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Exposure to Brominated Flame Retardants and Cognitive Function

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Exposure to Brominated Flame Retardants and Cognitive Function

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Bachelor of Science, Neuroscience

University of California Riverside

2019

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Abstract

Exposure to Brominated Flame Retardants and Cognitive Function

By Eduardo Monarrez

Background: Polybrominated Biphenyls (PBBs) are synthetic, bioaccumulative, compounds that were used as flame retardants. In 1973, a chemical factory incident resulted in the exposure of PBB through contamination of cattle feed and human consumption of by-products for years. The Michigan PBB Registry was created, and the occurrence of various health conditions have been obtained out of this cohort. No study has been done to look at the effect of PBB on mild cognitive impairment to date. In this longitudinal, cross-sectional study, we test the association between PBB exposure and abnormal scores on the cognitive functioning instrument.

Methods: Tertiles were created of serum PBB levels to evaluate their association with abnormal scores on the Cognitive Function Instrument. Serum PBB levels were also natural log transformed to evaluate their association with abnormal CFI scores. Logistic regression was performed to evaluate models including age and age at exposure, separately. Potential confounders included sex, age, age at exposure, cardiovascular conditions, smoking status, and alcohol use. The cognitive functioning instrument results were dichotomized for analysis. All statistical analyses were conducted in SAS version 9.4 and p-values less than 0.05 were considered significant.

Results: Among our 316 participants, no statistically significant association was found between log transformed PBB and abnormal scores on the Cognitive Function Instrument in both of our models. Our adjusted odds ratio for our log-transformed PBB was 1.087 (0.758, 1.558) controlling for age, and 1.074 (0.756, 1.527) controlling for age at exposure. However, we did find an association between PBB exposure and hypertension in our bivariate analysis (p-value=0.0011).

Conclusions: Exposure to PBBs was not significantly associated with abnormal scores on the cognitive function instrument in our study population. An association between PBB exposure and hypertension was found in our study population.

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Chapter I: Literature Review

Introduction to Polybrominated Biphenyls and other POPS

Polybrominated biphenyls (PBBs) are Brominated flame retardants with a long half-life. They are synthetic, fat-soluble, bioaccumulative and persistent organic pollutants (POPs). The most common PBB congener seen is hexabromobiphenyl, or PBB153. There are a multitude of other POPs that have been researched like polybrominated diphenyl ethers (PBDEs), another brominated flame retardant and polychlorinated biphenyls (PCBs), the latter of which is similar in structure to PBB. Historically, these synthetic compounds were used in mixing with various hard plastics as a flame retardant.

In 1973, the Velsicol Chemical plant (formerly Michigan Chemical) accidently mixed PBB-containing flame retardant Firemaster in place of the livestock feed Nutrimaster that was distributed across the state for nearly a year (1). Due to this incident, in 1976, the Michigan Long-Term PBB study was established by the Michigan Department of Health and Human Services (MDHHS), formally the Michigan Department of Public Health, with CDC and NIH as partners to determine any health effects caused by this exposure, which included participants with varying degrees of PBB exposure (2). During the period of exposure, thousands of livestock ingested the contaminated feed and Michigan residents consumed beef, milk, chicken and other products of the animals. Due to hydrocarbon's fat-soluble nature, accumulation in the body has been detected in humans, most notably in large concentrations through breastmilk, with PBB contamination passing from mother to infant, and crossing through the placenta(3). This is concerning that mothers are passing this contamination to their offspring, exposing them at an early age when their brains are developing. Additionally, a study conducted 40 years after the incident found that those who were former employees of Velsicol Chemical, family of former

chemical workers, individuals who lived or ate food from contaminated farms, and those born after the incident have higher than average PBB-153 levels compared to the rest of the United States (4). The persistent nature of PBB, and intergenerational pattern demonstrates the importance of exploring PBB-related health effects.

Impact of Polybrominated Biphenyls and POPS on Health

The Michigan Long-Term PBB Study, or PBB registry, has helped identify all possible occurrences of disease. Early studies of this cohort found that this population had affected mood (depression), prose recall, short-term memory, concentration, and cognitive flexibility (5, 6). As well as a higher prevalence of skin, neurological (headaches, blurred vision, dizziness, depression, fatigue, perception changes, nervousness, sleeplessness, sleepiness, weakness, trouble ambulating, paresthesia, clumsiness, and slowness), and musculoskeletal symptoms in Michigan residents compared to non-exposed control groups (7). Many years later studies have found that PBBs are Endocrine-Disrupting Chemicals (EDCs) and interfere with our body's regulation of hormones. Studies of offspring born to mothers with high levels of PBB, have discovered that PBB exposure is associated with earlier menarche, and development of pubic hair (8). Current studies have also shown that exposure to PBB have been associated with an increased rate of biological aging, and increased age acceleration has been associated with age related issues, like Alzheimer's Disease (9, 10).

Similarly, other POPs, like PBDEs and PCBs, have also shown to be associated with infertility, estrogen concentration, premature ovarian failure, less high-quality embryos, development of reproductive organs, earlier menarche, type II diabetes, obesity, and cardiovascular diseases (CVD) (11-14). Research on PBB and neurological conditions, cognitive

decline, and Alzheimer's Disease is lacking, both in the PBB cohort and generally. Initial studies were done looking at the association of neurological conditions and PBB exposure after the incident occurred but have not been done since. For example, in 1981, a case control study was done where no correlation was seen between PBB exposure and memory deficits when they compared 25 Michigan farm residents to 25 chemical factory workers, all of whom were exposed (15). This study was done only 8 years after the incident and lacked a comparable control group which was needed to consider the long-term effects of PBB exposure on memory. The study had a very small sample size, and should have tested any exposure against appropriate non-exposed individuals. Other studies were done on children looking at various neurological effects of PBB exposure but found no correlation which could be due to no baseline measurements immediately after the incident or insensitive tests. Due to when these studies were conducted, they could not look at long-term effects of PBB yet (16-18). This longitudinal cohort, the PBB registry, has now followed individuals for over 40 years, and PBB measurements have been assessed to study effects made on a wide range of health concerns. PBB exposure has resulted in the occurrence of various health concerns that could further be linked to cognitive decline and should be explored. A study looking at this relationship has not been done in decades and this relationship has not been further explored.

