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The Association between Exposure to a Flame-retardant Chemical and Autoimmune Disorders

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2016

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Abstract

The Association between Exposure to a Flame-retardant Chemical and Autoimmune Disorders

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Background: Polybrominated Biphenyls (PBBs) are synthetic halogenated compounds that were originally used as flame retardants. In 1973, individuals living in Michigan were exposed to PBBs through the consumption of contaminated meat and dairy products after an accidental substitution of a flame retardant containing PBB for animal feed. Previous studies on this cohort suggest that exposure to PBBs may be related to many health conditions. However, no studies have evaluated whether exposure to PBBs is associated with autoimmune disease. In this cross-sectional study, we evaluated this possibility and whether this association differs by sex and age at exposure.

Methods: Current serum PBB levels were natural log transformed and evaluated for their association with self-reported autoimmune disease. Logistic regression was performed to evaluate models stratified by sex and age at exposure. Potential confounders included sex, age at blood draw, BMI, and smoking status. All statistical analyses were conducted in SAS version 9.4 and p-values less than 0.05 were considered significant.

Results: Overall, current serum PBBs were not associated with lifetime prevalence of autoimmune disease. However, among 228 individuals exposed to PBBs in utero, women were over four times as likely to have an autoimmune disease compared to men after controlling for age at blood draw (OR=4.234 [1.872, 9.580]). In addition, increasing PBB was associated with a reduced risk of autoimmune disease and that association varied in magnitude, depending on the age at blood draw. Among 340 individuals exposed to PBBs as children, women were twice as likely to have an autoimmune disease compared to men (OR=2.167[1.245-3.772]]). Among 284 individuals exposed to PBBs as adults, women were nearly twice as likely to have an autoimmune disease compared to men (OR=1.911 [1.047, 3.490]). These results were statistically significant.

Conclusions: Exposure to PBBs was not significantly associated with autoimmune disease in the study population. Among those exposed in utero, the association between PBBs and autoimmune disease differed by age at blood draw. Exposed women were significantly more likely to have autoimmune disease than men. The magnitude of the effect of participant sex differed by whether participants were exposed in utero, as children, or as adults.

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Chapter I: Literature Review

Introduction to Polybrominated Biphenyls

Polybrominated Biphenyls (PBBs) are synthetic halogenated compounds produced through the bromination of biphenyl. Although several different congeners are possible depending on the position and number of bromine atoms that are substituted for hydrogen, the most common congener is hexabromobiphenyl. Due to their chemical structure, PBBs are biologically stable, fat-soluble chemicals that do not easily breakdown in the environment and may also bioaccumulate. These compounds were originally used as flame retardants and were incorporated in the manufacturing of electronics, plastics, carpets, and other home furnishings during the 1970s (1).

In 1973, residents of Michigan were exposed to PBBs when between 500 and 1000 pounds of Firemaster FF-1, a flame retardant containing PBB, was accidentally substituted for animal feed by the Michigan Chemical Company (2). As a result, the feed ingested by livestock in the months following the incident contained an estimated maximum chemical concentration ranging from 4,000 to 13,500 ppm (2). Humans were primarily exposed to the chemical through consumption of meat and dairy products from the contaminated food supply before the mistake was identified, but exposure also occurred among manufacturing workers. Although the production of PBBs in the United States was discontinued in 1976, many Michigan residents continue to have serum PBB levels that are significantly higher than the general population (3). Therefore, continued research on the health effects of PBBs is warranted.

Impact of Polybrominated Biphenyls on Health

Since the incident in Michigan in the 1970s, several studies have been conducted to understand the health effects associated with exposure to PBBs and other halogenated compounds. Before human studies were undertaken, early research relied on animal models. For example, an observational study in 1976 concluded that cows that had consumed the contaminated feed had a noticeable reduction in milk production, gave birth to stillborn calves, and had shrunken utters (4). In other animal models, exposure to PBBs has been associated with smaller than normal thyroid glands, delayed growth and development, altered kidney function, increased weight of the liver, and altered hormone levels (5-9). It is also known that PBBs accumulate in adipose tissue and can be transferred to nursing pups through breast milk (9). These animal studies suggest that PBBs can impact multiple organ systems, and continued research is needed to understand the full effect these chemicals can have on humans.

In 1976, the Michigan Long-Term PBB Study (now called the PBB Registry) was established by the Michigan Department of Public Health. This cohort originally contained 57 non-exposed and 3820 exposed individuals representing farm product recipients, quarantined farm residents, and chemical workers (10). This cohort has since been expanded to include the decedents of the original study participants with over 7000 participants ever enrolled. The PBB Registry has provided researchers the unique opportunity to study the effects of PBBs on humans directly exposed to the chemical as well as potential intergenerational health effects of PBBs.

Previous studies involving the Michigan Polybrominated Biphenyl (PBB) Registry suggest that exposure to PBBs may be related to a variety of health conditions.

For example, PBBs may influence lymphoma, breast, and digestive cancer incidence in this cohort (11,12). Another recent study points to the detrimental impact PBBs may have on reproductive outcomes, as women born to mothers who were directly exposed to the highest levels of PBBs were more likely to have a spontaneous abortion compared to women born to mothers with low levels of PBB exposure (13). Other biological effects of PBBs have been demonstrated, such as changes in DNA methylation patterns that vary by sex (14).

Furthermore, exposure to PBBs in utero may be related to changes in growth and pubertal development among children. Previous studies suggest that girls who were highly exposed to PBBs in utero and were subsequently breastfed had lower ages at first menarche than girls who were exposed to lower levels of PBB in utero and were not breastfed (15). Evidence of the impact of PBBs on children was also reported in a 2009 study that found that boys born to women that were highly exposed to PBBs were more likely than boys born to women in the lowest exposure category to report having genitourinary conditions (16). Finally, maternal exposure to PBBs has been associated with an increase in the odds of lower Apgar scores among infants (17).

Another area of extensive research has been on the association between exposure to endocrine disrupting chemicals, like PBBs, and thyroid function. However, studies have yielded mixed results. For example, results from a 2011 case control study of the Michigan Polybrominated Biphenyl (PBB) Registry by Yard et al. indicated that those with thyroid disease had similar PBB blood levels compared to controls (18). The authors concluded that thyroid disease incidence was not associated with serum PBB or PCB levels among the study population, which did not include exposed chemical workers (18).

By contrast, a 1981 study of 35 workers of a PBB manufacturing plant found four workers with hypothyroid disease, and no cases among 89 controls (19). These results are further supported by findings from a 2017 study by Jacobson et. al., in which women enrolled in the Michigan Polybrominated Biphenyl (PBB) Registry that were in the fourth quintile of PBB-153 serum levels had significantly higher odds of thyroid disease when compared to women in the first quintile of PBB-153 serum levels (20). Similarly, in previous studies exposure to PBBs has been associated with higher thyroid hormone levels among those exposed before age 16 (21). Due to the conflicting results, further research is needed to understand the complex relationship between PBB exposure and thyroid disease, as well as possible mechanisms that thyroid disease progresses or results in an autoimmune disorder. One of the most common autoimmune disorders is autoimmune thyroiditis, therefore it is possible that there is a connection between thyroid disorders and autoimmune disorders.

Introduction to Autoimmune Disorders

Autoimmune disorders arise when the body is unable to distinguish itself from foreign elements. Instead, the immune system degrades its own cells, molecules, tissues and organs through the action of lymphocytes or antibodies. Autoimmune disorders affect approximately 3-5% of the population, and conditions can impact single organs or multiple organ systems (22,23). Autoimmune disorders have varying ages of onset with type I diabetes and autoimmune thyroiditis being the most common of these diseases (22,23).

There are several known risk factors for autoimmune diseases. Many autoimmune diseases have a genetic basis, but disease onset is often triggered by environmental exposures (23). There is a known relationship between exposures to smoking, dietary iodine, low levels of vitamin d, infectious diseases and the onset of autoimmune disorders (22). However, the association and mechanism by which exposure to halogenated biphenyls may result in autoimmune disorders remains poorly understood.

