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AN ABSTRACT OF THE DISSERTATION OF

Jennifer Przybyla for the degree of Doctor of Philosophy in Public Health presented on November 28, 2016.

Title: Environmental Mixtures and Selected Health Outcomes in the US Population.

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Molly L. Kile

The human health effects of exposure to numerous single environmental contaminants have been well characterized. Yet, biomonitoring studies have detected multiple environmental chemicals in humans, highlighting the need to investigate the health effects of exposure to multiple environmental chemicals. Environmental data is highly complex, therefore current methods of defining environmental mixtures and examining relationships between exposure and health outcomes often do not capture the dynamic relationship between chemicals or take into account different modes of action. The introduction contextualizes the limitations in defining environmental mixtures and outlines the need for understanding the relationship between exposure to environmental mixtures and important health outcomes. Recursive partition mixture modelling (RPMM) was used to evaluate the US population's exposure to mixtures of environmental chemicals. Path analysis and structural equation modeling (SEM) were used to evaluate the relationship between exposure to environmental mixtures and cognitive functioning and thyroid hormone disruption, respectively.

The objective of the study presented in chapter 2 was to develop a novel approach to identify exposure profiles of 7 common environmental chemicals in a US population. This study provides evidence the US population has 8 mixture profiles of 7 environmental chemicals (lead,

cadmium, bisphenol-A (BPA), triclosan, benzophenone-3, 2,4-dichlorophenol, and 2,5-dichlorophenol). The findings also showed that different subpopulations in the US are exposed to different mixture profiles. Specifically, Non-Hispanic Black (NHB) or Other Hispanic (OH) males below the poverty index threshold were most likely to have the highest exposure. This study built on previous definitions of environmental mixtures by using biomarkers of exposure to report the dynamic relationship between chemicals of different classes and modes of actions.

The third chapter evaluated the relationship between exposure to a mixture of neurotoxins (lead, cadmium, 8 non-dioxin-like PCBs, and 4 dioxin like PCBs) and cognitive functioning in older US adults. The results indicate that lead and non-dioxin like PCBs, specifically PCB 146 has a negative relationship with cognitive functioning. This study confirmed that lead is negatively associated with cognitive functioning when controlling for exposure to other neurotoxin. Unlike previous studies using the same dataset that used an additive definition of PCBs mixtures it was found that non-dioxin-like PCBs were negatively associated with cognitive functioning, signifying different conclusion may be reached based on the definition of environmental mixtures.

The fourth chapter evaluated the association between exposure to a mixture of 11 endocrine disrupting compounds (EDCs) (7 phthalates, 3 phenols, and perchlorate), thyroid hormones (THs), and body mass index (BMI). We observed that exposure to multiple EDCs was associated with thyroid functioning in both males and females. A positive association between exposure to multiple EDCs and BMI directed through alterations in concentrations of thyroid stimulating hormone (TSH) was observed in females only. These results confirm that women may be more susceptible to higher BMI from exposure to individual EDCs, but is the first to suggest higher BMI is a result of TH alterations from exposure to multiple EDCs

The fifth chapter provides a summary and conclusions of the 3 studies. In addition to describing a more realistic environmental mixture exposure scenario in US subpopulations, these results provide evidence that exposure to multiple environmental chemicals is detrimental to important human health outcomes. Future studies should focus on incorporating a greater number of chemicals with different modes of action into environmental mixture profiles and use these profiles to direct future toxicology and epidemiology studies. Public health interventions, policies, and regulations can protect human health from the detrimental effects of environmental exposures by focusing on reducing exposure to common co-exposures or chemicals known to have synergistic effects. Overall, these results can guide future toxicology and epidemiology research into the health effects related to multiple environmental exposures.

Environmental Mixtures and Selected Health Outcomes in the US Population

by
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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Jennifer Przybyla, Author

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Environmental Mixtures and Selected Health Outcomes in the US Population.

Chapter 1-Introduction

Despite evidence that human populations are exposed to multiple environmental chemicals (1) there are limitations to how environmental mixtures are currently defined in human health risk assessments and environmental epidemiology studies. Also, because environmental data is highly correlated and has high dimensionality, barriers exists when using traditional methodology to understand the health effects of exposure to multiple chemicals. This research utilizes covariance data to describe the exposure profiles of environmental mixtures in US subpopulations in addition to understanding the effect of exposure to environmental mixtures on important health outcomes.

The objective of the first aim is to describe a more realistic definition of environmental mixtures in US subpopulations. To date, environmental mixtures have been defined in human health risk assessment or in epidemiology using additive or binary interaction methods. These methods have limitations that all chemicals act in a dose additive fashion on all endpoints and limited data exists on many binary interaction methods. Such limitations result in a definition of environmental mixtures that does not address the synergistic or antagonistic relationship of multiple chemicals. This research will provide a clearer definition of environmental mixtures by describing the exposure profiles between multiple chemicals while identifying subgroups of people with similar exposure profiles using Recursive Partition Mixture Modeling (RPMM). RPMM is a technique that can cluster together people with similar groups of exposure and was used in this body of work to identify exposure profiles for 7 environmental chemicals using

National Health and Nutrition Survey (NHANES) biomonitoring data from 2003 to 2012 data. Lead, cadmium, 2,4-dichlorophenol, 2,5-dichlorophenol, bisphenol A (BPA), triclosan, benzophenone-3 were used to construct exposure profiles and quasibinomial logistic regression was used to determine demographic variables associated with each profile. The 7 chemicals profiled in this analysis were chosen because they are both common and harmful to human health. The implications for the findings from this specific aim are the ability to prioritize mitigation and intervention efforts for subpopulations with high exposure and inform toxicology and epidemiology research with a more realistic mixture exposure profile for the US population.

The objective of the second specific aim is to examine the relationship between exposure to multiple neurotoxins and cognitive functioning in older adults. Cognitive decline is a major public health concern in the United States because of a rapidly aging population and the large economic and social impacts of cognitive decline (2). Identifying preventable environmental exposures that are related to neurodegenerative diseases has the potential to have high impact on public health by informing policy to prevent and mitigate exposure. Cognitive decline has been shown to be associated with exposure to multiple single environmental exposures including polychlorinated biphenyls (PCBs) (3), cadmium (4) and lead (5) yet no studies have been completed to determine the effect of environmental mixtures on this outcome. To begin to understand the effect of exposure to multiple neurotoxins, this research used path analysis to model the relationship between PCBs, lead, and cadmium and cognitive functioning using NHANES 1999-2002 data. Path analysis is a technique befitting the examination of the relationship between multiple neurotoxins and cognitive decline because it can estimate the relative magnitude of several highly correlated compounds. Through the use of path analysis, this research is the first to examine the effect of multiple exposures from different chemical

classes on cognitive functioning. The findings from this research has potential implications for policy as the research determined that exposure to non-dioxin-like PCBs, which are of less regulatory concern compared to dioxin-like PCBs, are negatively associated with cognitive decline.

The objective of the third aim is to examine the relationship between exposure to multiple endocrine disrupting compounds (EDCs), thyroid hormones (THs), and body mass index (BMI). Exposure to several individual EDCs such as phthalates (6), phenols (7), and perchlorate (8) have been shown to disrupt thyroid function, until now no studies have been completed to examine the relationship between exposure to multiple EDCs and thyroid function. EDCs are suspected "obesogens" or compounds that can lead to obesity. One way in which EDCs are suspected to affect metabolism is through disruption of thyroid hormone homeostasis. There is limited work describing the complex relationship between exposure to multiple EDCs, THs concentrations, and BMI. Structural equation modelling (SEM) is an approach that is well-suited to modeling not only multiple highly correlated compounds but also complex relationships such as those between EDCs, THs, and BMI. Using SEM with NHANES 2007-2008 data, this study is the first to describe the relationship between exposure to multiple EDCs compounds and TH concentrations and to demonstrate a sex-specific positive relationship between EDCs exposure, TSH concentrations, and BMI in women.

Human Biomonitoring Studies

Human biomonitoring (HBM) measures exposure to chemical substances by analyzing human biological media for chemicals or their biomarkers and are considered the "gold standard" for evaluating a population's exposure to environmental chemicals (9). The aim of HBM is to protect human health by determining exposure to chemical substances in a

population. HBM can be useful to monitor trends of exposure, identify emerging chemical exposures, monitor changes in exposures, identify vulnerable subpopulation with higher chemical exposures, and find associations between exposures and health outcomes. Advantages of using HBM include it is relatively inexpensive and measures all routes of exposure. A disadvantage of using HBM is that the process of collecting human biological media can be invasive. Currently, about 200 biomarkers of internal exposure are used in HBM, making HBM a value resource for assessing a population's exposure to multiple environmental chemicals (10).

In the United States, the National Biomonitoring Program (NBP), coordinated by the Center for Disease Control and Prevention (CDC)'s Division of Laboratory Sciences, is the main program to collect information on the US population's exposure to environmental chemicals and toxic substances. The NBP is conducted in conjunction with the National Health Nutrition Examination Survey (NHANES) to collect and analyze biospecimens from a non-institutionalized US representative population. In addition to collecting and analyzing environmental chemical biomarker, NHANES utilizes physical examinations and surveys to collect data on nutrition and health measurements on non-institutionalized populations, which is used to guide federal health programs and initiatives (11). Because of the extensive and diverse data provided in NHANES, it is an important database to generate hypotheses and test theories about environmental exposures and human health outcomes.

Exposure to Chemicals and Human Health

NHANES measures biomarkers of public health concern considering the measured chemicals are both commonly found in the environmental and have the potential to be harmful to human health. The health effects of the individual compounds measured in NHANES are well characterized. Yet little is known about synergistic or antagonistic effects on health outcomes

that may occur when humans are exposure to environmental mixtures. Below is a description of the human health effects of the individual chemicals used in this body of work.

Heavy Metals

Heavy metals, such as lead and cadmium, are potentially toxic dense metals that occur naturally in the environment. Humans can be exposed to heavy metals through the use or manufacturing of many consumer products like batteries, mining activities or through contaminated food or water (12). After the United States removed tetraethyl lead from gasoline the main sources of exposure to lead were contaminated food, water, house dust and occupational exposures like battery manufacturing and metal recycling (13). Lead exposure occurs mainly through ingestion or inhalation. The gastrointestinal adsorption of lead is dependent on many factors like age, gender and nutritional status. For instance, a calcium deficiency may result in increased lead absorption. Absorbed lead that is not retained is excreted through the urine and feces (14). After GI absorption, lead is distributed to blood plasma, soft tissues and eventually accumulates in bones. In blood and soft tissues the half-life of lead is 1 to 2 months and in bones the half-life is 10 to 30 years (15). Blood is the preferred biospecimen to assess the association between long-term lead exposure and health effects in humans when it is too evasive and costly to assess bone lead levels (14).

In both adults and children, lead targets the central nervous system. Lead can affect the central nervous system by causing oxidative stress, which is a systemic imbalance between reactive oxygen species and antioxidants. Oxidative stress can affect the central nervous system by damaging DNA or disrupting cellular signaling (16, 17). Weakness in ankles, wrists or fingers, may occur from lead exposure along with small increase in blood pressure and anemia. Severe damage to the kidneys and brain can also occur after chronic lead exposure (18). Blood

and bone levels in older adults were negatively associated with cognitive functioning (19). Adverse reproductive effects for both men and women have been reported after high lead exposure (18).

Cadmium can also affect the nervous system through oxidative stress (20). Humans can be exposed to cadmium through food, tobacco smoke and occupations such as battery manufacturing, welding or metal soldering (4). Cadmium is poorly absorbed in the GI tract and adsorption increases with calcium deficiency. Absorbed cadmium is mainly retained in tissues such as the kidneys or liver. Cadmium measured in blood has a half-life of over 1 and ½ years and reflects recent and cumulative exposure (21). Cadmium exposure has been associated with poorer cognitive functioning in adults and complaints of decreased concentrations, and reduced attention and memory (22). Beside affecting the central nervous system, exposure to cadmium can also cause kidney damage, decreased bone density, anemia and liver disease (23). The International Agency for Research on Cancer (IARC) classifies cadmium as a probable human carcinogen (24).

Polychlorinated biphenyls

Polychlorinated biphenyls (PCBs) are organic chlorine compounds used in the US as coolants fluids in electric equipment. PCBs were also used as plasticizers and solvents in adhesives and carbonless copy paper from 1930 to 1979. Production of PCBs in the US was banned in 1979 and in 2001 the use and production was banned worldwide. Despite these bans, the lipophilic nature of PCBs results in bioaccumulation of PCBs in marine life and biomagnification in food chain. Humans can be exposed to PCBs through old electrical and lighting devices that when in use get hot and release vaporized PCBs into the air (25). Humans can also be exposed to PCBs through food especially consumption of fish or by breathing air

contaminated with PCBs (26). Blood levels of PCBs have a half-life of years (27) and reflect cumulative past exposure (25). The base structure of a PCB is a biphenyl with chloride atoms replacing hydrogen atoms resulting in 209 configurations of PCB congeners. PCBs are divided into two groups based on their structure. The first group has the two phenyl rings in the same plane and are called coplanar PCBs (28). This group is also called dioxin like PCBs because they share a similar structure to dioxins and elicit similar responses to the aryl hydrocarbon receptor (AhR) (29). The AhR is the specific binding site for dioxins and dioxin like compounds and mediates the toxic effects of dioxins and dioxin like compounds (30). Non-coplanar PCBs do not active the AhR but are neuro- and immunotoxic through interference of dopamine production, intracellular signal transduction, and altered T-cell or macrophage function (31-34). PCBs are also classified as a human carcinogen by IARC (35).

Pesticides and Herbicides

2,4-Dichlorophenol

2,4-Dichlorophenol is a synthetic chemical that is used in the manufacturing of weed killers, such as 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorphenoxyacetic acid. 2,4-Dichlorophenol can be produced from the incineration or combustion of wood, coal, solid waste or as a byproduct when manufacturing chlorinated chemicals. It can also be produced as a byproduct from bleaching wood pulp and during chlorination of water. Humans can be exposed to 2,4-dichlorophenol by inhaling air or ingesting water or food contaminated with 2,4-dichlorophenol. 2,4-Dichlorophenol is highly lipophilic and easily absorbed through the skin after dermal exposure (36). Once 2,4-dichlorophenol enters the body it is rapidly metabolized and eliminated in the urine within hours as glucuronide conjugates (37). Pregnant mice exposed to 2,4-dichlorphenol had reduced body weight and litter size (38). In other animal studies, exposure to chlorophenols resulted in adverse effects to the liver and immune system (39).

2,5-Dichlorophenol

2,5-dichlorophenol is a metabolite of 1,4-dichlorobenzene (paradichlorobenzene). Paradichlorobenzene is used in the manufacturing of commercial products like room and toilet deodorizers, moth balls and insecticide fumigants. Paradichlorobenzene is also used in the manufacturing of industrial products like dyes, chemicals and resins (40). 2,5-Dichlorophenol can be also be formed during wood pulp processing, or during the burning of wood, municipal waste and coal or during wastewater treatment (41). The majority of exposure to paradichlorobenzene is through inhalation since it is highly volatile. Dermal exposure is high in occupations were 2,5-dichlorophenol is processed or manufactured. Once paradichlorobenzene enters the body it is metabolized to 2,5-dichlorphenol followed by conjugation to glutathione and excretion in the urine within days (42). Laboratory animals chronically exposed to paradichlorobenzene have been shown to develop kidney and liver damage. Offspring born to pregnant animals exposed to paradichlorobenzene showed no adverse effect, however the mothers had reduced weight gain (24). Workers exposed to high levels of paradichlorobenzene in the air, reported eye and respiratory irritation. Chronic exposure to paradichlorobenzene has also been shown to be associated with increase white blood counts and liver necrosis (43). IARC classifies paradichlorobenzene as a probable human carcinogen (24).

Endocrine Disrupting Compounds

Endocrine disrupting compounds (EDCs) are typically synthetic chemicals that can interfere with the body's endocrine system and disrupt hormonal function. EDCs act on the endocrine system by mimicking naturally occurring hormones, binding to receptors within the cell and thereby blocking the body's hormones from binding, or blocking the production of

hormones or their receptors. A wide variety of compounds are suspected endocrine disruptors including phenols, phthalates, and perchlorate (44).

Phenols

Phenols are synthetically derived compounds characterized by a hydroxyl group bonded to an aromatic hydrocarbon ring (45). Triclosan is a phenol used in many consumer products, such as personal care products, kitchenware and textiles for its antimicrobial properties (46). The main route of exposure to triclosan for humans is through dermal or oral exposure after use of products containing triclosan. Once absorbed triclosan can be conjugated to glucuronides and sulfates (47, 48) or excreted unchanged in the urine or feces (49, 50). Urinary triclosan measurements are reflective of recent exposure with the half-life of urinary triclosan being 2 to 3 days (49). However, repeat studies have suggested that spot urine samples are a good indicator of triclosan levels over time (51). The health effects of exposure to triclosan are still being investigated, but animal and human studies suggest triclosan modulates estrogen dependent responses (52) and is associated with higher BMI in humans (53).

BPA is a phenol used in the production of plastics including dental sealants, polycarbonate plastics and epoxy resins that coat the inside of food cans (54, 55). People are exposed to BPA through eating or drinking food or liquids that have been in contact with material containing BPA or through dental sealants which contain BPA (56). Depending on fasting time, the urinary half-life of BPA is estimated to be between 4.1 to 48 hours (57). Urinary BPA is a short term biomarker but studies have found that urinary spot samples concentrations are representative of long term exposure (58). Little is known about the human health effects of exposure to low doses of BPA. After occupational exposure to BPA, workers reported eye and skin irritation. In animal studies, animals exposed to high doses of BPA showed reproductive and

development alterations, including altered development of fetal prostate and mammary gland, alterations in neurodevelopment and inhibition of postnatal testosterone production (59-62).

Benzopheonone-3 is a phenol used as a UV filter in cosmetics and plastics (63, 64). Humans are exposed to benzophenone-3 dermally after application of sunscreens and cosmetic products (65-68). After absorption, benzophenone-3 is excreted in the urine as the glucuronidataed conjugate (66). Benzophenone-3 has a urinary half-life of 5 days and reflect recent exposure, however repeat measure studies suggest that urinary spot samples are adequate estimates of long term exposure (69). Using animal studies, benzopheonone-3 has been reported to have weak anti-androgenic and estrogenic activity (70, 71).

Phthalates

Phthalates are synthetically derived diesters of phthalic acid. Low molecular weights phthalates (3-6 carbon atoms in their alcohol chain) are used mainly in consumer products. High molecular weights phthalates (greater than 6 carbons in their alcohol chain) are used to add flexibility and soften to plastics (72). Humans are exposed to phthalates through the ingestion of food and water that is contaminated with phthalates and also through dermal exposure when using cosmetics are manufactured with phthalates. Exposure can also occur through inhalation of air polluted with phthalates (73). During phase I metabolism, both low and high molecular weight phthalates are metabolized to their hydrolytic monoesters. The high-molecular weight monoesters then undergo enzymatic oxidation to become a more hydrophilic oxidative metabolite. Both the monoesters and oxidative metabolites undergo glucuronidation during phase II biotransformation or be excreted in the urine or feces, unchanged (72, 73). Phthalates have a urinary half-life of a few days, representing short term exposure, however studies have indicated that urinary spot samples are good estimates of long term exposure (72). Toxicological studies

using both male and female animals studies suggest exposure to phthalates results in developmental and reproductive toxicities (72).

Perchlorate

Perchlorate is an inorganic chemical with the formal ClO_4^- . Small amounts of perchlorate are naturally occurring in the environment, but the greater amount of perchlorate found in the environment is from commercial manufacturing and use in fireworks, road flares, explosives and rocket fuel (74). Humans are exposed to perchlorate by eating food with high water content if those plants have taken up water contaminated with perchlorate or by drinking water or milk contaminated with perchlorate. People who have occupations such as manufacturing perchlorate-containing products are exposed to greater amounts of perchlorate compared to the general population (74, 75). The majority of orally ingested perchlorate enters the bloodstream where it passes into the kidneys. Once inside the kidneys, 90% of perchlorate is excreted within minutes in urine (75), however repeat measure studies suggest that urinary spot samples are good estimates of long term exposure (76). Exposure to high levels of perchlorate has been shown to effect the thyroid hormone levels in women with low iodine levels (77).

Evaluation of chemical mixtures on human health

Exposure to environmental chemicals does not happen in isolation, for example, air pollution is a complex mixture (78) and several toxic chemicals have been detected in the food supply (79). These chemicals have been shown to be persistent in the environment (80) and have been detected in humans through biomonitoring studies (81, 82). For instance, biomonitoring studies utilizing US representative populations have shown that 80 to 100% of the sample population using the NHANES dataset were exposed to environmental contaminants such as polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), pesticides

or heavy metals (83-86). Furthermore, in a study conducted in the United Kingdom analyzing 78 chemical contaminates in the blood serum of with 155 volunteer the range of the number of chemicals people were exposed to was 9 to 49 with the median being 27 chemicals detected (87). Despite the evidence that the human population is exposed to multiple environmental exposures, limitations exists to the current methods used to understand exposure to environmental mixtures and associated health outcomes.

Environmental mixtures are typically defined as substances that are mixtures themselves (i.e. Aroclor is made up of several PCBs), jointly emitted chemicals from a single source, or chemicals that occur in the same environmental media (i.e. food, water, soil) (88). Using biomarker data exposure to multiple compounds have been described by reporting the means of each chemical (89) or by reporting the frequency of detection (90). In risk assessment, chemical mixture toxicities are typically described using dose (concentration) addition or using the independent action method. An assumption with dose (concentration) addition is that all compounds in a mixture influence the joint effect in relation to their concentrations and potency (91). An example of dose (concentration) addition is the use of toxic equivalence factors (TEF) for PCB mixtures. TEF are derived from relative potency factor (RPF), which are determined through the use of *in vitro* bioassays. The Toxic Equivalent (TEQ) is a single quantity that describes the toxicity of the mixture and is determined by summing concentration of each individual component and multiplying each individual component by its TEF (92). In addition to PCBs, TEF have also been develop for PAHs (93) and PBDEs (94). When examining chemical mixtures using the independent action method, the risk for each chemical is evaluated individually determined by if the chemical is present in concentrations above or below threshold

levels. The independent action method is useful when chemicals have different mechanism of action (91).

In addition, several governmental agencies have put forth guidelines regarding the handling multiple environmental exposures for risk assessment. The preferred method is using data obtained from testing the actual mixture, however data exists only for a few mixtures like diesel exhaust (95, 96) and environmental weathering of the mixture is often not taken into account (95). Other methods include dose addition methodologies like the TEQ method described above, hazard index (HI) or response addition. The HI approach first starts with a calculation of the hazard quotient (HQ) for each chemical by dividing the concentration of the compound by the maximum acceptable dose of the compound such as the reference dose (RfD), each HQ for the components of the mixture are then summed to obtain the HI (95). Response addition sums the individual probabilistic risk of an outcome for each component in a mixture (97). Limitations to additive approaches include that they are specific to chemicals that have the same mechanism and endpoints and assumes dose additivity. Additionally synergistic or antagonistic interactions are not addressed using additive methods (97).

To address the issue of interactions several methods have been proposed. The interaction-based HI method uses a weight of evidence approach using data available from a binary mixtures database combined with judgment by the investigator in reference to direction, plausibility and relevance of interaction (98). The Agency for Toxic Substances and Disease Registry (ATSDR) has developed interaction profiles for 11 chemicals using data on binary interaction between the chemicals. Nine of the profiles are from commonly occurring chemicals at Superfund sites and the other 2 are of chemicals commonly occurring in breastmilk and fish (99). Limitations to the interaction-based HI approach is that there is limited data on binary mixture and it relies heavily

on investigator decisions which could lead to basis (100). Additionally, interaction may occur between more than 2 chemicals which are not accounted for in the profiles.

Traditional epidemiology methods are often not appropriate to address exposure to environmental mixtures due to the inherent correlations and high dimensionality of the data (101). Typically, in environmental epidemiology studies the issue of multiple exposures is either ignored or additive approaches like the TEF approach are utilized (102). More recently work has been done to describe the association of the body burden index (BBI) of chemicals and empirical wellness. Chemicals were assigned a biological pathway based on their mode of action and the BBI was constructed by summing the scores of 6 biological pathway-specific indices (103). Limitations to the BBI approach include that several chemicals may interact with more than one biological pathway. Several other methods have been proposed that use a sum weighted index representing a score of exposure which then can be included in a regression model (101, 104, 105). Limitations to the sum weighted index approaches are that only binary interactions are addressed.