Introduction to Cognitive Decline or Dementia

Mild Cognitive Impairment (MCI) can be described as the state before dementia, where the prevalence of MCI in adults over 60 is 6.7% to 25.2% (19,30). MCI can arise from other neurological conditions like Alzheimer's Disease (AD), Cerebral Vascular incidents (strokes), and Parkinson's Disease (PD). A decline in cognitive function can be referred to the mental processes that are needed to live a functional life. Dementia occurs from continuous abnormal

protein functioning due to misstructuring, where common symptoms are memory loss, confusion, mental decline, language deficits, personality changes, amongst others.

There are several risk factors for MCI and its progression to dementia. Studies have shown that MCI is highly correlated with diabetes, depression, and hypertension (20). Diagnosing dementia at earlier stages would be beneficial at the preclinical stage, and studies have expressed how sensitive tests would be needed to prevent further decline and progression of MCI to dementia (22). The Cognitive Functional Instrument (CFI) was developed by the Alzheimer's Disease Cooperative Study to detect early changes in cognition and functional abilities in non-demented individuals by scoring individuals on a 14-question survey. The CFI was validated in a study in which proved CFI effective in recognizing MCI amongst non-demented individuals (24). This study provided evidence that the CFI would be beneficial at detecting mild cognitive impairment and can be used to identify individuals at the pre-diagnosed stage which could further help reduce the risk of development to dementia. Studies have shown that CFI results are affected by depression, which is a known risk factor of MCI and is highly prevalent in this population

(25). Identifying this indirect path between PBB and MCI is worth further research, so that secondary preventative measures could be in place to reduce the risk of progression of MCI to Dementia. This research could help identify factors that affect MCI in this population and could be advantageous for detecting potential MCI at the earliest stage possible in this Michigan Cohort.

The role of Diabetes on Dementia

Various studies have been done looking at the possibility of diabetes being a risk factor for dementia. Several studies have found that there is an association between PCB and diabetes (32, 33). Given the similarity in structure between PCB and PBB, several studies were also done to test the association between PBB and diabetes. These studies that did not find an association for diabetes with PBB, but there are important implications for diabetes and Dementia (34, 35). Studies have shown that diabetes or prediabetes, is associated with progressing MCI to Dementia (36).

The role of Hypertension on Dementia

Previous research has shown that hypertension may be a risk factor for dementia. A recent study theorized that hypertension may play a role in autoregulatory deficits by repurposing vessel walls as a response to neurological changes (30). This study touches on the fact that these changes in brain structure and function can result in the risk for vascular strokes, and hence vascular dementia. More research is needed to understand the role of hypertension on dementia. In our study, the role hypertension plays on being a predictor for the development of dementia among our PBB exposed population remains unresearched and should be considered.

The role of Depression on Dementia

Depression is another risk factor of dementia. A review of studies was done on the effects of depression on dementia and found that late in life depression is associated with an increased risk for dementia, specifically, vascular dementia and dementia caused by Alzheimer's Disease (31). Early studies of the Michigan cohort showed that there were high rates of depression due to loss of their farms, or guilt over spreading contamination (5). Considering that depression is a

risk factor for the development of dementia, it is important to recognize that depression may be a part of the causal pathway between PBB exposure, MCI, and progression to dementia. Therefore, it is important to explore the association between depression and MCI in this PBB exposed cohort. As well as accounting for the association between depression and dementia.

POPS and Dementia

Several POPs have been associated with various neurological conditions, especially PCB and PBDE. Some epidemiological studies have found a relationship between POPs and neurological impairments like cognitive, motor and sensory impairments, including the relationship between PCBs and Attention Deficit Hyperactive Disorder (ADHD) and autism spectrum disorder (ASD) (26). An in vitro study found that cells exposed to PBDE, and PCB released signaling molecules that are involved in synaptic plasticity processes that have been linked to neurological diseases such as Alzheimer's (27). Other studies theorize the possibility of POPs and transition metals working synergistically to permeate the lipid bilayer further exacerbating the misstructuring of proteins that cause various disorders including neurological diseases (28), which is a hallmark observance in many neurological conditions, and dementia. A case control study looking at the prevalence of POPs, amongst people with Alzheimer's and non-diseased controls saw that AD individuals saw higher concentrations of several POPs (29). The POPs, PBBs, PCBs, and PBDEs are similar in structure and various research has been done on the latter two and neurodevelopment. It is imperative that we explore the association of PBB on these neurological conditions. This cross-sectional study will offer insight into the association of PBB and MCI, and all forms of dementia that have remained unexplored. We will utilize the CFI to score participants and determine their cognitive impairment status by scoring their individual self-reported responses and testing the association seen across this cohort. Given all the potential

theories and relationships of other persistent organic pollutants and neurological conditions it is deemed necessary to observe the relationship between Polybrominated Biphenyls and Mild Cognitive Impairment.

