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Interplay of Environmental Pollutants and Folate in the Etiology of Autistic Traits Analysis Using Multipollutant Approaches

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Interplay of Environmental Pollutants and Folate in the Etiology of Autistic Traits
Analysis Using Multipollutant Approaches

A Dissertation Presented

by

MICHAEL T. MASCARI

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
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DOCTOR OF PHILOSOPHY

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Department of Biostatistics and Epidemiology

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DEDICATION

To my patient and loving wife Chelsea, my son Miles and my future family, and my mom, Lisa.

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I want to thank Youssef Oulhote PhD, for guiding my learning process, helping me continue to achieve academically, and develop my skills as an environmental epidemiologist. I gained a significant amount of knowledge about research methods, epidemiology, and how to balance my life and my work. Thank you for all your tutelage.

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ABSTRACT

Interplay of Environmental Pollutants and Folate in the Etiology of Autistic Traits

Analysis Using Multipollutant Approaches

SEPTEMBER 2022

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Background: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by a spectrum of communication deficits and repetitive behaviors. The challenges in learning, thinking, and problem-solving abilities of people with ASD can be debilitating. There is currently no cure for ASD. Many environmental pollutants are suspected to contribute to the etiology of ASD and its associated traits, whereas folate and folate supplements have been shown to exhibit both protective and adjuvant roles. Little is known about the interplay of multiple environmental pollutants and folate in the etiology of ASD. Additionally, statistical approaches that consider the effects of pollutant mixtures instead single pollutant approaches and establishing the appropriate windows of susceptibility of these pollutants are needed when assessing the relationship between environmental pollutants and health outcomes.

Methods: In order to examine the interplay between environmental pollutants, folate, and autistic traits, ExWAS (Exposome Wide Association Study), BKMR (Bayesian Kernel Machine regression), and QgComp (Quantile G Computation) were used. These methods

consider many of the challenges associated with pollutants mixtures. The data sources used in this study were the NHANES (National Health and Nutrition Examination Survey) and GUSTO (Growing Up in Singapore Toward healthy Outcomes) cohort.

Results: Many environmental pollutants were inversely associated with decreased folate, and autistic traits. Additionally, many environmental pollutants were both positively and inversely associated with autistic traits, and these associations often depended on sex. Finally, folate did modify the associations between some environmental pollutant mixtures and autistic traits, including phthalates, PFAS, and benzophenones.

Conclusions: This study highlights the importance for continued research on the etiology of autism. The novel findings of this study inform future epidemiologic analyses of the importance of sex-specific associations between environmental pollutants and autistic traits, and the importance of pollutant mixtures approaches and nutrients when considering such associations. The exploration of new mechanistic pathways between environmental pollutants and autistic traits through folate opens new avenues for future research. Finally, this dissertation provides further evidence that harmful environmental pollutants remain ubiquitous pollutants and remain a threat to public health. Although the results of this dissertation are modest, population level-influence depends on the magnitude of its impact on health *and* distribution of the factor. Given the ubiquitous exposure to environmental pollutants these modest effect sizes may have a considerable impact on populations.

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PREFACE

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by a spectrum of communication deficits and repetitive behaviors. (Centers for Disease Control and Prevention [CDC], 2020). The challenges in learning, thinking, and problem-solving abilities of people with ASD can be debilitating. There is currently no cure for ASD, and ASD is a lifelong burden on both the person diagnosed as well as their family. (CDC, 2020). The prevalence of ASD has increased since 2000, and the most recent surveillance data from the Centers for Disease Control and Prevention (CDC) suggests that 1 in 54 children are diagnosed with autism. (CDC, 2020).

Many environmental pollutants (Pelch et al., 2019; Flores-Pajot et al., 2016; Gong et al., 2017; Dutheil et al., 2021; Jo et al., 2019; Pagalan et al., 2019; Testa et al., 2012; Oulhote et al., 2020; Haggerty et al., 2021; Miodovnik et al., 2011; Shin et al., 2020; Lyall et al., 2018; Shin et al., 2019; Braun et al., 2014; Barkowski et al., 2019; Hansen et al., 2021; kardas et al., 2015) are suspected to contribute to the etiology of ASD and its associated traits, whereas folate and folate supplements have been shown to exhibit both protective (Surén et al., 2012) and adjuvant (Ergova et al., 2020) roles. Environmental pollutants refer to pollutants that are present in air, water, food, soil, dust, or other environmental media such as consumer products (Keil et al., 2016). These pollutants are often ubiquitous in the environment, and some may persist for several years. More than 140,000 pollutants have been introduced into the environment (Kishi et al., 2020). Folate, and its synthetic form folic acid (FA), are inactive forms of vitamin B9. Folate is vital in

humans for several metabolic reactions that keep humans healthy and are quintessential in prenatal development.

Little is known about the interplay of multiple environmental pollutants and folate in the etiology of ASD. Additionally, statistical approaches that consider the effects of pollutant mixtures instead single pollutant approaches and establishing the appropriate windows of susceptibility of these pollutants are needed when assessing the relationship between environmental pollutants and health outcomes. For example, humans are not exposed to a single pollutant, but rather a mixture of pollutants. These pollutants may be correlated, interact, or have nonlinear effects that threaten the validity of traditional statistical methods.

Also, developing fetuses have critical windows of neurodevelopment. For example, the first six months of prenatal neurodevelopment is largely driven by genetics, but the third trimester represents a period of rapid growth in neurodevelopment with less dependence on genetic influence (Vasung et al., 2019). This makes later pregnancy sensitive to environmental insults and studying associations during this period may provide a more accurate representation the interplay between environmental pollutants and folate in the etiology of ASD.

To answer these questions and address the limitations in the current body of evidence, I conducted these three papers to examine the relationships between environmental pollutants, folate, and autistic traits.