The Role of Sex in the Development of Autoimmune Disorders

Autoimmune disorders are generally more common in women than in men. For example, the female to male ratio for rheumatoid arthritis is approximately 2:1, while for conditions such as Sjogren's syndrome and lupus it is as high as 9:1 (22). Several mechanisms have been proposed to explain the difference in prevalence of autoimmune disorders by sex including genetic factors related to the skewed inactivation of the x chromosome (23). However, sex hormones may also influence the development and progression of autoimmune diseases by selectively binding to hormone receptors on immune cells (24). Estrogens, found at higher levels in women than in men, may be related to the activation of antibody production and are an important predictor for autoimmunity.

Furthermore, studies suggest that autoimmune disease prevalence may be related to reproductive function. Evidence for this trend includes the documented decline in the female to male sex ratio for lupus to 5:1 among postmenopausal populations from 15:1 among premenopausal populations as estrogen levels become more similar between the sexes (24). In animal models, mice treated with estrogens at similar levels to those

produced by females during pregnancy showed improved autoimmune encephalomyelitis progression (25). This suggests that the biological consequences of pregnancy may be protective against certain autoimmune diseases. However, pregnancy is also associated with an increase in lupus symptoms, indicating that the role of estrogen in the development of autoimmune disease throughout the life course is complex and requires further study.

Additionally, environmental exposures that are important in the autoimmune pathway such as infectious agents, pesticides and vitamin d may vary among men and women. Environmental estrogens, or synthetic chemicals that act as endocrine disrupters, may also impact autoimmune disease development differentially by sex (23). According to a recent study by Curtis et. al, exposure to PBB, a known endocrine disrupter, is associated with variation in DNA methylation which indicates that PBB is acting similarly to estrogen and is associated with dysregulated immune system pathways (26). However, whether endocrine disrupters produce additive or antagonistic effects to natural estrogens remains unknown.

The Role of Obesity in the Development of Autoimmune Disorders

Previous research has demonstrated that obesity may also be a risk factor for the development and progression of autoimmune disorders such as lupus, psoriasis, multiple sclerosis, and rheumatoid arthritis (27, 28). In 2014, Versini et al. hypothesized that the association between obesity and autoimmune disease may be due to a state of chronic inflammation produced as a result of adipokine secretion from fat tissue (27). In addition, poor diet may lead to changes in the gut microbiome resulting in detrimental impacts to

immune function. Finally, obese individuals are more likely to be vitamin d deficient, which is a known risk factor for autoimmune disease. Whether obesity remains an important predictor for the development of autoimmune disorders among those exposed to PBBs remains unexplored.

PBBs and Autoimmune Disorders

Previous research on human populations has demonstrated an association between PBB exposure and the development of antithyroid antibodies. Interestingly, all four PBB exposed workers that were found to have hypothyroid disease in the study conducted by Bahn et al. also had elevated levels of antithyroid microsomal antibodies (19). This suggests that PBB simultaneously acts as an endocrine disrupter and influences the cell mediated immune response. Furthermore, hypothyroidism may be a manifestation of autoimmune disease of the thyroid. However, further studies need to be conducted on larger samples to confirm the connection between PBB exposure, hypothyroidism and autoimmune thyroiditis.

Furthermore, a 1979 study by Bekeski et al. of 55 adult dairy farmers that had consumed the PBB contaminated food supply showed a decrease in available blood lymphocytes and significant increase in the number of lymphocytes without membrane markers compared to non-exposed farmers from Wisconsin (29). Authors of this study hypothesized that PBB may selectively bind to T-lymphocytes, therefore blocking surface antigens from detection. According to results of the study, 18 of 55 farmers from Michigan had reductions in cell mediated immune responses (29). This suggests that PBB

may be detrimental to other immune system pathways beyond the thyroid through mechanisms such as cellular mimicry.

In 2009, Burek et al. hypothesized that the connection between PBB and autoimmune thyroiditis may be related to exposure to bromine. Researchers exposed mice to KBR as a proxy for PBB and observed a one and half fold increase in the likelihood of developing autoimmune thyroiditis among exposed mice, compared to unexposed mice (30). Similar findings have yet to be observed in human studies.

However, whether exposure to PBBs is associated with the development of other autoimmune disorders remains poorly understood. Previous studies linking halogenated compounds to autoimmune disorders yield inconsistent results. For example, according to a study of the Michigan PBB Registry conducted in 2006 by Vasiliu et al., higher blood levels of PBB were not found to be associated with an elevated risk for diabetes mellitus (31). However, in this cohort elevated polychlorinated biphenyl serum levels were associated with incidence of diabetes mellitus in women but not in men (31). The difference in disease mechanism between these two similarly structured endocrine disrupters remains poorly understood.

Furthermore, studies linking PBB exposure to specific autoimmune diseases, such as rheumatoid arthritis, crohn's disease, fibromyalgia, psoriasis, and colitis are missing from the literature. This indicates that more research is needed to understand the impact of PBB exposure on the development of autoimmune disorders. Moreover, it is well known that autoimmune disorders occur more frequently in women than in men. However, whether this relationship holds true in populations exposed to PBBs remains unexplored.

Chapter II: Manuscript

Abstract

Background: Polybrominated Biphenyls (PBBs) are synthetic halogenated compounds that were originally used as flame retardants. In 1973, individuals living in Michigan were exposed to PBBs through the consumption of contaminated meat and dairy products after an accidental substitution of a flame retardant containing PBB for animal feed. Previous studies on this cohort suggest that exposure to PBBs may be related to many health conditions. However, no studies have evaluated whether exposure to PBBs is associated with autoimmune disease. In this cross-sectional study, we evaluated this possibility and whether this association differs by sex and age at exposure.

Methods: Current serum PBB levels were natural log transformed and evaluated for their association with self-reported autoimmune disease. Logistic regression was performed to evaluate models stratified by sex and age at exposure. Potential confounders included sex, age at blood draw, BMI, and smoking status. All statistical analyses were conducted in SAS version 9.4 and p-values less than 0.05 were considered significant.

Results: Overall, current serum PBBs were not associated with lifetime prevalence of autoimmune disease. However, among 228 individuals exposed to PBBs in utero, women were over four times as likely to have an autoimmune disease compared to men after controlling for age at blood draw (OR=4.234 [1.872, 9.580]). In addition, increasing PBB was associated with a reduced risk of autoimmune disease and that association varied in magnitude, depending on the age at blood draw. Among 340 individuals exposed to PBBs as children, women were twice as likely to have an autoimmune disease compared to men (OR=2.167[1.245-3.772]]). Among 284 individuals exposed to PBBs as adults, women were nearly twice as likely to have an autoimmune disease compared to men (OR=1.911 [1.047, 3.490]). These results were statistically significant.

Conclusions: Exposure to PBBs was not significantly associated with autoimmune disease in the study population. Among those exposed in utero, the association between PBBs and autoimmune disease differed by age at blood draw. Exposed women were significantly more likely to have autoimmune disease than men. The magnitude of the effect of participant sex differed by whether participants were exposed in utero, as children, or as adults.

Introduction

Polybrominated Biphenyls (PBBs) are synthetic halogenated compounds produced through the bromination of biphenyl. These compounds were originally used as flame retardants and were incorporated in the manufacturing of electronics, plastics, carpets, and other home furnishings during the 1970s. Studies conducted on animal models and human groups suggest that exposure to PBBs may be related to a wide variety of health conditions including thyroid dysfunction, delayed growth and development among boys, accelerated pubertal development among girls, altered hormone levels, breast and digestive cancer, spontaneous abortion, and lower Apgar scores among infants.

To further research the health effects of PBBs on humans, we utilized the Michigan Polybrominated Biphenyl (PBB) Registry which is comprised of approximately 7500 individuals that were exposed to PBBs through the consumption of contaminated meat and dairy products after an accidental substitution of a flame retardant containing PBB for animal feed. Although the production of PBBs in the United States was discontinued in 1976, many Michigan residents continue to have serum PBB levels that are significantly higher than the general population due to the biological stability of the chemical (3). Therefore, continued research on the health effects of PBBs is warranted within this population.