POPs and Neurological Conditions

The etiology of neurological disorders has long been debated, and the evidence of POPs on the incidence of these conditions should be further explored. Dementia can arise due to other neurological conditions, which include Alzheimer's Disease, Parkinson's Disorder, and presence of Lewy bodies. The occurrence of strokes, and chronic headaches can also be indicators of some form of damage that has occurred in the brain and before further cognitive decline is seen.

Alzheimer's disease, the leading cause of dementia, is classified as progressive problems with episodic memory, difficulties with multitasking, loss of confidence, behavior change, impaired mobility, hallucinations and potentially seizures (40). In dementia caused by Alzheimer's Disease, some common risk factors are diabetes, depression, hypertension, low vitamin E & C intake, frailty, and low education (21). There have been studies that look at the association between pesticides and AD, in which it expresses how pesticides destroy dopaminergic neurons (41). These types of neurons are known to be affected in individuals with AD and PD. Studies have not been done looking at the other POPS, specifically PBB, and their association with AD outcomes. Future studies should be done looking closely at this relationship. Considering MCI can lead to different forms of dementia it is important for us to look at the relationship between MCI and PBB to potentially look further into other neurological conditions.

Parkinson's disease is a result of loss of dopaminergic neurons in the midbrain that results in symptoms like tremors, rigidity, stooping posture, and can also display depressive, and anxious behaviors (38). Especially important is that PD can lead to cognitive impairment and its association with PBB should be tested, and has not been done. Several studies have shown that there is an association between pesticides or herbicide exposure and PD (39). Again, relative few studies have investigated the association between POPs or EDCs and Parkinson's Disease. Future studies should focus on the association of PD and PBB and the prevalence of PD in our cohort will be included in our analysis. The prevalence of these neurological diseases in our PBB cohort can capture if our cohort experiences these conditions at a higher rate. Testing the association between MCI and PBB can also be an indicator of these other neurological conditions given that MCI can be a trademark sign of PD or AD. Our analysis is important to identify if there is a relationship between PBB and MCI, so that we may implement preventative measures to reduce the chance of MCI progressing to dementia.

Chapter II: Manuscript

Abstract

Exposure to Brominated Flame Retardants and Cognitive Function

By Eduardo Monarrez

Background: Polybrominated Biphenyls (PBBs) are synthetic, bioaccumulative, compounds that were used as flame retardants. In 1973, a chemical factory incident resulted in the exposure of PBB through contamination of cattle feed and human consumption of by-products for years. The Michigan PBB Registry was created, and the occurrence of various health conditions have been obtained out of this cohort. No study has been done to look at the effect of PBB on mild cognitive impairment to date. In this longitudinal, cross-sectional study, we test the association between PBB exposure and abnormal scores on the cognitive functioning instrument.

Methods: Tertiles were created of serum PBB levels to evaluate their association with abnormal scores on the Cognitive Function Instrument. Serum PBB levels were also natural log transformed to evaluate their association with abnormal CFI scores. Logistic regression was performed to evaluate models including age and age at exposure, separately. Potential confounders included sex, age, age at exposure, cardiovascular conditions, smoking status, and alcohol use. The cognitive functioning instrument results were dichotomized for analysis. All statistical analyses were conducted in SAS version 9.4 and p-values less than 0.05 were considered significant.

Results: Among our 316 participants, no statistically significant association was found between log transformed PBB and abnormal scores on the Cognitive Function Instrument in both of our models. Our adjusted odds ratio for our log-transformed PBB was 1.087 (0.758, 1.558) controlling for age, and 1.074 (0.756, 1.527) controlling for age at exposure. However, we did find an association between PBB exposure and hypertension in our bivariate analysis (p-value=0.0011).

Conclusions: Exposure to PBBs was not significantly associated with abnormal scores on the cognitive function instrument in our study population. An association between PBB exposure and hypertension was found in our study population.

Introduction

Polybrominated Biphenyls (PBBs) are synthetic, fat-soluble, bioaccumulative persistent organic pollutants (POPs) with a long half-life. The most common PBB congener seen is hexabromobiphenyl, or PBB153. These synthetic compounds were used in mixing with various hard plastics as a flame retardant. Due to an unfortunate accident at the Velsicol Chemical plant (formerly Michigan Chemical) in 1976, many residents of Michigan were exposed to PBBs and the creation of the Michigan Long-Term PBB study was established to monitor the effects caused by PBB exposure. A recent study found that those who were former employees of the Velsicol Chemical Plant, family of former chemical workers, individuals who lived on or ate food from contaminated farms, and those living in the vicinity of the former chemical plant have higher than average PBB-153 levels compared to the rest of the United States (4). Some health outcomes as a result of PBB exposure have been linked to associations with skin, neurological (headaches, blurred vision, dizziness, depression, fatigue, perception changes, nervousness, sleeplessness, sleepiness, weakness, trouble ambulating, paresthesia, clumsiness, and slowness), earlier menarche, and development of pubic hair (7, 8).

Due to the persistent, bioaccumulative nature of PBB further research needs to be done of this Cohort. The creation of the Michigan PBB Registry makes that possible as it consists of 7,500 individuals that have been exposed due to the Velsicol Company incident. Polychlorinated Biphenyls (PCBs), and Polybrominated Diphenyl Ethers (PBDEs) are similar in structure to PBBs, and have shown to be associated to neurological impairment, such as Attention Deficit Hyperactive Disorder, Autism Spectrum Disorder, and changes in synaptic plasticity processes (26, 27). After 40 years since the incident, it would be important to observe the long-term effects

of PBB on Mild Cognitive Impairment, including neurological conditions. Various studies have talked to the biological plausibility that POPs do have an association to neurological diseases, through exacerbation of misstructuring proteins (28).