The methods used in this body of work addresses several of the limitations associated with previous attempts of defining environmental mixtures by using the covariance structure of several environmental chemicals. By relying on the covariance structure when describing environmental mixtures an additive relationship is not assumed and a more dynamic relationship of exposure is presented. Covariance data can also be used to address the limitations in examining the relationship between exposure to multiple environmental chemicals and health outcomes by allowing the use of techniques, such as path analysis and SEM, which use covariance data to test the fit of an *a priori* hypothesis. Methods using covariance do not relay on

the assumptions associated with previous methods and instead are able to obtain the relative influence of each variable on health outcomes.

In summary, humans are exposed to multiple environmental chemicals that have been individually shown to be harmful to human health. Limitations in previous attempts to define environmental mixtures make it difficult to understand the human health effects of exposure to multiple environmental chemicals. This research used advanced statistical methods relying on the covariance structure of several environmental chemicals to address previous assumptions and limitations in defining and understanding the human health effects of exposure to environmental mixtures.

Specific Aims

The scope of this research is to identify common environmental mixture profiles and to investigate the impact of exposure to environmental mixtures on human health using a dataset that represents a US population. This goal was completed in three separate studies presented in the next three chapters. The first study described the exposure profiles of 7 common chemicals and associated sociodemographic features. The second study evaluated the association between exposure to multiple EDCs, thyroid hormones (THs), and BMI. The final study evaluated the association between exposure to several neurotoxins and cognitive functioning in older adults.

Specific Aim 1: Characterize the environmental mixture of 7 chemicals in Americans who are 6 years of age or older (2 heavy metals, 2 environmental pesticides and 3 environmental phenols) in NHANES cycles 2003-2004, 2005-2006, 2007-2008, 2009-2010 and 2011-2012. The mixture will be operationalized as the variance-covariance matrix of the chemicals after adjusting for potential confounders. Differences between NHANES cycles and select socio-demographic characteristics will be investigated.

Hypothesis 1: Covariation of the 7 chemicals under consideration (heavy metals, environmental pesticides, and environmental phenols) will differ between cycles after adjusting for potential confounders [e.g. age, ethnicity, gender, etc].

Hypothesis 2: Covariation of the 7 chemicals under consideration (heavy metals, environmental pesticides, and environmental phenols) will differ between selected socio-demographic groups [e.g. age, ethnicity, gender, etc] after adjusting accounting for differences among NHANES cycles.

Specific Aim 2: Evaluate the association between a mixture of metals (cadmium and lead) and non-dioxin like and dioxin like polychlorinated biphenyls (PCBs) in relation to cognitive functioning in adults 60 to 84 years of age using a Structural Equation Model (SEM). A latent variable representing environmental mixtures will be informed by blood serum concentrations of metals and PCBs. Cognitive functioning will be informed by scores from the Digit Symbol Coding (DSC) test from the Wechsler Adult Intelligence Scale (WAIS-III).

Hypothesis: There will be a negative association between a latent variable that represents the biological burden of environmental chemicals and cognitive functioning after controlling for other confounders.

Specific Aim 3: Evaluate the association between a mixture of endocrine disrupting compounds (phenols, plasticizers, and phthalates) in relation to thyroid functioning in adults 20 to 84 years of age using a SEM. A latent variable representing an environmental mixture of endocrine disruptors will be informed by urinary concentrations of phenols, plasticizers and phthalates. The relationship between a mixture of endocrine disrupting compounds, thyroid functioning and body

mass index (BMI) will also be examined. The association between a mixture of endocrine disrupting compounds will also be examined with clinical definitions of hyperthyroidism and hypothyroidism.

Hypothesis: There will be a significant association between a latent variable that represents the biological burden of environmental endocrine disrupting compounds and thyroid hormone concentrations after controlling for other confounders. There will be a significant association between a latent variable that represents the biological burden of environmental endocrine disrupting compounds and thyroid hormone concentrations and BMI after controlling for other confounders. There will be a significant association between a latent variable that represents the biological burden of environmental endocrine disrupting compounds and clinical definitions of thyroid disease after controlling for other confounders.

Chapter 2 – First Manuscript

Title: Description of exposure profiles for 7 environmental chemicals in a US population using recursive partition mixture modeling (RPMM).

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Abstract

Biomonitoring studies have shown that humans are exposed to numerous environmental chemicals. Previous works use additive or binary interactions methods to describe multiple exposure, but these approaches provide limited insights into the dynamic relationship between different chemicals within a population. The objective of this study is to develop an analytical method identifying exposure profiles of 7 common environmental chemicals. This study will also determine how exposure profiles differ by sociodemographic groups and NHANES cycle year using National Health and Nutrition Examination Survey (NHANES) 2003-2012 data. Each NHANES cycle measured lead, cadmium, 2,4-dichlorophenol, 2,5-dichlorophenol, bisphenol A (BPA), triclosan, and benzophenone-3 in the same individuals aged ≥6 years. We used recursive partition mixture modeling (RPMM) to define classes of the population with similar exposure profiles. Additionally, quasibionomial logistic regression was used to examine the association between each class and selected demographic characteristics. Eight exposure profiles were identified. Individuals who clustered together and had the highest chemical exposures were more likely to be older, to be Non-Hispanic Black (NHB) or Other Hispanic (OH), more likely to live below the poverty line, more likely to be male, and more likely to have participated in the older

NHANES cycle (2003-2004). The developed method described the dynamic relationship between chemicals and demonstrated the relationship is different for subpopulations based on their sociodemographic characteristics. The exposure profiles can be used in toxicological and epidemiology studies as a more realistic exposure scenario.

Introduction

Biomonitoring studies have shown that people are exposed to multiple environmental chemicals (1, 106). These biomarkers of exposure are typically highly correlated and have high dimensionality making it inherently difficult to characterize exposure to multiple environmental chemicals and assess the association between these mixtures and health outcomes (101). Many attempts to define environmental mixtures have, therefore, taken a forthright approach and have neglected to present real-world exposure scenarios.

Environmental epidemiology studies and human health risk assessment often define environmental mixtures using additive methods such as summing the relative potency factors (RPF), toxic equivalency factor (TEF) or hazard quotients for structurally similar compounds (98, 102). TEF report the toxicity of a single compound in relation to a reference chemicals and are determined using REP established from toxicity assays for each chemical (94, 107). Another method used to assess the risk of chemical mixtures is the interaction-based hazard index (HI) method. This method uses a weight of evidence approach using data available from binary mixture databases combined with judgment by the investigator in reference to direction, plausibility and relevance of interaction (100). Recently, in environmental epidemiology, weighted index approaches have also been proposed which provides a weighted sum scale of environmental chemicals (103-105). Limitations to these methods are the assumption of a dose additive nature for compounds of similar classes, which may not be true. Additionally, these

methods are limited to examining binary interactions and don't capture the synergistic or antagonistic effects of multiple chemicals.

When examining the human health effects of environmental chemicals, it is important to incorporate mixtures and the dynamic relationships between chemicals in the exposure assessment process. This study used a hierarchical version of the Finite Mixture Model (FMM), called the Recursively-Partitioned Mixture Model (RPMM) to create real world exposure profiles. RPMM is a likelihood-based hierarchical clustering method which support "fuzzy" clustering, i.e. it accounts for the uncertainty in the classification of some subjects. Clustering methods provide a natural means for grouping together subjects that are similar in exposure, and thus are well-suited to describing exposure profiles of environmental chemicals. We chose RPMM over other data reduction methods for the following reasons: (1) Principal component analysis (PCA) and related Factor Analysis (FA) methods produce factor loadings that are more difficult to interpret than cluster-specific exposure patterns; (2) conventional FMM approaches (e.g. Mclust) do not naturally support the inclusion of case weights into the modeling scheme (as does RPMM). We applied RPMM to data collected in five cycles of the National Health and Nutrition Examination Survey (NHANES) using 7 chemicals that had been assessed in the same individual. We then used quasibionomial logistic regression to examine whether the exposure profiles differed by socio-demographic characteristics or cycle year.

Methods

Study design and population

NHANES is a cross-sectional survey that collects data on nutrition and health measures from a US non-institutionalized representative sample population by utilizing physical examinations, specimen collection and surveys (11). NHANES is conducted by the National Center for Health Statistics (NCHS) of the Center for Disease Control (CDC). NHANES utilizes

a complex, multistage probability sampling design and data is publicly available. Protocol is described elsewhere (108) and was approved by the NCHS Research Ethics Review Board and documented consent was obtained from all participants. Data from cycle years 2003-2012 was included in this analysis. Not all environmental chemicals are measured in every NHANES participant. For instance, urinary phenols and pesticides concentrations were assessed in a randomly selected subsample of participants 6 years of age and older, thereby limiting the sample population to those individuals 6 years and older who had their urine analyzed for pesticides or phenols in each cycle. Additionally, the type and number of chemicals measured are different from cycle to cycle. Subsequently we identified 7 chemicals that were measured within the same individual and in multiple cycles. This approach allowed us to collect covariance data throughout the 5 NHANES cycles. It should be noted that NHANES is not a longitudinal study and covariance data for the 7 chemicals were available for different subgroups throughout the 5 cycles. Out of the 12,793 participants that had their blood specimens analyzed for the 7 analytes used in this analysis, 963 were missing data on family poverty income ratio (PIR) and 865 individuals were missing biomarker data leaving 10,964 people with complete data.

Chemical Exposures

Cadmium and lead were measured in blood samples using inductively coupled plasma mass spectrometry (ICP-MS) (109). Environmental phenols and pesticides were measured using solid phase extraction (SPE) coupled on-line to high performance liquid chromatograph (HPLC) and tandem mass spectrometry (MS/MS) (110, 111). For chemical values detected below the LOD, NCHS imputes values equal to the limit of detection divide by the square root of 2 ($LOD/\sqrt{2}$) into the dataset. All chemicals that are included in this analysis all have a detection frequency of 60% or greater.

Covariates

We evaluated several socio-demographic factors including: race/ethnicity, age, socioeconomic status (SES), and sex. Other Hispanic (OH) and Other Race including Multi-Racial categories were combined to provide a sufficient sample size to obtain accurate estimates (112). Race/ethnicity was categorized as Mexican American (MA), OH, Non-Hispanic White (NHW) and Non-Hispanic Black (NHB). Age was categorized as 6-19, 20-39, 40-59 and greater than or equal to 60 years of age. Poverty Index Ratio (PIR) was used as an indicator of SES and is calculated by NHANES for that cycle year using the U.S. Department of Health and Human Services poverty guidelines (112). PIR was categorized as 0-0.99 and greater than or equal to 1. Sex was categorized as male and female.

Statistical Analysis

Descriptive statistics were calculated for demographic and chemical data for NHANES 2003-2004, 2005-2006, 2007-2008, 2009-2010 and 2011-2012 cycles merged using Stata for Windows (version 14, StataCorp LP, www.stata.com). Survey variables including stratification, clustering (PSU) and sample weights corresponding to the weights of the subsample who had their urine analyzed for phenols and pesticides were used to account for the complex sampling design of the NHANES (112).

We used the RPMM package (https://cran.r-project.org/web/packages/RPMM/RPMM.pdf) in R (https://www.r-project.org/) to perform clustering on the matrix of the environmental chemicals. RPMM uses a divisive or "top down" approach to clustering, where all the subjects begin in one cluster and based upon similar correlations of chemical biomarkers split into smaller groups. In this analysis, the 7 chemicals were clustered together to form latent classes of individuals with similar exposure profiles. Bayesian information criterion (BIC) were compared to determine splits for the latent classes.

The classes were named after the splits that occurred, where a right split is equivalent to a "1" and a left split is equivalent to a "0". For instance, class 000, represents three left splits. Normalized NHANES subsample weights were normalized by multiplying the number of participants by NHANES sampling weights divided by the sum of weights, which was used as inputs in the RPMM analysis. Although RPMM supports automatic determination of the number of classes (i.e. pruning of the hc tree). We chose to prune at 3 levels (8 classes) in order to provide sufficient sample size for downstream analysis. The outcome of RPMM is a matrix of class membership weights, with each row corresponding to a subject, each column corresponding to a class, and the values of each row summing to one. The "classes" represent groups of people with similar exposure patterns we refer to as "exposure profiles'. The class membership weights are posterior membership probabilities which can be visually represented in a heat map by Pearson correlations that takes into account NHANES sampling weights. Next, to identify sociodemographic variables associated with the exposure profile of each class, the classes were used as dependent variables in a generalized linear model (glm) with family as quasibinomial; this setting accounts for continuous responses falling between 0 and 1. Other than RPMM, all analysis was completed using the Survey package in R to account for NHANES' complex multi-stage sampling design (https://cran.r-project.org/web/packages/survey/survey.pdf).

Results

Descriptive Statistics

The descriptive characteristics of the population are shown in Table 1. A chi-squared analysis was conducted to determine if the subsample used in this analysis differed from those excluded in this analysis. The subsample used in this analysis was younger and was more likely to be NHW and in the less recent cycle years than the population who did not have their

biospecimens analyzed for the 7 chemicals used in this analysis. The average concentrations for the 7 analytes measured in this population are shown in Table 2. Of the heavy metals, lead had the highest geometric mean of 1.20 µg/L (95% Confidence Interval (95% CI): 1.17, 1.23). Of the pesticides used in this analysis, 2,5-dichlorphenol had the highest geometric mean of 7.66 µg/L (95% CI: 6.86, 8.59). Whereas, benzophenone-3 had the highest geometric of 21.14 ng/mL (95% CI: 19.05, 23.45) for the phenols.

The clustering profiles generated by RPMM are presented in Table 3. The correlations of chemicals within the exposure profiles are represented by weighted Pearson correlations. Heatmaps were used to characterize the correlation structure between different classes where the greatest positive correlation is represented as light blue and the greatest negative correlation is light yellow (Figure 1). The strength of correlations per class describes the relationship between the 7 biomarkers of exposure between and within a class. For instance, 2,5-dichlorophenol and 2,4-dichlorophenol had the highest correlations with class 111, with correlations of 0.55 and 0.50, respectively, while the other 5 chemicals were not highly correlation with class 111. This can be interpreted as out of all the clusters, individuals in cluster 111 have the highest exposure to 2,5-dichlrophenol and 2,4-dichlorophenol and low exposure to other chemicals. Lead and BPA had the greatest correlation with class 100 of 0.35 and 0.26, respectively. Class 101 had the greatest correlation for benzopheonone-3 (0.24) and triclosan (0.49). Cadmium was most correlated with class 010 (0.38) (Table 3).

The odds ratios and 95% CI generated from the quasibionomial logistic regression models are presented in Figures 2-6. We examined the association between each class and sociodemographic factors and cycle year to determine their association with each classes' exposure profiles. These estimates can be interpreted as the log odds of that selected

demographic in comparison to the reference group for that class. For instance, being 20-39 year old increased the log odds of being in class 100 by 0.55 (95% CI: 0.43, 0.67, pvalue <0.001) compared to 6-19 year olds from class 100 (Table 4 and Figure 2). Class 100 is significantly more likely to be older, to be NHB, more likely to have a PIR of below 1, more likely to be male and more likely to be in the 2003-2004 survey year (Table 4, Figure 3, Figure 4, Figure 5, and Figure 6). In contrast, those in class 101 are significantly younger and are more likely to have a PIR of greater than 1 (Table 5, Figure 2, and Figure 4). Older NHWs in survey year 2007-2008 are more likely to be in class 010 (Table 6, Figure 2, and Figure). Younger subjects, female subjects, those who are NHW, and those who have a PIR greater than 1, and were in the more recent survey years are significantly more likely to be in class 011 (Table 7, Figure 2, Figure 4, Figure 5, and Figure 6). Those in class 000 are significantly more likely to be older, be NHW and in the more recent survey years (Table 8, Figure 2, Figure 3, and Figure 6). For class 001, individuals are significantly more likely to be younger, NHW, have a higher PIR, be female, and had their biospecimens analyzed in the more recent survey years (Table 9, Figure 2, Figure 3, Figure 4, and Figure 6). Older MAs, OHs, and NHBs having a PIR of less than 1 and in recent survey years are significantly more likely to be in class 110 (Table 10, Figure 3, Figure 4 and Figure 6). Those in class 111, which have high exposure to 2,4-dichlorophenol and 2,5-dichlorophenol are significantly more likely to be 20-39 year olds and be MA, OH or NHB and in the more recent survey years (Table 11, Figure 2, Figure 3, Figure 6).

Discussion

The developed analytical method successfully builds on previous methods of defining environmental mixtures by identifying 8 exposure profiles that describe a more realistic relationship between several different types of chemicals using biomarker data from a US population. These exposure profiles address several of the limitations of previous definitions of

environmental mixtures. Firstly, this exposure assessment approach models the dynamic relationship between each chemical for the subpopulations based on the correlations between compounds. Therefore, assigning individual exposure using this approach provides an alternative to creating a summed score of individual compounds. This offers several advantages since additive methods assume that chemicals with similar structure or common modes of action have an additive nature with a specific endpoint. However, there is evidence that for some chemicals this is not true and additive responses are often species and outcome dependent (92, 113) or the individual chemicals produce a synergistic or agonistic effect (114, 115). While fewer studies have examined the effects of mixtures of chemicals with different modes of action, the few studies that have been completed suggest combination effects (116, 117). Our method of defining environmental mixtures, is able to account for structurally different chemicals without assuming similar modes of action or additivity. Secondly, previous definitions of environmental mixture tend to be derived using substances that are complex mixtures themselves (i.e. diesel exhaust), chemicals in a single product, or chemicals emitted from a single source. Defining environmental mixtures as such does not represent real-world exposure scenarios, as weathering of individual chemicals is not addressed nor are individual behavioral patterns or geographical locations which can affect exposure (95, 118, 119). Our use of biomonitoring data to define environmental mixtures provides a more realistic profile of exposures than combinations of chemicals derived from single sources or environmental media.

The developed methodology allows the identification of common co-exposures, indicated by clusters of chemicals. As expected, the personal care and consumer products (triclosan, BPA, and benzophenone-3), metals (lead and cadmium) and pesticides/herbicides clustered together. Clustering of chemicals suggests common exposure pathways. For instance, lead and cadmium

exposure occurs through food, tobacco smoke and occupational exposures such as battery manufacturing (14, 23). Similarly, we found most of the co-exposures specific to certain subpopulations are consistent with findings from research examining exposure to single chemicals. For example, the greatest correlations for 2,4-dichlorophenol and 2,5-dichlorophenol were observed for older participants of minority race in recent survey years with a PIR less than the poverty threshold. Higher exposures to 2,4-dichlorophenol in racial groups of Non-Hispanic Black and Other Hispanic and in those with a PIR less than the poverty threshold is consistent with findings from NHANES 2005-2008 data (120). Similarly, Non-Hispanic Black and Other Races had higher urinary concentrations of 2,5-dichlorophenol in Non-Hispanic Black and Other Races, as did those having a lower household income (120). Despite our findings that show younger individuals are more likely to have greater correlation with triclosan, researchers using NHANES data found those in age range of 20-59 years of age had the highest triclosan LSGM concentrations (46).

This study applied a novel exposure assessment approach that can be utilized to study the health effects of chemical mixtures at a population-level. However, it has several limitations. Specifically, NHANES does not measure all compounds in the same individuals. Hence, we could only analyze the covariance of 7 chemicals in the 5 data cycles. A further limitation is that the cross-sectional nature of the data and that the biomarkers have different half-lives. Lead and cadmium have half-lives of 1 to 2 months and 1.5 years, respectively (15, 21). Hence, blood levels of these two metals represent more long term cumulative exposure. Whereas, the organic compounds measured in the urine have short half-lives of hours to days and represent short term exposure, although studies suggest that for some of the chemicals, urinary spot samples represent long term exposure (58, 72). Strengths of this study, include the generation of exposure profiles

which can be used to guide prevention or interventions efforts in vulnerable subpopulations. Additionally, unlike describing exposure through chemical concentration means, correlations tend not to be influenced by single data points and are interpretable even if chemicals are measured on different scales. Because of data limitations, we were only able to model 7 chemicals, however this method can be used with high-dimensional data sets that contain biomarker data on hundreds of chemicals. Finally, the chemicals used to develop the mixtures are ubiquitous in the environment and have been shown to cause detrimental health effects.

Conclusion

We applied a clustering algorithm more commonly utilized in molecular epidemiology to identify 8 different exposure profiles for 7 important environmental chemicals and the sociodemographic variables associated with each mixture profile. Future works should include expanding on the number of chemicals used to construct the profiles. Additionally, toxicology and epidemiology studies should integrate the mixture profiles into research examining health effects associated with these mixtures.

Table 2.1. Descriptive Characteristics of the study population of 6-85 year olds who participated in NHANES 2003-2012. Chi-square tests were used to compare selected characteristics for individuals included in this analysis and those who were missing exposure data

Variables	Subpopulation who had biospecimens analyzed N= 12,793 Proportion (N)[a]	Population who did not have biospecimens analyzed N= 31,413 Proportion (N)	Chi-square (p-value)
Age (years)			10.75 (0.01)
Geometric Mean (95% CI)	40.12 (39.51, 40.73)	29.08 (28.84, 29.32)	
6-19	19.73 (4,201)	33.86 (10,636)	
20-39	30.12 (3,034)	22.83 (7,7172)	
40-59	30.91 (2,687)	20.59 (6,469)	
≥60	19.23 (2,871)	22.72 (7,136)	
Missing (%)	0	0	
Sex			0.66 (0.42)
Male	48.68 (6,355)	49.14 (15,435)	
Female	51.32 (6,438)	50.86 (15,978)	
Missing	0	0	
Race			140.75 (0.00)
Mexican	9.00 (2,568)	19.92 (6,259)	
Other Hispanic	11.45 (1,949)	16.19 (5,085)	
White	67.50 (5,227)	39.35 (12,362)	
Black	12.05 (3,049)	24.53 (7,707)	
Missing	0	0	
PIR			2.79 (0.10)
Geometric Mean (95% CI)	2.90 (2.82, 2.98)	1.75 (1.73, 1.76)	
≤ 0.99	15.65 (2,945)	25.16 (7,279)	
≥1	84.34 (8,885)	74.84 (21,656)	
Missing	8.14 (963)	8.56 (2,478)	
Survey Year			3.4×10^3 (<0.001)
2003-2004	19.64 (2,567)	18.36 (5,766)	
2005-2006	18.22 (2,236)	18.38 (5,774)	
2007-2008	20.37 (2,670)	18.20 (5,717)	
2009-2010	20.69 (2,777)	19.08 (5,995)	
2011-2012	21.09 (2,543)	25.98 (8,161)	
Missing	0	0	

[a]Weighted proportion

Table 2.2. Description of the chemical concentrations measured in 6-85 year olds who had their biospecimens analyzed for 7 chemicals, NHANES 2003-2012 and were included in this analysis.