1. Exploratory analysis of associations between environmental pollutants and red blood cell folate.

- a. Hypothesis 1: Environmental pollutants will be inversely associated with red blood cell folate.
 - b. Hypothesis 2: These association are significantly modified by child sex with stronger effects in boys.
 - c. Hypothesis 3: The cumulative association of these pollutants on folate is higher than the sum of their individual associations.
2. Analysis to better understand the association between pollutant mixtures and autistic traits.
- a. Hypothesis 1: Environmental pollutants will be positively associated with autistic traits.
 - b. Hypothesis 2: These association are significantly modified by child sex with stronger effects in boys.
 - c. Hypothesis 3: The cumulative association of these pollutants on autistic traits is higher than the sum of their individual associations.
3. Analysis to help determine the mediation/modification role of folate in the association between pollutant mixtures and autistic traits.
- a. Hypothesis 1: Circulating folate levels play both a mediator and modifier role in the association between environmental pollutants and autistic traits.

CHAPTER 1

Exposure to Multiple Environmental Pollutants and Red Blood Cell Folate Concentrations

1.a Specific Aims and Hypotheses

Specific Aim 1: To evaluate the associations between environmental pollutant concentrations and RBC folate concentrations in a nationally representative sample of adults from the United States (U.S).

Hypothesis 1: Among U.S. adults, there will be a negative association between environmental pollutant levels and red blood cell folate.

Specific Aim 2: Assess whether these associations are modified by sex.

Hypothesis 2: The associations between environmental pollutants and RBC folate concentrations will be differential by sex with stronger effects among boys.

Specific Aim 3: Compare evidence from multiple approaches investigating joint and independent associations between environmental pollutants and low RBC folate.

Hypothesis 3: The joint association of environmental pollutants with RBC folate concentrations is stronger than the sum of their individual associations.

1.b Background and Significance

1.b.i Public Health Impact of Low Folate Levels

Folate, and its synthetic form folic acid (FA), are inactive forms of vitamin B9. A recent NHANES biomonitoring study suggested that, although blood folate concentrations in the US population have not decreased recently, folate insufficiency rates are about 20% of the US population. (Pfeiffer et al., 2019). Folate is vital in humans for several metabolic reactions involved in the formation and transfer of a single carbon atom. These metabolic

reactions in humans include: biosynthesis of purines and thymidine, amino acid homeostasis of glycine, serine, and methionine, epigenetic maintenance, homocysteine remethylation, hematopoiesis, and immune responses (Ducker & Rabinowitz, 2017; Fowler, 2001). These processes are important for DNA synthesis, silencing or inhibiting gene expression, maintain safe levels of homocysteine, red blood cell formation, and providing responses to foreign substances. Folate deficiency has been linked to anemia (Ducker & Rabinowitz, 2017; Fowler, 2001) and hyperhomocystinemia (Son & Lewis 2020), which has been associated with increased cardiovascular, cerebrovascular, and thromboembolic diseases. There is also epidemiologic evidence to suggest RBC folate is inversely associated with various cancers including: lung, oropharynx, esophagus, stomach, colorectal, pancreas, cervix, ovary, prostate, and breast cancers as well as leukemia (Keil et al., 2016; Kim, 1999; Kim, 2005).

Folate is also critical for prenatal development of fetuses (Kim, 2005; Greenberg et al., 2011). Birth outcomes that have been associated with low RBC folate include neural tube defects, neurodevelopment, child cognition, fetal development, recurrent pregnancy loss, still birth, and preterm birth (Botto et al., 1999; Czeizel et al., 2013; George, 2002; Liu et al., 2015; Nanick et al., 2019; Watanabe et al., 2008; Zhu et al., 2018). According to the CDC, approximately 3,000 pregnancies are affected by neural tube defects every year, about 1 in 100 is affected by stillbirth, and 10% were affected by preterm birth (CDC, 2021). These birth outcomes constitute financial and emotional burdens and alter the quality of life of affected individuals and their families. Although mandatory inclusion of folic acid was implemented in the United States, the rate of adverse birth outcomes has increased from 2014-2017 (CDC, 2021).

Folate has been measured in multiple biological matrices. Folate has been measured in plasma, serum, and red blood cells. Red blood cell folate is a better indicator of tissues stores, making it less susceptible to rapid changes in diet (Galloway, 2003). Although the bioassays to enumerate red blood cell folate are less cost efficient than plasma, or serum folate, its robustness makes red blood cell folate a preferred option for representing a person's folate level.

There are both modifiable and non-modifiable factors associated with low folate levels. Established modifiable factors affecting folate levels include dietary intake of certain medications, alcohol use, and dietary intake of folate and folic acid supplements, and smoking (Weggemans et al., 1997; Sansvisens et al., 2017; Zhou et al., 2018). Pregnant women are at risk of folate deficiency because more folate is required for the developing fetus, and there is an increased demand for folate during lactation periods (Stamm et al., 2013). One non-modifiable risk factor for low folate levels is genetic mutations (Tan et al., 2017; Wang et al., 2015).

1.b.ii Ubiquity and Impact of Environmental Pollutants

Another potential modifiable risk factor for low folate levels is exposure to environmental pollutants. Environmental pollutants refer to pollutants that are present in air, water, food, soil, dust, or other environmental media such as consumer products (Zhu et al., 2018). These pollutants are often ubiquitous in the environment, and some may persist for several years. Many of these pollutants had limited testing for their effects on human health and even less is known about the combined exposure of many environmental pollutants, which is the realistic setting on how most humans come into contact with environmental pollutants (CDC, 2017).

There are currently more than 140,000 industrial pollutants in circulation in the U.S (Kishi & Grandjean, 2020). Examples of environmental pollutants include phthalates, metals, per- and polyfluroalkyl substances (PFAS), parabens, polycyclic aromatic hydrocarbons (PAH), and pollutants released by tobacco smoke. Many of these pollutants are ubiquitous in the environment and their full impact on health outcomes is unknown (Braun et al., 2016; Carpenter et al., 1998).