Mild Cognitive Impairment (MCI) is described as the state before dementia, in which can arise from other neurological conditions like Alzheimer's Disease (AD), Cerebral Vascular Incidents (strokes), and Parkinson's Disease (PD) (19, 30). Common symptoms of dementia could be memory loss, confusion, mental decline, language deficits, personality changes, amongst others. Diagnosing dementia at earlier stages would be vital to prevent further decline and progression of MCI to Dementia and would require sensitive tests to capture these stages (22). The Cognitive Functional Instrument (CFI) was developed by the Alzheimer's Disease Cooperative Study to detect early changes in cognition and functional abilities in non-demented individuals by scoring individuals on a 14-question survey, which has been validated in subsequent studies (24). Identifying the association between PBB and MCI is worth researching as preventative measures could help reduce the risk of the development of MCI to dementia. Identifying the risk factors for the progression of MCI to dementia would be important to capture in the Michigan PBB Cohort.

Given the biological plausibility, history of Persistent Organic Pollutants and their association with neurological conditions we deem it necessary to observe the relationship between Polybrominated Biphenyls and Mild Cognitive Impairment.

Methods

Study Design and Population

This cross-sectional, longitudinal, data comes from the PBB Registry which consists of about 7500 individuals with measured serum PBB levels, health outcome data and biospecimens collected over the past 40 years. This cohort's data initiated by the Michigan Department of Health and Human Services, CDC, and NIH enrolled thousands of exposed individuals into the then long-term Environmental Epidemiology Cohort (EEC). Participants additionally completed several questionnaires about their demographic information, health outcomes and provided a blood sample. In this study, we utilized the data from the Comprehensive Health Questionnaire (CHQ), which was completed by 332 participants 18 years of age or older between 2017-2019 which included health related conditions. Participants were recruited for PBB measurements and health updates through community meetings throughout Michigan. Members of the registry were invited through mailed invitations and media advertisements. The inclusion criteria to participate in the study included being current PBB registers, residents in Michigan between 1973 and 1974, or descendants of those who lived in Michigan within the time frame.

Cognitive Function Instrument

The CHQ asked a wide range of health outcomes and included questions from the Cognitive Function Instrument (CFI) (24). The CFI consists of 14 questions that asks about clinical assessments of aging and dementia which include questions about memory decline, assessment of cognitive difficulties, and functional abilities. Responses were coded as Yes = 1, No = 0, and Maybe = 7 which were then summed together individually to create a total score for each participant. The Maybe selection was converted to a 0.5 value for summation and

interpretation purposes. Each point was derived from a positive answer to each of the 14 questions and higher scores indicate greater subjective cognitive complaints. Participants who did not answer all 14 questions were set to a null value and excluded from analysis.

Covariates

Beyond the CFI, the CHQ asked questions about doctor diagnosed conditions, including our health conditions of interest. Questions ask, "Has a doctor ever told you that you have any of the following conditions?" for various areas of health. The neurological conditions included Mild Cognitive Impairment (MCI), attention deficit disorder (ADD), Attention Deficit Hyper-Activity Disorder (ADHD), autism spectrum disorder (ASD), Dementia, Parkinson's disease, Amyotrophic Lateral Sclerosis/Lou Gehrig's Disease, Multiple Sclerosis, Myasthenia Gravis, Tremors, Seizures, Epilepsy, Narcolepsy, and Migraines. Answer choices consisted of Yes, No, or I don't Know and included spaces for indicating age at diagnosis, if they are taking medications, and which medications if so. If a participant selected Yes for these conditions, it would prompt additional questions. For example, if a participant selected Yes for Doctor diagnosed dementia, then they would be asked "What type of dementia do you have?", and Alzheimer's Disease, Vascular Dementia, Dementia with Lewy Bodies, or Other dementia were asked in a similar format. Another important group of conditions that were asked were prevalence of Cardiovascular Conditions, such as Doctor diagnosed High Blood Pressure, Tremors, High Cholesterol, Coronary Artery Disease, Blood clots, and Other Cardiovascular Conditions. A combined Cardiovascular condition variable was created as a summation of these variables to indicate the number of cardiovascular conditions reported, and also dichotomized for analysis as yes or no to any cardiovascular condition.

Additionally, the CHQ also asked questions regarding exposure information like whether the participant had occupational exposure, residential exposure, or was a descendant of an exposed person. Demographics included questions about race, sex, smoking status, drinking status, family history, highest education attained, and income. Additionally, further questions asked status of diabetes, mental health status, and cardiovascular conditions. For participants born prior to the start of exposure (July 1st, 1973), age when exposed to PBB was calculated from Date of Birth and this date. We created a categorical age at exposure variable, combining those born after the start of exposure into one category, and then separating those born before the start of exposure into two categories, exposed when they were younger than 16 years of age, and exposed when they were older than 16 years of age. The date of when a participant was interviewed, and when blood was drawn was also recorded in order to determine age at the time of analysis.

Laboratory Analytical Methods for serum PBB-153

PBB analysis was conducted using Gas Chromatography Tandem Mass Spectrometry (GC-MS-MS) with methods previously used to analyze PBB, including how those with measures below the limit of detection (LOD) were treated (42). We only measured PBB-153, which is the predominant congener of PBBs, and will reference this congener for the remainder of this paper. The PBB levels were log transformed and treated as a continuous variable for analysis below. For those that were below the limit of detection, they were imputed with the LOD (different by batch, described below) divided by the square root of 2, in order to include them in the log transformation analysis. If a sample was collected in 2017 through 2018 the LOD was 0.020 ng/mL, in 2019 through 2020 samples analyzed with 1 mL serum with LOD 0.050 ng/mL, and

for samples in 2019 through 2020 with samples analyzed with greater than 1 mL serum with LOD 0.005 ng/mL. PBB was also categorized into tertiles of PBB exposure.