Chemical	Geometric Mean	95% CI	Range	Above LOD (%)	Missing (n)	Present (n)	Percent Missing (%)
Pb (ug/dL)	1.20	1.17, 1.23	0.18-43.52	94.01	760	12,033	5.94
Cd (ug/L)	0.32	0.31, 0.32	0.1-10.8	89.12	760	12,033	5.94
2,5-dichlorophenol (ug/L)	7.66	6.86, 8.59	0.07-473.00	96.47	234	12,559	1.83
2,4-dichlorophenol (ug/L)	0.88	0.83, 0.93	0.14-15.90	86.73	234	12,559	1.83
BPA (ng/mL)	1.94	1.87, 2.01	0.28-9.65	90.48	242	12,551	1.89
Benzophenone-3 (ng/mL)	21.14	19.05, 23.45	0.2-929.00	95.43	242	12,551	1.89
Triclosan (ng/mL)	14.46	13.72, 15.24	1.6-58.90	75.18	242	12,551	1.89

Table 2.3. Weighted Pearson correlations by class for 7 chemicals, 6-85 year olds who had their biospecimens analyzed for 7 chemicals, NHANES 2003-2012

Class	Pb	Cd	Triclosan	BPA	Benzophenone-3	2,5-dichlorophenol	2,4-dichlorophenol
111	0.02	-0.003	0.005	0.08	-0.01	0.50	0.55
110	0.07	0.04	0.02	0.07	-0.04	0.50	0.49
101	-0.34	-0.41	0.49	0.22	0.24	0.15	0.29
100	0.35	0.31	0.26	0.26	-0.04	0.29	0.32
011	-0.41	-0.44	-0.01	-0.05	0.17	-0.24	-0.26
010	0.29	0.38	-0.17	-0.02	-0.09	-0.17	-0.28
001	0.0009	0.05	-0.32	-0.30	-0.13	-0.42	-0.53
000	0.02	0.09	-0.36	-0.25	-0.13	-0.36	-0.30

Table 2.4. Log odds for being in class 100 and select demographic variables, participants 6-85 years who had their biomarkers analyzed for 7 chemicals, NHANES 2003-2012

		Log Odds	95 % Confidence Interval	pvalue
Age				
	6-19	Ref	Ref	Ref
	20-39	0.55	0.43, 0.67	<0.001
	40-59	0.81	0.70, 0.93	<0.001
	≥60	1.08	0.94, 1.22	<0.001
Race				
	NHW	Ref	Ref	Ref
	MA	0.32	0.16, 0.48	<0.001
	OH	0.31	0.20, 0.42	<0.001
	NHB	0.63	0.54, 0.73	<0.001
PIR				
	≤ 0.99	Ref	Ref	Ref
	≥ 1.00	-0.25	-0.35, -0.15	<0.001
Sex				
	Male	Ref	Ref	Ref
	Female	-0.42	-0.50, -0.34	<0.001
Survey Year				
	2003-2004	Ref	Ref	Ref
	2005-2006	-0.14	-0.31, 0.03	0.11
	2007-2008	-0.31	-0.50, -0.13	0.002
	2009-2010	-0.51	-0.66, -0.36	<0.001
	2011-2012	-0.92	-1.09, -0.75	<0.001

Table 2.5. Log odds for being in class 101 and select demographic variables, participants age 6-85 years who had their biomarkers analyzed for 7 chemicals, NHANES 2003-2012

		Log Odds	Confidence Interval	pvalue
Age				
	6-19	Ref	Ref	Ref
	20-39	-0.22	-0.33, -0.11	<0.001
	40-59	-0.81	-0.95, -0.66	<0.001
	≥60	-1.30	-1.44, -1.15	<0.001
Race				
	NHW	Ref	Ref	Ref
	MA	-0.12	-0.28, 0.04	0.16
	OH	0.004	-0.13, 0.14	0.95
	NHB	0.09	-0.03, 0.21	0.13
PIR				
	≤ 0.99	Ref	Ref	Ref
	≥ 1.00	0.34	0.23, 0.44	<0.001
Sex				
	Male	Ref	Ref	Ref
	Female	-0.07	-0.18, 0.03	0.16
Survey Year				
	2003-2004	Ref	Ref	Ref
	2005-2006	0.19	0.002, 0.39	0.05
	2007-2008	0.10	-0.10, 0.31	0.31
	2009-2010	0.06	-0.11, 0.23	0.52
	2011-2012	0.02	-0.15, 0.20	0.81

Table 2.6. Log odds for being in class 010 and select demographic variables, participants age 6-85 years who had their biomarkers analyzed for 7 chemicals, NHANES 2003-2012

	Log Odds Ratio	Confidence Interval	pvalue
Age			
6-19	Ref	Ref	Ref
20-39	0.62	-0.33, -0.11	<0.001
40-59	0.98	-0.95, -0.66	<0.001
≥60	1.24	-1.44, -1.15	<0.001
Race			
NHW	Ref	Ref	Ref
MA	0.09	-0.28, 0.04	0.21
OH	-0.22	-0.13, 0.14	<0.001
NHB	-0.17	-0.03, 0.21	0.01
PIR			
≤ 0.99	Ref	Ref	Ref
≥ 1.00	-0.24	0.23, 0.44	<0.001
Sex			
Male	Ref	Ref	Ref
Female	-0.05	-0.18, 0.03	0.21
Survey Year			
2003-2004	Ref	Ref	Ref
2005-2006	0.09	0.002, 0.39	0.22
2007-2008	0.24	-0.10, 0.31	0.007
2009-2010	0.10	-0.11, 0.23	0.14
2011-2012	-0.10	-0.15, 0.20	0.16

Table 2.7. Log odds for being in class 011 and select demographic variables, participants age 6-85 years who had their biomarkers analyzed for 7 chemicals, NHANES 2003-2012

	Log Odds Ratio	95 % Confidence Interval	pvalue
Age			
6-19	Ref	Ref	Ref
20-39	-0.48	-0.63, -0.34	<0.001
40-59	-1.05	-1.19, -0.91	<0.001
≥60	-1.73	-1.90, -1.56	<0.001
Race			
NHW	Ref	Ref	Ref
MA	-0.44	-0.60, -0.30	<0.001
OH	-0.36	-0.53, -0.19	<0.001
NHB	-0.67	-0.80, -0.54	<0.001
PIR			
≤ 0.99	Ref	Ref	Ref
≥ 1.00	0.40	0.28, 0.52	<0.001
Sex			
Male	Ref	Ref	Ref
Female	0.18	0.07, 0.29	0.001
Survey Year			
2003-2004	Ref	Ref	Ref
2005-2006	0.32	0.18, 0.46	<0.001
2007-2008	0.52	0.36, 0.69	<0.001
2009-2010	0.64	0.50, 0.78	<0.001
2011-2012	0.81	0.68, 0.95	<0.001

Table 2.8. Log odds for being in class 000 and select demographic variables, participants age 6-85 years who had their biomarkers analyzed for 7 chemicals, NHANES 2003-2012

		Log Odds	95 % Confidence Interval	pvalue
Age				
	6-19	Ref	Ref	Ref
	20-39	-0.28	-0.41, -0.15	<0.001
	40-59	0.01	-0.12, -0.15	0.85
	≥60	0.16	0.02, 0.30	0.03
Race				
	NHW	Ref	Ref	Ref
	MA	-0.22	-0.41, -0.04	0.02
	OH	-0.23	-0.41, -0.06	0.01
	NHB	-0.60	-0.72, -0.47	<0.011
PIR				
	≤ 0.99	Ref	Ref	Ref
	≥ 1.00	-0.02	-0.14, 0.10	0.70
Sex				
	Male	Ref	Ref	Ref
	Female	-0.04	-0.13, 0.05	0.40
Survey Year				
	2003-2004	Ref	Ref	Ref
	2005-2006	0.20	-0.08, 0.47	0.17
	2007-2008	0.38	0.08, 0.68	0.02
	2009-2010	0.55	0.26, 0.84	<0.001
	2011-2012	0.79	0.53, 1.06	<0.001

Table 2.9. Log Odds for being in class 001 and select demographic variables, participants age 6-85 years who had their biomarkers analyzed for 7 chemicals, NHANES 2003-2012

		Log Odds	Confidence Interval	pvalue
Age				
	6-19	Ref	Ref	Ref
	20-39	0.26	0.06, 0.47	0.01
	40-59	0.42	0.22, 0.62	<0.001
	≥60	0.33	0.11, 0.55	0.004
Race				
	NHW	Ref	Ref	Ref
	MA	-0.12	-0.39, 0.15	0.39
	OH	-0.75	-1.01, -0.49	<0.001
	NHB	-1.43	-1.65, -1.21	<0.001
PIR				
	≤ 0.99	Ref	Ref	Ref
	≥ 1.00	0.02	-0.18, 0.21	0.86
Sex				
	Male	Ref	Ref	Ref
	Female	0.58	0.44, 0.71	<0.001
Survey Year				
	2003-2004	Ref	Ref	Ref
	2005-2006	-0.27	-0.65, 0.10	0.16
	2007-2008	-0.60	-0.92, -0.27	<0.001
	2009-2010	-0.14	-0.45, 0.16	0.36
	2011-2012	0.17	-0.10, 0.44	0.22

Table 2.10. Log odds for being in class 110 and select demographic variables, participants age 6-85 years who had their biomarkers analyzed for 7 chemicals, NHANES 2003-2012

		Log Odds Ratio	Confidence Interval	pvalue
Age				
	6-19	Ref	Ref	Ref
	20-39	-0.09	-0.31, 0.12	0.40
	40-59	0.23	-0.004, 0.47	0.06
	≥60	0.32	0.15, 0.50	<0.001
Race				
	NHW	Ref	Ref	Ref
	MA	0.48	0.17, 0.80	0.004
	OH	1.13	0.86, 1.40	<0.001
	NHB	1.29	1.05, 1.52	<0.001
PIR				
	≤ 0.99	Ref	Ref	Ref
	≥ 1.00	-0.40	-0.56, -0.22	<0.001
Sex				
	Male	Ref	Ref	Ref
	Female	-0.04	-0.18, 0.10	0.59
Survey Year				
	2003-2004	Ref	Ref	Ref
	2005-2006	-0.46	-0.74, -0.09	0.02
	2007-2008	-0.26	-0.56, 0.04	0.10
	2009-2010	-0.53	-0.60, -0.20	0.002
	2011-2012	-0.73	-1.08, -0.37	<0.001

Table 2.11. Log Odds ratio for being in class 111 and select demographic variables, participants age 6-85 years who had their biomarkers analyzed for 7 chemicals, NHANES 2003-2012

		Log Odds	Confidence Interval	pvalue
Age				
	6-19	Ref	Ref	Ref
	20-39	-0.28	-0.51, -0.05	0.02
	40-59	-0.21	-0.53, 0.11	0.20
	≥60	0.18	-0.10, 0.45	0.21
Race				
	NHW	Ref	Ref	Ref
	MA	1.15	0.61, 1.70	<0.001
	OH	1.65	1.22, 2.08	<0.001
	NHB	1.74	1.35, 2.13	<0.001
PIR				
	≤ 0.99	Ref	Ref	Ref
	≥ 1.00	-0.14	-0.40, 0.13	0.33
Sex				
	Male	Ref	Ref	Ref
	Female	-0.003	-0.26, 0.26	0.98
Survey Year				
	2003-2004	Ref	Ref	Ref
	2005-2006	-0.26	-0.79, 0.27	0.34
	2007-2008	-0.31	-0.76, 0.14	0.18
	2009-2010	-0.61	-1.25, 0.04	0.07
	2011-2012	-0.77	-1.22, -0.33	0.001

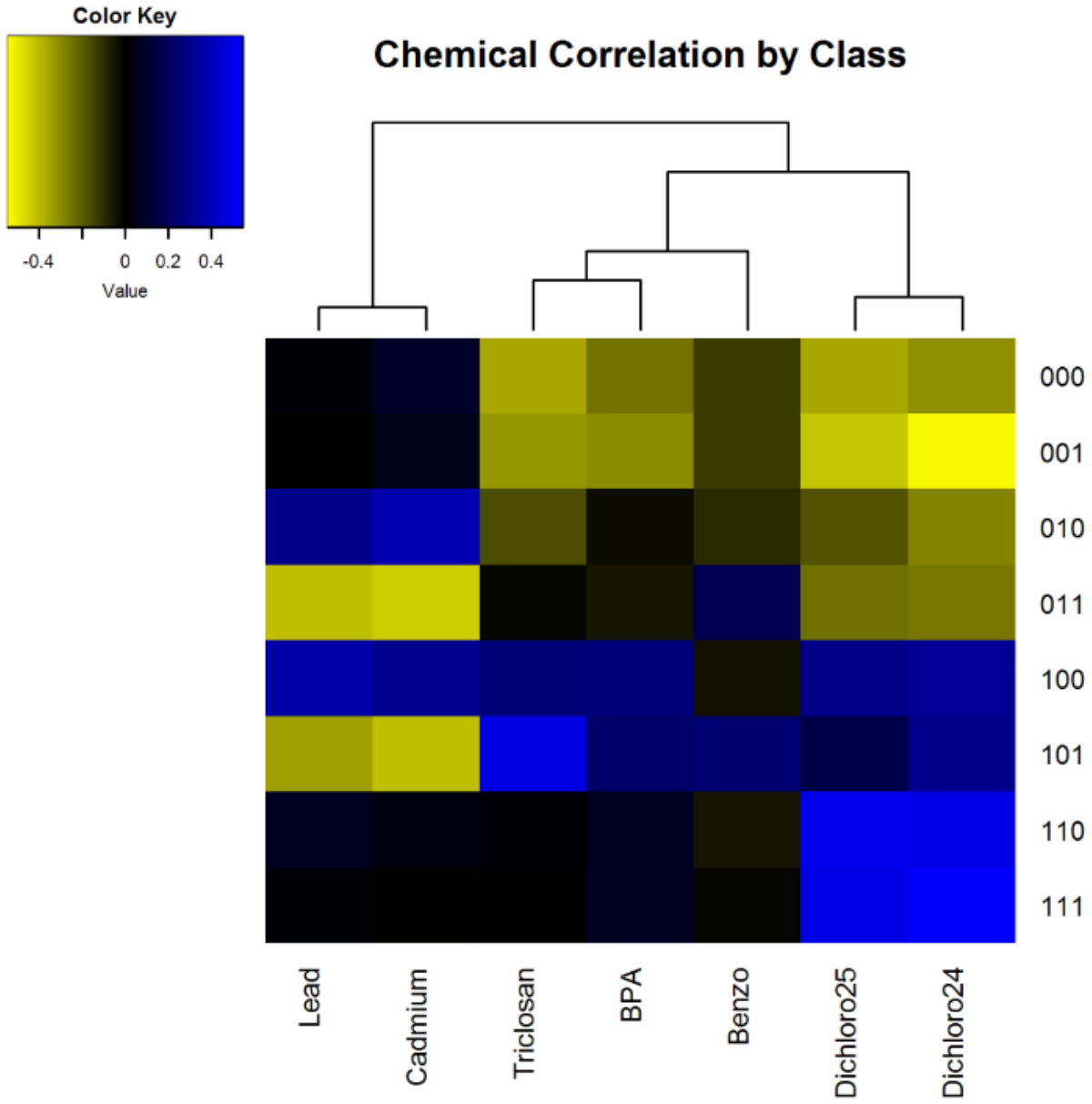

Figure 2.1. Heat map depicting correlation and clustering of 7 environmental chemicals and 8 classes using Recursive Partition Mixture Modelling (RPMM).

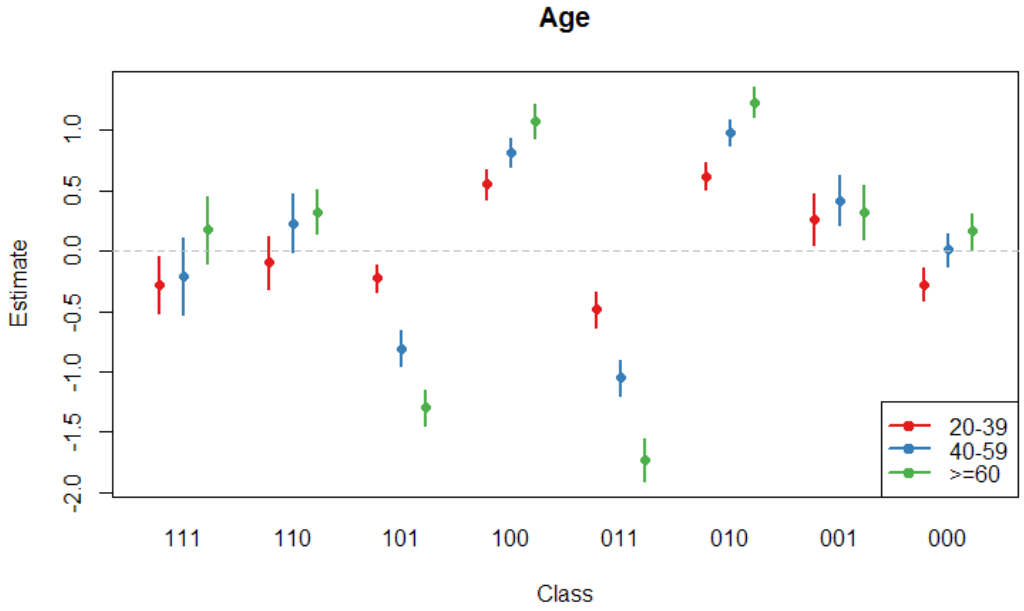

Figure 2.2. Estimates and 95% CI for class membership and age, participants age 6-85 years who had their biomarkers analyzed for 7 chemicals, NHANES 2003-2012

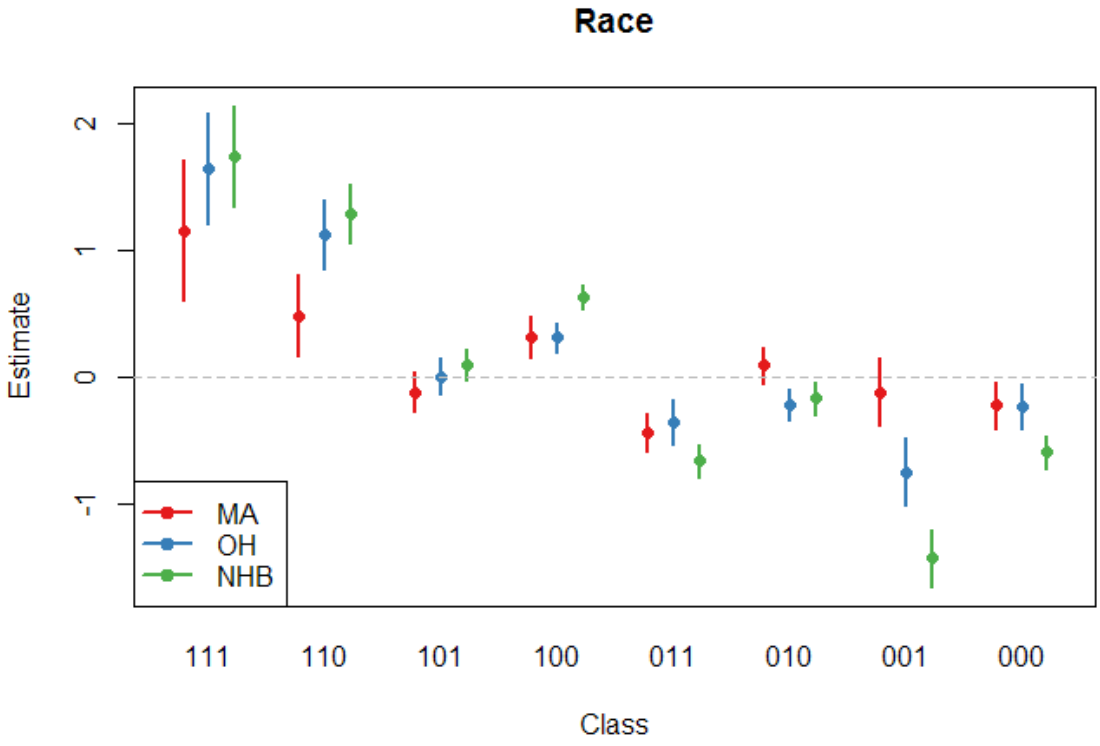

Figure 2.3. Estimates and 95% CI for class membership and race, participants age 6-85 years who had their biomarkers analyzed for 7 chemicals, NHANES 2003-2012

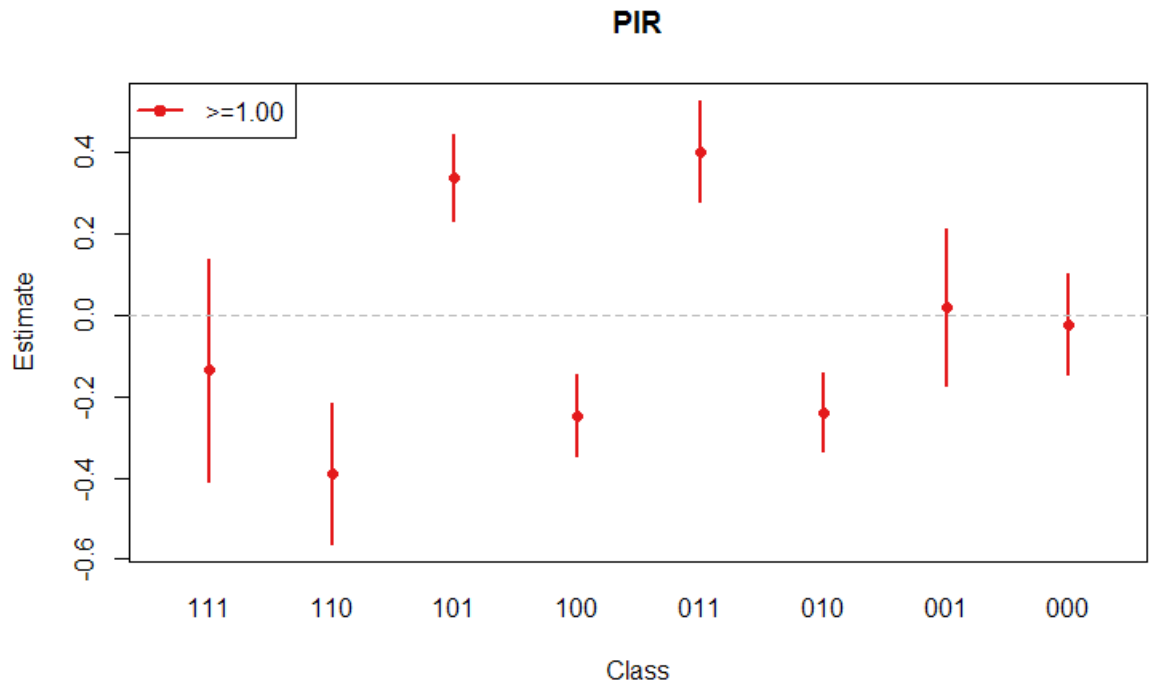

Figure 2.4. Estimates and 95% CI for class membership and PIR, participants age 6-85 years who had their biomarkers analyzed for 7 chemicals, NHANES 2003-2012

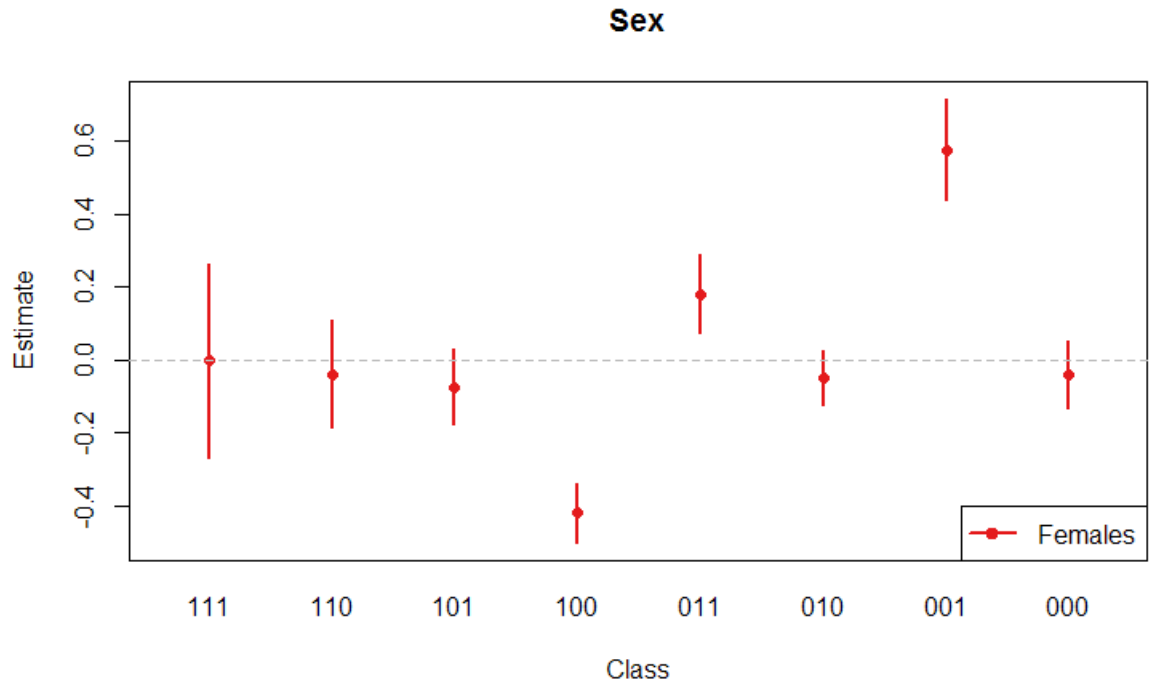

Figure 2.5. Estimates and 95% CI for class membership and race, participants age 6-85 years who had their biomarkers analyzed for 7 chemicals, NHANES 2003-2012

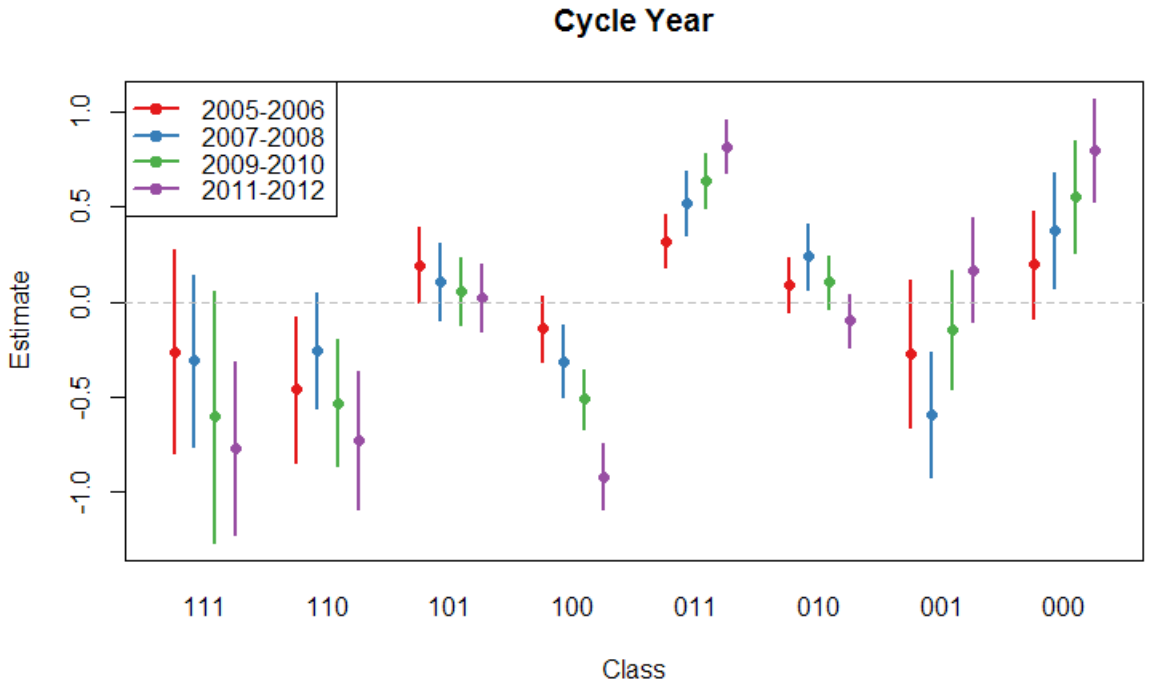

Figure 2.6. Estimates and 95% CI for class membership and race, participants age 6-85 years who had their biomarkers analyzed for 7 chemicals, NHANES 2003-2012

Chapter 3 – Second Manuscript

Title: A pathway analysis of multiple neurotoxic chemicals and cognitive functioning in older US adults (NHANES 1999-2002)

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Abstract

Polychlorinated biphenyls (PCBs) and heavy metals (lead and cadmium) are neurotoxic and affect neurobehavioral performance. Yet little is known about the association between exposure to multiple neurotoxins and cognitive functioning in older adults.