There are multiple exposure routes for environmental pollutants. Sources of PFAS exposure include drinking contaminated water, eating fish caught from water that was contaminated, packaged foods, stain resistant carpeting, non-stick pans, and other consumer products, and by ingesting contaminated soil or dust (CDC, 2021). People are exposed to phthalates by eating and drinking foods that have come into contact with phthalates and by breathing in air contaminated by phthalate particles (CDC, 2021). Metal exposure can come from diet, or occupational exposure. Additionally, some metals are used in paint, in batteries, and as plastic stabilizers (Jarrett et al., 2012). Parabens can be absorbed through the skin, and consumer products, such as makeup, shaving cream, moisturizers contain parabens (CDC, 2017). PAH exposure can occur from breathing in air contaminated by motor vehicle exhaust, smoke from wood or cigarettes, and asphalt road fumes. Additionally, PAH exposure can occur from eating charred food (CDC, 2017).

Although most studies analyze environmental pollutants individually, people are often exposed to a suite of pollutants, rather than a single pollutant. Because of this, there is an increased demand for the analysis of environmental pollutant mixtures. However, there are challenges to overcome when analyzing the health effects of pollutant mixtures that traditional single-pollutant approaches can rarely address (Bobb et al., 2018). These

issues include 1) non-additive and non-linear relationships between pollutant exposures and health outcomes, 2) the number of comparisons between each metabolite and the outcome of interest can increase the chance for a spurious associations, 3) the components of the mixture may be highly correlated yielding inflated variances and even reverse estimates due to multicollinearity, and 4) lack of inference for both individual effects of each component of the mixture as well as the joint effect of the mixture as a whole (Bobb et al., 2018). Methods to examine mixtures of exposures exist and using these methods when examining association between environmental pollutants and health outcomes is critical to increasing the validity of the findings from environmental epidemiology studies.

1.b.iii Physiology of the Relationship between Environmental Pollutants and Low Folate Levels

The physiological relationship between environmental pollutants and folate are unclear and may vary depending upon the pollutant. These differences can also differ by biological sex because one carbon metabolism processes differ significantly by sex (Sadre-Marandi et al., 2018). Furthermore, this relationship may change when examining a single exposure or a mixture of exposures, as the multiple exposures may interact with each other in a synergistic way. However, there is some physiological evidence to suggest that environmental pollutants may lower folate levels.

The folate cycle is a metabolic process that has been suggested to be critical for pregnancy (Fowler, 2001; GreenBerg et al., 2011). Folate, or folic acid, a synthetic derivative of folate, enters the cycle and undergoes enzymatic reactions to convert to the biologically active *L*-methylfolate by the enzyme methylenetetrahydrofolate reductase (MTHFR) (GreenBerg, 2011). *L*-methylfolate is critical for humans because it is necessary

as a resource for one-carbon transfer reactions for purine/pyrimidine synthesis during deoxyribose nucleic acid and ribose nucleic acid (RNA) assembly (Fowler, 2001; GreenBerg et al., 2011). Folate may also be used to protect against the adverse health effects of environmental pollutants. This use of folate may extinguish the available supply of folate for DNA/RNA assembly during pregnancy.

Additionally, homocysteine, an intermediate in methionine metabolism, has been shown to be inversely associated with bioavailable folate levels, and to be affected by pollutant exposures (Blom & Smulders, 2011). During one carbon metabolism, folate and cobalamin are used for the synthesis of methionine from homocysteine. When there are insufficient levels of folate, buildup of homocysteine can occur. Many pollutants have been positively associated with increased homocysteine levels including heavy metals, pollutants associated with air pollution, PFAs, and PAHs (Ledda et al., 2019; Park et al., 2008; Min et al., 2012; Alhamdow et al, 2018). The biological mechanisms by which environmental pollutant affect homocysteine and folate are not well characterized, but the positive associations between homocysteine and many environmental pollutants, and the inverse association between homocysteine and folate levels suggests that environmental pollutants and folate levels may be inversely related.

1.b.iv Epidemiology of the Relationship between Environmental Pollutants and Low Folate Levels

To our knowledge, there have been no prior studies evaluating the association between a mixture of environmental pollutants and folate concentrations. However prior studies have evaluated the relationship between individual components of a pollutant mixtures and the consequences of low folate and have found positive associations (Gao et

al., 2019; Jankowska et al., 2019; Ferguson et al., 2019; Qian et al., 2019; Liao et al., 2018; Vuong et al., 2019; Manea et al., 2020; Harris et al., 2018; Spartlen et al., 2020; Sutler et al., 2019; Perera et al., 2015; Tang et al., 2006; Perera et al., 2008; Aung et al., 2018; Barkowski et al., 2019; Baker et al., 2020; Shah-Kulkarni et al., 2020; Wehby et al., 2011; Prasodjo et al., 2014). For example, studies have evaluated the relationship between phthalates and the outcomes impacted by low folate, such as neurodevelopment, fetal development, recurrent pregnancy loss, and preterm birth (Gao et al., 2019; Jankowska et al., 2019; Ferguson et al., 2019; Qian et al., 2019; Lia et al., 2018). Studies have also examined the association between PFAs (Vuong et al., 2019; Manea et al., 2020; Harris et al., 2018; Spartlen et al., 2020), PAHs (Sutler et al., 2019; Perera et al., 2015; Tang et al., 2006; Perera et al., 2008), parabens (Aung et al., 2018; Barkowski et al., 2019; Baker et al., 2020), heavy metals (Shah-Kulkarni et al., 2020), and cotinine (Wehby et al., 2011; Prasodjo et al., 2014) and have found significant positive associations between these pollutants and decreased neurodevelopment and fetal development, and increased recurrent pregnancy loss and preterm birth.

