* was upregulated six-fold in our model when comparing F0 PBDE_F1 PBS and the F0 PBDE_F1 HDM, has been seen to correlate with childhood asthma, and could play a similar role as *Bdnf* in lung cell innervation. *Sfrp3*'s gene expression data had no trend in between the F0-Oil_F1-PBS and the F0-PBDE47_F1-PBS, or between the F0-Oil _F1-HDM and the F0- PBDE47_F1-HDM. *Sfrp1*, which is highly expressed in allergic asthma and alters airway inflammation, increased three-fold in the male mice when adding the allergen modification of HDM exposure within the oil and PBDE subgroups.

Siah1, which is seen as a marker of neuron regulation in allergic asthma, was only modified through HDM exposure. When comparing the F0-Oil_F1-PBS and the F0-PBDE47_F1-PBS, or the F0-Oil _F1-HDM and the F0- PBDE47_F1-HDM, there was no difference observed. Finally, *Socs5* saw almost a four-fold

decrease between the F0-Oil_F1-PBS and the F0-PBDE_F1-PBS. Albeit not statistically significant (p=0.08), this increase observed would indicate increased cytokine signaling, increasing inflammation response in asthma.

Of note in this study is that for many of the genes RRBS gene list, HDM served as a much stronger modifier of gene expression than PBDE-47 exposure did (such as *Pparg*, *Ptk2* and *Sfrp1*). HDM is a strong inducer of allergic response, so while this is expected, observed gene expression changes was also expected to be correlated with PBDE-47, based on the results of the RRBS. Two reasons most likely exist for this discrepancy between the RRBS and the collected data. The first is that these genes are epigenetically modified through a mechanism other than through 5mC methylation, which could be overriding potential methylation observed. Examples of this include low RNA stability, which could lead to low observed transcription level. Other possibilities include alterations in binding of transcription factors or the binding of histones, which could interfere with transcription despite methylation status changing. Finally, induction of miRNAs could also degrade higher RNA we would otherwise measure. Outside of an epigenetic rationale, some trends observed may be confirmed to be significant with a higher sample size, particularly in the genes *Bdnf, Tnfa*, *Itpr3,* and *Socs5*. Being limited to smaller sample sizes, especially ones with outliers that overlap in value with another subgroup as seen in our data, make it difficult to obtain results of statistical significance.

Overall, the data suggests that maternal PBDE exposure impacts the methylation of genes related to inflammatory pathways, such as *Bdnf* and *Tnfa*, as well as alter gene expression in certain genes, such as *Bdnf, Tnfa*, *Itpr3,* and *Socs5*. This supports the hypothesis that epigenetic regulation of asthma pathogenesis in response to PBDE in an experimental asthma model. More data needs to be obtained to see whether similar methylation alterations can be observed in other genes modified by PBDE-47 exposure.

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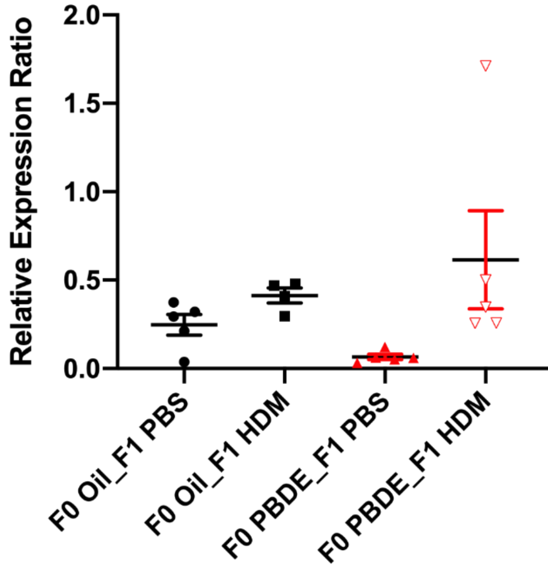