Using data from two consecutive cycles of the National Health and Nutrition and Examination Survey (1999-2002), path analysis was used to simultaneously evaluate the association between blood serum concentrations of 14 neurotoxins and cognitive functioning measured by the Digit Symbol Coding Test of the Weschler Adult Intelligence Scale, 3rd Edition in participants 60-84 years of age (N=498). Models were stratified by age (above/below the mean) to test for effect modification. The final path model fit 5 compounds (i.e. PCB 74, PCB 118, PCB 146, PCB 153, and lead). After controlling for co-exposures and confounders, PCB 146 (β = -0.17, 95% CI: -0.32, -0.02, p=0.03) and lead (β = -0.08, 95% CI: -0.17, 0.01, p = 0.09) was negatively associated with DSC scores in 60-84 year olds. Whereas, PCB 153 was positively associated with DSC scores (β =0.21, 95%CI: 0.0.05, 0.36; p=0.01). This cross-sectional analysis which controlled for collinear exposure to several neurotoxic compounds demonstrated an association between non-

dioxin like polychlorinated biphenyl exposure, specifically PCB 146, and lower cognitive functioning, in older adults. Lead exposure was also weakly associated with lower cognitive functioning. Additional studies are needed to determine the causality of the observed associations.

Background

Age-related decreases in cognitive functioning is typical as life progression. Normal age-related cognitive impairment is characterized by increasing difficulties with memory, learning new information, speed of processing information, language recall and other cognitive functions (121). Mild cognitive impairment (MCI) is a greater than average age-related changes in cognitive functioning. MCI has been shown to be a risk factor for other neurodegenerative diseases like Alzheimer's disease (122). Findings from the Aging, Demographic and Memory Study (ADAMS) indicated that the prevalence of cognitive impairment, dementia and Alzheimer's disease is 60.4, 33.3 and 22.9 per 1,000 person years, respectively. Further it was estimated that over a 6 year period 3.4 million individual will develop dementia and out of these people who developed dementia, 2.3 million will develop Alzheimer's disease (123). These numbers are especially dire as the population of the United States is rapidly aging (2). Economically speaking, in 2010 it was estimated that costs related to dementia and Alzheimer's disease was between $159 to $215 billion dollars and by 2040 those cost could increase to $379 to $500 billion dollars (123, 124). Further it has been shown that the health of family caregivers of people with dementia or Alzheimer's disease declines(125), indicating that cognitive impairment negatively affects more than the elderly.

Exposure to certain industrial chemicals such as lead, cadmium, and polychlorinated biphenyls (PCBs) can affect the central nervous system (CNS) and result in alterations in neurobehavioral performance (126-128). Once absorbed in the body lead, cadmium, and PCBs can influence brain functioning by affecting neural cells, inducing oxidative stress, and lowering dopamine concentrations (17, 129-131). Because age-related cognitive impairment are predicted

to have a high societal and economic impact (123-125), identifying preventable environmental exposures is important to ensure a healthy aging population.

Considerable evidence shows that the developing brain is vulnerable to environmental pollutants (132-136). There is also evidence that the brain is vulnerable to environmental toxics during the later stages of life due to behavioral, metabolic and physiological changes that occur with aging (137). These studies are often restricted to examining the effect of single environmental exposures on neurocognitive outcomes in older adults. For instance, the Normative Aging Veterans Affair (VA) cohort demonstrated that blood lead levels were a significant negative predictor of performance on speed memory, spatial copying and vocabulary (n=141) from a battery of 8 cognitive tests (138). Lead was associated with higher odds of having a Mini Mental State Exam (MMSE) score (n=1,031) less than 24, which is an indication of increased risk of dementia in elderly men (139). Cadmium also had a negative association with cognitive functioning using Symbol Digit Substitution Test (SDST) in US adults 20-59 (n=5,572) (4). Whereas another study with Chinese subjects > 65 years of age (n=1,016) observed a negative relationship between cadmium exposure and cognitive functioning using a composite score of the Community Screening Instrument for Dementia (CSID), the Consortium to Establish a Registry for Alzheimer's Disease (CERAD), Word List Learning Test, the CERAD Word List Recall Test, the Indiana University (IU) Story recall, Animal Fluency test and the IU Token test (140). Total serum polychlorinated biphenyls (PCBs) have also been associated with decreased measures of memory and learning as measured by the California Verbal Learning Test trial and increase depression as measured by the Beck Depression Inventory in participants (n=253) aged 55-74 years of age (141).

Yet very little is known about how exposure to multiple environmental toxins affect cognition in adults despite the fact that humans are often exposed to mixtures from several different chemical classes. Attempts have been made to examine the effect of PCB mixtures on cognitive functioning, either summing PCB concentrations or using toxic equivalency factors (TEFs). TEF report the toxicity of a single PBC congener in relation to the most toxic dioxin, 2,3,7,8-TCDD (107). TEFs are determined using relative effect potencies (REP) established using World Health Organization (WHO) criteria of a compound's binding capacity and ability to elicit toxic responses from the AhR, persistence in the environment, and accumulation in food chain (94). Summing concentrations of different PCBs congeners assumes an additive nature of the chemicals specific to the endpoint, which in the case of PCBs and cognitive functioning may or may not be true. Additionally using the TEFs to summarize the potency of PCBs mixtures must be done with caution considering PCBs' TEFs are both species and response dependent (92).

Therefore, we employed path analysis to examine the association between multiple chemical exposures from different chemical classes on cognitive functioning in older adults. Path analysis is a technique well-suited to modeling multiple collinear environmental exposures because of the ability to determine the magnitude and significance of the relationship between several exposures and an outcome, simultaneously (142). We hypothesized that exposure to multiple environmental chemicals would be negatively associated with cognitive functioning in older U.S. adults as measured by the Digit Symbol Coding (DSC) test from the Wechsler Adult Intelligence Scale, 3rd edition (WAIS-III).

Methods

Study design and population

Data from the National Health and Nutrition Examination Survey (NHANES) continuous cycles 1999-2000 and 2001-2002 were merged for this analysis. NHANES is funded and conducted by the National Center for Health Statistics (NCHS), which is part of the Center for Disease Control (CDC). NHANES is the main tool used to gather data to guide federal health programs and initiatives. NHANES collects data by utilizing physical examinations, specimen collection and surveys to collect data on nutrition and health measurements from a US non-institutionalized representative sample population (11).

This study focused on older adults aged ≥60 years. For confidentiality reasons, NHANES top-codes age at 85 years of age. Therefore, to eliminate outliers due to extreme age and to be consistent with previous studies (102) this sample consists of women and men aged 60-84 years of age who had their blood serum analyzed for lead, cadmium and PCBs and completed the WAIS-III DSC module (n=870). Since having a stroke is a major reason for cognitive dysfunction (143), participants were excluded (n=57) from the analysis if they answered yes to the question “Has a doctor or other health professional ever told you that you had a stroke?”

Of the 813 individuals aged 60-84 year in the subsample that had their blood serum analyzed for PCBs and reported not having a stroke, 715 individuals also had information on DSC scores. However, 102 individuals were missing information on sociodemographic variables with 95, 6, and 1 individuals missing data on poverty index ratio (PIR), smoking status, and education, respectively. Among participants with measured PCB, 115 individuals were excluded due to potential contamination or inadequate biospecimen sample size leaving 498 individuals with complete data.(144).

Exposure

PCBs were measured in blood serum using high-resolution gas chromatography/isotope-dilution. Cadmium and lead were measured in blood samples using inductively coupled plasma mass spectrometry (ICP-MS) (109). The methods for detecting the environmental chemicals did not change from the 1999-2000 to the 2001-2002 cycles. All analytes were detected at a frequency of 75% or above. For chemical values detected below the limit of detection (LOD), NCHS imputes values equal to the limit of detection divide by the square root of 2 ($LOD/\sqrt{2}$) into the dataset.

Cognitive Functioning

Cognitive functioning was assessed using the DSC Module of the WAIS-III. Participants were given a key with symbols corresponding to letters. It was ensured the participants had an adequate writing area, glasses (if needed) and could complete the test without distraction before they were allowed to begin the test. Practice sheets were given to ensure the concept of the test was understood before proceeding with the test. Participants were then given the test sheet which showed numbers and asked to draw as many of the corresponding symbols as they could in 120 seconds. The number of symbols correctly draw were then summed with a maximum score of 133. Cognitive scores were not provided for participants who refused to take the test, could not complete the test due to distraction, had cognitive or physical limitations, or did not complete the test in the given time limit. Examiners were given extensive instruction on how to score the symbol drawing and 10% of the test were scored twice as quality assurance (145). Cognitive testing procedure did not change from the 1999-2000 to the 2001-2002 cycles.

Covariates

We explored a variety of sociodemographic covariates based on prior literature showing that they were related to cognitive functioning and/or environmental chemical exposure including: race/ethnicity (Mexican American [MA], Other Hispanic [OH], Non-Hispanic White

[NHW], Non-Hispanic Black [NHB]), age (continuous), education level (less than 9th grade, 9-11th grade, high school diploma or GED, some college, college graduate or above), PIR (≤ 0.99 and ≥ 1.00) and sex (male/female) (102, 146-148). Since cigarette smoke is a source of cadmium and lead (149) and has been associated with decreased cognitive functioning (150), smoking status was explored as a covariate. Participants with blood cotinine levels $\leq$10 ng/L were categorized as non-smoking and participants with blood cotinine levels of >10 ng/L were categorized as smokers (151).

Statistical Analysis

All serum chemical exposures were natural log transformed to address skewness. Descriptive statistics were calculated using a 4 year sampling weight to account for using two consecutive cycles of NHANES using Stata for Windows (version 14, StataCorp LP, www.stata.com). Survey variables including stratification, clustering (PSU) and sample weights corresponding to the 4 year weights of the subsample who had their serum analyzed for PCBs were used to account for the complex sampling design of the NHANES (112).

Path analysis was conducted using MPlus (version 7.4, Muthén and Muthén; www.StatModel.com) to determine the standardized path coefficients, although Stata was used to construct the path diagram. Survey variables and sampling weights were included in the path analysis. The path coefficients in a standardize model can be used to compare the relative influence among variables that have different measurements scales and are similar to beta coefficients in regression. In path analysis the term "effect" refers to statistical effect not causal effect. Complete case analysis was used.

Initially 14 chemicals from the chemical groups of heavy metal (lead and cadmium), non-dioxin like PCBs (PCB 74, PCB 99, PCB 138, PCB 146, PCB 153, PCB 170, PCB 180, PCB 187), and dioxin-like PCBs (PCB 118, PCB 126, PCB 156, PCB 169) were including in the

a priori path analysis models (Figure 1). A more parsimonious model was then fit by removing environmental chemicals that were not significantly (p-value ≥ 0.05) associated with cognitive functioning. Model were adjusted for PIR, education, race, age, sex and smoking status. Analysis was stratified by age (above mean/below mean) because previous studies have reported differences in associated between PCBs and neuropsychological functioning by age (152).

We conducted a sensitivity analysis by re-analyzing the final models using non-lipid adjusted PCBs instead of lipid-adjusted PCBs. This is because the serum samples collected in this study were non-fasting which can influence the concentration of lipophilic compounds (153).

Results

The descriptive characteristics of the population are shown in Table 1. We compared the characteristics of the subpopulation included in this analysis with those who were excluded due to missing data. We observed that the individuals included in this analysis were more likely to be Mexican American, Other Hispanic or Non-Hispanic White. Otherwise, the characteristics of the subsample included in this analysis were similar to those with missing data.

The mean concentrations for each of the biomarkers are shown in Table 2. The mean concentration of lead and cadmium was 2.17 µg/dL (95% Confidence Interval (95% CI): 2.07, 2.28 µg/dL) and 0.49 µg/dL (95% CI: 0.46, 0.52 µg/dL), respectively. For the lipid adjusted and non-lipid adjusted non-dioxin like PCBs, the highest concentration was for PCB 153 with a geometric mean of 66.99 ng/g (95% CI: 63.66, 70.50 ng/g) and 0.44 ng/g (95% CI: 0.42, 0.47 ng/g), respectively. For the dioxin like PCBs, PCB 118 had the highest lipid adjusted and non-lipid adjusted geometric mean of 20.16 ng/g (95% CI: 18.43, 22.05 ng/g) and 0.13 ng/g (95% CI: 0.12, 0.14 ng/g), respectively.

Our *a priori* model included all 14 environmental chemicals in the model. Not all environmental chemicals were significantly associated with cognitive functioning and subsequently dropped from the final path model, which included 5 different chemicals. For all participants (aged 60-84), PCB 146 had the strongest negative association with cognitive functioning scores with a path coefficient of -0.17 (95% CI: -0.32, -0.02). This effect size can be interpreted as an increase in 1 standard deviation (SD) in the exposure of PCB 146 is associated with a decrease in DSC score of 0.17 points after controlling for other chemical exposures, race and education. Blood lead levels were also associated with a slightly lower DSC score although the strength of this association was weaker (β = -0.08, 95% CI: -0.17, 0.01, p = 0.09). We also observed that PCB 153 had a strong positive association with cognitive functioning scores with a path coefficient of 0.21 (95% CI: 0.05, 0.36) (Figure 2, Table 3).

When analyzing the population stratified by age, the directions of the associations for PCB 146, lead, and PCB 153 were similar but the strength of the association was weakened and these associations were no longer significant (Table 4). This suggested that there was no effect modification by age. Additionally, we observed no substantial differences in the observed associations when PCBs were modeled using non-lipid adjusted PCBs.

Discussion

In this cross sectional study of the U.S. population we observed that chemical exposures were associated with cognitive functioning in 60-84 year olds. After controlling for co-exposures, we identified PCB 146 as having a negative relationship with cognitive functioning. This is important because non-coplanar PCBs do not active the arylhydrocarbon receptor (AhR) (132) and subsequently are less of a concern to regulatory bodies. We also observed that lead was weakly associated with lower cognitive scores in this older adult population which is not surprising given that lead is a known neurotoxin (154, 155). This association has also been

demonstrated in previous studies (138, 139, 156-158). Lead can pass through the blood-brain barrier (BBB) by substituting for calcium ions and affect the brain through oxidative stress, altering neurotransmission, or inducing neural cells cell death (17, 128, 130, 131). There is also evidence from mouse studies that exposure to lead results in the accumulation of amyloid beta protein (159), which accumulates in the brain of Alzheimer's patients (160). Interestingly, when we were building our model, lead was very strongly associated with lower cognitive scores until we controlled for smoking status which attenuated the strength of the association. This was not unexpected as tobacco smoke is a source of lead and has been shown to be a predictor of cognitive decline (128, 149, 161).

Surprisingly, we did not find an association between cadmium and cognitive functioning despite cadmium being known neurotoxicant (162). However, the relationship between cadmium and cognitive function in adults is not clear. For instance, several studies using various tests to assess cognitive functioning have found a negative relationship between cadmium exposure and cognitive functioning (4, 140). In contrast, a study using the MMSE in Malaysian 60-72 year olds (n=54) found a non-significant positive relationship between cadmium exposure and cognitive functioning (163). These discrepancies could be due to different effects of cadmium at different ages, controlling for different confounders, or study design. Further research is needed to determine the effect of cadmium on cognitive functioning in adults and the elderly.

Similar to previous studies using toxic equivalents, total PCBs or total dioxin like PCBs, we found a significant negative relationship between non-dioxin like PCB 146 and cognitive functioning after controlling for co-exposure to four other PCB congeners (102, 141, 152, 164, 165). Unlike dioxin like PCBs which active the aryl hydrocarbon receptor (AhR) non-dioxin like PCBs do not active the AhR (29). Instead, epidemiology and animal toxicological studies

suggest non-dioxin like PCBs act on the CNS by decreasing dopamine production (31, 32, 166, 167). Dopamine is an important neurotransmitter and its production is not related to the AhR (31), therefore summing all PCBs congeners or modeling only dioxin like PCBs may overestimate the risk of PCBs on cognitive functioning.

Interestingly, we also observed that PCB 153 had a significant positive association with cognitive functioning. We are unaware of a biological mechanism that can explain this findings, although PCB 153 has a less potent Neurotoxic Equivalent (NEQ) than PCB 146. NEQs are determined using REPs that are derived using *in vitro* experiments with neurotoxic outcomes. NEQs were developed to account for the neurotoxicity of PCB congeners, such as ortho-substituted non-coplanar PCBs, which are not included in the TEF scheme (33). Similarly to modeling sum PCBs, modeling PCB toxic equivalents could overestimate or underestimate risk of altered cognitive functioning by not addressing PCBs' neurotoxic mode of action.

There are several strength to this study. Specifically, we were able to simultaneous control for the multiple collinear chemical exposures representing different classes of persistent environmental pollutants and other confounders. However, there are several limitations that are worth mentioning. NHANES is a cross-sectional study which prevents us from understanding the temporality between the exposures and the outcomes. Although, the environmental exposure used in this analysis have relatively long half-lives on the order of months to years (15, 23, 168) and therefore represent long-term exposure. Additionally, the DSC scores have proven to be a strong predictor of cognitive functioning even when assessed at only one time point (169). Also we were unable to account for several important covariates. For instance, data was not available on neurotoxic and neuroprotective factors such as methylmercury (170) and omega-3 fatty acids (171), respectively. NHANES only measures a subset of chemicals in each participant which

limits the number of compounds that can be included in this analysis. Therefore, we were unable to model additional chemicals which may play a synergistic or antagonistic role in the association between environmental exposures and cognitive functioning. Additionally, because NHANES samples non-institutionalized individuals, cognitively impaired elderly individuals in retirement or assistance living facilities were not captured in this subpopulation, which could result in an underestimate of associations. Finally, findings may not be representative of the general population because the analyzed population had a different racial composition compared to population that did not have missing data.

Conclusion

In this sample of older US adults, we observed a dose-depended effect between lower cognitive functioning and non-dioxin like PCBs and heavy metals, specifically PCB 146 and lead. Additional animal toxicity and epidemiology are needed to confirm the role of non-dioxin like PCBs and declines in cognitive function. Continued development and incorporation of the recently proposed NEQs (Simon et al. 2007) will allow the impact of non-dioxin like PCBs on neurotoxicity to be properly addressed. Since neurodegenerative disease have a high economic and social burden and many environmental exposures are preventable the potential impact of exposure to neurotoxins in late life is great and warrants further investigation to guide prevention and intervention efforts.

Table 3.1 Descriptive characteristics for adults 60-84 years of age who had their serum analyzed for PCBs, lead and cadmium and did not have a stroke (NHANES 1999-2002).