There have been a few studies examining the association between a single exposure and low folate levels. For example, A few studies suggest decreased circulating folate in relation to smoking (Okumura et al., 2011; McDonald et al., 2002; Walmsley et al., 1999; Piyathilake et al., 1994; Mannino et al., 2003; Ortega et al., 2004; Matthews et al., 2000; Yanbaeva et al., 2007; Ulvik et al., 2010). Elevated lead exposure levels have also been associated with decreased circulating folate concentrations in two studies (Büyükşekerci et al., 2015; Mehrpour et al., 2020) whereas another study found a negative association

between higher copper intake and folate concentrations (Tamura et al., 2004). Whether folate concentrations are associated with other environmental pollutants is unknown.

1.c Study Design and Methods

1.c.i Study Design

Using a cross-sectional design, we assessed the relationship between environmental pollutants and low RBC folate levels among National Health and Nutrition Examination Survey (NHANES) participants using data from the 2007 to 2016 NHANES data collection cycles. The NHANES is a series of studies designed for the assessment of the health and nutritional status of both adults and children in the United States (National Health and Nutrition Examination Survey [NHANES], 2020). Their collection systems include both demographic and laboratory examinations. The laboratory component includes assessment of biomarkers and occurs yearly at mobile examination centers (MECs). These centers contain the medical equipment necessary to collect urine and blood specimens. The MECs are located at convenient locations near NHANES locations and transportation is provided to MEC facilities. The NHANES uses a multistage probability sampling design to examine a nationally representative sample of about 5,000 persons every year. This sample is derived from sampling 15 locations across the United States (NHANES, 2020)

1.c.ii Study Population

The total study population for cycles from 1999-2016 has approximately 92,062 potential adult study participants aged 18-80. Informed consent was provided by each participant in accordance with section 308(d) of the Public Health Act (42 U.S.C. 242m). Participants were excluded if they do not have a recorded red blood cell folate level. Of the 50,588 individuals from the 2007-2016 NHANES cycles, we excluded individuals who

lacked RBC folate measurements (n=9155). We additionally excluded all participants under the age of 18 (n=13,495). These exclusions left 27,938 individuals for our analysis.

1.c.iii Assessment of Environmental Pollutants

We analyzed six environmental pollutant families in this study: Phthalates, heavy metals, PFAS, phenols and parabens, PAHs, and cotinine. These environmental pollutant families include 41 environmental pollutant biomarkers and metabolites. The NHANES does not include every participant from each cycle for BPA, parabens, PAHs, PFAS, and phthalates. Instead, they utilize one third of the total sample cycle for analysis of these pollutant families. Phthalates, arsenic, phenols and parabens, and PAH were measured in spot urine samples collected at mobile examination centers MECs (NHANES, 2020). PFAS and cotinine were measured in serum samples collected at MECs, whereas metals were measured in whole blood samples.

Phthalates (ng/mL)

There were 11 phthalate metabolites included in this study: Mono(carboxyisooctyl) phthalate (MCOP), Mono-2-ethyl-5-carboxypentyl phthalate (MECPP), Mono-n-butyl phthalate (MBP), Mono-(3-carboxypropyl) phthalate (MCP), Mono-ethyl phthalate (MEP), Mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), Mono-(2-ethyl)-hexyl phthalate (MEHP), Mono-isobutyl phthalate (MiBP), Mono-isononyl phthalate (MNP), Mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and Mono-benzyl phthalate (MBzP). Urine samples for the quantification of phthalate metabolites were stored at -20°C until they arrived at the National Center for Environmental Health for testing. They were quantified using high performance liquid chromatography-electrospray ionization-tandem mass spectrometry (HPLC-ESI-MS/MS)

Heavy Metals

Heavy metals measured in blood included cadmium (ug/L), lead (ug/dL), manganese (ug/L), mercury (ug/L), and selenium (ug/L). Blood samples for the quantification of heavy metals were stored at -30°C until they arrived at the National Center for Environmental Health for testing. Samples of total urinary arsenic samples (ng/ml) were stored at -30°C until they arrived at the National Center for Environmental Health for testing. They were quantified using inductively coupled plasma-mass spectrometry (ICP-MS).

PFAS (ng/mL)

There were 8 PFAS compounds included in this study: Perfluorooctanoic acid (PFOA), Perfluorooctane sulfonic acid (PFOS), Perfluorononanoic acid (PFNA), Perfluoroundecanoic acid (PFUA), Perfluorohexane sulfonic acid (PFHxS), Perfluorodecanoic acid (PFDeA), Perfluorododecanoic acid (PFDoA), and 2-(N-methylperfluorooctanesulfonamido)acetic acid (Me-PFOSA-AcOH). Serum samples for the quantification of PFAs were stored at -30°C until they arrived at the National Center for Environmental Health for testing. They were quantified using solid phase extraction couple to high performance liquid chromatography-turbo ion spray ionization-tandem mass spectrometry (on-line SPE-HPLC-TIS-MS/MS).

Phenols and parabens (ng/mL)

There were 2 phenols and 3 parabens measured in this study: bisphenol A (BPA), triclosan, methyl paraben (MBP), butyl paraben (BPB), and propyl paraben (PBP). Urine samples for the quantification of phenols and parabens were stored at -20°C until they arrived at the National Center for Environmental Health for testing. They were quantified

using on-line solid phase extraction coupled to high performance liquid chromatography and tandem mass spectrometry (on-line SPE-HPLC-MS/MS).

PAH (ng/L)

There were 10 PAHs measured in this study: 1-hydroxynaphthalene, 2-hydroxynaphthalene, 3-hydroxyfluorene, 2-hydroxyfluorene, 3-hydroxyphenanthrene, 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 1-hydroxypyrene, 9-hydroxyfluorene, and 4-phenanthrene. Urine samples for the quantification of PAH were stored at -20°C until they arrived at the National Center for Environmental Health for testing. They were quantified using on-line SPE-HPLC-MS/MS.

Cotinine (ng/mL)

Samples of serum cotinine were stored at -20°C until they arrived at the National Center for Environmental Health for testing. Cotinine was measured by isotope-dilution high-performance liquid chromatography/atmospheric pressure pollutant ionization tandem mass spectrometric (ID HPLC-ACPI MS/MS) method.