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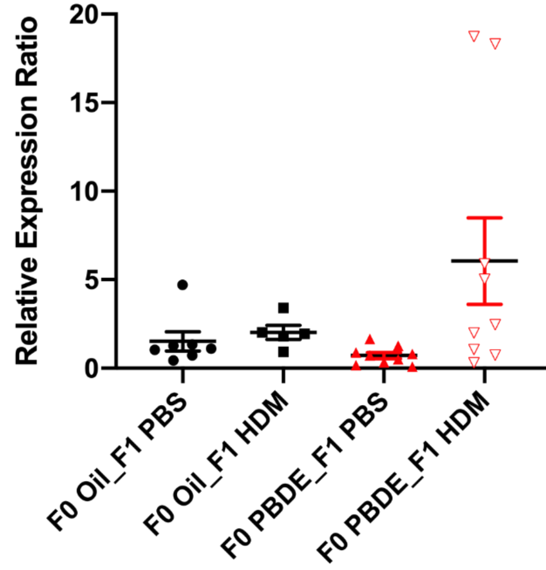

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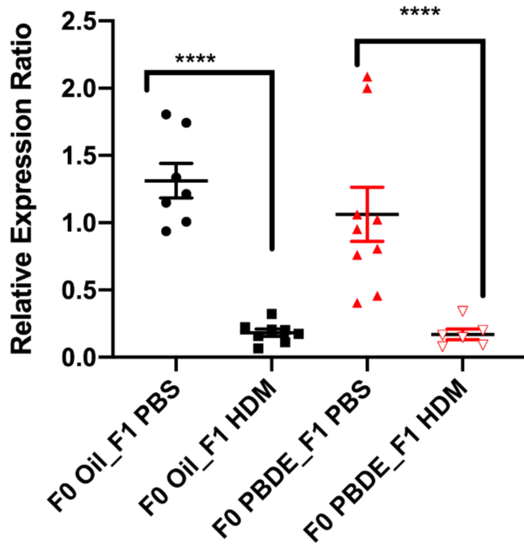

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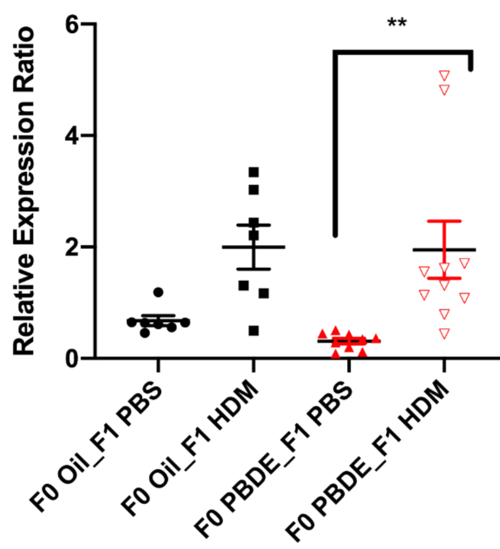

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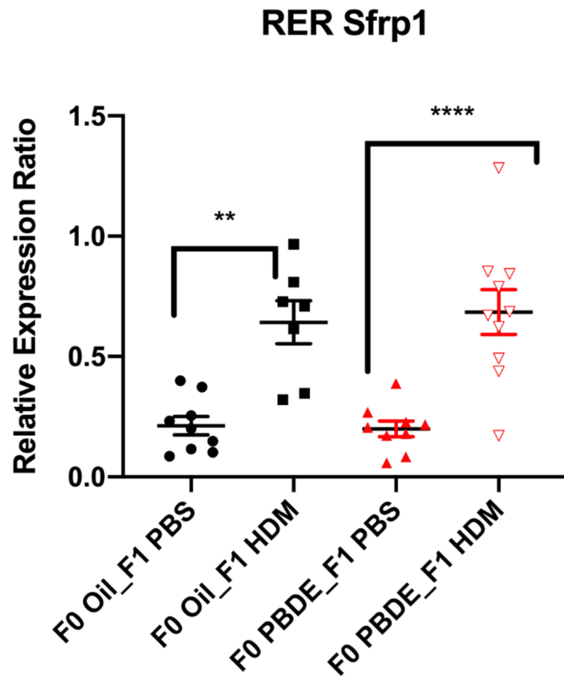