Variable	Males and females aged 60-84, who had completed data N (%[a])	Males and females aged 60-84, who did not have complete data N (%[a])	Chi-square (pvalue)
Age (years)			0.12 (0.73)
60-69	250 (50.24)	162 (54.00)	
70-84	248 (49.76)	153 (46.00)	
Race			8.77 (0.03)
MA	106 (2.98)	76 (.53)	
OH	35 (8.24)	18 (6.78)	
NHW	293 (82.02)	160 (78.97)	
NHB	64 (6.75)	61 (10.71)	
PIR			2.23 (0.14)
≤0.99	74 (11.39)	40 (7.45)	
≥1.00	424 (88.61)	166 (57.48)	
Missing	NA	109 (35.07)	
Sex			0.12 (0.73)
Male	231 (43.45)	150 (43.00)	
Female	267 (56.55)	165 (57.01)	
Smoking Status			0.08 (0.77)
Smoker	413 (81.01)	253 (80.28)	
Non-smoker	85 (18.99)	55 (17.87)	
Missing	NA	7 (1.85)	
Education			6.81 (0.15)
Less than 9th Grade	120 (13.09)	91 (12.90)	
9-11th Grade	80 (16.01)	58 (18.70)	
High School Grad/ GED	121 (30.25)	63 (25.83)	
Some College or AA degree	96 (21.39)	65 (25.41)	
College Graduate or above	81 (19.26)	37 (16.93)	
Missing	NA	1 (0.22)	
Cognitive Functioning			7.27 (0.06)
1st Quartile	131 (15.88)	56 (10.22)	
2nd Quartile	128 (26.20)	43 (14.88)	
3rd Quartile	129 (27.87)	51 (19.12)	
4th Quartile	110 (30.06)	67 (30.69)	
Missing	NA	98 (25.08)	

[a]Weighted proportion

Table 3.2. Description of the concentration of EDCs including geometric mean, 95% confidence interval (95% CI), percent above LOD, percent who were included in this analysis (NHANES 1999-2003)

Chemical	Above LOD (%)	Missing (%)	Geometric Mean (95% CI)	Range	Geometric Mean (95% CI)	Range
Heavy Metals µg/dL						
Lead (µg/dL)	100	0	2.17 (2.07, 2.27)	0.4-16.4	NA	NA
Cadmium (µg/L)	86	0	0.49 (0.46, 0.52)	0.2-4.7	NA	NA
Non-Dioxin Like (ng/g)			Lipid-adjusted		Non lipid-adjusted	
PCB 74	92	0.70	17.78 (16.23, 19.49)	2.6-144	0.12 (0.11, 0.13)	0.02-0.89
PCB 99	80	2.10	10.65 (9.88, 11.48)	2.5-132	0.07 (0.07, 0.08)	0.02-0.80
PCB 138	92	0.56	44.28 (41.40, 47.37)	5.9-310	0.29 (0.27, 0.31)	0.04-4.01
PCB 146	74	1.26	7.86 (7.39, 8.35)	1.6-68.6	0.05 (0.05, 0.05)	0.02-0.69
PCB 153	95	0.29	66.99 (63.66, 70.50)	3.5-433	0.44 (0.42, 0.47)	0.02-5.26
PCB 170	91	4.76	20.59 (19.76, 21.46)	2.6-129	0.14 (0.13, 0.14)	0.02-1.74
PCB 180	97	0.70	51.79 (49.13, 54.60)	4.4-397	0.34 (0.32, 0.36)	0.02-3.65
PCB 187	94	0.14	14.93 (14.25, 15.64)	2.5-178	0.10 (0.09, 0.10)	0.02-1.16
Dioxin Like (ng/g)			Lipid-adjusted		Non lipid-adjusted	
PCB 118	93	0.42	20.16 (18.43, 22.05)	2.6-361	0.13 (0.12, 0.14)	0.02-1.95
PCB126[a]	75	11.19	33.55 (29.98, 37.55)	1.8-402	223.05 (199.26, 249.68)	14-8,188
PCB 156	82	1.68	10.14 (9.47, 10.87)	1.6-62.1	0.07 (0.06, 0.07)	0.02-1.03
PCB 169[a]	84	11.19	31.66 (29.66, 33.79)	3.3-172	210.45 (197.19, 224.60)	22.7-3,340.4

[a]Measured in fg/g

Table 3.3. Standardized path coefficients depicting the relationship between 5 chemicals, cognitive functioning and covariates for all participants (NHANES 1999-2002)

Path	Path coefficient (95% CI)	p-value
All Participants (N=498)		
PCB 74 → DSC	0.07 (0.07, 0.20)	0.31
PCB 118 → DSC	-0.09 (-0.24, 0.07)	0.27
PCB 146 → DSC	-0.17 (-0.32, -0.02)	0.03
PCB 153 → DSC	0.21 (0.05, 0.36)	0.01
Lead → DSC	-0.08 (-0.17, 0.01)	0.09
[a]PIR→ DSC	0.13 (0.05, 0.21)	0.001
[b]ED1→ DSC	0.16 (0.03, 0.28)	0.02
[c]ED2 → DSC	0.39 (0.25, 0.54)	<0.001
[d]ED3 → DSC	0.38 (0.27, 0.49)	<0.001
[e]ED4 → DSC	0.46 (0.32, 0.57)	<0.001
[f]MA → DSC	-0.09 (-0.14, -0.04)	0.001
[g]OH → DSC	-0.12(-0.20, -0.04)	0.002
[h]NHB → DSC	-0.17 (-0.32, -0.11)	<0.001
Age → DSC	-0.34 (-0.42, -0.25)	<0.001
Sex → DSC	0.09 (0.01, 0.18)	0.04
Smoke → DSC	-0.03 (-0.13, 0.07)	0.54

[a] PIR: Poverty Index Ratio
[b]Ed1: 9-11th Grade
[c]Ed2: High School Grad/GED
[d]Ed3: Some College or AA degree
[e]Ed4: College Graduate or above
[f]MA: Mexican American
[g]OH: Other Hispanic
[h]NHB: Non-Hispanic Black

Table 3.4 Standardized path coefficients depicting the relationship between 5 chemicals, cognitive functioning and covariates for participants 60-69 and 70-84 years old, (NHANES 1999-2002)

Path	Path coefficient (95% CI)	p-value
Age 60-69 (N=250)		
PCB 74 → DSC	0.09 (-0.12, 0.30)	0.40
PCB 118 → DSC	-0.05 (-0.32, 0.22)	0.73
PCB 146 → DSC	-0.18 (-0.42, 0.05)	0.13
PCB 153 → DSC	0.24 (-0.02, 0.51)	0.07
Lead → DSC	-0.12 (-0.26, 0.03)	0.12
[a]PIR→ DSC	0.06 (-0.01, 0.14)	0.09
[b]ED1→ DSC	0.11 (-0.09, 0.31)	0.28
[c]ED2 → DSC	0.40 (0.16, 0.63)	0.001
[d]ED3 → DSC	0.44 (0.24, 0.64)	<0.001
[e]ED4 → DSC	0.47 (0.28, 0.65)	<0.001
[f]MA → DSC	-0.11 (-0.19, -0.03)	0.008
[g]OH → DSC	-0.13 (-0.25, 0.00)	0.05
[h]NHB → DSC	-0.19 (-0.27, -0.12)	<0.001
Age → DSC	-0.18 (-0.28, -0.07)	0.002
Sex → DSC	0.12 (0.01, 0.23)	0.03
Smoke → DSC	-0.02 (-0.16, 0.13)	0.83
Age 70-84 (N=248)		
PCB 74 → DSC	0.001 (-0.20, 0.20)	0.99
PCB 118 → DSC	-0.14 (-0.31, 0.03)	0.11
PCB 146 → DSC	-0.10 (-0.39, 0.20)	0.51
PCB 153 → DSC	0.16 (-0.06, 0.37)	0.15
Lead → DSC	-0.07 (-0.18, 0.04)	0.21
PIR → DSC	0.25 (0.11, 0.39)	<0.001
ED1→ DSC	0.20 (0.07, 0.33)	0.003
ED2 → DSC	0.37 (0.19, 0.54)	<0.001
ED3 → DSC	0.28 (0.15, 0.43)	<0.001
ED4 → DSC	0.41 (0.25, 0.57)(	<0.001
MA → DSC	-0.08 (-0.15, -0.01)	0.02
OH → DSC	-0.15 (-0.25, -0.06)	0.001
NHB → DSC	-0.19 (-0.29, -0.09)	<0.001
Age → DSC	-0.21 (-0.32, -0.10)	<0.001
Sex → DSC	0.09 (-0.05, 0.22)	0.20
Smoke → DSC	-0.04 (-0.15, 0.08)	0.54

[a] PIR: Poverty Index Ratio
[b]Ed1: 9-11th Grade
[c]Ed2: High School Grad/GED
[d]Ed3: Some College or AA degree
[e]Ed4: College Graduate or above
[f]MA: Mexican American
[g]OH: Other Hispanic
[h]NHB: Non-Hispanic Black

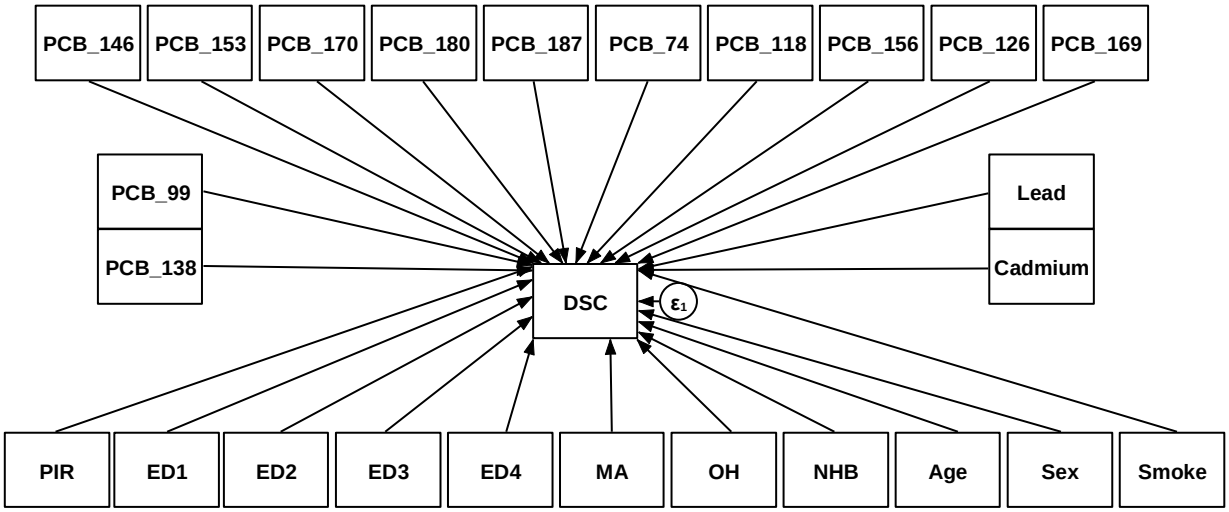

Figure 3.1 A priori model of 14 neurotoxic compounds and DSC scores and confounding factors. PIR is poverty index ratio, Ed1 is less than 9th grade, Ed2 is High School Grad/GED, Ed3 is Some College or AA degree, Ed4 is College Graduate or above, MA is Mexican American, OH is Other Hispanic and NHB is Non-Hispanic Black

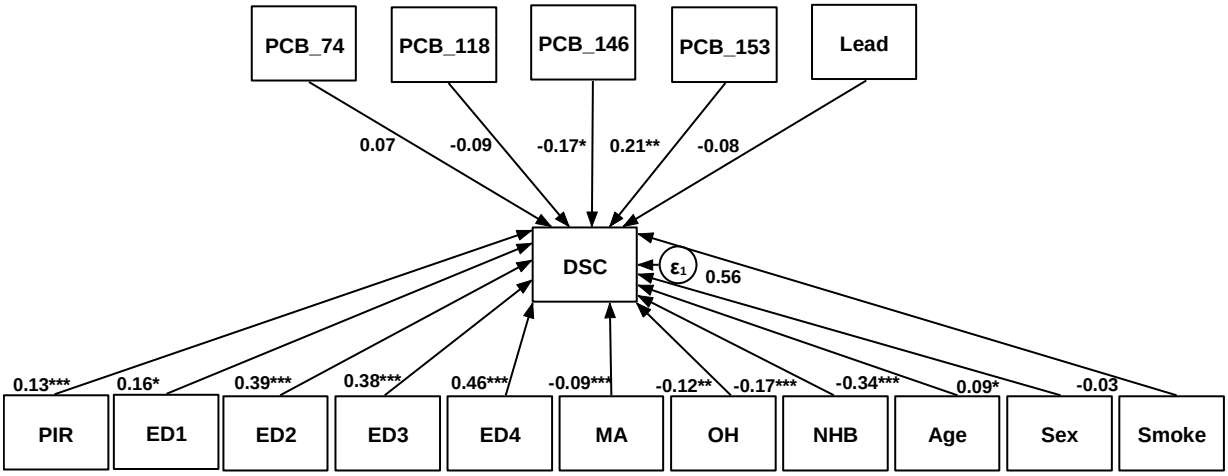

Figure 3.2. Path analysis of relationship between DSC scores and environmental chemicals after adjusting for confounding factors. Males and females 60-84 years of age, NHANES 1999-2002. Ed1 is less than 9th grade, Ed2 is High School Grad/GED, Ed3 is Some College or AA degree, Ed4 is College Graduate or above, MA is Mexican American, OH is Other Hispanic and NHB is Non-Hispanic Black.

Chapter 4 –Third Manuscript

Title: A cross sectional study of exposure to endocrine disrupting compounds on thyroid hormones and body mass index in US adults using structural equation models

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Abstract

Endocrine disrupting compounds (EDCs) affect thyroid hormone levels. Disruption of thyroid hormone homeostasis alters metabolism which can affect weight. To test the hypothesis that exposure to EDCs is associated with thyroid hormones and body mass index (BMI) using structural equation models. Using data from the National Health and Nutrition and Examination Survey (NHANES, 2007-2008), we fit a latent variable for EDCs utilizing urinary measurements of 9 compounds in females (i.e. perchlorate, bisphenol A, benzophenone-3, mono-2ethyl5carboxypentyl phthalate, mono-n-butyl phthalate, mono-(3-carboxypropyl) phthalate, mono(2ethyl5hydroxyhexyl) phthalate, mono-benzyl phthalate, and mono-isobutyl phthalate) and 8 compounds in males (i.e., without benzophenone-3). The association of this latent variable with serum thyroid hormones (Total T3, Total T4, and Thyroid Stimulating Hormones [TSH])

and BMI was assessed in females (N=676) and males (N=812) over the age of 12 controlling for age, race, creatinine, iodine, and parity (female only). EDCs were associated with higher TSH in females (β: 0.13, p <0.001) and lower Total T4 serum concentrations in males [β: -0.18, p = 0.013]. In females, EDCs were indirectly associated with higher BMI via TSH (β: 0.01, p = 0.05). No associations were observed between EDCs and BMI in males. This cross-sectional analysis demonstrated sex specific associations of EDC exposure on thyroid hormones and BMI. Specifically, exposure to multiple EDCs was positively associated with BMI in females, and this relationship was directed through TSH levels. In males, exposure to multiple EDCs was associated with lower Total T4 levels, but there was no indirect relationship with BMI.

Introduction

The neuroendocrine system is susceptible to environmental toxicants called endocrine disrupting chemicals (EDCs) (172). EDCs are exogenous compounds including many synthetic compounds such as phthalates, bisphenol A (BPA), polyhalogenated compounds, alkylphenols, and perchlorates (173). Once absorbed in the body, EDCs can influence the hypothalamic-pituitary-adrenal axis and thyroid homeostasis by binding to transport molecules or receptors (174), mimicking thyroid hormones (THs), or blocking iodine from entering the thyroid (175). For instance, if an EDC acts as a TH antagonist, the lower levels of THs prompt the hypothalamus to secrete thyrotropin releasing hormone (TRH) which informs the pituitary gland how much thyroid stimulating hormone (TSH) can be secreted (176). TSH stimulates follicular cells in the thyroid gland to produce thyroxine (T4) which is dependent on iodine concentrations (177). T4 is converted to triiodothyronine (T3) within cells (178). Once THs are produced, they interact with almost every cell in the body by binding to the thyroid hormone receptor (TR) and are important for metabolism, growth, bone and heart health, respiratory and nervous systems (178-182). Additionally, THs play an important role in energy balance and alterations in TH

levels—especially—hypothyroidism, which can lead to weight gain or difficulty losing weight (183-185).

Several studies using National Health and Nutrition Examination Survey (NHANES) data have explored the association between exposure to single EDCs and TH concentrations in the United States general population. These studies show that, among adults (n=1,346), urinary metabolites of di(2-ethylhexyl) phthalate (DEHP) were inversely related to total T4 and potentially associated with lower total T3, free T4, and thyroglobulin, and increased TSH (6). Blount and colleagues (77) found a significant positive association between urinary perchlorate and TSH levels in women (n=1,111) and a negative asociation between urinary perchlorate and total T4 among women with iodine levels <100 ug/L. They found no significant associations in men. Additionally, data from NHANES have been used to examine the relationship between EDCs and BMI. For instance, Hatch and colleagues (2008) reported that urinary phthalate metabolite concentrations were positively associated with BMI, although the strength of the association differed by sex and age. Another cross-sectional analysis (n=4,037) showed that triclosan was positively associated with BMI (53) and that people with the highest exposure to BPA (n=2747) had a higher odds of being classified as obese (186).

Individuals, however, are exposed to a mixture of EDCs. The combined effect of these exposures on thryroid hormones and BMI has not been examined. Structural equation modelling (SEM) is a technique well-suited to examining multiple highly correlated environmental exposures on complex biological pathways. SEM is heavily used in the social sciences, and the ability of SEM to test a priori hypotheses by modelling complex relationships between exposures and outcomes is resulting in SEM gaining popularity in biological and health sciences (187). Subsequently, this study aimed to test the hypothesis that exposure to multiple EDCs expressed

as a latent variable would be associated with thryoid hormone levels which in turn would be associated with BMI in the general population. Furthermore, we hypothesized that the strength of this association would be sex-specific. It should be noted that in SEM the term “effect” refers to statistical and not causal effect and we use the former meaning throughout the rest of the manuscript.

Methods

Study design and population

Data from the 2007-2008 cycle of the NHANES were used in this analysis. This cross-sectional survey utilized a complex, multistage probability sampling design that collected data on nutrition and health measures from a U.S. non-institutionalized representative sample population by utilizing physical examinations, specimen collection and surveys (11). Conducted by the National Center for Health Statistics (NCHS) of the Center for Disease Control (CDC), the data are publicly available. All protocols were approved by NCHS Research Ethics Review Board (ERB) and documented consent was obtained from all participants (108).

Subjects were included in this analysis if data were available on: i) urinary concentrations of the phenols and phthalates, and ii) serum thyroid hormones within the same individual. These measurements were assessed only in 1/3 of participants aged 12 and above. Of the 1,160 females who had their urinary EDCs and blood TH levels measured, we excluded pregnant women (n=20), women who were breastfeeding (n=10), participants with self-reported thyroid problems (n=58), and individuals taking medication for a thyroid problem (n=91), resulting in 981 females who were not at risk for thyroid dysfunction. Additionally, 305 females were missing data on a covariate (urinary perchlorate, thyroid hormones, urinary iodine, serum cotinine, BMI, caloric intake, sedentary time, menopausal status, or poverty-income ratio). This left 676 females with complete data that were used in this analysis.

Of the 1,018 males who had their urine analyzed for EDCs and had blood serum analyzed for THs, we excluded men who reported taking thyroid hormone modifiers (n=23) or reported a history of thyroid disease (n=14). These restrictions resulted in 1,095 men who were not at risk for thyroid dysfunction. Of those 283 were missing data on a covariate (urinary perchlorate, T3, T4, iodine, cotinine, BMI, caloric intake, sedentary time, or poverty-income ratio). This left in 812 males with complete data that were used in this analysis.

Measurement of chemical biomarkers in urine

Urinary concentrations of phthalates, phenols (BPA, triclosan, and benzophenone-3) and perchlorate were measured in the same participants. Phenol analytes were measured using gas chromatograph-mass spectrometry (GC-MS (111), phthalates were measured using high performance liquid chromatograph (HPLC) with electrospray ionization (ESI) combined with MS/MS (188), and perchlorate was analyzed with ion chromatography (IC) coupled with electrospray tandem MS (189). NCHS recommends using chemical data that are above 60% frequency detection (112). Therefore only 7 of the 15 urinary phthalate metabolites were retained for use in this study (i.e. mono-2ethyl5carboxypentyl phthalate [MECPP], mono-n-butyl phthalate [MnBP], mono-(3-carboxypropyl) phthalate [MCPP], mono-ethyl phthalate [MEP], mono(2ethyl5hydroxyhexyl) phthalate [MEHHP], mono-benzyl phthalate [MBzP], mono-isobutyl phthalate [MiBP]). For individuals who had biomarker concentrations below the limit of detection (LOD), NCHS inserts the LOD divided by the $\sqrt{2}$. Due to concerns that substituting values with the LOD/$\sqrt{2}$ may lead to bias, we performed a sensitivity analysis using multiple imputation to substitute values for left-censored chemical data (190). Briefly, EDC concentrations falling below the LOD were coded as missing and Markov Chain Monte Carlo (MCMC) simulations were used to create 20 complete data sets with imputed values for

concentrations below the LOD. Estimates for the SEM were then made using data drawn from these 20 datasets and compared to estimates using non-imputed data. In children BMI can be influenced by age and sex, therefore a sensitivity analysis was performed for adults aged 20 and above on each sex strata.

Thyroid Hormones and Thyroid Disease Outcomes

Total T3 (pg/mL), total T4 (ng/dL) and TSH (mIU/L) was measured in serum using various immunoenzymatic assays described elsewhere (191). Hyperthyroidism was defined by TSH values below 0.3 mIU/L while hypothyroidism was define by TSH values above 3.0 mIU/L (192).

Body Mass Index Outcome

BMI was calculated by dividing the weight of the participant in kilograms by the height of the individual in meters (193) and was used as a continuous variable in this analysis.

Covariates

Several variables were examined as potential confounders. Demographic variables that were explored included poverty index ratio (PIR) (at or below 0.99 and at or above 1.00), race (Mexican American (MA), Other Hispanic (OH) including multi-racial, non-Hispanic White (NHW), non-Hispanic Black (NHB)), and age (continuous). Caloric intake (continuous), sedentary time in minutes (continuous), energy expenditure (quartiles) and smoking status were assessed because of their potential relationship with BMI. We determined energy expenditure by multiplying self-reported minutes of vigorous work, minutes of moderate work, minutes of walking/biking for transportation, minutes of vigorous recreation, and minutes of moderate recreation by their respective metabolic equivalent score (MET) (4 for moderate activities and walking/biking for transport and 8 for vigorous activity). The values were then summed for total energy expenditure (194). Smoking status was determined using cotinine values, where ≤ 10

ng/L cotinine was classified as a non-smoker and >10ng/L cotinine was classified as a smoker (151). For women, parity (nulliparous, one live birth, or greater than one live birth) and menopausal status (pre or post-menopausal) were assessed. Iodine is needed for the production of TH, therefore urinary iodine concentrations were investigated as a covariate. For all questions, responses of "don't knows" or refused responses were coded as missing.

Statistical Modeling

All urinary chemical exposures, iodine, creatinine, BMI, THs, sedentary time, and caloric intake were natural log transformed to address skewness. Descriptive statistics were calculated using sampling weights for the subsample who had their biospecimens analyzed for phenols and phthalates using Stata for Windows (version 14, StataCorp LP, www.stata.com). Complete case analysis was utilized. SEMs were used to evaluate our *a priori* hypothesis that EDC exposure, defined as a latent variable, would have a direct effect on THs as well as the potential for the latent exposure variable to indirectly effect BMI through THs (Figures 1 and 2). Models were stratified by sex because there are important covariates (parity and menopause) that are unique to females. Model building and testing of the SEM was performed using Mplus (version 7.4, Muthén and Muthén; www.StatModel.com), although Stata was used to construct the SEM diagram and to calculate the weighted descriptive statistics of the selected populations. Survey variables, including stratification, clustering (PSU), and sample weights corresponding to the weights of the subsample who had their urine analyzed for EDC were used to account for the complex sampling design of the NHANES (112). Monte Carlo estimates for the indirect effects confidence intervals (CI) were determined using Rstudio (https://www.rstudio.com/).

In SEM diagrams the latent, or unmeasured, variables are represented by ovals. Measured variables are represented by rectangles. In this analysis, the *Exposure* latent variable is indicated by the measured EDCs variables. Because latent variables are unmeasured and have no inherent

metric, the measurement scale needs to be set during model building. In this analysis the scale was set by fixing the unstandardized loading of perchlorate to 1. Single headed arrows represent loadings - or regressions - between variables. Subsequently, the path coefficients in a standardized model can be used to compare the relative influence among variables and are similar to beta coefficients computed in regression analysis. Intercepts are indicated by the number in the lower right of the measured variables. In SEM, a model is specified based on the hypothesized associations between variables and is tested using empirical data. Models were specified by initially including all hypothesized associations, and then removing non-significant associations based on their strength of association (i.e., a p value ≥ 0.05) until the most parsimonious model was achieved. Due to the concern that BPA concentrations might be a result of external contamination (195), models were run with and without BPA. Because no substantial differences were observed between these models, we retained BPA in the EDC mixture.

To adjust for urinary dilution, creatinine was covaried out of the individual urinary EDCs and urinary iodine. Additionally, metabolites that shared the same phthalate parent compound were correlated (e.g. MEHHP with MECPP, MnBP with MBzP, MiBP with MBzP). Finally, perchlorate was correlated with iodine because of its ability to inhibit iodide uptake from the bloodstream (196). The small circles designated as ε_x are residuals, or unexplained variance.

A double-headed arrow between the residuals indicates that the residuals are correlated. Global model fit was evaluated using the following indices: Comparative Fit Index (CFI), Tucker-Lewis Index (TLI), Standardized Root Mean Square Residual (SRMR), Root Mean Square Error of Approximation (RMSEA), and the RMSEA 90% confidence interval (RMSEA 90% CI). Multinomial logistic regression was used to model the relationship between *Exposure*

and thyroid disease. Fit indices are no available for SEM's utilizing multinomial logistic regression.

Results

The descriptive characteristics of the population are shown in Table 4.1. Males who were included in this analysis were more likely to be NHW than males who were excluded due to missing data. Whereas, females who were included in this analysis were more likely to be older, NHW, overweight, and have greater than one birth than females who were excluded due to missing data. The mean concentrations of T3, T4, and TSH in males was 114.55 ng/dL (112.11, 117.03 ng/dL), 7.32 µg/dL (7.18, 7.45 µg/L), 1.63 mIU/mL (1.54, 1.72 mIU/mL). In females, the mean for concentration of T3, T4 and TSH was 111.79 ng/dL (107.63, 114.05 ng/dL), 7.57 µg/L (7.36, 7.78 µg/L), and 1.61 mIU/mL (1.44, 1.79 mIU/mL).