Because each NHANES cycle contains different amounts of missing data for each pollutant, and not all pollutants were collected for each NHANES cycle, multivariable imputation by chained equations (MICE) was implemented. MICE is an imputation method that creates multiple imputed datasets in order to consider the uncertainty of relying on imputed data and generate more accurate standard error values (Melissa et al., 2011). Performing MICE methods first imputes the mean for every missing value in the dataset. Then these imputed means are set to missing. Next the observed values are regressed on all other variables in the model. The missing values from the regressed model are replaced by imputed values. This process is repeated for each missing variable. Finally, this entire

process is repeated based on a number of predetermined iterations. Based on previous research, it is suggesting that the number of iterations for a model is related to the average percent of iterations (Zhu, 2014). In this study, there will be 70 iterations for the MICE imputation process. We assumed that the missingness did not depend on unobserved data. Environmental pollutants were natural log transformed during analysis to reduce the influence of outliers.

1.c.iv Validity of the Assessment of Environmental Pollutants

The NHANES uses a quality control protocol, detailed laboratory protocols, annual retraining, repeat testing on 2% of all specimens, and investigates all departures from trends to monitor the validity of all laboratory analyses. Specific data about the quantification methods for each environmental pollutant can be obtained from the NHANES laboratory methods:

wwwn.cdc.gov/nchs/nhanes/ContinuousNhanes/labmethods.aspx?BeginYear=2007,

wwwn.cdc.gov/nchs/nhanes/ContinuousNhanes/labmethods.aspx?BeginYear=2009,

wwwn.cdc.gov/nchs/nhanes/ContinuousNhanes/labmethods.aspx?BeginYear=2011,

wwwn.cdc.gov/nchs/nhanes/ContinuousNhanes/labmethods.aspx?BeginYear=2013,

wwwn.cdc.gov/nchs/nhanes/ContinuousNhanes/labmethods.aspx?BeginYear=2015.

1.c.v Assessment of RBC Folate Levels

RBC Folate levels were calculated by assessing both whole blood folate and serum total folate. Whole blood folate levels were assessed by a microbiological assay. Whole blood was added to an assay medium with *Lactobacillus rhamnosus*, and all of the nutrients necessary for this bacterium to grow, besides folate. The turbidity of the assay medium is then measured and is proportional to the amount of total folate present in the blood sample.

Serum total folate was calculated as the sum of 5 folate forms. These forms were measured by Liquid Chromatography with Tandem Mass Spectrometry (LC-MS/MS). A hematocrit value for the serum total folate was created by taking the ratio of the volume of red blood cells and the total volume of blood to correct the serum folate level (CDC, 2016).

To calculate the RBC folate levels, the whole blood folate levels are subtracted from serum folate values multiplied by a hematocrit value. This value is then divided by the hematocrit value to yield red blood cell folate. Dilution factors are considered for whole blood as multiplication factors (CDC, 2016).

We used one folate variable, and it is parameterized as a continuous variable in the form of red blood cell folate in ng/mL. RBC folate was natural log transformed during analysis to reduce the influence of outliers.

1.c.vi Validity of the Assessment of Folate Levels

The same procedures as described in the environmental pollutant section were used to assess the validity of red blood cell folate levels. The NHANES chose to repeat any analyses of red blood cell folate that had a percent coefficient of variation (%CV) greater than 15%.

1.c.vii Covariate Assessment

Using a directed acyclic graph (DAG), we identified potential confounders from prior research that were associated with environmental pollutants and RBC folate. Questionnaire data were collected for participant gender, age, race, education, and family income to poverty ratio (FPR) at the in-home visit. Urinary creatinine (mg/dL) was assessed from laboratory data. Diet, in the form of the healthy eating index (HEI) was calculated as a composite score of nutrition data captured from 24-hour dietary recall

interviews at the MECs. The HEI is a 100-point scale, with a higher score indicating a better quality of overall diet. Finally, NHANES cycle was also chosen as a confounder and included in all models.

1.c.viii Data Analysis Plan

Participant urinary/blood concentrations of environmental pollutants were calculated as well as RBC folate concentrations. The geometric mean (GM), geometric standard deviation (GSD), interquartile range (IQR), and maximum values were calculated. The percent distribution or mean value of each covariate and/or basic summary statistics were calculated where appropriate and included.

Spearman correlation between the natural log of each environmental pollutant and red blood cell folate concentration were calculated using spearman correlation coefficients. Additionally, bivariate analyses between RBC folate and important study characteristics were performed. These analyses include a student's t test or a one-way analysis of variance (ANOVA) test depending on the study characteristic. If the comparison between RBC folate and a study characteristic includes a dichotomous variable, student's t test was used and if the variables is instead a variable with more than two levels, ANOVA was used.

To consider multiple pollutants and control for multiple comparisons of these pollutants, Exposome Wide Association Study (ExWAS) was used. This method corrects multiple comparison by performing traditional linear regression and applying a threshold for effective tests (TEF) for significance testing (Warembourg et al., 2019). The TEF is calculated based on the number of between exposures.

To investigate the joint associations of the mixture of environmental pollutants with RBC folate concentrations, a quantile-based g-computation (qGComp) was used. The first

step of qGComp is to transform the exposures into quantized versions (Keil et al., 2020). Then a linear model is fit and can be extended to polynomial terms for the quantized exposures or a model that uses indicator variables depending on the nature of the variables included in the model (Keil et al., 2020).

In a supplemental analysis, both the ExWAS and quantile g computation were repeated using a complete-case analysis approach. These results, as well as the distribution of RBC folate per covariate were compared to the MICE analyses results.

Both the ExWAS and quantile g computation were used for the MICE dataset, as well as sex-stratified datasets.