The mechanisms by which exposure to PBBs may contribute to the development of autoimmune diseases remains poorly understood. One possible mechanism is through the development of hypothyroid disease and the subsequent elevation of antithyroid microsomal antibodies, as described by Bahn et al. (19). It has also hypothesized that

PBB may selectively bind to T-lymphocytes, therefore blocking surface antigens from detection and affecting body systems through cellular mimicry (29). In 2009, Burek et al. hypothesized that the connection between PBB and autoimmune thyroiditis may be related to exposure to bromine itself (30).

Furthermore, although it is known that autoimmune disorders occur more frequently among women than men, the mechanisms by which sex impacts the development of autoimmune disorders over the life course warrant further investigation. It is hypothesized that differences in sex chromosomes, microRNA expression, and gut microbiota by sex may influence this trend (24). Furthermore, according to Ortona et al., the presence of estrogens may impact the progression and severity of autoimmune disorders due to the selective binding of estrogens to hormone receptors on immune cells (24). The extent to which exposure to PBBs could influence these biological mechanisms remains undetermined, although PBBs are known endocrine disrupters.

To our knowledge, no studies have evaluated whether exposure to PBBs is associated with the development of lifetime autoimmune disease. Further, studies linking PBB exposure to specific autoimmune diseases, such as rheumatoid arthritis, crohn's disease, fibromyalgia, psoriasis, and colitis are missing from the literature. Although it is known that autoimmune diseases are related to environmental exposures and can occur at varying ages across the lifespan, whether the association holds true in populations exposed to PBBs remains unexplored (22, 32, 33). Further, research is lacking as to whether the relationship varies by age of exposure to an environmental contaminant.

Methods

Study Design and Population

Data for this study came from the Michigan PBB Registry, which consists of approximately 7,500 participants exposed to PBB that have been followed longitudinally over the last 40 years. The study sample was restricted to adults aged 18 or older that had serum PBB levels collected between 2012-2018. In addition, participants in this study completed at least one of three online questionnaires about their demographic characteristics and health outcomes including autoimmune diseases. Questionnaires were completed close to the time that blood samples were collected. The three guided questionnaires included a generalized health questionnaire (GHQ) completed between 2012-2015, an in-depth questionnaire (IDQ) completed between 2012-2015 and a comprehensive health questionnaire (CHQ) completed between 2017-2019.

Description of Covariates

All three questionnaires asked, “Has a doctor ever told you that you had any of the following conditions?” The conditions posed to the study participants for the GHQ were non-specific and stated as “autoimmune disease”. If the participant answered yes, a follow up question was asked regarding the type of autoimmune disease that the participant had. Prior to harmonizing the data from three surveys, the autoimmune type variable from the GHQ was cleaned such that separate variables were created for each autoimmune disease reported from participants, if they were in fact autoimmune diseases. If participants stated that they had a condition that was not an autoimmune disease, their response to the non-specific autoimmune disease question was changed to ‘no’.

By contrast, the IDQ and CHQ asked about doctor diagnosis of specific autoimmune diseases by name. 374 (43.9%) participants completed more than one questionnaire. If responses were conflicting with regard to covariates of interest, the response to the more detailed questionnaire was kept. Responses to the CHQ were kept if conflicting answers were provided to questions on the CHQ and the IDQ, and responses to the IDQ were kept if conflicting answers were provided to questions on the IDQ and the GHQ.

In order to evaluate data from three questionnaires, combined variables for each autoimmune disease of interest were created. Responses such as "I may have this condition but have not been diagnosed by a doctor" and "Don't know" were considered no responses and compared to the responses of other surveys to determine the coding of the combined variable for that particular autoimmune disease. Variables were created using these methods for the following conditions: rheumatoid arthritis, crohn's disease, ulcerative colitis, multiple sclerosis, connective tissue disease, celiac disease, alopecia, eczema, psoriasis, fibromyalgia, lupus, graves' disease, sarcoidosis, psoriatic arthritis, and Hashimoto's thyroiditis.

Each autoimmune disease was further categorized according to the body system most impacted, to allow for analysis of the association between PBB exposure and type of autoimmune disease. The following conditions were considered autoimmune diseases of the skin and/or joints: rheumatoid arthritis, alopecia, eczema, psoriasis, vitiligo, and juvenile dermatitis. Furthermore, ulcerative colitis, crohn's disease, and celiac disease were considered diseases of the digestive system. Autoimmune diseases of the thyroid included Hashimoto's thyroiditis and graves' disease. Additionally, fibromyalgia and

multiple sclerosis were considered neurological autoimmune diseases. Finally, conditions that affect multiple body systems were coded as “other” including lupus, sarcoidosis, and Sjogren’s syndrome.

PBB levels were obtained from blood samples provided by participants close to the time of survey completion. One blood sample was collected per questionnaire completed. For participants that completed more than one questionnaire during the data collection period and therefore provided more than one blood sample, their PBB level was calculated by averaging the PBB levels from each blood sample. PBB level was natural log transformed before conducting subsequent analyses. For participants that had PBB levels below the limit of detection (LOD), levels were imputed with LOD/SQRT(2), as follows: 0.001/ 1.4142 for serum blood samples collected between 2012-2015, and 0.02/1.4142 for serum blood samples collected between 2017-2018.

Demographic variables were created for Body Mass Index (BMI), age at blood draw, and age at exposure. Participants’ self-reported heights and weights were used to calculate BMI on each survey. For those participants that completed more than one questionnaire, their BMI was calculated by averaging their height and weight from each questionnaire. Age at blood draw was calculated from a participant’s date of birth and the date their blood sample was collected. For participants with more than one blood sample, their most recent age was used in the analysis. Each participant’s age at exposure to PBB was calculated using their date of birth and the date July 1, 1973 as a proxy for the PBB contamination event. Age at exposure was further categorized into groups by those born after July 1, 1973, those that were less than or equal to 18 years old at the time of the

contamination event and those that were greater than 18 years old at the time of the contamination event.

To create a summary variable for smoking status, participant responses were combined across questionnaires, and participants were considered smokers if they indicated an affirmative response on at least one survey. If there were conflicting responses, responses from the most recent questionnaire were kept. A variable for race was created by combining responses across questionnaires. If a participant indicated that they were a specific race on at least one questionnaire they were considered as being the race of interest. If a participant indicated more than one race, they were considered multiracial. For education, participant responses were also combined across questionnaires. If there were conflicting responses, the highest education level was kept. Lastly, for income, participant responses were combined across questionnaires in a similar way as previously described. If there were conflicting responses, the most recent survey response was kept.

Statistical Analysis

PBB levels were natural log transformed and categorized into tertiles of PBB exposure. Tertiles of PBB exposure were also created within age at exposure categories. Descriptive analyses were conducted for the variables of interest. For categorical variables, counts and percentages were reported. For continuous variables, mean values and standard deviations were reported (Table 1 and Table 2). During bivariate analyses, chi square and fisher's exact tests were used to assess statistical differences in the distribution of demographic and outcome variables among participants in each level of PBB exposure (Table 3 and Table 7). These tests were also performed to assess statistical

differences in the distribution of demographic variables among participants by autoimmune disease diagnosis status (Table 6). For continuous variables, t-tests and anova tests were used to assess statistical differences between mean values among participants by autoimmune diagnosis status and in each level of PBB exposure (Table 4 and Table 5).

Logistic regression was performed to determine if current serum PBBs were associated with the occurrence of lifetime autoimmune disease. Both crude and adjusted models were evaluated. Potential confounders included sex, age at blood draw, age at exposure, BMI, and smoking status. Models stratified by sex and age at exposure were also evaluated (Table 8). Finally, an interaction assessment was conducted to determine if the association between exposure to PBBs and autoimmune disease was modified by age at blood draw, sex, or smoking status within the applicable stratified model. Sub analyses were conducted and included running similar models using types of autoimmune diseases or specific autoimmune diseases as the outcome (Table 9 and Table 10), and a sensitivity analysis to determine if results differed among groups with varying reasons for study participation. All statistical analyses were conducted in SAS version 9.4 and p-values less than 0.05 were considered significant. Emory University IRB reviewed and approved this study. In addition, all participants consented to participate in the blood draw and questionnaires.