We initially created a latent variable based on 11 urinary EDCs which met our criteria of being measured in the same individual and where detected in over 60% of the sample. However, not all EDCs loaded on the latent variable. Additionally, the EDCs which loaded on the latent variable were different depending on sex. Thus, the latent variable was comprised of 9 EDCs for females and 8 EDCs for males. The mean concentrations and 95% confidence intervals for these urinary analytes are described in Table 4.2. The geometric mean for perchlorate was 4.40 ng/L (95% CI: 3.97, 4.87 ng/L) and 3.45 ng/L (95% CI: 3.03, 3.94 ng/L) for males and females, respectively. The highest geometric mean of the phenols for men and women was benzophenone-3 with a geometric mean of 12.14 ng/mL (95% CI: 9.05, 16.29 ng/mL) and 28.56 ng/mL (95% CI: 19.69, 41.43 ng/mL), respectively. Mono (2-ethyl-5-hydroxyhexyl) phthalate had the highest geometric mean of the phthalates with geometric mean concentrations of 23.37 ng/mL (95% CI: 19.28, 28.33 ng/mL) and 21.06 ng/mL (95% CI: 17.34, 25.57 ng/mL) for males and females, respectively.

Figures 4.3 and 4.4 show the fitted SEM for the associations between *Exposure* and THs for males and females 12-85 years of age in NHANES 2007-2008. In females MCPP had the highest loading on the latent *Exposure* variable of 0.51 (95% CI: 0.42, 0.59), which can be interpreted as an increase in 1 standard deviation (SD) in the exposure of multiple EDCs is associated with an increase of 0.51 SD of MCPP after controlling for being NHB. *Exposure* was positively correlated with TSH (0.13, 95% CI: 0.06, 0.20) among women after adjusting T3 and TSH for age (Table 4.3). For males, MCPP had the highest loading of 0.50 (95% CI: 0.31, 0.63) onto *Exposure*. *Exposure* was negatively associated with T4 (-0.18, 95% CI: -0.31, -0.05) in males (Table 4.4).

The influence of the *Exposure* on BMI, mediated through THs, can be determined by quantifying the indirect and total effects. Among females, an increase of 1 SD in the exposure of mutliple EDCs is associated with an increase of 0.01 SD (95% CI: 0.0007, 0.03) of log BMI as directed through TSH, after controlling for parity, race, age, and iodine (Figure 4.5, Table 4.5). The standardized estimates for Figure 4.5 are summarized in Table 4.6. Among males, TSH was positively associated with BMI, but exposure to EDCs had no direct or indirect impact on this association (Figure 4.6, Table 4.7). Fit indices showed that the all models are well above acceptable criteria (Table 4.8).

Figure 4.8 depicts the relationship of *Exposure* to thyroid disease in females. The standardized estimates for Figure 4.8 are summarized in Table 4.9. After controlling *Exposure* for being NHB, and thyroid disease for urinary iodine concentrations, age and PIR, the odds of hypothyroidism (0.93, 95% CI: 0.20, 4.21) or hyperthyroidism ((0.02, 95% Ci: 0.00, 2.95) was not significantly associated with *Exposure* in females (Table 4.11). For males, after controlling thyroid disease for iodine, age and being NHB, *Exposure* was not significantly associated with

either hypothyroidism (0.29, 95% CI: 0.02, 2.82) or hyperthyroidism (13.38, 95% CI: 0.04, 1,755.66) (Figure 4.9, Table 4.11). The standardized estimates for males for Figure 4.9 are summarized in Table 4.10.

There was no substaintal difference in model estimates or model fit when performing the sensitivity analysis by using multiple imputation for values below the LOD. Additionally, there was no substaintal diffrences in model estimates or model fit when using data for males aged ≥20 years old compared to using all male participarts. Nor were there any substantial differences in indirect or direct effects when using females aged aged ≥20 years old compared to using all female participants.

Discussion

In this cross-sectional study of the U.S. population, we observed that exposure to multiple EDCs was associated with differences in THs in both men and women aged 12 years and older. Specifically, exposure to multiple EDCs was postively associated with TSH in females and negatively associated with Total T4 in males. Furthermore, we observed that exposure to multiple EDCs was positvely associated with BMI in females and that this association appeared to be directed through TSH levels. These observations support the hypothesis that exposure to multiple EDCs may alter thyroid hormone levels and influence health outcomes related to thryroid homeostatis in the general population, but in a sex-specific manner

We observed that both females and males showed the expected negative association between TSH and T4 and positive association between T4 and T3, as examplified by the fact that TSH signals the production of T4 and T4 is converted to T3. However, we also observed sex-specific associations between *Exposure* and THs. While we do not know what caused the observed sex-specific effects, there are several possible explanations. First, exposure profiles and the body burden of enviornmental chemicals differ by sex (197-199). In our models, different

exposure profiles were exemplified by differences in loadings on individuals EDCs on *Exposure*. Specifically, males exhibited a stronger association between *Exposure* and phthalates. Second, hormonal loads (200) and the binding capacity of thyroid hormones may differ by sex (201). For example, the negtive association we observed between *Exposure* and T4 in males could be due to a stronger association between perchlorates, phthalates and *Exposure*, which are thought to disrupt T4 production by inhibiting the sodium-iodide symporter (NIS), thereby suppressing the uptake of iodine into the cells (113, 174, 196).

Additionally, our study showed that exposure to multiple EDCs was associated with a slightly higher BMI, as directed through TSH, in females but not males. These findings are consistent with reports that exposure to individual urinary phthalate metabolites are associated with higher BMI and larger waist circumference in the U.S. general population (194, 202). Additionally, several studies report a positive relationship between TSH concentrations and BMI (203, 204). Although limited knowledge exists on how EDCs can disrupt the thyroid feedback loop, it is possible that the transportation of T4 to the hypothalamus after exposure to EDCs is compomised. This would result in the increased production of TSH. This theory is based on data from experimental studies which show that phthalates and phenols (205, 206) can bind to transthyretin (TTR), which is the transportation protein for T4. This in turn could decrease transport of T4 to the hypothalamus which could obstruct the negative feedback loop, resulting in increased production of TSH. Furthermore, there is evidence that TSH can induce differentiation of preadipocytes into mature adipocytes, which increases adipose tissue (adipogenesis) by binding to receptors in adipose tissue (207).

Additionally, we observed that *Exposure* did not have significant indirect effects on BMI, directed through TSH and T4, or significant total effects. These null findings suggest the main

mechanism behind EDC's association with increase BMI was driven by increased TSH levels. Interestingly, the indirect effect from perchlorate to BMI was not significant, highlighting the importance of studying multiple exposures. Finally our findings indicate that exposure to multiple EDCs does not increase the odds of thyroid disease. These findings are similar to a study that found no difference in the prevalence of thyroid disease in Clark County, Nevada, where perchlorate drinking water levels are high (4 to 24 µg/L) over a 2 year period compared to other counties in Nevada that do not have levels of perchlorate in their drinking water (148).

This study has several strengths, including the use of a large nationally representative population which allowed us to control for multiple confounders. By utilizing SEM we were able to extend on previous research by modelling multiple EDCs and examine the associations between multiple EDC exposure, TSH, and BMI which have not been elucidated previously. Additionally, NHANES employs a rigorous quality control program which provides high quality environmental and biological measurements. There were also limitations to this study including the cross-sectional nature of NHANES. Although the urinary environmental biomarkers used in this analysis represent relatively short term exposure, evidence does suggests that spot urine samples are a moderate representation of long term exposure for perchlorates, phthalates, and BPA (58, 72). Another limitation is due to data constraints: we were unable to model all EDCs because of the limited number of chemicals NHANES measures in the same individual. Additionally, our *a priori* hypothesis only examined the pathway between EDCs and BMI via THs and did not capture all the ways in which EDCs might affect the outcome variables. For example, in addition to altering thyroid hormone concentrations, BPA is also thought to contribute to being overweight by affecting glucose transportation (208). Furthermore, this study only examined EDCs. Other chemicals may play a synergistic or antagonistic role in the

association between THs and BMI that were not examined in this analysis. Lastly, previous studies have suggested a confounding association between urinary biomarker concentrations and health outcomes due to differences in urinary flow rate (UFR), body weighted-adjusted (UFR), and osmolality by demographic characteristics (209) that we were unable to capture in this study. Specific to this study, osmolality increased with increasing BMI. Additionally, BPA and 2,5-dichlorophenol were shown to have positive associations with BMI, which attenuated when the association between BPA and BMI was adjusted for osmolality and creatinine and when the association between 2,5-dichlorophenol was adjusted for creatinine. Although we did adjust for creatinine, osmolality data were not available for this cycle year. There were no substantial differences in the findings when analyses were performed without BPA, suggesting the robustness of the findings despite being unable to adjust for potential confounders associated with urinary biomarker concentrations. Additionally, due to the cross-sectional nature of the data the possibility of reverse causation should be acknowledge as BMI has been shown to be a predictor of some phthalates and phenols (210, 211), although model fit and modification indices didn't support a regression arrow from BMI to phthalates or phenols. Finally, our definition of thyroid disease may not be comparable to what laboratories are currently flagging as abnormal thyroid levels. Our less restrictive definition may result in an overestimate of the prevalence of thyroid disease, however the cutoff values we used are guidelines suggested by the American Association of Clinical Endocrinologists (AACE). Using AACE cutoff values allowed us to have the largest cell size when examining the relationship between EDCs and thyroid disease and despite the less restrictive definition of thyroid disease estimates still may be unstable due to small sample sizes for hyperthyroidism.

Conclusion

Our study determined that exposure to multiple EDCs is associated with alterations in TH serum concentrations and BMI directed through TSH and that these associations were sex specific. By incorporating exposure to mixtures of environmental chemicals we were able to model a more realistic exposure scenario. Future works should focus on modelling other environmental chemicals which may have a synergistic or antagonistic effects on the EDCs used in this analysis. Further, a longitudinal study with a larger sample that collects repeat measures of the biomarkers and anthropometric data used in this analysis would reduce some of the causal uncertainty presented in this study.

Table 4.1. Descriptive characteristics for males and females who had their serum analyzed for multiple EDCs that were included in this analysis compared to those that were excluded due to missing data (NHANES 1999-2002).

Variable	Males who had complete data (n=812) N (%)	Males who did not have complete data (n=269) N (%)	Chi-square (p-value)	Females who had complete data (n=676) N (%)	Females who did not have complete data (n=305) N (%)	Chi-square (p-value)
Age (years)			2.09 (0.56)			7.76 (0.05)
Geometric Mean (95% CI)	38.37 (36.91, 39.89)	36.41 (33.96, 39.03)		37.34 (35.82, 38.92)	37.40 (35.03, 39.94)	
12-19	154 (14.66)	58 (14.42)		128 (14.32)	65 (17.60)	
20-39	215 (33.74)	77 (36.03)		178 (30.77)	81 (34.42)	
40-59	205 (33.41)	65 (33.60)		207 (37.12)	69 (22.34)	
≥60	238 (18.19)	69 (15.95)		163 (17.79)	90 (25.64)	
Race			12.23 (0.007)			11.12 (0.01)
MA	146 (9.60)	45 (10.56)		144 (8.83)	54 (7.76)	
OH	108 (9.26)	47 (13.09)		100 (9.57)	65 (14.56)	
NHW	400 (71.13)	105 (60.54)		275 (68.97)	102 (60.37)	
NHB	158 (10.00)	72 (15.89)		157 (12.62)	84 (17.31)	
PIR			2.20 (0.14)			2.21 (0.14)
Geometric Mean (95% CI)	1.74 (1.71, 1.78)	1.65 (1.58, 1.72)		1.71 (1.67, 1.74)	1.72 (1.66, 1.78)	
≤0.99	161 (12.11)	43 (12.91)		155 (15.41)	60 (14.29)	
≥1.00	651 (88.00)	130 (51.43)		521 (84.59)	155 (60.06)	
Missing	NA	96 (35.66)		NA	90 (25.65)	
BMI (kg/m^2)[a]			2.50 (0.47)			7.57 (0.06)
Geometric Mean (95% CI)	27.42 (27.61, 27.89)	27.08 (26.29, 27.89)		27.36 (26.87, 27.86)	27.08 (26.29, 27.89)	
Underweight	6 (0.45)	3 (0.43)		10 (1.39)	8 (2.94)	
Normal	254 (30.61)	85 (32.68)		234 (40.93)	110 (41.43)	
Overweight	276 (35.20)	93 (34.33)		2087 (27.99)	66 (19.53)	
Obese	276 (33.74)	74 (26.22)		224 (29.70)	105 (32.50)	
Missing	NA	14 (6.34)		NA	16 (3.59)	
Energy Expenditure[b] (minutes*MET)			4.01 (0.26)			8.07 (0.04)
Geometric Mean (95% CI)	268.44 (221.30, 325.63)	213.09 (147.03, 308.81)		79.12 (63.06, 99.28)	45.89 (32.09, 65.62)	
1st quartile	144 (14.04)	59 (19.08)		199 (25.80)	133 (31.42)	
2nd quartile	172 (21.41)	52 (16.18)		227 (32.79)	81 (29.43)	
3rd quartile	220 (28.33)	60 (22.29)		146 (23.81)	64 (23.38)	
4th quartile	276 (36.23)	93 (40.85)		104 (17.60)	39 (14.57)	
Missing	NA	5 (1.60)		NA	8 (1.20)	
Energy Intake (kcal)[b]			0.89 (0.83)			2.45 (0.48)
Geometric Mean (95% CI)	2,133 (2,066, 2,203)	2,045 (1,907, 2,192)		1,581 (1,527, 1,637)	1,570 (1,475, 1,670)	
1st quartile	134 (11.72)	41 (10.69)		233 (28.38)	82 (23.75)	
2nd quartile	173 (18.29)	45 (15.03)		203 (31.45)	68 (23.63)	
3rd quartile	212 (25.98)	57 (21.57)		155 (25.64)	66 (21.43)	
4th quartile	293 (44.00)	73 (28.92)		85 (14.52)	38 (14.14)	
Missing	NA	53 (23.79)		NA	51 (17.05)	
Sedentary Time (minutes)[b]			0.95 (0.81)			3.36 (0.34)
Geometric Mean (95% CI)	261.62 (247.89, 276.09)	262.60 (239.63, 287.78)		253.50 (237.17, 270.00)	256.53	
1st quartile	271 (29.55)	85 (31.73)		227 (30.37)	102 (29.41)	
2nd quartile	194 (22.23)	67 (28.28)		144 (19.04)	50 (15.08)	
3rd quartile	190 (25.47)	54 (15.65)		176 (29.79)	89 (35.12)	
4th quartile	157 (22.75)	52 (21.68)		129 (20.80)	54 (17.92)	
Missing	NA	11 (2.67)			10 (2.47)	
Smoking Status			1.66 (0.20)			3.90 (0.05)
Smoker	225 (29.10)	64 (30.67)		534 (78.42)	37 (12.28)	
Non-smoker	587 (70.90)	134 (48.79)		142 (21.58)	207 (69.56)	
Missing	NA	71 (20.53)		NA	61 (18.17)	
Parity			NA			61.09 (<0.001)
Nulliparious	NA	NA		211 (30.96)	174 (58.69)	
One Birth	NA	NA		94 (15.08)	18 (4.74)	
Greater One Birth	NA	NA		371 (53.96)	113 (36.56)	
Missing	NA	NA		NA	NA	
Menopausal Status			NA			2.22 (0.34)
Pre-menopause	NA	NA		402 (63.56)	106 938.17)	
Post-menopause	NA	NA		274 (36.44)	92 (25.27)	
Missing	NA	NA		NA	107 (36.56)	

[a] BMI categories for males and females aged 20 and above were determined using the CDC's definition. BMI < 18.5 is underweight, BMI ≥ 18.5 and ≤ 24.9 is normal, BMI ≥ 25.0 and ≤ 29.9 is overweight, and BMI ≥ 30.0 is obese (212). BMI definition for males and females aged 12-19 are determined using percentiles calculated from the CDC growth charts (213).

[b]Description of quartiles can be found in Appendix Table A.1.

Table 4.2. Description of the concentration of EDCs in males and females including geometric mean, 95% confidence interval (95% CI), percent above LOD, and percent missing (NHANES 2007-2008).

Chemicals	Males (n=812) Geometric Mean (95 % CI)	Above LOD (%)	Percent Missing (%)	Females (n=676) Geometric Mean (95% CI)	Above LOD (%)	Percent Missing (%)
Perchlorate (ng/L)	4.40 (3.97, 4.87)	100	4.35	3.45 (3.03, 3.94)	100	6.72
Bisphenol A (ng/mL) (BPA)	2.20 (1.93, 2.50)	94	2.04	2.00 (1.78, 2.24)	92	3.57
Benzophenone-3 (ng/mL)	12.14 (9.05, 16.29)	96	2.04	28.56 (19.69, 41.43)	97	3.57
Mono-2ethyl5carboxypentyl phthalate (ng/mL) (MECPP)	34.47 (28.77, 41.30)	99	2.04	31.55 (25.95, 38.37)	96	3.57
Mono-n-butyl phthalate (ng/mL) (MnBP)	17.85 (16.25, 19.61)	99	2.04	19.87 (16.90, 23.35)	99	3.57
Mono-(3-carboxypropyl) phthalate (ng/mL) (MCPP)	2.92 (2.58, 3.30)	98	2.04	2.44 (2.09, 2.86)	93	3.57
Mono(2ethyl5hydroxyhexyl) phthalate (ng/mL) (MEHHP)	23.37 (19.28, 28.33)	97	2.04	21.06 (17.34, 25.57)	96	3.57
Mono-benzyl phthalate (ng/mL) (MBzP)	7.35 (6.50, 8.30)	99	2.04	6.82 (5.71, 8.13)	97	3.57
Mono-isobutyl phthalate (ng/mL) (MiBP)	7.38 (6.61, 8.25)	98	2.04	6.67 (6.00, 7.42)	98	3.57
Total T3 (ng/dL)	114.55 (112.11, 117.03)	NA	8.32	110.79 (107.63, 114.05)	NA	8.26
Total T4 (ug/L)	7.32 (7.18, 7.45)	NA	8.41	7.57 (7.36, 7.78)	NA	8.26
TSH (mIU/mL)	1.63 (1.54, 1.72)	NA	8.32	1.61 (1.44, 1.79)	NA	8.26
Iodine (ug/L)	169.19 (156.20, 183.25)	NA	7.31	145.77 (134.16, 158.39)	NA	9.89
Creatinine	124.02 (117.10, 131.36)	NA	1.76	86.46 (78.70, 94.98)	NA	2.65

Table 4.3. Standardized estimates from the SEM depicting the relationship between 9 EDCs, THs, and covariates in females 12-85 years of age, NHANES 2007-2008. These values are depicted in Figure 4.3.

Loadings/effects	Standard Estimates (95% CI)	Standard Errors	p-value
Exposure → Perchlorate	0.24 (0.13, 0.35)	0.06	<0.001
Exposure → BPA	0.22 (0.13, 0.31)	0.05	<0.001
Exposure → Benzophenone-3	0.22 (0.09, 0.35)	0.07	0.001
Exposure → MECPP	0.46 (0.34, 0.59)	0.06	<0.001
Exposure → MnBP	0.28 (0.19, 0.38)	0.05	<0.001
Exposure → MCPP	0.51 (0.42, 0.59)	0.04	<0.001
Exposure → MEHHP	0.49 (0.38, 0.60)	0.06	<0.001
Exposure → MBzP	0.24 (0.15, 0.33)	0.05	<0.001
Exposure → MiBP	0.26 (0.14, 0.39)	0.06	<0.001
NHB → Exposure	-0.21 (-0.32, -0.11)	0.06	<0.001
Exposure → TSH	0.13 (0.06, 0.20)	0.04	<0.001
Creatinine → Perchlorate	0.57 (0.49, 0.64)	0.04	<0.001
Creatinine → BPA	0.63 (0.52, 0.74)	0.06	<0.001
Creatinine → Benzo	0.27 (0.19, 0.36)	0.04	<0.001
Creatinine → MECPP	0.59 (0.52, 0.66)	0.04	<0.001
Creatinine → MnBP	0.71 (0.66, 0.77)	0.03	<0.001
Creatinine → MCPP	0.68 (0.60, 0.76)	0.04	<0.001
Creatinine → MEHHP	0.59 (0.51, 0.66)	0.04	<0.001
Creatinine →MBzP	0.70 (0.64, 0.75)	0.04	<0.001
Creatinine → MiBP	0.71 (0.66, 0.76)	0.03	<0.001
TSH → T4	-0.19 (-0.29, -0.08)	0.05	<0.001
Iodine→ T4	0.04 (-0.03, 0.10)	0.04	0.31
T4 → T3	0.50 (0.42, 0.58)	0.04	<0.001
Iodine→ T3	0.03 (-0.05, 0.11)	0.04	0.45
Age → T3	-0.32 (-0.42, -0.22)	0.05	<0.001
Age → TSH	0.20 (0.13, 0.27)	0.04	<0.001
Creatinine → Iodine	0.38 (0.29, 0.47)	0.05	<0.001
Perchlorate → Iodine	0.37 (0.29, 0.45)	0.04	<0.001
Residual variances			
e.Perchlorate	0.63 (0.54, 0.72)	0.05	<0.001
e. BPA	0.56 (0.45, 0.68)	0.06	<0.001
e. Benzophenone-3	0.88 (0.81, 0.96)	0.04	<0.001
e. MECPP	0.46 (0.31, 0.60)	0.08	<0.001
e. MnBP	0.43 (0.35, 0.50)	0.04	<0.001
e. MCPP	0.31 (0.21, 0.42)	0.06	<0.001
e. MEHHP	0.44 (0.31, 0.57)	0.07	<0.001
e. MBzP	0.47 (0.39, 0.56)	0.04	<0.001
e. MiBP	0.44 (0.37, 0.52)	0.04	<0.001
e.TSH	0.94 (0.92, 0.97)	0.01	<0.001
e.T3	0.63 (0.57, 0.69)	0.03	<0.001
e.T4	0.96 (0.93, 1.00)	0.02	<0.001
e.Iodine	0.57 (0.49, 0.65)	0.04	<0.001
e.Exposure	0.96 (0.91, 1.00)	0.02	<0.001
Covariance			
MEHPP ↔ MECPP	0.90 (0.87, 0.93)	0.02	<0.001
MnBP ↔ MiBP	0.43 (0.35, 0.51)	0.04	<0.001
MnBP ↔ MBzP	0.38 (0.28, 0.47)	0.05	<0.001
MBzP ↔ MiBP	0.29 (0.20, 0.38)	0.05	<0.001
Creatinine ↔ NHB	0.10 (0.11, 0.25)	0.03	<0.001
Creatinine ↔ Age	-0.34 (-0.41, -0.28)	0.03	<0.001

Table 4.4. Standardized estimates SEM depicting the relationship between 8 EDCs, THs, and covariates in males 12-85 years of age, NHANES 2007-2008. These values are depicted in Figure 4.4.