Statistical Analysis

Descriptive analyses were conducted for the variables of interest including counts, and means and standard deviations, where appropriate, which were all reported (Tables 1 & 2). Bivariate analyses were conducted using Fisher's exact and chi-square tests to assess the relationship between demographics variables and health outcomes of interests by PBB tertiles (Table 3). The continuous CFI variable was transformed into a binary variable for the bivariate analyses. All those who scored above 4 were coded as 1 for an abnormal score, else they were scored 0, for a normal score. Based on the distribution of the CFI variable, 4 was decided to be the cut point as it was the 75th percentile. Fisher's and chi-square tests were also used to assess the relationship between the demographic variables by the dichotomous CFI variables, normal versus abnormal (Table 4). Only those who answered all 14 questions were included in the analysis, which consisted of 316 participants.

The association between serum PBB levels and the dichotomized Cognitive Function Instrument were tested using a Logistic Regression model and potential confounders were evaluated via a backwards elimination strategy. The model was tested for effect modification using the likelihood ratio test. Potential confounders were also tested, variables were included for sex, age, age at exposure, smoking status, alcohol drinking status, and cardiovascular conditions. The covariates were selected and tested based on their association with MCI and PBB based on the literature (12, 30) and the lack of testing between the association of PBB with CVD, like hypertension.

This study was approved by the Emory University IRB and informed consent was obtained prior to all participants providing both the blood draws and filling of questionnaires. All statistical analysis were completed in SAS version 9.4 and results considered statistically significant if p-values were less than 0.05.

Results

There were 332 participants in our final study sample. Of which, 324 (98%) identified as white. 210 participants (63%) identified as natal-born females. The average age of participants is 59 years of age (+/- 12.75 years). Two-hundred and five participants drank alcohol in the last 6 months, with an average of 3.5 drinks per week. Additionally, 107 (32%) smoked on a regular basis at any point in time, with 33 (31%) being current smokers. Out of 317 participants who answered, 80 (25%) participants have a family history of dementia, family history defined as either a sibling or parent. Likewise, out of 325 participants, 244 (75%) reported family history of cardiovascular conditions such as high blood pressure, high cholesterol, heart attack, or stroke. Also, 285 (87%) of participants were alive and lived in Michigan during the PBB incident, 136 participants worked with livestock feed that were contaminated with PBB, 148 lived with a farm worker who worked with contaminated livestock feed, and 173 who reported they definitely ate contaminated food. Other demographic and exposure data is displayed on Table 1.

Participants were questioned whether they were diagnosed by a physician for various health conditions, of which, 11 reported being diagnosed with Mild Cognitive Impairment, 2 reported being diagnosed with an intellectual disability, 10 with a learning disability, 1 with developmental delay, 4 diagnosed with dementia (2 dementia with lewy bodies, and 2

unspecified), 12 with tremors, and 3 diagnosed with Parkinson's Disease. Other health diagnoses included 12 reported being diagnosed with Tremors, 130 with hypertension, 143 with High Cholesterol, 22 with blood clots, 19 with Coronary Artery Disease, 17 diagnosed with heart attacks, 9 with strokes, and 65 with diabetes (Table 2).

Participants were also asked about mental health problems. Out of 330 participants who answered, 63 (19%) reported experiencing a mental health problem, of which 60 reported depression, 43 reported anxiety, and 42 reported being diagnosed with both (Table 2).

As seen in table 3, the distribution of serum PBB concentrations was significantly different by sex ($p<0.0001$) with more men with higher levels of PBB compared to women, with women having lower concentrations of PBB. Distribution of serum PBB concentrations was significantly different by age ($p<0.0001$), and age of exposure ($p<0.0001$). In table 3, lower concentrations of PBB were found for those under the age of 50 compared to the other age groups. Individuals between the ages of 50 and 67 had higher concentrations across the medium and high concentrations than the younger age group. Finally, the individuals over the age of 67 had a higher proportion of participants with higher concentrations of PBB compared to the younger age groups. The distribution of serum PBB concentrations was also significantly different by hypertension ($p<0.0011$), with 130 participants reporting hypertension which increased with higher PBB levels. The distribution of age was significantly different by CFI score ranges ($p<0.0074$), with an inverse relationship seen of individuals under the age of 50 scoring abnormally on the CFI (Table 4). We generated a correlation matrix between our covariates and observed that our age and age at exposure variables were highly correlated ($r=0.99$, p-value <0.001). As were our cardiovascular conditions, most notably Heart Attacks and

Coronary Artery Disease (r=0.40, p-value < 0.001), and CAD with Hypertension (r=0.25, p-value < 0.001).

A bivariate analysis was done on the association of CFI and PBB that showed no difference across groups (Table 5).

To account for collinearity between age at exposure and age at blood draw we ran two separate models excluding the other age variable. We ran logistic regression of our tertial PBB, as lower concentrations of PBB as the reference in both models. In our first model (table 6), we observed an OR for our medium concentration tertial of PBB of 0.903 which was insignificant, 95%CI (0.461, 1.768) after adjusting for age. Our higher PBB tertial, we saw an OR of 1.153 which was also insignificant, 95%CI (0.574, 2.314) after adjusting for age. In model 2 (table 7), We observed an OR for our medium concentrations of PBB of 0.949 which was not significant, 95%CI (0.473, 1.905) adjusting for age at exposure (Table 7). We observed an OR for higher concentrations of PBB of 1.171, which was also insignificant, 95%CI (0.573, 2.393) adjusting for age at exposure (Table 7).