Results

There were 852 participants in the final study sample. Of these, 797 (95.2%) identified as White. Data on the other demographic characteristics are presented in Table

1. Fifty-six percent of participants were female (n=480). Additionally, 504 participants (59.5%) were non- smokers, and 428 participants (50.3%) were overweight or obese. Seventy-three percent of participants (n=624) were alive at the time of the PBB contamination event in Michigan and fifty-six percent of the sample were between 30-60 years old at the time of blood draw. Because a majority of the sample were exposed to PBB in childhood or adulthood, age at exposure and age at blood draw were positively correlated in this sample ($r=0.99463$, $p<0.0001$). Additionally, a quarter of participants (24.1%) reported having at least one autoimmune disease (n=205). The majority of autoimmune disease diagnoses were skin and joint autoimmune diseases, which comprised 19.8% of the total sample (Table 2).

The proportion of individuals with autoimmune disease diagnoses did not differ significantly across tertiles of increasing serum PBB concentration ($p=0.6176$). However, significantly more males were highly exposed to PBB compared to females ($p<0.0001$) (Table 3). Additionally, the distribution of smoking status and BMI did not vary significantly across tertiles of increasing serum PBB level ($p=0.5024$ and $p=0.4180$, respectively) (Table 3). Furthermore, the mean age at blood draw increased across tertiles of increasing serum PBB concentration ($p<0.0001$) (Table 4). The distribution of age at exposure among those born before 7/1/1973, also varied significantly across tertiles of serum PBB concentration ($p<0.0001$) (Table 4). Mean serum PBB levels were lower but not significantly different among those that reported having at least one autoimmune disease compared to those that did not report having an autoimmune disease ($p=0.1690$) (Table 5).

The distribution of sex was significantly different by autoimmune disease diagnosis status ($p<0.0001$); 148 (30.9%) females reported being diagnosed with at least one autoimmune disease, compared to 57 (15.3%) males (Table 6). The distribution of predictors such as smoking status, BMI, age at blood draw, age at exposure and serum PBB concentration were not significantly different across autoimmune disease diagnosis status. Likewise, our outcomes of interest did not vary significantly across tertiles of PBB concentration within age at exposure categories (Table 7).

To account for collinearity between age at blood draw and age at exposure, as well as the differing PBB levels among age at exposure categories, we ran logistic regression models stratified by age at exposure and controlled for age at blood draw and sex within each age at exposure stratum. In the final sample, there were 228 individuals exposed to PBBs in utero, 340 individuals exposed to PBBs in childhood, and 284 individuals exposed to PBBs in adulthood. Among those exposed to PBBs in utero and in childhood, the association between current serum PBBs and lifetime prevalence of autoimmune disease was negative, while the association was positive among those exposed to PBBs in adulthood. Comparing across strata, the effect of current serum PBBs on the odds of autoimmune disease increased as the age at exposure increased. However, this association was statistically significant among those exposed as children only (OR=0.795 [0.654, 0.966]) (Table 8).

We tested for interaction between current serum PBBs and sex as well as current serum PBBs and age at blood draw within each age at exposure strata. We found no significant interaction except between current serum PBBs and age at blood draw among those exposed in utero. Within this subgroup, increasing PBB was associated with a

reduced risk of autoimmune disease and that association varied in magnitude, depending on the age at blood draw (p=0.00656 by likelihood ratio test). The odds ratios among those exposed in utero were 0.5430 [0.3637, 0.8105] and 0.8359 [0.6868, 1.0172] for individuals at age 18 and age 30, respectively (Table 8).

Additionally, the impact of sex on the risk of autoimmune disease varied across age at exposure strata. As age at exposure increased, the impact of sex decreased. Among those exposed to PBBs in utero, women were over four times as likely to have an autoimmune disease compared to men after controlling for the interaction between PBBs and age at blood draw (OR=4.234 [1.872, 9.580]). Among those exposed to PBBs as children, women were twice as likely to have an autoimmune disease compared to men (OR=2.167[1.245, 3.772]]), when adjusted for age at blood draw. Among those exposed to PBBs as adults, women were nearly twice as likely to have an autoimmune disease compared to men (OR=1.911 [1.047, 3.490]), when adjusted for age at blood draw. These results were statistically significant (Table 8).

We performed additional analyses using data from women in our sample to study the association between current serum PBBs and outcomes such as skin and joint autoimmune diseases and eczema. After running models stratified by age at exposure and controlling for age at blood draw among 480 women, the association between current serum PBBs and skin and joint autoimmune diseases was negative in all strata and reached statistical significance among those exposed in childhood only (OR=0.729 [0.571, 0.932]) (Table 9). Similarly, among 344 females with data on whether or not they were ever diagnosed with eczema, the association between current serum PBBs and

eczema was negative in all strata, and only reached statistical significance among those exposed to PBBs in childhood (OR=0.744 [0.556, 0.994]) (Table 10). There was no evidence of significant interaction in any model. As age at exposure increased, the magnitude of the association between serum PBBs and eczema decreased.

Discussion

The overall prevalence of autoimmune disease in our study population (24.1%) was considerably higher than the estimated 3-5% prevalence in the United States population (22). In addition, approximately 72% of autoimmune diagnoses in our population were among women. This aligns with the current literature, since it is known that women are more likely than men to develop autoimmune disease. For example, rheumatoid arthritis, the most prevalent condition in our sample, has a 2:1 sex ratio (22).

Our results show that overall, current serum PBBs are not associated with lifetime prevalence of autoimmune disease. We hypothesize that this may be due to PBBs mimicking estrogen in the body, and subsequent biological feedback mechanisms in which women exposed to PBBs produce less natural estrogen (34). Estrogen is a known risk factor for autoimmune disease, so it is plausible that with less natural production of the hormone, exposure to PBBs is negatively associated with the development of autoimmune disease.

Additionally, multivariable logistic regression analyses indicate that the impact of sex on the risk of autoimmune disease differs in this population depending on when individuals were exposed to the flame retardant chemical. Women exposed in utero were over four times as likely as men exposed in utero to have an autoimmune disease, while

women exposed as adults were less than twice as likely than men in the same exposure group to have an autoimmune disease. It is worth noting that age at exposure and age at blood draw are highly correlated in this population. As a result, those exposed in utero are also the youngest members of this cohort. Therefore, the impact of sex on the development of autoimmune disease decreases as age increases among those exposed to PBBs.

The trend we observed with decreasing sex ratios as age increases supports study results referenced by Ortona et. al. in which sex ratios for the risk of lupus were between 8:1 and 15:1 in the pre-menopausal population and declined to 5:1 after menopause (24). The proposed biological mechanism for this trend involves decreasing estrogen production in women after menopause. As women age, their estrogen levels become more similar to their age-matched male counterparts and the difference in autoimmune risk by sex is less pronounced at older ages. Our results provide evidence supporting this phenomenon.

Furthermore, we observed a statistically significant interaction between serum PBB levels and age at blood draw among those exposed to PBBs in utero only. There was no evidence of statistically significant interaction in the other strata. Among those exposed to PBBs in utero, increasing PBB levels was associated with a reduced risk of autoimmune disease and that association varied in magnitude, depending on the age at blood draw.

We conducted a sensitivity analysis to assess if selection bias influenced our study results. We restricted our study population by removing participants from our sample that indicated in the questionnaire that their participation was due to concerns about exposure

to PBBs or about specific health conditions. 416 individuals were removed from the sample on the basis of these reasons for participating. Participants that did not answer questions about why they participated in the study were excluded from the sensitivity analysis. The remaining population, comprised of individuals that did not express concerns with health conditions or PBB exposures as reasons for participating in the study, was limited to 155 participants in total; of which 65 individuals were exposed to PBBs in utero, 68 individuals were exposed in childhood and 22 individuals were exposed as adults. We compared the distribution of serum PBBs in the restricted population (n=155) with the distribution of serum PBBs among those that participated due to health or exposure concerns (n=416) and analyzed the proportion of autoimmune diagnoses by age at exposure categories within each population. We also ran multivariable logistic regression models stratified by age at exposure with serum PBBs, age at blood draw, and sex as covariates among the restricted study population (n=155). However, these models were unstable, due to small sample sizes, and could not be compared to the results of models run using our full sample (n=852).