Loadings/effects	Standard Estimates (95% CI)	Standard Errors	p-value
Exposure → Perchlorate	0.15 (0.06, 0.23)	0.04	0.001
Exposure → BPA	0.19 (0.09, 0.29)	0.05	<0.001
Exposure → MECPP	0.40 (0.30, 0.51)	0.05	<0.001
Exposure → MnBP	0.44 (0.36, 0.52)	0.04	<0.001
Exposure → MCPP	0.50 (0.31, 0.63)	0.07	<0.001
Exposure → MEHHP	0.48 (0.35, 0.60)	0.06	<0.001
Exposure → MBzP	0.40 (0.33, 0.47)	0.04	<0.001
Exposure → MiBP	0.33 (0.23, 0.43)	0.05	<0.001
Exposure → T4	-0.18 (-0.31, -0.05)	0.07	0.008
Creatinine → Perchlorate	0.49 (0.42, 0.56)	0.04	<0.001
Creatinine → BPA	0.57 (0.51, 0.63)	0.03	<0.001
Creatinine → MECPP	0.56 (0.49, 0.63)	0.04	<0.001
Creatinine → MnBP	0.62 (0.53, 0.71)	0.05	<0.001
Creatinine → MCPP	0.59 (0.51, 0.67)	0.04	<0.001
Creatinine → MEHHP	0.54 (0.48, 0.61)	0.03	<0.001
Creatinine →MBzP	0.61 (0.56, 0.67)	0.03	<0.001
Creatinine → MiBP	0.66 (0.60, 0.72)	0.03	<0.001
TSH → T4	-0.13 (-0.21, -0.06)	0.04	<0.001
Iodine → T4	-0.04 (-0.12, 0.04)	0.04	0.30
Iodine → Creatinine	0.34 (0.25, 0.42)	0.04	<0.001
Iodine → Perchlorate	0.29 (0.21, 0.37)	0.04	<0.001
T4 → T3	0.29 (0.23, 0.35)	0.03	<0.001
Iodine → T3	-0.002 (-0.07, 0.07)	0.04	0.95
Age → T3	-0.44 (-0.48, -0.41)	0.02	<0.001
Age → TSH	0.09 (0.01, 0.17)	0.04	0.02
Residual variances			
e.Perchlorate	0.74 (0.66, 0.82)	0.04	<0.001
e. BPA	0.64 (0.55, 0.73)	0.04	<0.001
e. MECPP	0.52 (0.40, 0.65)	0.06	<0.001
e. MnBP	0.43 (0.35, 0.50)	0.04	<0.001
e. MCPP	0.40 (0.33, 0.48)	0.04	<0.001
e. MEHHP	0.48 (0.34, 0.62)	0.07	<0.001
e. MBzP	0.46 (0.39, 0.54)	0.04	<0.001
e. MiBP	0.45 (0.35, 0.56)	0.05	<0.001
e.T3	0.72 (0.68, 0.76)	0.02	<0.001
e.T4	0.95 (0.90, 1.00)	0.03	<0.001
e.TSH	0.99 (0.98, 1.01)	0.01	<0.001
e.Iodine	0.71 (0.61, 0.81)	0.05	<0.001
Covariance			
MEHPP ↔ MECPP	0.92 (0.89, 0.94)	0.01	<0.001
MnBP ↔ MiBP	0.41 (0.26, 0.57)	0.08	<0.001
MnBP ↔ MBzP	0.27 (0.17, 0.36)	0.05	<0.001
MiBP ↔ MBzP	0.19 (-0.02, 0.39)	0.10	0.07
Creatinine ↔ Age	-0.18 (-0.23, -0.13)	0.03	<0.001

Table 4.5. Indirect and Total Loadings of Exposure to Multiple EDCs on BMI for Females 12-85 years of age, NHANES 2007-2008. These values correspond to Figure 4.5.

	Exposure → TSH → BMI	Exposure → TSH → T4 → BMI	Per → Iodine → T4 → BMI	Exposure → BMI
Loadings type	Indirect	Indirect		Total
Std. Loadings	0.01	-0.004	0.001	0.008
Standard Errors	0.006	0.003	0.001	0.005
Confidence Interval	0.0007, 0.03	-0.02, 0.04	-0.0006, 0.002	-0.002, 0.02
p-value	0.05	0.16	0.29	0.13

Table 4.6. Standardized estimates SEM depicting the relationship between 9 EDCs, THs, BMI, and covariates in females 12-85 years of age, NHANES 2007-2008. These values are depicted in Figure 4.5

Loadings/effects	Standard Estimates (95% CI)	Standard Errors	p-value
Exposure → Perchlorate	0.24 (0.13, 0.35)	0.06	<0.001
Exposure → BPA	0.22 (0.13, 0.31)	0.05	<0.001
Exposure → Benzophenone-3	0.22 (0.09, 0.35)	0.07	0.001
Exposure → MECPP	0.46 (0.34, 0.59)	0.06	<0.001
Exposure → MnBP	0.28 (0.19, 0.38)	0.05	<0.001
Exposure → MCPP	0.51 (0.42, 0.59)	0.04	<0.001
Exposure → MEHHP	0.49 (0.38, 0.60)	0.06	<0.001
Exposure → MBzP	0.24 (0.15, 0.32)	0.05	<0.001
Exposure → MiBP	0.26 (0.14, 0.39)	0.06	<0.001
NHB → Exposure	-0.21 (-0.32, -0.11)	0.05	<0.001
Exposure → TSH	0.13 (0.06, 0.20)	0.04	<0.001
Creatinine → Perchlorate	0.57 (0.49, 0.64)	0.04	<0.001
Creatinine → BPA	0.63 (0.52, 0.74)	0.06	<0.001
Creatinine → Benzo	0.27 (0.19, 0.36)	0.04	<0.001
Creatinine → MECPP	0.59 (0.52, 0.66)	0.04	<0.001
Creatinine → MnBP	0.71 (0.66, 0.77)	0.03	<0.001
Creatinine → MCPP	0.68 (0.60, 0.76)	0.04	<0.001
Creatinine → MEHHP	0.59 (0.51, 0.66)	0.04	<0.001
Creatinine →MBzP	0.70 (0.64, 0.75)	0.03	<0.001
Creatinine → MiBP	0.71 (0.66, 0.76)	0.03	<0.001
TSH → T4	-0.19 (-0.29, -0.08)	0.05	<0.001
Iodine→ T4	0.04 (-0.03, 0.10)	0.04	0.31
T4 → T3	0.50 (0.42, 0.58)	0.04	<0.001
Iodine → T3	0.03 (-0.05, 0.11)	0.04	0.45
Age → T3	-0.31 (-0.41, -0.21)	0.05	<0.001
TSH → BMI	0.09 (0.02, 0.16)	0.04	0.01
T4 → BMI	0.17 (0.08, 0.25)	0.05	<0.001
Onechild → BMI	0.12 (0.01, 0.23)	0.06	0.04
Gonechild → BMI	0.22 (0.14, 0.30)	0.04	<0.001
MA → BMI	0.05 (0.01, 0.10)	0.02	0.02
NHB → BMI	0.20 (0.08, 0.33)	0.06	<0.001
Age → TSH	0.20 (0.13, 0.27)	0.03	<0.001
Creatinine →Iodine	0.38 (0.29, 0.47)	0.05	<0.001
Perchlorate → Iodine	0.37 (0.29, 0.45)	0.04	<0.001
Residual variances			
e.Perchlorate	0.63 (0.54, 0.72)	0.05	<0.001
e. BPA	0.56 (0.45, 0.68)	0.05	<0.001
e. Benzophenone-3	0.88 (0.81, 0.96)	0.04	<0.001
e. MECPP	0.46 (0.31, 0.61)	0.07	<0.001
e. MnBP	0.43 (0.35, 0.50)	0.04	<0.001
e. MCPP	0.31 (0.21, 0.42)	0.05	<0.001
e. MEHHP	0.44 (0.31, 0.57)	0.06	<0.001
e. MBzP	0.47 (0.39, 0.55)	0.04	<0.001
e. MiBP	0.44 (0.37, 0.52)	0.05	<0.001
e.TSH	0.95 (0.92, 0.97)	0.02	<0.001
e.T3	0.64 (0.58, 0.70)	0.04	<0.001
e.T4	0.96 (0.92, 1.00)	0.02	<0.001
e.BMI	0.89 (0.82, 0.95)	0.03	<0.001
Iodine	0.57 (0.49, 0.65)	0.04	<0.001
e.Exposure	0.96 (0.91, 1.00)	0.02	<0.001
Covariance			
MEHPP ↔ MECPP	0.90 (0.87, 0.93)	0.02	<0.001
MnBP ↔ MiBP	0.43 (0.35, 0.51)	0.04	<0.001
MnBP ↔ MBzP	0.38 (0.28, 0.47)	0.05	<0.001
MBzP ↔ MiBP	0.29 (0.20, 0.38)	0.05	<0.001
Creatinine ↔ NHB	0.18 (0.12, 0.25)	0.03	<0.001
Creatinine ↔ Age	-0.22 (-0.28, -0.16)	0.03	<0.001
Creatinine ↔ MA	-0.03 (-0.09, 0.04)	0.03	0.44
Age ↔ Onechild	0.27 (0.17, 0.38)	0.04	0.02
Age ↔ GOneChild	0.47 (0.40, 0.55)	0.04	<0.001
Onechild ↔ GOneChild	-0.46 (-0.53, -0.38)	0.04	<0.001

Table 4.7. Standardized estimates SEM depicting the relationship between 8 EDCs, THs, BMI, and covariates in males 12-85 years of age, NHANES 2007-2008. These values are depicted in Figure 4.6.

Loadings/effects	Standard Estimates (95% CI)	Standard Errors	p-value
Exposure → Perchlorate	0.15 (0.06, 0.23)	0.04	0.001
Exposure → BPA	0.19 (0.09, 0.29)	0.05	<0.001
Exposure → MECPP	0.40 (0.30, 0.51)	0.05	<0.001
Exposure → MnBP	0.44 (0.36, 0.52)	0.04	<0.001
Exposure → MCPP	0.50 (0.37, 0.63)	0.07	<0.001
Exposure → MEHHP	0.48 (0.35, 0.60)	0.06	<0.001
Exposure → MBzP	0.40 (0.33, 0.47)	0.04	<0.001
Exposure → MiBP	0.33 (0.23, 0.43)	0.05	<0.001
Exposure → T4	-0.18 (-0.31, -0.05)	0.07	0.008
Creatinine → Perchlorate	0.49 (0.42, 0.56)	0.04	<0.001
Creatinine → BPA	0.57 (0.51, 0.63)	0.03	<0.001
Creatinine → MECPP	0.56 (0.49, 0.63)	0.04	<0.001
Creatinine → MnBP	0.62 (0.53, 0.71)	0.05	<0.001
Creatinine → MCPP	0.59 (0.51, 0.67)	0.04	<0.001
Creatinine → MEHHP	0.54 (0.48, 0.61)	0.03	<0.001
Creatinine →MBzP	0.61 (0.56, 0.67)	0.03	<0.001
Creatinine → MiBP	0.66 (0.60, 0.72)	0.03	<0.001
TSH → T4	-0.13 (-0.21, -0.06)	0.04	<0.001
Iodine → T4	-0.04 (-0.12, 0.04)	0.04	0.30
T4 → T3	0.29 (0.23, 0.35)	0.03	<0.001
Iodine → T3	-0.002 (-0.07, 0.07)	0.04	0.95
Age → T3	-0.44 (-0.48, -0.41)	0.02	<0.001
TSH → BMI	0.14 (0.06, 0.22)	0.04	<0.001
MA → BMI	0.04 (0.002, 0.08)	0.02	0.04
Age → BMI	0.27 (0.20, 0.35)	0.04	<0.001
Age → TSH	0.09 (0.01, 0.17)	0.04	0.02
Creatinine → Iodine	0.34 (0.25, 0.42)	0.04	<0.001
Perchlorate → Iodine	0.29 (0.21, 0.37)	0.04	<0.001
Residual variances			
e.Perchlorate	0.74 (0.66, 0.82)	0.04	<0.001
e. BPA	0.64 (0.55, 0.72)	0.05	<0.001
e. MECPP	0.52 (0.40, 0.65)	0.06	<0.001
e. MnBP	0.43 (0.35, 0.50)	0.04	<0.001
e. MCPP	0.40 (0.33, 0.48)	0.04	<0.001
e. MEHHP	0.48 (0.34, 0.62)	0.07	<0.001
e. MBzP	0.46 (0.39, 0.44)	0.04	<0.001
e. MiBP	0.45 (0.35, 0.46)	0.05	<0.001
e.T3	0.72 (0.68, 0.76)	0.02	<0.001
e.T4	0.95 (0.90, 1.00)	0.03	<0.001
e.TSH	0.99 (0.98, 1.01)	0.01	<0.001
e.BMI	0.90 (0.86, 0.94)	0.02	<0.001
e.Iodine	0.71 (0.61, 0.81)	0.05	<0.001
Covariance			
MEHPP ↔ MECPP	0.92 (0.89 0.94)	0.01	<0.001
MnBP ↔ MiBP	0.41 (0.26, 0.57)	0.08	<0.001
MnBP ↔ MBzP	0.27 (0.17, 0.36)	0.05	<0.001
MiBP ↔ MBzP	0.19 (-0.02, 0.39)	0.10	0.07
Creatinine ↔ MA	-0.02 (-0.08, 0.04)	0.03	0.48
Creatinine ↔ Age	-0.18 (-0.23, -0.13)	0.03	<0.001

Table 4.8. Comparative Fit Index (CFI), Tucker Lewis Index (TLI), Standardize Root Mean Square Residual (SRMR), Root Mean Square Error of Approximation (RMSEA), and 90% Confidence Interval (90% CI) SRMR for Figures 4.3, 4.4, 4.5, and 4.6.

	Figure 4.3	Figure 4.4	Figure 4.5	Figure 4.6	Criteria for acceptable fit
CFI	0.97	0.96	0.95	0.95	0.90-.95
TLI	0.96	0.94	0.94	0.93	0.90-.95
SRMR	0.04	0.04	0.07	0.05	≤ 0.08
RMSEA	0.04	0.05	0.04	0.05	≤0.06
RMSEA 90% CI	0.04, 0.05	0.04, 0.06	0.04, 0.05	0.04, 0.06	Upper bound 0.08

Table 4.9. Standardized estimates SEM depicting the relationship between 8 EDCs, thyroid disease, and covariates in females 12-85 years of age, NHANES 2007-2008. These values are depicted in Figure 4.8.

Loadings/effects	Standard Estimates (95% CI)	Standard Errors	pvalue
Exposure → Perchlorate	0.25 (0.15, 0.35)	0.05	<0.001
Exposure → BPA	0.21 (0.13, 0.30)	0.05	<0.001
Exposure → Benzophenone-3	0.21 (0.08, 0.35)	0.07	0.002
Exposure → MECPP	0.46 (0.34, 0.59)	0.07	<0.001
Exposure → MnBP	0.29 (0.19, 0.38)	0.05	<0.001
Exposure → MCPP	0.51 (0.42, 0.59)	0.04	<0.001
Exposure → MEHHP	0.48 (0.37, 0.60)	0.06	<0.001
Exposure → MBzP	0.24 (0.15, 0.32)	0.05	<0.001
Exposure → MiBP	0.27 (0.14, 0.39)	0.07	<0.001
NHB → Exposure	-0.20 (-0.31, -0.10)	0.05	<0.001
Exposure → Hypo	-0.03 (-0.67, 0.60)	0.32	0.92
Exposure → Hyper	-0.59 (-0.95, -0.24)	0.18	<0.001
Creatinine → Perchlorate	0.56 (0.49, 0.64)	0.04	<0.001
Creatinine → BPA	0.63 (0.52, 0.74)	0.06	<0.001
Creatinine → Benzo	0.27 (0.19, 0.35)	0.04	<0.001
Creatinine → MECPP	0.59 (0.52, 0.66)	0.04	<0.001
Creatinine → MnBP	0.71 (0.66, 0.77)	0.03	<0.001
Creatinine → MCPP	0.67 (0.59, 0.76)	0.04	<0.001
Creatinine → MEHHP	0.59 (0.51, 0.66)	0.04	<0.001
Creatinine →MBzP	0.69 (0.63, 0.75)	0.03	<0.001
Creatinine → MiBP	0.71 (0.66, 0.76)	0.03	<0.001
Creatinine →Iodine	0.38 (0.29, 0.47)	0.05	<0.001
Perchlorate → Iodine	0.37 (0.29, 0.45)	0.04	<0.001
Hypo → Iodine	0.55 (-0.04, 1.15)	0.30	0.07
Hypo → Age	0.89 (0.57, 1.20)	0.16	<0.001
Hypo → PIR	0.32 (0.08, 0.56)	0.12	0.008
Hyper → Iodine	0.67 (0.45, 0.89)	0.11	<0.001
Hyper → Age	-0.39 (-0.67, -0.11)	0.14	0.006
Hyper → PIR	0.05 (-0.33, 0.43)	0.19	0.78
Residual variances			
e.Perchlorate	0.63 (0.54, 0.72)	0.05	<0.001
e. BPA	0.57 (0.45, 0.69)	0.06	<0.001
e. Benzophenone-3	0.89 (0.81, 0.96)	0.04	<0.001
e. MECPP	0.46 (0.31, 0.61)	0.08	<0.001
e. MnBP	0.43 (0.35, 0.50)	0.04	<0.001
e. MCPP	0.31 (0.21, 0.42)	0.05	<0.001
e. MEHHP	0.45 (0.31, 0.58)	0.07	<0.001
e. MBzP	0.48 (0.39, 0.56)	0.04	<0.001
e. MiBP	0.44 (0.37, 0.52)	0.04	<0.001
e.Iodine	0.57 (0.49, 0.65)	0.04	<0.001
e.Exposure	0.96 (0.92, 1.00)	0.02	<0.001
Covariance			
MEHPP ↔ MECPP	0.90 (0.87, 0.93)	0.02	<0.001
MnBP ↔ MiBP	0.43 (0.35, 0.51)	0.04	<0.001
MnBP ↔ MBzP	0.38 (0.29, 0.47)	0.05	<0.001
MBzP ↔ MiBP	0.29 (0.19, 0.38)	0.05	<0.001
Creatinine ↔ NHB	0.18 (0.11, 0.25)	0.03	<0.001
Creatinine ↔ Age	-0.34 (-0.41, -0.28)	0.03	<0.001

Table 4.10. Standardized estimates SEM depicting the relationship between 8 EDCs, thyroid disease, and covariates in males 12-85 years of age, NHANES 2007-2008. These values are depicted in Figure 4.9

Loadings/effects	Standard Estimates (95% CI)	Standard Errors	pvalue
Exposure → Perchlorate	0.15 (0.07, 0.23)	0.04	<0.001
Exposure → BPA	0.19 (0.09, 0.29)	0.05	<0.001
Exposure → MECPP	0.40 (0.29, 0.50)	0.05	<0.001
Exposure → MnBP	0.45 (0.37, 0.53)	0.04	<0.001
Exposure → MCPP	0.50 (0.37, 0.63)	0.07	<0.001
Exposure → MEHHP	0.47 (0.35, 0.60)	0.06	<0.001
Exposure → MBzP	0.40 (0.33, 0.46)	0.03	<0.001
Exposure → MiBP	0.33 (0.23, 0.44)	0.06	<0.001
Exposure → Hypo	-0.36 (-0.98, 0.27)	0.32	0.26
Exposure → Hyper	-0.39 (-0.63, 1.42)	0.52	0.45
Creatinine → Perchlorate	0.49 (0.43, 0.55)	0.03	<0.001
Creatinine → BPA	0.57 (0.53, 0.61)	0.02	<0.001
Creatinine → MECPP	0.56 (0.50, 0.62)	0.03	<0.001
Creatinine → MnBP	0.62 (0.54, 0.70)	0.04	<0.001
Creatinine → MCPP	0.59 (0.53, 0.66)	0.04	<0.001
Creatinine → MEHHP	0.55 (0.49, 0.60)	0.03	<0.001
Creatinine →MBzP	0.62 (0.56, 0.68)	0.03	<0.001
Creatinine → MiBP	0.66 (0.62, 0.71)	0.02	<0.001
Creatinine →Iodine	0.34 (0.26, 0.41)	0.04	<0.001
Perchlorate → Iodine	0.29 (0.21, 0.37)	0.04	<0.001
Hypo → Iodine	0.37 (-0.19, 0.92)	0.28	0.20
Hypo → Age	0.77 (0.36, 1.19)	0.21	<0.001
Hypo → NHB	-0.42 (-0.77, -0.07)	0.18	0.02
Hyper → Iodine	-0.40 (-1.17, 0.38)	0.40	0.32
Hyper → Age	0.60 (-0.28, 1.49)	0.45	0.60
Hyper → NHB	0.59 (-0.24, 1.43)	0.43	0.59
Residual variances			
e.Perchlorate	0.74 (0.68, 0.79)	0.03	<0.001
e. BPA	0.64 (0.57, 0.71)	0.04	<0.001
e. MECPP	0.53 (0.41, 0.64)	0.06	<0.001
e. MnBP	0.42 (0.34, 0.50)	0.04	<0.001
e. MCPP	0.40 (0.30, 0.49)	0.05	<0.001
e. MEHHP	0.48 (0.35, 0.61)	0.07	<0.001
e. MBzP	0.46 (0.38, 0.55)	0.04	<0.001
e. MiBP	0.45 (0.36, 0.54)	0.05	<0.001
e.Iodine	0.71 (0.62, 0.80)	0.05	<0.001
Covariance			
MEHPP ↔ MECPP	0.92 (0.89, 0.95)	0.01	<0.001
MnBP ↔ MiBP	0.41 (0.26, 0.55)	0.07	<0.001
MnBP ↔ MBzP	0.26 (0.17, 0.35)	0.05	<0.001
MBzP ↔ MiBP	0.18 (-0.03, 0.40)	0.11	0.09

Table 4.11. Odds ratio of hypothyroidism and hyperthyroidism given exposure to multiple EDCs in males and females aged 12-85 years of age, NHANES 2007-2008.

	Females		Males	
Disease	N	Odds Ratio	N	Odds Ratio
Normal	569	Ref	701	Ref
Hypothyroidism	97	0.93 (0.20, 4.21)	107	0.29 (0.02, 2.82)
Hyperthyroidism	10	0.02 (0.00, 2.95)	3	13.38 (0.04, 1,755.66)

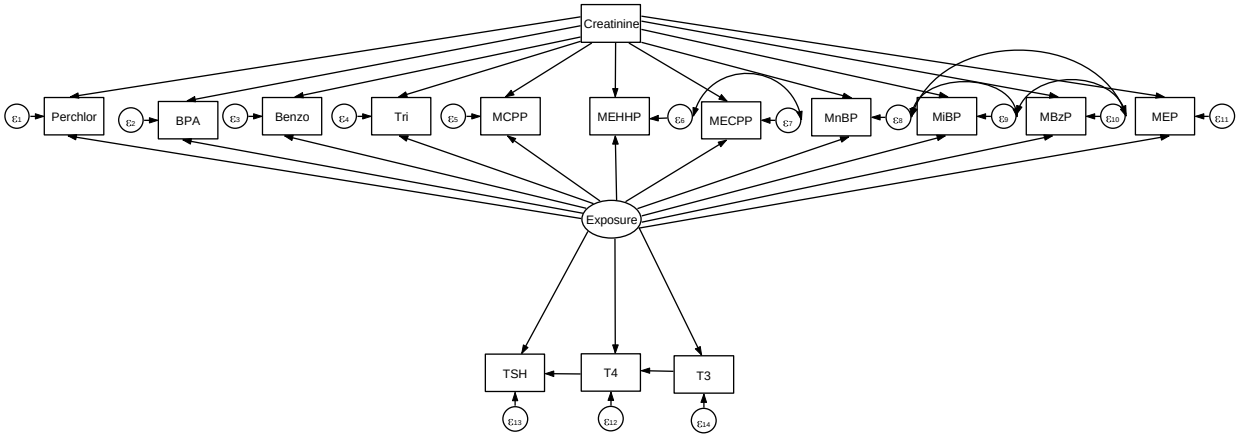

Figure 4.1. A priori model of relationship between 11 EDC compounds and THs after adjustment for creatinine. The latent Exposure variable represent exposure to a mixture of EDCs. Perchlorate is represented by “Perchlor”, benzophenone-3 is represented by “Benzo”, and triclosan is represented by “Tri”, mono-2ethyl5carboxypentyl phthalate is represented by “MECPP”, mono-n-butyl phthalate is represented by “MnBP”, mono-(3-carboxypropyl) phthalate is represented by “MCPP”, mono-ethyl phthalate is represented by “MEP”, mono(2ethyl5hydroxyhexyl) phthalate is represented by “MEHHP”, mono-benzyl phthalate is represented by “MBzP”, and mono-isobutyl phthalate is represented by “MiBP”.