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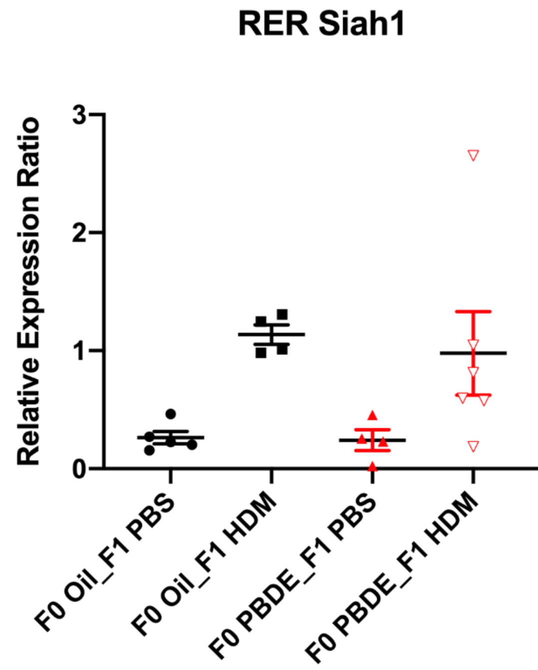

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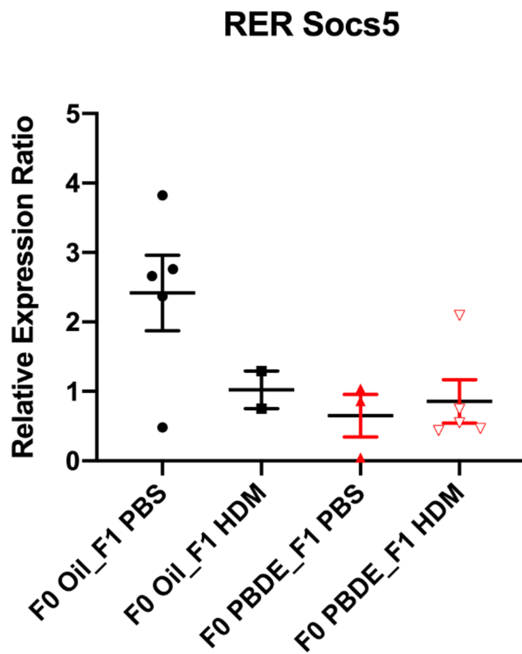

Figure 3: Effect of maternal PBDE-47 exposure and adult HDM exposure on mRNA level of Differential Methylated Genes Identified from RRBS. Relative gene expression ratios for *Adcy2* (A), *Itpr3* (B), *Pparg* (C), *Pcdh1* (D), *Ptk2* (E), *Sfrp3* (F), *Siah1* (G), *Socs5* (H) were observed in F1 offspring. The transcription levels of gene of interests were examined by real-time PCR and normalized to the housekeeping gene, *Rpl19*. Relative Expression Ratio (RER) values were calculated by a 2-ddCt method, in which RER was assigned a value of 1.00 for mouse universal RNA. Data were shown as mean values ± SEM. Statistical analyses conducted were ordinary one-way ANOVAs with Tukey's multiple comparisons. * = P<0.05, ** = P<0.01, *** = P<0.001, **** = P<0.0001.

Table 5: Statistical analysis of the effect of PBDE and HDM on promoter methylation of differential methylated genes identified from RRBS

Gene	**F0 Oil_F1 PBS (A)** Mean, SEM	**F0 Oil_F1 HDM (B)** Mean, SEM, **P value vs (A)**	**F0 PBDE_F1 PBS (C)** Mean, SEM, **P value vs (A)**	**F0 PBDE_F1 HDM (D)** Mean, SEM, **P value vs (B)**, **P value vs (C)**
Adcy2	0.25 0.06	0.41 0.04 **0.88**	0.07 0.02 **0.82**	0.61 0.28 **0.80** **0.08**
Itpr3	1.52 0.54	2.02 0.40 **>0.99**	0.72 0.171 **0.98**	6.05 2.44 **0.32** 0.05
Pparg	1.31 0.13	0.18 0.03 **<0.0001#**	1.06 0.20 **0.56**	0.170 0.04 **>0.99** <**0.001#**
Ptk2	0.68 0.09	2.00 0.39 **0.10**	0.31 0.05 **0.89**	1.95 0.51 **>0.99** **<0.01#**
Sfrp1	0.21 0.038	0.64 0.09 <**0.001#**	0.20 0.03 **>0.99**	0.69 0.09 **0.98** **<0.0001#**