Members of our study that participated due to concerns over health conditions or PBB exposures had higher average serum PBB levels (geometric mean=0.14 ng/mL) than those that participated in our study due to other reasons (geometric mean=0.12 ng/mL), although these averages were not significantly different (p=0.4633). Additionally, the proportion of participants with PBB levels below the limit of detection was lower among members of our study that participated due health or PBB exposure concerns (10.6%) compared to those that participated in our study due to other reasons (12.9%). Furthermore, the proportion of autoimmune diagnoses was higher among those that

participated due to health or exposure concerns, compared to those that participated due to other reasons for each strata of age at exposure. Among those that participated due to health concerns or concerns over PBB exposures and were exposed to PBBs in utero, 30.1% reported an autoimmune diagnosis, compared to 12.3% in the restricted population that participated for other reasons and were in the same age exposure group. Among those exposed as children, the proportion of autoimmune diagnoses was 33.5% versus 14.7% for those that participated due to the concerns noted and those that participated for other reasons, respectively. Finally, among those exposed as adults, the proportion of autoimmune diagnoses was 32.1% versus 13.6% for those that participated due to the concerns noted and those that participated for other reasons, respectively. Our sensitivity analysis results indicate that the increased participation among those with concerns over PBB exposure and current health conditions biased our sample by overrepresenting individuals with higher proportions of autoimmune disease and higher PBB levels. This may have biased our study results away from the null.

As noted previously, the concentration of PBBs in blood is influenced by a number of biological factors including the presence of adipose tissue and the rate of expenditure of the flame retardant. Therefore, an individual's serum PBB levels can change over time (35). To further study the association between exposure to PBBs and lifetime autoimmune disease, we ran multivariable logistic regression models stratified by age at exposure with enrollment serum PBB levels (collected 1975-1979), age at enrollment, and sex as covariates among those that had blood drawn at the time of enrollment into the PBB Registry (n=238). We tested for interaction between our exposure of interest and sex, as well as between the exposure and age at enrollment.

There was no evidence of statistically significant interaction in any strata. Additionally, we were unable to obtain odds ratios for our association of interest among those exposed in utero, due to a limited number of individuals in this subgroup that had enrollment PBB levels. Among those exposed to PBBs in childhood, enrollment serum PBBs were negatively associated with lifetime autoimmune disease (OR=0.981 [0.685,1.385]) though this result did not reach statistical significance. By contrast, among those exposed to PBBs in adulthood, enrollment serum PBBs were positively associated with lifetime autoimmune disease (OR=1.108 [0.776,1.584]), although these results also did not reach statistical significance. The magnitude of the association between enrollment serum PBBs and lifetime autoimmune disease was greater in both strata when compared to our main analysis using serum PBBs collected between 2012-2018. This suggests the need for a longitudinal study using serum PBBs collected at different points in the life course as well as the need to account for the change in PBB levels over time in subsequent analyses to accurately approximate the association between exposure to PBBs and lifetime autoimmune disease.

Chapter III: Extended Conclusions

Strengths and Limitations

The overall sample size (n=852) was a strength of this study. Our study included several cases of various autoimmune diseases, which comprised nearly a quarter of our sample. Our sample also included participants of varying ages and ages at exposure to PBBs. This allowed for comparisons of the relationship between PBBs and lifetime autoimmune disease throughout the life course.

Furthermore, the measurement and use of serum PBB levels as a predictor in logistic regression models added scientific rigor to the classification of our exposure variable. In this cohort, exposure to PBBs occurred prior to the collection of data about our health outcome of interest. Therefore, temporality is well established in this study and is an additional strength, although current serum PBB levels are influenced by a number of biological factors, that we may not have accounted for.

Despite the strengths of this research study, there were also several limitations. Although current PBB levels were measured from blood samples drawn around the time of outcome data collection, the instruments used to measure serum PBB concentrations were subject to limits of detection. As described in the methods section, the PBB level for some participants was imputed using a calculation based on the limit of detection. Therefore, the true PBB level for these individuals is unknown. However, due to the small percentage of participants whose PBB levels fell below the limit of detection, this is unlikely to have significantly impacted the results of the study.

Furthermore, autoimmune diagnoses were self-reported by participants on at least one of three questionnaires. On the GHQ, participants were asked to specify their condition if they responded positively to having at least one autoimmune disease. However, some participants reported having conditions that are not classified as autoimmune diseases. As a result, these responses were recoded as 'no' in the final dataset. It is possible that participants were unsure about what classifies as an autoimmune disease, incorrectly recalled their diagnoses, or were not aware that they had an autoimmune condition at the time of survey completion. Further, because certain autoimmune conditions are more common at older ages it is possible that individuals may not have developed autoimmune diseases at the time of data collection. These circumstances could have resulted in misclassification of the outcome and biased estimates toward the null.

In addition, obesity is a known predictor in the development of and progression of autoimmune diseases such as rheumatoid arthritis, multiple sclerosis, lupus, and psoriasis (27). The presence of adipose tissue and weight fluctuation also impacts the rate of PBB expenditure into the blood (36). However, because only a select portion of our final study sample had recorded heights and weights, we were unable to include BMI as a predictor in the full logistic regression analyses. We felt that the limited sample would not accurately predict the impact of current serum PBBs on lifetime autoimmune diagnoses. Future studies should be conducted with BMI as a predictor using larger sample sizes.

Public Health Implications

Our results suggest that exposure to PBBs is not significantly associated with lifetime autoimmune disease. However, exposed women in our study were more likely than exposed men to develop an autoimmune condition. Therefore, our study highlights the importance of increased autoimmune disease testing among women exposed to flame retardant chemicals. In addition, our results point to the importance of stratifying analyses of autoimmune disease by sex in future studies. Furthermore, the effect of sex on the development of autoimmune disease differed by whether participants were exposed in utero, as children, or as adults. Among those exposed to the flame retardant chemical in utero, increasing PBB level was associated with a reduced risk of autoimmune disease and that association varied in magnitude, depending on the age at blood draw.

Future Directions

Additional studies are necessary to further quantify the association between exposure to PBBs and the occurrence of lifetime autoimmune disease. In 2019, several PBB registry participants answered at least one questionnaire and provided a blood sample for the detection of current serum PBBs. These samples were not processed by the laboratory at the time of this analysis and therefore, were not included in the final study sample. Future studies would benefit from including these additional participants in the analysis to increase the sample size and strengthen the precision of the results.

Furthermore, future studies on this cohort could include BMI as a predictor in logistic regression models. As previously noted, obesity is a known predictor of autoimmune diseases, yet height and weight measurements were only available for a

select portion of our final study sample. Sub analyses with BMI included as a predictor could more accurately predict the association between exposure to PBBs and lifetime autoimmune disease.

Additionally, this analysis could be repeated over time as a longitudinal study due to the possibility that a person's serum PBB level could vary with time. This is due to several complex biological interactions including bioaccumulation, expenditure of PBB from adipose tissue and the interaction of PBBs and estrogen at various ages. Therefore, it would be beneficial to collect serum PBB levels at multiple times throughout the life course and account for the change in PBB levels over time to accurately reflect the risk of developing autoimmune disease after exposure to PBBs.

As noted in the discussion section, our results support the hypothesis that PBBs may be mimicking estrogen in the body and influencing biological mechanisms that result in women producing less natural estrogen. Additional studies could investigate this hypothesis further by exploring an interaction of estrogen levels and PBB exposure in the relationship with autoimmune disease.