All statistical analyses were conducted using R version 3.5.2. (R Core Team 2018), and SAS version 9.4 (SAS Institute, Inc).

This study was approved by the Institutional Review Board (IRB) of the University of Massachusetts Amherst.

1.d Results

1.d.i Characteristics of the Study Population

NHANES participants in our sample were predominantly male (48.68%), aged 35 to 64 years (48.86%), non-Hispanic white (%), had some college or an associate in arts degree (28.90%), had not smoked in the past 30 days (79.46%), had a family income to poverty ratio in the highest quartile (22.91%), and had urinary creatinine values in the highest quartile (23.15%) (Table 1).

1.d.ii Levels of RBC Folate

The geometric mean RBC folate concentration in our imputed sample was 469.49 ng/mL (geometric standard deviation = 1.53 ng/mL). RBC folate concentrations were

highest among females, participants aged 65 years and older, non-Hispanic whites, participants that had at least a college degree, participants that had not smoked in the past 30 days, participants in the highest family income to poverty ratio, participants in the lowest quartile of urinary creatinine values, and in the highest quartile of the HEI (Table 1).

1.d.iii Levels of Environmental Pollutants

Geometric mean concentrations were 11.34 ng/mL for MCOP, 15.19 ng/mL for MECPP, 11.83 ng/mL for MBP, 2.10 ng/mL for MCPP, 61.78 ng/mL for MEP, 9.68 ng/mL for MEHHP, 1.61 ng/mL for MEHP, 7.49 ng/mL for MiBP, 1.05 ng/mL for MNP, 5.96 ng/mL for MEOHP, 4.88 ng/mL for MBzP, 0.36 µg/L for cadmium, 1.20 µg/dL for lead, 9.38 µg/L for manganese, 0.90 µg/L for mercury, 192.83 µg/L selenium, 8.35 ng/mL for total arsenic, 0.23 ng/mL for PFDeA, 1.39 ng/mL for PFHxS, 0.16 ng/mL for me-PFOSA-AcOH, 0.91 ng/mL for PFNA, 0.15 ng/mL for PFUA, 2.34 ng/mL for PFOA, 7.49 ng/mL for PFOS, 1.53 ng/mL for BPA, 10.93 ng/mL for triclosan, 0.25 ng/mL for butyl paraben, 59.09 ng/mL for methyl paraben, 7.33 ng/mL for propyl paraben, 0.31 ng/mL for cotinine, 2235.54 ng/L for one hydroxynaphthalene, 4474.30 ng/L for two hydroxynaphthalene, 104.31 ng/L for three hydroxyfluorene, 261.61 ng/L for two hydroxyfluorene, 79.04 ng/L for three hydroxyphenanthrene, 123.95 ng/L for one hydroxyphenanthrene, 68.29 ng/L for two hydroxyphenanthrene, 118.89 ng/mL for one hydroxypyrene, 304.70 ng/L for nine hydroxyfluorene, and 21.81 ng/L for four phenanthrene (Table 2).

1.d.iv Correlations between RBC Folate and Environmental Pollutants

Environmental pollutants within the same family were moderately correlated (Figure 1). Correlation between pollutant families varied (Figure 1). The metal, PAH, and PFAS were weakly correlated with RBC folate (Figure 1).

1.d.v Results from the ExWAS

For the overall ExWAS analysis, the threshold for effective test (TEF) was $1.93e^{-3}$. After adjustment for sex, age, race, education, smoking status, FPR, urinary creatinine, diet, and NHANES cycle, 26 environmental pollutants were significantly inversely associated with RBC folate, and manganese and selenium were significantly positively associated with RBC folate (Figure 2). For the ExWAS analysis restricted only to males, the TEF was $1.89e^{-3}$. Among males, after adjustment for age, race, education, smoking status, FPR, urinary creatinine, diet, and NHANES cycle, 21 environmental pollutants were significantly inversely associated with RBC folate, and selenium was significantly positively associated with RBC folate (Figure 2). For the ExWAS analysis restricted only to females, the TEF was $1.99e^{-3}$. Among females, after adjustment for age, race, education, smoking status, FPR, urinary creatinine, diet, and NHANES cycle, 20 environmental pollutants were significantly inversely associated with RBC folate, and manganese was significantly positively associated with RBC folate (Figure 2).

1.d.vii Results from qGComp

A quantile increase in overall mixture was significantly inversely associated with RBC folate (-0.121 95%CI: -0.140,-0.103) after adjustment (Figure 3). Among boys, a quantile increase in the overall mixture was significantly inversely associated with RBC folate (-0.083, 95%CI: -0.112, -0.055). Among girls, a quantile increase in the overall mixture was significantly associated with RBC folate (-0.011 95%CI: -0.029, -0.008). A

quantile increase in the overall metal (-0.095 95%CI -0.114, -0.076), PFAS (-0.064 95%CI: -0.074, -0.054), and PAH (-0.016 95%CI: -0.027, -0.006) mixtures were significantly inversely associated with RBC folate. A quantile increase in the overall bisphenols and parabens was significantly positively associated with RBC folate (0.011 95%CI: 0.001, 0.021) (Figure 3).

A quantile increase in the overall mixture was significantly inversely associated with RBC folate among males (-0.083 95%CI: -0.012, -0.055) but not females (-0.011 95%CI: -0.029, 0.008) (Figure 3). A quantile increase in the phthalate mixture was significantly positively associated with RBC folate among males (0.018 95%CI: 0.004, 0.031), but inversely associated with RBC folate among females (-0.011 95%CI -0.025, 0.004). A quantile increase in the metal mixture was significantly inversely associated RBC folate among males (-0.074 95%CI: -0.088, -0.061) and females (-0.102 95%CI: -0.117, -0.086). Similarly, a quantile increase in the PFAS and PAH mixtures was significantly inversely associated with RBC folate among males ((-0.053 95%CI: -0.067, -0.040) for PFAS and (-0.020 95%CI: -0.035, -0.006) for PAH) and females ((-0.070 95%CI: -0.084, -0.056) for PFAS and (-0.018 95%CI: -0.033, 0.003) for PAH (Figure 3).