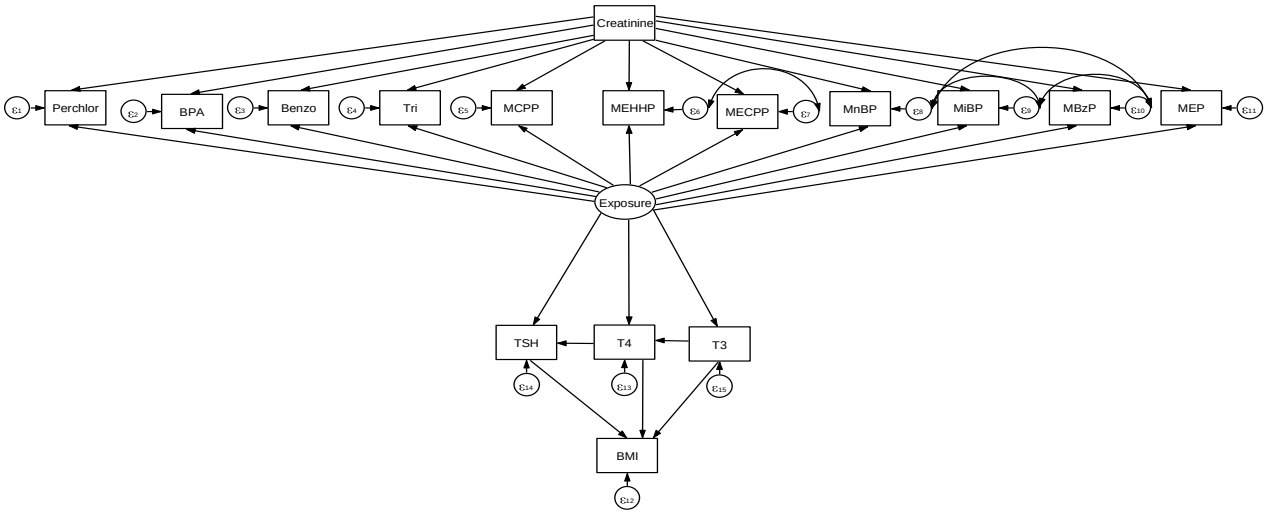

Figure 4.2. A priori model of relationship between 11 EDC compounds, THs and BMI loading after adjustment of EDCs for creatinine.

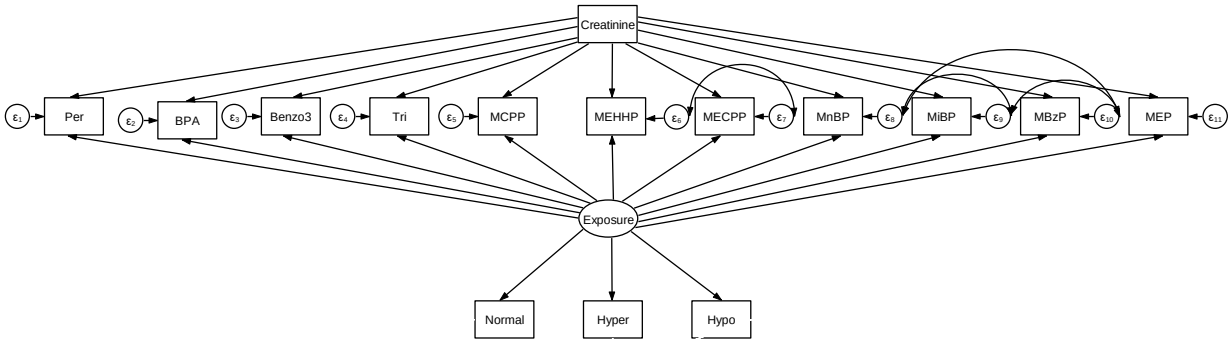

Figure 4.3. A priori model of relationship between 11 EDC compounds and thyroid disease loading after adjustment of EDCs for creatinine.

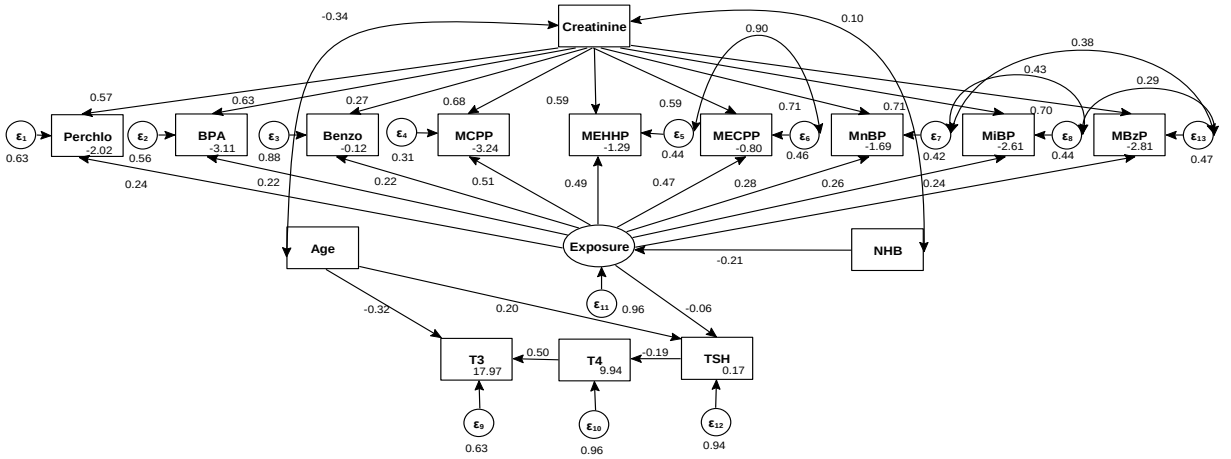

Figure 4.4. SEM of relationship between EDC and THs in females after adjusting T3 and TSH for age, T3 and T4 for Iodine, EDCs and Iodine for creatinine, and Exposure for being Non-Hispanic Black (NHB).

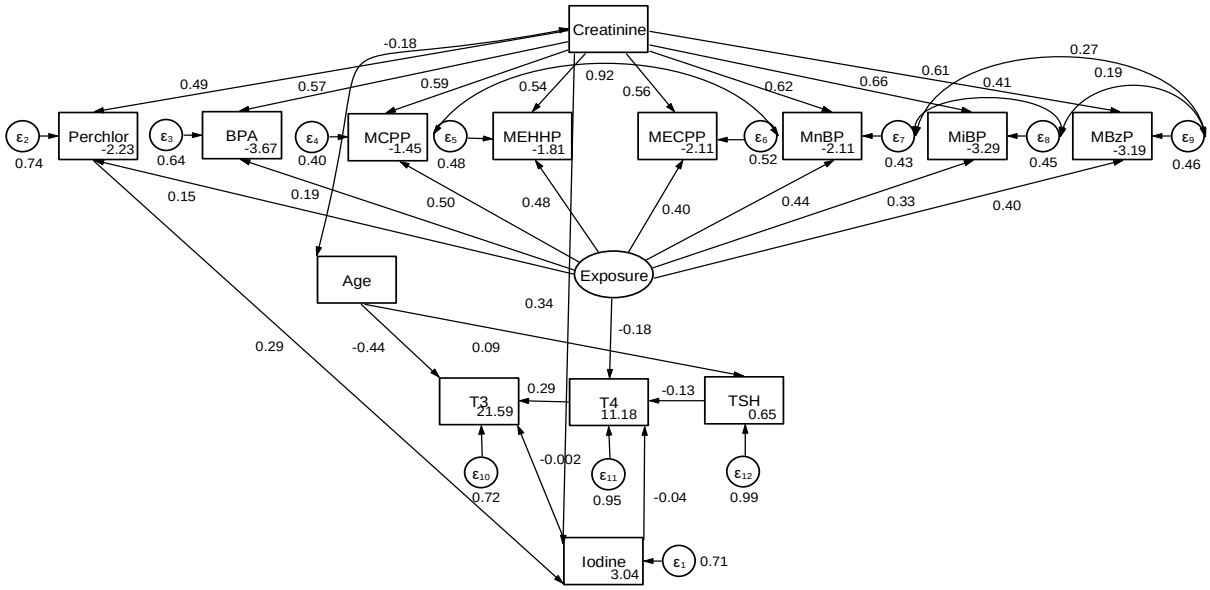

Figure 4.5. SEM of relationship between EDC and THs in males after adjusted EDCs and Iodine for creatinine, T3 and TSH for age, and T3 and T4 for Iodine.

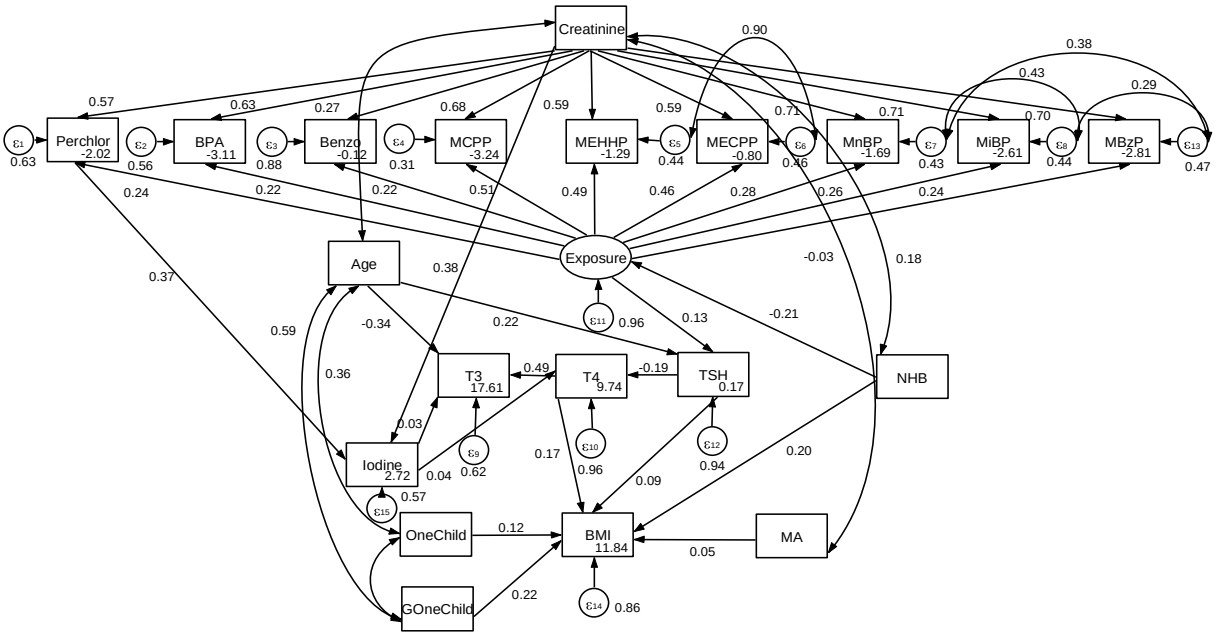

Figure 4.6. SEM of relationship between EDC, THs, and BMI in females after adjusting EDCs and Iodine for creatinine, Exposure for being NHB, T3 and T4 for age and Iodine, BMI for parity and being Mexican American (MA).

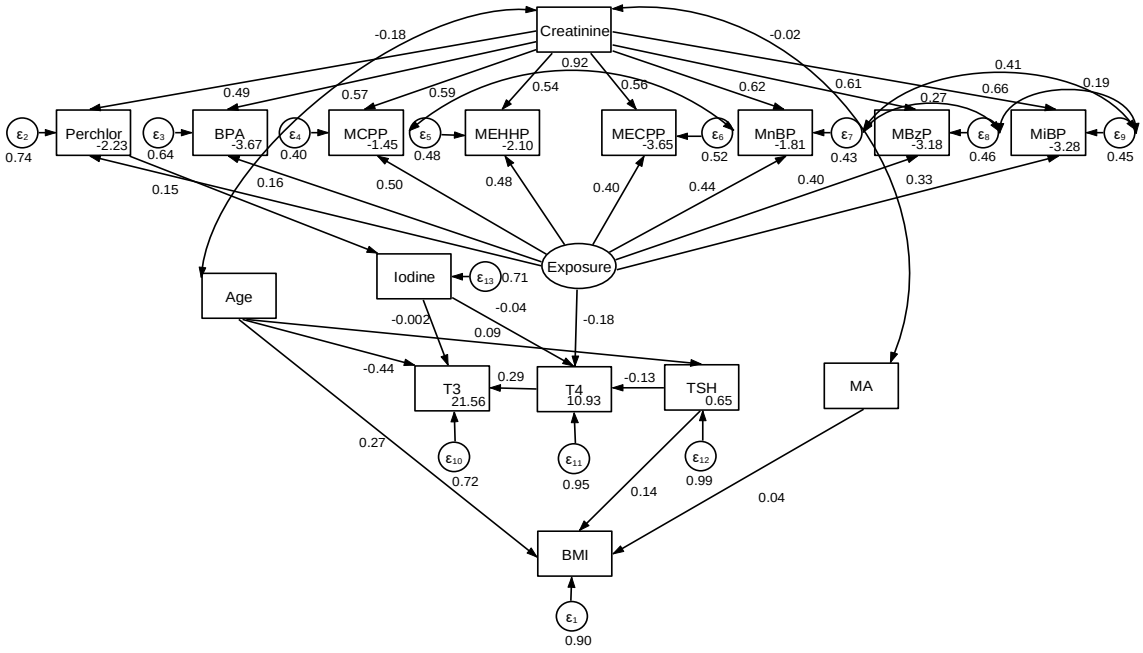

Figure 4.7. SEM of relationship between EDC, THs, and BMI in males after adjusting EDCs and Iodine for creatinine, T3 and T4 for iodine, T3 and TSH for age, and BMI for being MA.

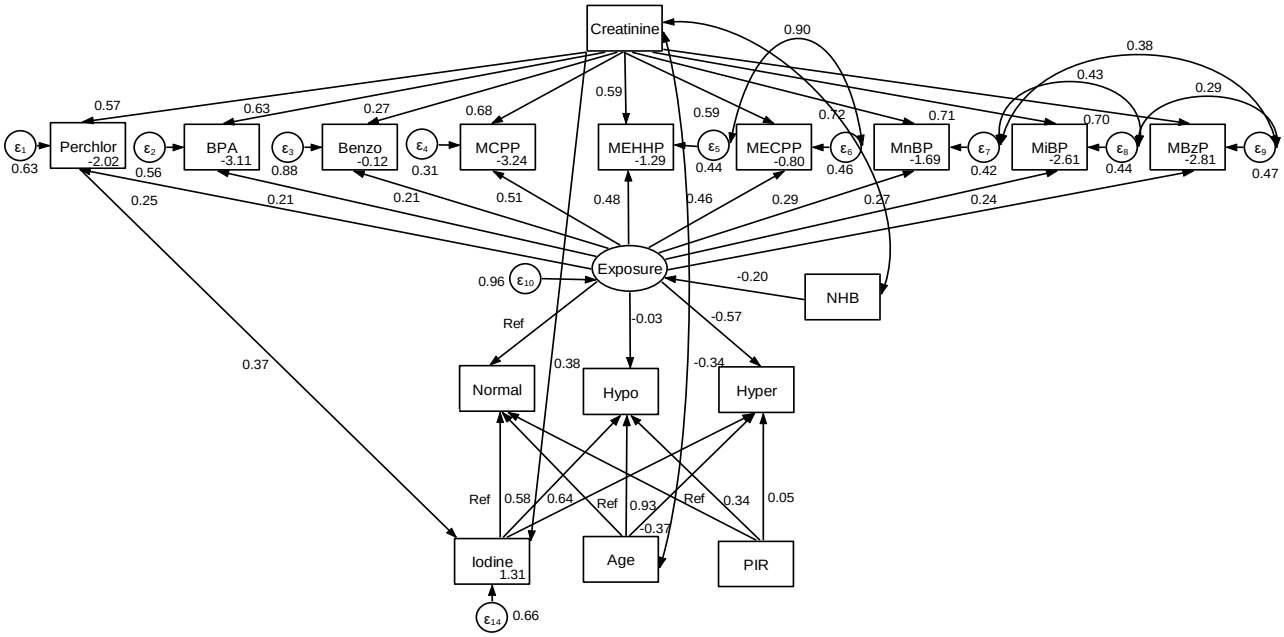

Figure 4.8. SEM of relationship between EDC, and thyroid disease in females after adjusting EDCs and iodine for creatinine, EDCs for being NHB and thyroid disease for iodine, age, and poverty index ratio (PIR)

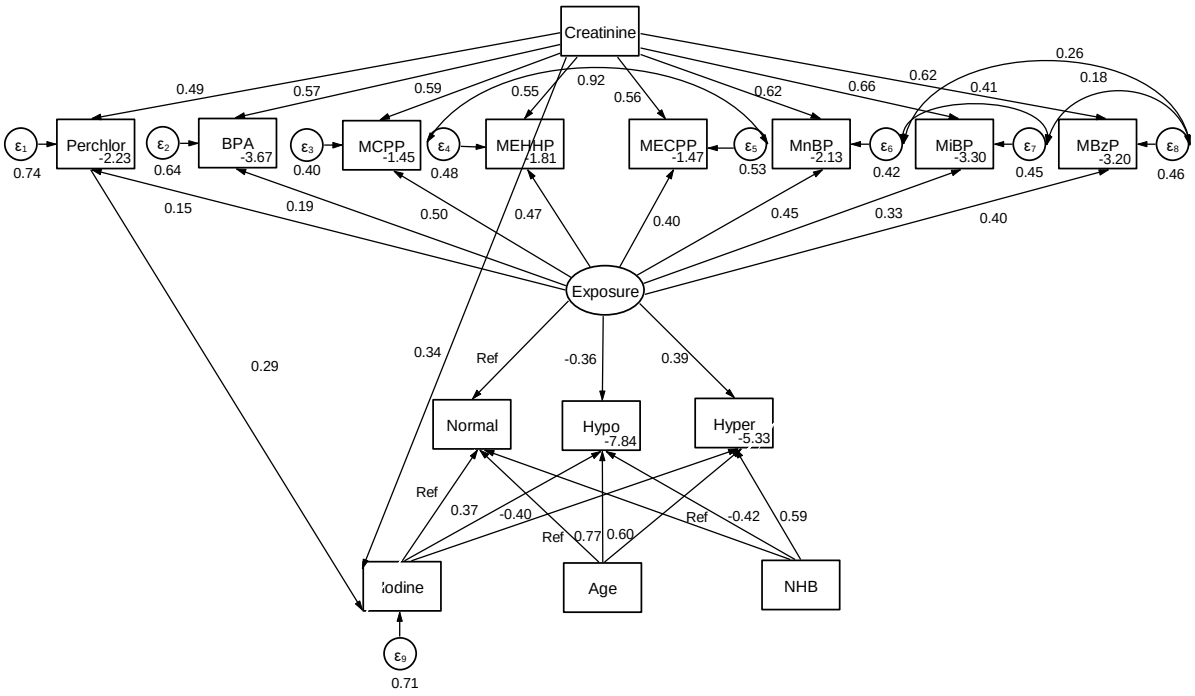

Figure 4.9. SEM of relationship between EDC, and thyroid disease in males after adjusting EDCs and iodine for creatinine and thyroid disease for iodine, age, and Non-Hispanic Black (NHB)

Chapter 5 - Summary and Conclusions

Heavy metals, EDCs, and PCBs are common environmental contaminants and have been individually shown to cause detrimental effects to human health. Determining the exposure profiles of these chemicals is of public health interest because the profile identified represent more realistic mixtures and can be used in risk assessment and environmental epidemiology studies. Identification of vulnerable subpopulation to high levels of chemicals in the exposure profiles can prioritize public health intervention and mitigation efforts.

Current methods of establishing relationships between environmental mixtures and health outcomes have some limitations due to the high correlation and dimensionality of environmental data. Using advance analytical methods to address these challenges this research defined a more realistic definition of environmental mixtures in addition to determining associations between environmental mixtures and important health outcomes. The conclusions from each proposed specific aim are summarized below:

Aim 1

The first aim was to characterize the exposure profile of 7 environmental chemicals in a US population. We also determined which sociodemographic factors were associated with each exposure profile. This aim was realized using a nationally representative sample population from NHANES (see Chapter 1).

Our findings support the hypothesis that different exposure profiles exist in the US population. We found 8 different exposure profiles using 7 common chemicals. As expected, heavy metals clustered together as did personal care products and pesticides. Additionally, our findings supported the hypothesis that different exposure profiles are associated with certain sociodemographic characteristics. Specifically we observed that older American males of minority races, with a PIR less than the poverty threshold in survey year 2003-2004 had the

highest exposure levels with the strongest correlations of lead and BPA, and moderately strong correlations of cadmium, triclosan, 2,4-dichlorophenol and 2,5-dichlorophenol. Our results suggest that regulatory efforts aimed at protecting human health from environmental chemicals would benefit from taking into account co-exposures. Further efforts aimed at protecting human health can customize intervention efforts to the subpopulation experiencing the highest exposure for certain chemicals. Future works should incorporate the exposure profiles into epidemiology and risk assessment studies.

Aim 2

The second aim evaluated the relationship between exposure to multiple neurotoxins (lead, cadmium, and PCBs) and cognitive functioning in US older adults. This aim was addressed in the second study using the NHANES dataset (see Chapter 1).

Our results showed a negative relationship between a non-dioxin like PCB and a heavy metal and cognitive functioning in US older adults. Specifically we observed a dose-dependent effect between lower cognitive functioning and PCB 146 and lead. Our findings are in contrast to another study using NHANES data that found a negative association between sum dioxin-like PCBS and cognitive functioning in US older adults. The differences between this study and our observations could be due to how PCBs exposure was defined and accounted for co-exposures, highlighting the importance of a uniformed method of defining environmental mixtures. The findings from this research confirm previous research that shows non-dioxin-like PCBs affect the central nervous system by altering dopamine production. In addition, our findings were the first to examine the relationship of individual multiple environmental neurotoxins while controlling for other neurotoxins. Future work should focus on understanding the relationship between mixtures of neurotoxins and cognitive functioning in older adults as well as other in age groups.

Aim 3

The third and final specific aim evaluates the relationship between exposure to multiple EDCs and thyroid hormones. In addition we evaluated the relationship between exposure to multiple EDCs and BMI as mediated through thyroid hormones. This aim was accomplished using the NHANES, see Chapter 1.

Our observations support the hypothesis that exposure to multiple EDCs is associated with thyroid hormone functioning. Additionally we observed a sex-specific positive association between exposure to EDCs and BMI as directed through TSH. Our findings are consistent with studies examining the relationship between single EDCs, THs, and BMI. Specifically we found that although exposure to multiple EDCs affect serum THs concentrations in both males and females, females may be more susceptible to higher BMI due to alterations of THs from exposure to EDCs. This study was the first to not only examine the association between exposure to multiple EDCs and THs but also the complex relationship between multiple EDCs, THs, and BMI. Due to limitations in this research, future work should focus on (1) incorporating other EDCs when examining the association between EDCs, THs, and BMI and (2) Incorporating other modes of action besides THs, such as glucose metabolism when examining the relationship between exposure to environmental chemicals and BMI.

Summary

Our results show different subpopulations are exposed to different environmental mixture profiles. We showed that exposure to multiple environmental neurotoxins is associated negatively with cognitive functioning in older adults. We also demonstrated that exposure to multiple EDCs I associated with thyroid functioning and is positively associated with BMI as directed through TSH in females. Based on the knowledge that humans are exposed to multiple

environmental contaminants and the health effects of exposure to multiple chemicals may be synergistically detrimental it is a priority to understand the health effects of multiple environmental exposures. Once the human effects to multiple environmental exposure are understood, public health priority can then focus on preventing environmental exposures.

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Appendix A: Supplemental Material for Chapter 4

Table A.1 Description of Quartiles for energy expenditure, energy intake and sedentary time for males and females, NHANES 2007-2008

	Males who had complete data (n=812)	Males who did not have complete data (n=269)	Females who had complete data (n=676)	Females who did not have complete data (n=305)
Energy Expenditure (minutes*MET)				
1st quartile	1-1	0-1	0	1-1
2nd quartile	40-480	40-480	40-480	40-480
3rd quartile	481-1,281	481-1,201	481-1,261	481-1,281
4th quartile	1,321-8,640	1,440-8,680	1,320-9,000	1,320-6,360
Energy Intake (kcal)				
1st quartile	476-1,342	0-1,332	89-1,346	229-1,341
2nd quartile	1,350-1,870	1,358-1,854	1,347-1,870	1,347-1,864
3rd quartile	1,875-2,575	1,900-2,566	1,875-2,578	1,874-2,558
4th quartile	2,582-13,133	2,584-6,059	2,590-8,485	2,580-5,291
Sedentary Time (minutes)				
1st quartile	1-180	20-180	1-180	0-180
2nd quartile	240-300	240-300	240-300	240-300
3rd quartile	360-480	360-480	360-480	360-480
4th quartile	540-1,080	540-1,020	540-960	540-1,080