References

1. Safe S, Hutzinger O. Polychlorinated Biphenyls (PCBs) and Polybrominated Biphenyls (PBBs): Biochemistry, Toxicology, and Mechanism of Action. *CRC Critical Reviews in Toxicology*. 1984;13(4):319-395. doi:10.3109/10408448409023762
2. Di Carlo FJ, Seifter J, DeCarlo VJ. Assessment of the hazards of polybrominated biphenyls. *Environ Health Perspect*. 1978;23:351-365. doi:10.1289/ehp.7823351
3. Chang CJ, Terrell ML, Marcus M, et al. Serum concentrations of polybrominated biphenyls (PBBs), polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in the Michigan PBB Registry 40 years after the PBB contamination incident. *Environ Int*. 2020;137:105526. doi:10.1016/j.envint.2020.105526
4. Kay K. Polybrominated biphenyls (PBB) environmental contamination in Michigan, 1973-1976. *Environ Res*. 1977;13(1):74-93. doi:10.1016/0013-9351(77)90006-8
5. Kasza L, Collins WT, Capen CC, Garthoff LH, Friedman L. Comparative toxicity of polychlorinated biphenyl and polybrominated biphenylin the rat thyroid gland: light and electron microscopic alterations after subacute dietary exposure. *J Environ Pathol Toxicol*. 1978;1(5):587-599.
6. McCormack KM, Braselton WE Jr, Sanger VL, Hook JB. Residual effects of polybrominated biphenyls following perinatal exposure in rats. *Toxicol Appl Pharmacol*. 1980;53(1):108-115. doi:10.1016/0041-008x(80)90387-7

7. Lambrecht LK, Barsotti DA, Allen JR. Responses of nonhuman primates to a polybrominated biphenyl mixture. *Environ Health Perspect*. 1978;23:139-145. doi:10.1289/ehp.7823139
8. Deng P, Barney J, Petriello MC, Morris AJ, Wahlang B, Hennig B. Hepatic metabolomics reveals that liver injury increases PCB 126-induced oxidative stress and metabolic dysfunction. *Chemosphere*. 2019;217:140-149. doi:10.1016/j.chemosphere.2018.10.196
9. McCormack KM, Melrose P, Rickert DE, Dent JG, Gibson JE, Hook JB. Concomitant dietary exposure to polychlorinated biphenyls and polybrominated biphenyls: tissue distribution and arylhydrocarbon hydroxylase activity in lactating rats. *Toxicol Appl Pharmacol*. 1979;47(1):95-104. doi:10.1016/0041-008x(79)90076-0
10. Landrigan PJ, Wilcox KR Jr, Silva J Jr, Humphrey HE, Kauffman C, Heath CW Jr. Cohort study of Michigan residents exposed to polybrominated biphenyls: epidemiologic and immunologic findings. *Ann N Y Acad Sci*. 1979;320:284-294. doi:10.1111/j.1749-6632.1979.tb56611.x
11. Terrell ML, Rosenblatt KA, Wirth J, Cameron LL, Marcus M. Breast cancer among women in Michigan following exposure to brominated flame retardants. *Occup Environ Med*. 2016;73(8):564-567. doi:10.1136/oemed-2015-103458
12. Hoque A, Sigurdson AJ, Burau KD, Humphrey HE, Hess KR, Sweeney AM. Cancer among a Michigan cohort exposed to polybrominated biphenyls in 1973. *Epidemiology*. 1998;9(4):373-378.

13. Small CM, Murray D, Terrell ML, Marcus M. Reproductive outcomes among women exposed to a brominated flame retardant in utero. *Arch Environ Occup Health*. 2011;66(4):201-208. doi:10.1080/19338244.2010.539640

14. Curtis SW, Gerkowicz SA, Cobb DO, et al. Sex-specific DNA methylation differences in people exposed to polybrominated biphenyl. *Epigenomics*. 2020;12(9):757-770. doi:10.2217/epi-2019-0179

15. Blanck HM, Marcus M, Tolbert PE, et al. Age at menarche and tanner stage in girls exposed in utero and postnatally to polybrominated biphenyl. *Epidemiology*. 2000;11(6):641-647. doi:10.1097/00001648-200011000-00005

16. Small CM, DeCaro JJ, Terrell ML, et al. Maternal exposure to a brominated flame retardant and genitourinary conditions in male offspring. *Environ Health Perspect*. 2009;117(7):1175-1179. doi:10.1289/ehp.0800058

17. Terrell ML, Hartnett KP, Lim H, Wirth J, Marcus M. Maternal exposure to brominated flame retardants and infant Apgar scores. *Chemosphere*. 2015;118:178-186. doi:10.1016/j.chemosphere.2014.08.007

18. Yard EE, Terrell ML, Hunt DR, et al. Incidence of thyroid disease following exposure to polybrominated biphenyls and polychlorinated biphenyls, Michigan, 1974-2006. *Chemosphere*. 2011;84(7):863-868. doi:10.1016/j.chemosphere.2011.06.020

19. Bahn AK, Mills JL, Snyder PJ, et al. Hypothyroidism in workers exposed to polybrominated biphenyls. *N Engl J Med*. 1980;302(1):31-33. doi:10.1056/NEJM198001033020105

20. Jacobson MH, Darrow LA, Barr DB, et al. Serum Polybrominated Biphenyls (PBBs) and Polychlorinated Biphenyls (PCBs) and Thyroid Function among Michigan Adults Several Decades after the 1973-1974 PBB Contamination of Livestock Feed. *Environ Health Perspect*. 2017;125(9):097020. Published 2017 Sep 26. doi:10.1289/EHP1302

21. Curtis SW, Terrell ML, Jacobson MH, et al. Thyroid hormone levels associate with exposure to polychlorinated biphenyls and polybrominated biphenyls in adults exposed as children. *Environ Health*. 2019;18(1):75. Published 2019 Aug 23. doi:10.1186/s12940-019-0509-z

22. Wang L, Wang FS, Gershwin ME. Human autoimmune diseases: a comprehensive update. *J Intern Med*. 2015;278(4):369-395. doi:10.1111/joim.12395

23. Ngo ST, Steyn FJ, McCombe PA. Gender differences in autoimmune disease. Front Neuroendocrinol. 2014 Aug;35(3):347-69. doi: 10.1016/j.yfrne.2014.04.004. Epub 2014 May 2. PMID: 24793874.

24. Ortona E, Pierdominici M, Maselli A, Veroni C, Aloisi F, Shoenfeld Y. Sex-based differences in autoimmune diseases. *Ann Ist Super Sanita*. 2016;52(2):205-212. doi:10.4415/ANN_16_02_12

25. Haghmorad D, Salehipour Z, Nosratabadi R, et al. Medium-dose estrogen ameliorates experimental autoimmune encephalomyelitis in ovariectomized mice. *J Immunotoxicol*. 2016;13(6):885-896. doi:10.1080/1547691X.2016.1223768

26. Curtis SW, Cobb DO, Kilaru V, et al. Exposure to polybrominated biphenyl (PBB) associates with genome-wide DNA methylation differences in peripheral blood. *Epigenetics*. 2019;14(1):52-66. doi:10.1080/15592294.2019.1565590

27. Versini M, Jeandel PY, Rosenthal E, Shoenfeld Y. Obesity in autoimmune diseases: not a passive bystander. *Autoimmun Rev*. 2014;13(9):981-1000. doi:10.1016/j.autrev.2014.07.001

28. de Hair MJ, Landewé RB, van de Sande MG, et al. Smoking and overweight determine the likelihood of developing rheumatoid arthritis. *Ann Rheum Dis*. 2013;72(10):1654-1658. doi:10.1136/annrheumdis-2012-202254

29. Bekesi JG, Roboz J, Anderson HA, et al. Impaired immune function and identification of polybrominated biphenyls (PBB) in blood compartments of exposed Michigan dairy farmers and chemical workers. *Drug Chem Toxicol*. 1979;2(1-2):179-191. doi:10.3109/01480547908993189

30. Burek CL, Talor MV. Environmental triggers of autoimmune thyroiditis. *J Autoimmun*. 2009;33(3-4):183-189. doi:10.1016/j.jaut.2009.09.001

31. Vasiliu O, Cameron L, Gardiner J, Deguire P, Karmaus W. Polybrominated biphenyls, polychlorinated biphenyls, body weight, and incidence of adult-onset diabetes mellitus. *Epidemiology*. 2006;17(4):352-359. doi:10.1097/01.ede.0000220553.84350.c5

32. Tzioufas AG, Kapsogeorgou EK, Moutsopoulos HM. Pathogenesis of Sjögren's syndrome: what we know and what we should learn. *J Autoimmun*. 2012;39(1-2):4-8. doi:10.1016/j.jaut.2012.01.002