1.e Discussion

1.e.i Summary of Findings and Significance

The overall mixture, as well as the metal, PFAS, and PAH mixtures were significantly inversely associated with RBC folate levels in the overall sample, as well as among males and females among NHANES participants. Additionally, the bisphenol and paraben mixture was significantly positively associated with RBC folate. Among these pollutant families, cadmium, lead, mercury, PFDeA, PFNA, PFUA, PFOS, one

hydroxynaphthalene, two hydroxynaphthalene, three hydroxyfluorene, two, hydroxyfluorene, three hydroxyphenanthrene, one hydroxyphenanthrene, two hydroxyphenanthrene, one hydroxypyrene, nine hydroxyfluorene, four phenanthrene, and cotinine were identified to be significantly inversely associated with RBC folate in the overall samples as well as among males and females. Manganese, in the overall analysis and among males, and selenium, in the overall analysis and among males, were also identified as having a positive association with RBC folate.

The results from ExWAS and QgComp when stratified by sex were similar. One exception was the associations between phthalate mixtures and RBC folate. When stratified by sex, the association was significantly inverse among males and positive among females. These results were consistent in the individual analyses, although not statistically significant. Phthalates are known endocrine disruptors and block testosterone action. Although no other studies have examined the relationship between phthalates and folate, there have been sex specific associations between phthalates and the consequences of low folate.

Our results suggest significant relationships between exposures of environmental pollutants and RBC folate and these findings have substantial implications for neurodevelopment associated with folate. These findings are modest and may not seem clinically relevant, however their impact on public health is significant. First, a ubiquitous and continuous source of a pollutant mixture that has a modest inverse association with folate can have potent implications for public health. There is a paucity of the public health implications of small reductions in folate, however, folate plays a critical role in neurodevelopment and has been associated with cognitive ability. Low cognitive ability

has been shown to have profound implications for public health at even modest levels. This is because population impact of a risk factor is dependent on both the effect size as well as its distribution. Second, vulnerable populations with insufficient folate levels are further burdened by a preventable factor that reduces folate levels. Pregnancy requires more folate, and there are other populations that either can't obtain adequate folate or are genetically predisposed to metabolizing folate and these vulnerable populations are further burdened by an environmental pollutant mixture.

1.e.ii Correlations between pollutants

There was high positive correlation between pollutants that shared the same pollutant family. There was also modest positive correlation between some pollutants not in the same family. Some pollutants within the same family are derived from other members and this may explain their correlation. Additionally, pollutants from either within the same family or not, may be correlated because they share similar exposure routes. For example, the CDC suggests that many of the exposure routes of these pollutants involve water or food.

1.e.iii Potential Biologic Mechanisms

Limited laboratory research suggests possible biological mechanisms for the inverse associations between these environmental pollutants and RBC folate. PFAS have been shown to effect thyroid hormone levels (Ballesteros et al. 2017; Lee and Choi 2017) and thyroid stimulating hormones have been suggested to predict folate levels (Catargi et al., 1999). A vitamin B complex, including folate, has been shown to reduce cadmium and lead levels in rats, possibly by preventing absorption or facilitating increased excretion (Tandon et al., 1984). Mercury and PAHs are detoxified through the glutathione

detoxification system and folate is responsible for the metabolic processing of cysteine, a precursor of glutathione (Kim et al., 2020; Aspera-Werz et al., 2018). Additionally, selenium has been shown to enhance concentrations of glutathione, and would help to explain the positive associations between selenium and RBC folate observed in our study (Richie et al., 2012).

1.e.iv Strengths and Limitations

This study has several key limitations. First, many of the exposures are biomarkers of exposures and represent the true exposure. Because of the reliance on biomarkers instead of the true exposure, the exposure status of individuals may be misclassified. This issue is compounded by the cross-sectional study design with only one measurement representing past exposure status. Pollutants that have long a long half-life, including PFAS and metals, are less of a concern because their exposure status on collection day may more accurately represent their past exposure status. Phthalates, BPA, parabens, and PAH have a half-life that ranges from a few hours to a few weeks. A study comparing results from phthalates collected over several days found the phthalate levels to be similar. It has been suggested that using conjugates of parabens and the unconjugated BPA may more accurately represent exposure. We attempted to minimize the effects of this limitation by using the conjugated forms of parabens and unconjugated form of BPA.

Additionally, these data were collected cross-sectionally, and a temporal relationship between environmental pollutants and folate will be unclear. Although internal endogenous measures of environmental pollutants have increased measurement accuracy over proxy exposure methods, there is an increased concern for reverse causation (Weisskopf et al., 2017). Measuring environmental pollutants within the body can be

compromised by unrecognized effects of the outcome on the measurement of the exposure. This may be true for folate and some or all environmental pollutants, and our study design limits the ability to examine the potential for reverse causation.

This study utilized imputed environmental pollutant data. On average, 67% of the environmental pollutant data was missing. The NHANES does not complete a laboratory profile of all participants. Instead, the NHANES chooses a section of total participants for pollutant measure ascertainment. Because of this, there are about 2/3 of the data missing, and the missing is assumed to be random. In order to improve the accuracy of the imputed variables, the MICE method was chosen. Even with using multiple imputation and the variables assumed to be missing at random, a cautious interpretation of the results is appropriate due to the number of iterations. The analyses of this paper were repeated using complete case analysis. The results of ExWAS and qGComp were similar to the imputed results in terms of direction, but the magnitude and statistical significance of the associations in some pollutant families and the overall mixture differed.