Our log transformed PBB logistic regression output resulted in an OR of 1.087 adjusting for age, which was insignificant, 95% CI (0.758, 1.588) in model 1 (table 8). In model 2, we observed an OR of 1.074 which was insignificant, 95%CI (0.756, 1.527) (table 8).

We tested our tertial PBB for interaction in our Age model and found no interaction present across our model (p=0.1596, using the Likelihood Ratio Test). The covariates tested for interaction included age, gender, cardiovascular conditions, smoking, and alcohol use. We tested for interaction in our age at exposure model and found no interaction present across our model

(p=0.2733, using the Likelihood Ratio Test). The covariates tested for interaction included age at exposure, gender, cardiovascular conditions, smoking, and alcohol use.

Discussion

We observed very few neurological conditions, and our outcome of interest, in our population with only 11 (3%) experiencing Mild Cognitive Impairment, 3 (<1%) with Parkinson's Disease, and 4 (1%) with Dementia. For dementia, we had 2 people report having dementia with Lewy Bodies, 1 who reported unspecified dementia, and 1 who did not know what type. We did observe that amongst the 316 participants who answered all 14 Cognitive Function Instrument questions, 77 (24%) of those who scored above 4 reported being diagnosed with mild cognitive impairment. Interestingly, we observed that amongst the 11 participants who self-reported having diagnosed MCI, 8 of them scored abnormally on the CFI, 2 scored within the normal range, and 1 did not complete the CFI questions.

We ran Logistic Regression models testing the association of PBB with the Cognitive Functioning Instrument that identifies pre-diagnosed Mild Cognitive Impairment in our study. Due to collinearity of age at exposure with age, we ran each model separately and observed similar Odds Ratios (Tables 6-8). Our reference group was low PBB concentrations and compared to Medium and High concentrations. We consider this reference group to be a comparable group as average NHANES PBB levels fall withing our low exposure PBB concentration ranges (43).

It was surprising to see that there seemed to be an inverse relationship between CFI scores and Age, which was not anticipated. We anticipated that CFI scores would be positively correlated with age. PBB has also been associated with an increased rate of biological aging,

which may complicate interpretation of chronological age (9, 10). We do see for those who scored above 4 on the CFI, our cut point, the median age was 55, and ranged from as low as 25 years old up to 86 years of age. It is noteworthy to mention that there seemed to be an association of Hypertension in our cohort with higher levels of PBB (p-value=0.0011). This is important considering that risk factors for Dementia, and progression of MCI to Dementia include diabetes, depression, and hypertension (20, 21). In our cohort, we observe that 65 (20%) have diabetes, 60 (18%) have depression, and 130 (40%) have hypertension. Other POPs have shown to be associated with cardiovascular diseases (11-14). Depression is not only a risk factor for dementia, but other studies have observed that CFI scores can be affected by depression as well (25). Alternatively, Parkinson's disease typical symptoms include tremors, rigidity, and can display depressive, and anxious behaviors (38).

Due to the now long-term study of PBB, we know that several potential confounders exist, including sex and age at exposure (3). These were tested for interaction and confounding, which we found no interaction amongst either variable. We did, however, find confounding with age at exposure and age in each model, which we adjusted for and presented in our models (Tables 6-8). We additionally tested cardiovascular conditions for interaction and confounding, of which we found none. This was important to do as the test of this association has to our knowledge, never been done between PBB and CVD.

One limitation of our study was our small sample size of 332 participants, of these only 316 were used in our final models. We only included those that completely answered all 14 CFI questions. Those who could not complete the survey may have cognitive issues interfering with survey completion and exclusion of these individuals could have biased our measure of association towards the null. The CFI was originally designed to be completed with a partner

which is a limitation in our study as this was a self-reported survey. Self-reporting has proven to be an accurate measure of pre-diagnosed MCI at early enough stages and we do not anticipate much bias (24). Another limitation was how in our log transformed PBB model, our PBB levels below the LOD, as mentioned in the methods, were treated. The true level of PBB for these individuals is unknown as they are set to zero. Considering only very few of those had PBB levels below those LOD (25 samples), we do not anticipate those affecting our results in our log-transformed model in Table 8. This was irrelevant in our tertial PBB exposure models, as they were used in our low PBB concentration tertial.

Although we did not observe an association of our outcome of interest, Abnormal CFI scores and PBB levels, we did see an association between PBB levels and Hypertension which would be important to explore. We did pose the question of whether participants had family with history of any cardiovascular conditions, which 244 (75%) responded Yes. We recommend future studies with a larger sample size to capture more precise measure of the association between CFI and PBB, and to test the association between Hypertension and CFI. Hypertension could potentially be a part of an intermediary pathway between PBB and CFI, or dementia. Lastly, future studies could observe levels of PBB prior to diagnoses of these neurological conditions, or PBB levels closer to the time of exposure, in this cohort as individuals have provided serum in the PBB registry for years.