33. Peng J, Narasimhan S, Marchesi JR, Benson A, Wong FS, Wen L. Long term effect of gut microbiota transfer on diabetes development. *J Autoimmun*. 2014;53:85-94. doi:10.1016/j.jaut.2014.03.005

34. Howards PP, Terrell ML, Jacobson MH, et al. Polybrominated Biphenyl Exposure and Menstrual Cycle Function. *Epidemiology*. 2019;30(5):687-694. doi:10.1097/EDE.0000000000001045

35. Sweeney AM, Symanski E, Burau KD, Kim YJ, Humphrey HE, Smithci MA. Changes in serum PBB and PCB levels over time among women of varying ages at exposure. *Environ Res*. 2001;86(2):128-139. doi:10.1006/enrs.2001.4261

36. Chevrier J, Dewailly E, Ayotte P, Mauriège P, Després JP, Tremblay A. Body weight loss increases plasma and adipose tissue concentrations of potentially toxic pollutants in obese individuals. *Int J Obes Relat Metab Disord*. 2000;24(10):1272-1278. doi:10.1038/sj.ijo.0801380

Tables

Table 1. Characteristics of PBB Registry Participants (2012-2018)

Variable		Full Sample (n=852) n (%)
Sex		
	Male	372 (43.7)
	Female	480 (56.3)
Race/Ethnicity		
	White	797 (95.2)
	Hispanic	9 (1.1)
	American Indian	2 (0.2)
	Mixed Race	28 (3.4)
	Other	1 (0.1)
Educational Attainment		
	Some high school (Grades 9 through 11)	10 (1.2)
	High school graduate or have a GED	127 (22.3)
	Some college or technical school	220 (38.6)
	College graduate	141 (27.7)
	Some graduate school or graduate degree	72 (12.6)
Smoking Status		
	Non-Smoker	504 (59.5)
	Ever Smoker	343 (40.5)
Income		
	Less than $10,000	54 (9.6)
	$10,000-less than $35,000	138 (24.6)
	$35,000- less than $50,000	85 (15.2)
	$50,000-less than $75,000	118 (21.1)
	$75,000-$100,000	87 (15.5)
	More than $100,000	78 (13.9)
BMI		
	Underweight or Normal	147 (17.3)
	Overweight	193 (22.7)
	Obese	235 (27.6)
	Missing	277
Age at Blood Draw		
	<30	103 (12.1)
	30-60	477 (56.0)
	>60	272 (31.9)
Age at Exposure		
	In Utero*	228 (26.8)
	≤18	340 (39.9)

	>18	284 (33.3)
Any Autoimmune Disease	No	647 (75.9)
	Yes	205 (24.1)
Skin or Joint Autoimmune Disease	No	681 (80.2)
	Yes	168 (19.8)
Autoimmune Disease of the Digestive System	No	825 (97.5)
	Yes	21 (2.5)
Neurological Autoimmune Disease	No	805 (94.8)
	Yes	44 (5.2)
Autoimmune Disease of the Thyroid	No	671 (98.8)
	Yes	8 (1.2)
Other Autoimmune Disease	No	561 (98.3)
	Yes	10 (1.8)

* Born after 7/1/1973

Table 2. Characteristics of PBB Registry Participants by Autoimmune Disease Type (2012-2018)

Autoimmune Disease Group		Full Sample (n=852) n (%)
Skin or Joint Autoimmune Disease*		168 (19.8)
	Rheumatoid Arthritis	70 (10.5)
	Connective Tissue Disease	8 (1.0)
	Alopecia	6 (1.1)
	Eczema	68 (12.1)
	Psoriasis	33 (5.8)
	Psoriatic Arthritis	12 (1.8)
Autoimmune Disease of the Digestive System*		21 (2.5)
	Crohn's Disease	8 (1.0)
	Ulcerative Colitis	11 (1.3)
	Celiac Disease	5 (0.9)
Neurological Autoimmune Disease*		44 (5.2)
	Multiple Sclerosis	8 (0.9)
	Fibromyalgia	37 (4.4)
Autoimmune Disease of the Thyroid		8 (1.2)
	Grave's Disease	3 (0.4)
	Hashimoto's Thyroiditis	5 (0.7)
Other Autoimmune Disease		10 (1.8)
	Lupus	7 (1.3)
	Sarcoidosis	2 (0.6)
	Sjogren's Syndrome	1 (0.2)

* Frequencies for individual conditions do not sum to the total frequency for the autoimmune disease group due to participants having more than one autoimmune condition within the disease group

Table 3. Characteristics of Participants by Serum PBB Concentration (2012-2018)

Variable		Low Serum PBB Concentration (n=284)	Medium Serum PBB Concentration (n=286)	High Serum PBB Concentration (n=282)	P
		n (%)	n (%)	n (%)	
Sex					
	Male	102 (27.4)	105 (28.2)	165(44.4)	*<0.0001
	Female	182 (37.9)	181 (37.7)	117 (24.4)	
Smoking Status					
	Non-Smoker	175 (34.7)	164 (32.5)	165 (32.7)	0.5024
	Ever-Smoker	106 (30.9)	120 (35.0)	117 (34.1)	
BMI					
	Underweight or Normal	66 (44.9)	37 (25.2)	44 (29.9)	0.4180
	Overweight	76 (39.4)	59 (30.6)	58 (30.1)	
	Obese	84 (35.7)	78 (33.2)	73 (31.1)	
Age at Blood Draw					
	<30	95 (92.2)	7 (6.8)	1 (1.0)	*<0.0001
	30-60	172 (36.1)	180 (37.7)	125 (26.2)	
	>60	17 (6.3)	99 (36.4)	156 (57.4)	
Age at Exposure					
	In Utero^{+}	207(90.8)	16 (7.0)	5 (2.2)	*<0.0001
	≤18	59 (17.4)	162 (47.7)	119 (35.0)	
	>18	18 (6.3)	108 (38.0)	158 (55.6)	
Any Autoimmune Disease	No	210 (32.5)	221 (34.2)	216(33.4)	0.6176
	Yes	74 (36.1)	65 (31.7)	66 (32.2)	

Skin or Joint	No	221 (32.5)	232 (34.1)	228 (33.5)	0.5459
Autoimmune Disease	Yes	62 (36.9)	54 (32.1)	52 (31.0)	
Autoimmune Disease of					
the Digestive System	No	274 (33.2)	278 (33.7)	273 (33.1)	0.5749
	Yes	9 (42.9)	7 (33.3)	5 (23.8)	
Neurological		270 (95.1)	272 (95.1)	263 (93.3)	
Autoimmune Disease	No	270 (33.5)	272 (33.8)	263 (32.7)	0.7008
	Yes	14 (31.8)	13 (29.6)	17 (38.6)	
Autoimmune Disease of					
the Thyroid	No	234 (34.9)	236 (35.2)	201 (30.0)	1.000
	Yes	3 (37.5)	3 (37.5)	2 (25.0)	
Other Autoimmune					
Disease	No	226 (40.3)	170 (30.3)	165 (29.4)	0.4430
	Yes	2 (20.0)	4 (40.0)	4 (40.0)	

* Statistically significant at a 0.05 significance level by chi square test
+ Born after 7/1/1973
Low Serum PBB Concentration **≤ 0.125 ng/mL**
Medium Serum PBB Concentration**>0.125 and ≤0.467 ng/mL**
High Serum PBB Concentration **>0.467 ng/mL**

Table 4. Characteristics of Participants by Serum PBB Concentration (2012-2018)

Variable	Low Serum PBB Concentration (n=284)	Medium Serum PBB Concentration (n=286)	High Serum PBB Concentration (n=282)	P
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	
BMI	29.2 (7.3)	30.5 (6.7)	29.5 (6.5)	0.2014
Age at Blood Draw in years	36.7 (12.5)	55.8 (11.6)	61.5 (11.1)	*<0.0001
Age at Exposure in years+	11.9 (9.4)	16.2 (10.1)	20.3 (10.3)	*<0.0001