Siah1	0.26 0.05	1.14 0.08 **0.10**	0.24 0.09 **>0.99**	0.98 0.35 **0.96 0.17**
Socs5	2.42 0.54	1.02 0.27 **0.29**	0.65 0.305 **0.08**	0.85 0.314 **>0.99 0.99**

Note: Gene expression was examined by real-time PCR and normalized to the housekeeping gene, Rpl19. Relative Expression Ratio values were calculated by a 2-ddCt method, in which RER was assigned a value of 1.00 for mouse universal RNA. Data were shown as mean values ± SEM. Statistical analyses used were ordinary one-way ANOVAs with Tukey's multiple comparisons. Adcy2: Adenylate cyclase 2. Itpr3: Inositol 1,4,5-triphosphate receptor 3. Pcdh1: Protocadherin 1. Pparg: Peroxisome proliferator-activated receptor gamma. Ptk2: Protein tyrosine kinase 2. Rpl19: ribosomal protein L19. Sfrp1: Secreted frizzled-related protein 1. Siah1: Siah E3 ubiquitin protein ligase 1B. Socs5: Suppressor of cytokine 5 signaling. **#** indicates statistical significance.

CHAPTER 4: Discussion, Implications and Future Directions

In this study, our mouse model was able to demonstrate the ability of PBDE-47 in mediating epigenetic changes underpinning altered gene expression in the genes *Tnfa* and *Bdnf*. The goal of this study was to fill the black box between PBDE exposure and asthma, given no confirmed mechanism of toxic action. From the results found in our study, an epigenetic mechanism of action for the primary genes of interest is plausible. While more remains to be done to confirm our findings, it can be reasonable to assume there is an epigenetic element of regulation from PBDE-47 exposure in important asthma related genes.

Given the understanding that upregulated *Tnfa* and *Bdnf*, caused through PBDE-47 exposure are implicated in asthma phenotype, it becomes even more critical to recommend protection from PBDEs during pregnancy, as exposure during this window of susceptibility, based on this study, is likely to modulate asthma risk. This can come with the recommendation to avoid using furniture imported from countries without pentaPBDE bans, and avoid using furniture made in the U.S. before 2005, when the manufacturing of products containing penta and octa-PBDE species was banned. The data suggests that U.S. law regarding PBDE species should be updated to become more similar to laws already adopted in the European Union, where there is already full bans on all PBDE species and bans on recycling of products that could contain PBDEs, due to PBDEs' persistent nature in the environment.

Experimentally, the next step forward for the multi-dose PBDE model is to investigate other genes included in the RRBS study and observe if their promoter methylation changes matches their gene expression, particularly in the genes with statistical significance (*Adcy2, Itpr3, Pparg, Ptk2, Sfrp1* and *Siah1*). Following up with promoter methylation data for these

genes would strength the argument of epigenetic control of asthma related response due to PBDE exposure. Additionally, since our multi dose experiment focused on exposing during multiple points of development, it is unclear whether or not every point of exposure truly had an impact on the final outcomes of gene expression and methylation. Parsing out which points are most important, or if it truly requires every dose administered, is the next logical step for follow up experiments. In particular, we could separate each major exposure timepoint: pre-conception, in utero, and post natal, to see which have the largest impact or if each dose is needed. Since Chapter 2 already begins to discuss the effect of post natal exposure during lung development, I think looking at in-utero exposures on its own would be most appropriate, exposing during 1st and 2nd week of gestation only. Further, it could be useful to determine whether any of the gene expression and promoter methylation was modified by factors such as sex and genetic background (those with high asthma risk or genetic knockout of inflammatory genes). This would be important to ascertain if their were confounding factors that caused specific genes and promoter regions to show significant changes. Finally, we could also alter the dosage amounts, to see if there is a threshold dosage for epigenetic effects or if even miniscule doses can lead to a larger adverse health outcome. This would help with determining the level of control needed over these chemicals from a regulatory standpoint.