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Tables

Table 1: Demographics N=332

Variable	N (%, S.D)
Age	59 (+/- 12.75)
Age at Exposure	17.2 (+/- 9.6)
Sex	
Male	122 (37%)
Female	210 (63%)
Race	
White	324 (98%)
Other	8 (2%)
Exposure	
Lived In Michigan between 73-74	
Yes	285 (87%)
Lived Quarantined Farm	93 (28%)
Lived in Non-Quarantined	53 (16%)
Not on a Farm	132 (40%)
Not sure	7 (2%)
No	5 (1%)
Born After 1975	39 (12%)
Worked at Velsicol	
Yes	9 (3%)
No	322 (97%)
Lived with worker	11 (3.3%)
Handled PBB livestock	
Yes	136 (42%)
No	155 (47%)
Lived with Farm worker	
Yes	148 (45%)
No	146 (45%)
Born After 1975	34 (10%)
Worked with Other PBB Exposure	
Yes	16 (5%)
No	287 (89%)
Don't Know	20 (6%)
Ate Contaminated food	
Definitely	173 (54%)
Probably	79 (25%)
Probably not	15 (5%)
Definitely not	7 (2%)
Don't Know	45 (14%)
Alcohol	

Alcohol past 6 mo.	205 (62%)
Average weekly drinks	3.5 (+/- 4)
Smoked on regular basis	
Yes	107 (32%)
Smoke now	
Yes	33 (31%)
Average daily smoke	14 (+/- 10)
Not Now	74 (69%)
Average past daily smoke	15.4 (+/- 9)
Never Smoked	224 (68%)
Education completed	
Some High School	2 (1%)
High School Graduate/GED	71 (22%)
Some College	108 (33%)
College Grad	80 (25%)
Graduate School	64 (20%)
Income	
Less than $10,000	11 (4%)
$10k-$29k	60 (19%)
$30k-$34k	14 (4%)
$35k-$49k	59 (19%)
$50k-$74k	57 (18%)
$75k-$99k	47 (15%)
$100k +	64 (21%)
Family History	
Dementia (n=317)	
Yes	80 (25%)
Cardiovascular Conditions (n=325)	
Yes	244 (75%)

Source: PBB Registry

Table 2: Health Conditions

Dr. Diagnosed Conditions	N (%)
MCI	
Yes	11 (3%)
No	305 (94%)
Don't know	9 (3%)
Intellectual Disability	
Yes	2 (<1%)
No	322 (99%)
Don't know	1 (<1%)
Learning Disability	
Yes	10 (3%)
No	316 (96%)
Don't know	2 (1%)
Developmental Delay	
Yes	1 (1%)
No	322 (98%)
Don't Know	4 (1%)
Dementia	4 (1%)
Yes	4 (1%)
Alzheimer's	
No	*0*
Don't Know	*1*
Vascular Dementia	
No	1
Don't know	1
Dementia with Lewy Bodies	
Yes	2
No	1
Don't know	1
Unspecified Dementia	0
No	1
Don't Know	1 (<1%)
No	324 (98%)
Parkinson's Disease	
Yes	3 (<1%)
No	323 (99%)
Don't know	1 (<1%)
Tremor	
Yes	12 (4%)

No	311 (95%)
Don't Know	2 (1%)
High Cholesterol	
Yes	143 (44%)
No	182 (55%)
Don't Know	2 (1%)
Blood Clots	
Yes	22 (7%)
No	297 (92%)
Don't Know	2 (1%)
Other Cardiovascular Conditions	
Yes	60 (20%)
No	236 (77%)
Don't Know	8 (3%)
Coronary Artery Disease	
Yes	19 (6%)
No	301 (93%)
Don't Know	5 (1%)
Migraines	
Yes	83 (25%)
No	238 (73%)
Don't know	7 (2%)
Hypertension	
Yes	130 (40%)
No	193 (59%)
Don't know	3 (1%)
Heart Attack	
Yes	17 (5%)
No	307 (94%)
Don't know	2 (1%)
Stroke	
Yes	9 (2%)
No	311 (96%)
Don't know	2 (1%)
Diabetes	
Yes	65 (20%)
Diabetes Type I	4
Don't know	3
Diabetes Type 2	28
Don't know	2

Unspecified	29
No	259 (78%)
Don’t Know	7 (2%)
Mental Health problems (n=330)	
Yes	63 (19%)
Depression	60
Anxiety	43
Don’t know	2
Both	42
No	262 (79%)
Don’t Know	5 (2%)
Total	

Table 3: Characteristics by Serum PBB Concentrations

Variable	Low Serum PBB Concentrations (N= 108)	Medium Serum PBB Concentrations (N=105)	High Serum PBB Concentrations (N=103)	P-Value
Sex				
Male	19 (16%)	44 (36%)	59 (48%)	<0.0001
Female	91 (43%)	68 (32%)	51 (24%)	
Age				
<50	54 (67%)	19 (23%)	8 (10%)	<0.0001
50-67	47 (27%)	68 (39%)	60 (34%)	
>67	9 (12%)	25 (33%)	42 (55%)	
Age at Exposure				
Born after 1973	46 (87%)	5 (9%)	2 (4%)	<0.0001*
≤ 16	38 (87%)	58 (42%)	42 (30%)	
> 16	26 (28%)	49 (35%)	66 (47%)	
Hypertension				
Yes	28 (22%)	55 (42%)	47 (36%)	0.0011
No	78 (40%)	54 (28%)	61 (32%)	
Don't Know	2 (67%)	1 (33%)	0	
Coronary Artery Disease				
Yes	3 (16%)	6 (32%)	10 (53%)	0.1347*
No	101 (34%)	103 (34%)	97 (32%)	
Don't Know	3 (50%)	1 (25%)	1 (25%)	
Migraines				
Yes	33 (40%)	29 (35%)	21 (25%)	0.1660
No	73 (31%)	80 (34%)	85 (36%)	
Don't Know	3 (42%)	2 (29%)	2 (29%)	
Heart Attack				
Yes	5 (29%)	3 (18%)	9 (53%)	0.1827*
No	100 (33%)	107 (35%)	100 (33%)	
Don't Know	2 (100%)	0	0	
Stroke				
Yes	5 (56%)	4 (44%)	0	0.0705*
No	100 (32%)	106 (34%)	105 (34%)	
Don't Know	1 (50%)	0	1 (50%)	
Tremor				
Yes	4 (33%)	3 (25%)	5 (42%)	0.7666*
No	101 (33%)	107 (34%)	103 (33%)	
Don't Know	2 (100%)	0	0	