* Statistically significant at a 0.05 significance level by anova test
+ Includes participants born before 7/1/1973
Low Serum PBB Concentration **≤ 0.125 ng/mL**
Medium Serum PBB Concentration**>0.125 and ≤0.467 ng/mL**
High Serum PBB Concentration **>0.467 ng/mL**

Table 5. Participant Characteristics by Autoimmune Disease Diagnosis (2012-2018)

Variable	No Autoimmune Disease Diagnosis (n=647)	Autoimmune Disease Diagnosis (n=205)	P
	Mean (SD)	Mean (SD)	
BMI	29.4 (6.8)	30.5 (7.1)	0.0730
Age at Blood Draw in years	51.3 (16.2)	51.6 (14.6)	0.8120
Age at Exposure in years+	10.2 (15.8)	10.1(14.3)	0.9056
Serum PBB Concentration in ng/mL*	0.20(8.8)	0.15 (9.5)	0.1690

+ Includes participants born before 7/1/73

* Geometric means and standard deviations reported

Table 6. Characteristics of Participants by Autoimmune Disease Diagnosis (2012-2018)

Variable		No Autoimmune Disease Diagnosis (n=647)	Autoimmune Disease Diagnosis (n=205)	P
		n (%)	n (%)	
Sex				
	Male	315 (84.7)	57 (15.3)	*<0.0001
	Female	332 (69.2)	148 (30.9)	
Smoking Status				
	Non-Smoker	381 (75.6)	123 (24.4)	0.7919
	Ever-Smoker	262 (76.4)	81 (23.6)	
BMI				
	Underweight or Normal	114 (77.6)	33(22.5)	0.2501
	Overweight	139 (72.0)	54 (28.0)	
	Obese	164 (69.8)	71 (30.2)	
Age at Blood Draw				
	<30	84 (81.6)	19 (18.5)	0.1148
	30-60	350 (73.4)	127 (26.6)	
	>60	213 (78.3)	59 (21.7)	
Age at Exposure				
	In Utero+	179 (78.5)	49 (21.5)	0.1837
	≤18	247 (72.7)	93 (27.4)	
	18	221 (77.8)	63 (22.2)	
Serum PBB Concentration (ng/mL)				
	Low Serum PBB Concentration	210 (73.9)	74 (26.1)	0.6176
	Medium Serum PBB Concentration	221 (77.3)	65 (22.7)	
	High Serum PBB Concentration	216 (76.6)	66 (23.4)	

* Statistically significant at a 0.05 significance level by chi square test
+ Born after 7/1/1973
Low Serum PBB Concentration **≤ 0.125 ng/mL**
Medium Serum PBB Concentration**>0.125 and ≤0.467 ng/mL**
High Serum PBB Concentration **>0.467 ng/mL**

Table 7. Autoimmune Disease Groups by Serum PBB Concentration within Different Age at Exposure Categories

	In Utero (n=228)				≤18 (n=340)			
Autoimmune Disease Group	**Low Serum PBB Concentration (n=77)**	**Medium Serum PBB Concentration (n=76)**	**High Serum PBB Concentration (n=75)**	**P**	**Low Serum PBB Concentration (n=114)**	**Medium Serum PBB Concentration (n=112)**	**High Serum PBB Concentration (n=114)**	**P**
	n (%)				**n (%)**			
Any Autoimmune Disease	20 (40.9)	15 (30.6)	14 (28.6)	0.4940	38 (40.9)	31 (33.3)	24 (25.8)	0.1145
Skin or Joint Autoimmune Disease	16 (41.0)	11 (28.2)	12 (30.8)	0.5654	33 (45.2)	21 (28.8)	19 (26.0)	0.0575
Autoimmune Disease of the Digestive System	3 (42.9)	1 (14.3)	3 (42.9)	0.6366	2 (20.0)	4 (40.0)	4(40.0)	0.6710
Neurological Autoimmune Disease	2 (22.2)	5 (55.6)	2 (22.2)	0.4770	10 (35.7)	10 (35.7)	8 (28.6)	0.8550
Autoimmune Disease of the Thyroid	0 (0.0)	1 (100.0)	0 (0.00)	0.6458	2 (33.33)	3 (50.0)	1 (16.7)	0.8740
Other Autoimmune Disease	0 (0.0)	0 (0.0)	2 (100.0)	0.0938	1 (12.5)	3 (37.5)	4 (50.0)	0.5453

Low Serum PBB Concentration **≤ 0.012 ng/mL**
Medium Serum PBB Concentration **>0.012 and ≤0.0362 ng/mL**
High Serum PBB Concentration **>0.0362 ng/mL**

Low Serum PBB Concentration **≤ 0.234 ng/mL**
Medium Serum PBB Concentration **>0.234 and ≤0.48762 ng/mL**
High Serum PBB Concentration **>0.48762 ng/mL**

	>18 (n=284)			
Autoimmune Disease Group	**Low Serum PBB Concentration (n=95)**	**Medium Serum PBB Concentration (n=95)**	**High Serum PBB Concentration (n=94)**	**P**
	n (%)			
Any Autoimmune Disease	20 (31.8)	17 (27.0)	26 (41.3)	0.2572
Skin or Joint Autoimmune Disease	19 (33.9)	14 (25.0)	23 (41.1)	0.2275
Autoimmune Disease of the Digestive System	2 (50.0)	1 (25.0)	1 (25.0)	1.000
Neurological Autoimmune Disease	1 (14.3)	4 (57.1)	2 (28.6)	0.5116
Autoimmune Disease of the Thyroid	0 (0.0)	1 (100.0)	0 (0.00)	0.6222
Other Autoimmune Disease	0 (0.0)	0 (0.0)	0 (0.0)	

Low Serum PBB Concentration **≤ 0.33 ng/mL**
Medium Serum PBB Concentration **>0.33 and ≤0.92038 ng/mL**
High Serum PBB Concentration **>0.92038 ng/mL**

Table 8. Adjusted Odds Ratios (OR) and 95% Confidence Intervals for the Association between PBB Exposure and Autoimmune Disease Stratified by Age at Exposure

	OR	95% CI	
Exposed in Utero[^]			
Log PBB at Age 18	0.5430*	0.3637	0.8105
Log PBB at Age 30	0.8359	0.6868	1.0172
Sex[+]	4.234*	1.872	9.580
Exposed as Children			
Log PBB	0.795*	0.654	0.966
Sex[+]	2.167*	1.245	3.772
Age at Blood Draw	0.996	0.949	1.044
Exposed as Adults			
Log PBB	1.056	0.858	1.300
Sex[+]	1.911*	1.047	3.490
Age at Blood Draw	0.998	0.958	1.040

*Statistically significant at a 0.05 significance level

[^]Odds Ratios for this group presented at different ages due to evidence of statistically significant interaction between log PBB and age at blood draw by likelihood ratio test (p=0.0066) among those exposed in utero only

[+]Males as reference category

Table 9. Adjusted Odds Ratios (OR) and 95% Confidence Intervals for the Association between PBB Exposure and Skin and Joint Autoimmune Disease Stratified by Age at Exposure Among Females

	OR	95% CI	
Exposed in Utero			
Log PBB	0.897	0.672	1.042
Age at Blood Draw	1.025	0.962	1.091
Exposed as Children			
Log PBB	0.729*	0.571	0.932
Age at Blood Draw	1.009	0.949	1.074
Exposed as Adults			
Log PBB	0.899	0.638	1.266
Age at Blood Draw	1.023	0.967	1.082

*Statistically significant at a 0.05 significance level

Table 10. Adjusted Odds Ratios (OR) and 95% Confidence Intervals for the Association between PBB Exposure and Eczema Stratified by Age at Exposure Among Females

	OR	95% CI	
Exposed in Utero			
Log PBB	0.814	0.621	1.066
Age at Blood Draw	1.025	0.948	1.109
Exposed as Children			
Log PBB	0.744*	0.556	0.994
Age at Blood Draw	1.021	0.944	1.106
Exposed as Adults			
Log PBB	0.633	0.321	1.248
Age at Blood Draw	0.985	0.870	1.115

*Statistically significant at a 0.05 significance level