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EDUCATION

Johns Hopkins Bloomberg School of Public Health **August 2018 – May 2020**

- Master of Science Candidate in Environmental Health Toxicology
- Expected Graduation: May 2020

Johns Hopkins University **August 2014 – May 2018**

- Bachelor of Arts with Honors in Behavioral Biology, Bachelor of Arts in the History of Science Medicine and Technology

RESEARCH EXPERIENCE

Research Assistant **November 2018 – Present**

Tang Epigenetics Lab: Johns Hopkins Bloomberg School of Public Health

- Investigated the effect of flame retardants (PBDEs) on epigenetic regulation of asthma in utero.
  Manuscript in preparation: *Epigenetics Insights*
  Maternal Exposure to Polybrominated Diphenyl Ether Increases Asthma Susceptibility in Adult Offspring
  Bonnie H.Y. Yeung, Joan Lee, Qinying Sun, Kelly Griffiths, Rohan Kuruvilla, Jairus C. Pulczinski, Jeffrey M. Loube, Wayne Mitzner, Robert Y.S. Cheng and Wan-yee Tang
- Observed the role of house dust mite on lung functions (pathological and physiological measurements)

School Liaison **January 2018 – Present**

Cool Green Schools Environmental Health Project: Johns Hopkins University

- Collaborate with Baltimore City high school students through helping them identify health hazards in their schools and work to reduce health risks for students.

Research Assistant **January 2016 – May 2018**

Comparative Neural Systems and Behavior Laboratory: Johns Hopkins University

- Monitored the behavioral and seasonal patterns of endangered bat species.
- Recorded hippocampal and flight data on *Eptesicus Fuscus* bats in navigation experiments.

MEDICALLY RELATED EXPERIENCE

Clinical Shadowing **May 2019 – June 2019**

University of Maryland School of Medicine: Baltimore, MD

- Shadowed Dr. Stella Hines at the Division of Occupational and Environmental Medicine. Provided toxicology background and performed literature reviews alongside residents in the OEM residency program.

Hospital Volunteer **May 2017 – Present**

Mercy Medical Center: Baltimore, MD

- Volunteered in the Post Anesthesia Care Unit, assisting patients recovering from general surgery and anesthesia. Also volunteered in "Joint Journey," an orthopedic surgery rehabilitation unit for patients recovering from knee and hip replacements.

Clinical Shadowing **July 2018 – August 2018**

Shady Grove Allergy and Asthma Center

- Shadowed Dr. Leo Shue as he treated patients affected by severe allergies and asthma. Learned basic immunology and the interplay between immune response and allergic asthma.

Clinical Shadowing **January 2018**

National Hospital Abuja: Abuja, Nigeria

- Shadowed at a nephrology unit within the largest hospital in Abuja, Nigeria. Learned about tropical medicine and differences in the healthcare systems of developing nations.

WORK AND COMMUNITY SERVICE EXPERIENCE

Assistant Instructor **June 2018 – August 2019**

Summer Stem Academy: JHU Center for Educational Outreach

- Served as an assistant instructor for a free summer STEM program, designed to help underprivileged students in the Baltimore area gain exposure to engineering principles and develop a lifelong passion for the sciences.

Robotics Head Coach **September 2018 – Present**

Barclay Elementary School: Baltimore, MD

- Helped educate and coach elementary school students in the basics of engineering design and computer programming, designing projects using the program "LEGO Mindstorms".

Teaching Assistant **August 2019 – January 2020**

Public Health Toxicology: Bloomberg School of Public Health: Baltimore, MD

- Served as the lead teaching assistant for Public Health Toxicology, the introductory level toxicology course. Responsible for grading assignments, hosting review sessions, and answering questions.

President **September 2015 – May 2018**

Alpha Phi Omega Community Service Fraternity

- Led the largest student group at Johns Hopkins off over three hundred members, worked with dozens of faculty members and over forty non-profits to provide student run community service volunteering programs in Baltimore City.

Director of Recruitment **January 2017 – May 2018**

Baltimore First Community Service Program

- Founding member, helped grow organization from a dozen individuals to overs fifty.
- Volunteered with the AmeriCorps program, “Civic Works” at an urban farm since Spring 2016. Also researched food insecurity in Baltimore City.