High Cholesterol				
Yes	38 (27%)	53 (37%)	52 (36%)	0.3940
No	69 (38%)	57 (31%)	56 (31%)	
Don't Know	1 (50%)	0	1 (50%)	
Blood Clots				
Yes	4 (18%)	10 (45%)	8 (36%)	0.6190*
No	100 (34%)	100 (34%)	97 (32%)	
Don't Know	2 (100%)	0	0	
Other Cardiovascular Conditions				
Yes	16 (27%)	23 (38%)	21 (35%)	0.4325
No	83 (35%)	76 (32%)	77 (33%)	
Don't Know	6 (76%)	1 (12%)	1 (12%)	
Smoking				
Yes	32 (30%)	44 (41%)	31 (29%)	0.1509
No	77 (34%)	68 (30%)	79 (35%)	
Alcohol				
Yes	73 (36%)	71 (35%)	61 (30%)	0.1894
No	36 (29%)	40 (32%)	49 (39%)	

Chi-square test
Fisher's Exact test* for 2x2 tables with values less than or equal to 5

Table 4: Characteristics by CFI (N=316)

Variable	Normal CFI (N=239)	Abnormal (N=77)	P-Value
Sex			
Male	87 (76%)	28 (24%)	0.9952
Female	152 (76%)	49 (24%)	
Age			
<50	50 (63%)	29 (37%)	0.0074
50-67	130 (78%)	37 (22%)	
>67	59 (84%)	11 (16%)	
Age at Exposure			
Born after 1973	34 (67%)	17 (33%)	0.1430
≤16	99 (74%)	34 (26%)	
> 16	106 (80%)	26 (20%)	
Hypertension (N=311)			
Yes	92 (75%)	30 (25%)	0.8511
No	142 (76%)	44 (24%)	
Don't Know	1 (33%)	2 (67%)	
High Cholesterol (N=312)			
Yes	103 (76%)	32 (24%)	0.8596
No	132 (75%)	43 (25%)	
Don't Know	1 (50%)	1 (50%)	
Blood Clots (N=306)			
Yes	14 (70%)	6 (30%)	0.5167
No	217 (76%)	67 (24%)	
Don't Know	0	2 (100%)	
Other Cardiovascular Diseases (N=289)			
Yes	44 (76%)	14 (24%)	0.8823
No	172 (77%)	52 (23%)	
Don't Know	2 (29%)	5 (71%)	
Tremors (N=310)			
Yes	6 (60%)	4 (40%)	0.2553
No	229 (77%)	69 (23%)	
Don't Know	1 (50%)	1 (50%)	
Coronary Artery Disease (N=310)			
Yes	12 (67%)	6 (33%)	0.3264
No	222 (77%)	67 (23%)	
Don't Know	0	3 (100%)	
Migraines (N=313)			

Yes	54 (68%)	25 (32%)	0.0721
No	178 (78%)	49 (22%)	
Don't Know	5 (71%)	2 (29%)	
Heart Attack (N=311)			
Yes	12 (80%)	3 (20%)	1.0000*
No	223 (76%)	71 (24%)	
Don't Know	0	2 (100%)	
Stroke (N=307)			
Yes	8 (89%)	1 (11%)	0.6919*
No	224 (76%)	72 (24%)	
Don't Know	0	2 (100%)	
Smoking (N=315)			
Yes	74 (74%)	26 (26%)	0.6613
No	164 (76%)	51 (24%)	
Alcohol (N=314)			
Yes	153 (77%)	45 (23%)	0.3341
No	84 (72%)	32 (28%)	
Total			

Chi-square test
Fisher's Exact test* for 2x2 tables with values less than or equal to 5
CFI Normal Scores summation ≤ 4
CFI Abnormal Scores summation > 4

Table 5: Distribution of CFI by Serum PBB Concentrations

Variable	Low Serum PBB Concentrations (N= 108)	Medium Serum PBB Concentrations (N=105)	High Serum PBB Concentrations (N=103)	P-Value
Normal CFI	77 (32%)	83 (35%)	79 (33%)	0.4005
Abnormal CFI	31 (40%)	22 (29%)	24 (31%)	

Table 6: Adjusted Odds Ratio (OR) and 95% Confidence Intervals for the Association between PBB Exposure and Abnormal CFI adjusting for Age of Exposure (N=316)

	OR	95% CI
Low PBB	Ref	--
Medium PBB	0.903	(0.461, 1.768)
High PBB	1.153	(0.574, 2.314)

Table 7: Adjusted Odds Ratio (OR) and 95% Confidence Intervals for the Association between PBB Exposure and Abnormal CFI adjusting for Age (N=316)

	OR	95% CI
Low PBB	Ref	--
Medium PBB	0.949	(0.473, 1.905)
High PBB	1.171	(0.573, 2.393)

Table 8: Adjusted Odds Ratio (OR) and 95% Confidence Interval for the Association between Log Transformed PBB and Abnormal CFI adjusting for Age or Age at Exposure (N=316)

	OR	95% Confidence Interval
Model 1		
Log PBB (Age)	1.087	(0.758, 1.558)
Model 2		
Log PBB (Age at Exp)	1.074	(0.756, 1.527)