To consider the potential impact of the imputations, the ExWAS and qGComp analyses were repeated using only complete cases. For the ExWAS analysis, there were differences in statistical significance, minor differences in magnitude, and no differences in direction of the association between the environmental pollutants and RBC folate. Lead, PFDeA, PFNA, PFUA, PFOS, and cotinine were significantly inversely associated with RBC folate in the overall analysis, as well as among males and females for both the imputed analysis and the complete case analysis (Supplemental Table 1). For the qGComp analysis, their differences in statistical significance, minor differences in magnitude, and no differences in direction of the association between environmental pollutant mixtures and

RBC folate (Supplemental Table 2). The metal and pfas mixtures were significantly inversely associated with RBC folate in the overall analysis as well as among males and females for both the imputed analysis and the complete case analysis. Sample sizes for the complete case analysis are available in supplemental table 3.

Finally, the pollutant mixture included in this list is comprehensive, but not exhaustive. People are exposed to thousands of pollutants daily and the NHANES has limitations when considering the analysis of pollutant mixtures. For example, different pollutants have varying sample sizes, collection age ranges, and the number of pollutants assessed changes per cycle. These attributes restrict the formation of a complete pollutant mixture based on the entire NHANES dataset available.

Strengths of this study include: the breadth of environmental pollutants available through the NHANES dataset, consideration of effect modification by sex, and the adoption of modern statistical approaches to analyze both mixture and individual associations between environmental pollutants and RBC folate.

1.f Conclusion

This study, to our knowledge, was the first study to examine the association between a pollutant mixture and RBC folate. Although modest, the inverse association between a mixture of environmental pollutants, including: PFAS, PAHs, BPA, parabens, metals, and phthalates, and RBC folate has important public health implications. Small shifts in outcome levels lead to bigger shifts in population levels when the exposure is ubiquitous (Day et al., 2021; Bellinger, 2012) . This study's results should be interpreted cautiously due to the amount of imputations on key variables. Future studies can expand

these preliminary findings by considering a mixtures approach when assessing the association between pollutant mixtures and RBC folate.

CHAPTER 2

Bayesian Kernel Machine Regression for in utero Environmental Pollutant Mixtures and Autistic Traits

2.a Specific Aims and Hypotheses

Specific Aim 1: To evaluate the associations between environmental pollutant concentrations and Autistic Traits in the Growing Up in Singapore Towards Healthy Outcomes cohort.

Hypothesis 1: will be a positive association between environmental pollutant levels and autistic traits.

Specific Aim 2: Asses whether these associations are modified by sex.

Hypothesis 2: The associations between environmental pollutants and autistic traits will be differential by sex with stronger effects among boys.

Specific Aim 3: Compare evidence from Joint and independent associations between environmental pollutants and autistic traits.

Hypothesis 3: The joint association of environmental pollutants with autistic traits is stronger than the sum of their individual associations.

2.b Background and Significance

2.b.i Public Health Impact of Autism Spectrum Disorder

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder characterized by a spectrum of communication and social deficits, and repetitive behaviors (Centers for Disease Control and Prevention [CDC], 2020). The learning, thinking, and problem-

solving abilities of people with ASD can be debilitating. There is currently no cure for ASD, and ASD is a lifelong burden on both the person diagnosed as well as their family (Centers for Disease Control and Prevention [CDC], 2020). The prevalence of ASD has increased since 2000, and the most recent surveillance data from the Centers for Disease Control and Prevention (CDC) suggests that 1 in 54 children are diagnosed with autism (Figure 5) (Centers for Disease Control and Prevention [CDC], 2020).

The total costs per year for children with ASD in the United States (U.S.) were estimated to be between \$11.5 billion to \$60.9 Billion in 2011 (Pelch et al., 2019). The significant portion of these costs are due to lifelong impairments including medical care, special education, and lost parental productivity (Centers for Disease Control and Prevention [CDC], 2020). In the United States, the total ASD-attributable costs are projected to rise to over \$450 billion by 2025. These costs will likely exceed those of diabetes, stroke, and hypertension (Flores-Pajot et al., 2016).

2.b.ii Ubiquity and Impact of Environmental Pollutants

Although ASD has been shown to be heritable, there has been increasing evidence that environmental pollutants may be positively associated with ASD (Gong et al., 2017). Environmental pollutants refer to pollutants that are present in air, water, food, soil, dust, or other environmental media such as consumer products (Flores-Pajot et al., 2016). These pollutants are often ubiquitous in the environment, and some may persist for several years (gong et al., 2017). Many of these pollutants had limited testing for their effects on human health and even less is known about the combined exposure of many environmental pollutants, which is how most humans come into contact with these pollutants (Flores-Pajot et al., 2016).

Examples of environmental pollutants include phthalates, per- and polyfluoroalkyl substances (PFAS), parabens, benzophenones, and the pollutants present in air pollution. Many of these pollutants are ubiquitous in the environment and their full impact on health outcomes is unknown (Dutheil et al., 2021; Jo et al., 2019). There are multiple exposure routes for environmental pollutants. Sources of PFAS exposure include Drinking contaminated water, eating fish caught from water that was contaminated, packaged foods, stain resistant carpeting, non-stick pans, and other consumer products, and by ingesting contaminated soil or dust (Pagalan et al., 2019). People are exposed to phthalates by eating and drinking foods that have come into contact with phthalates and by breathing in air contaminated by phthalate particles (Testa et al., 2012). Parabens can be absorbed through the skin, and are found in consumer products, such as makeup, shaving cream, moisturizers contain parabens (Oulhote et al., 2020). Benzophenones are commonly used as a drying agent for food packaging, and they can enter the body by migrating from the packaging to food (Haggerty et al., 2021).

People are often exposed to a suite of pollutants, rather than a single pollutant. There are challenges to overcome when analyzing the health effects of pollutant mixtures that traditional single-pollutant approaches can rarely address (Miodovnik et al., 2011). These issues include 1) non-additive and non-linear relationships between the exposure and outcome, 2) the number of comparisons between each metabolite and the outcome of interest can increase the chance for a spurious association, 3) the components of the mixture may be highly correlated yielding inflated variances, and 4) lack of inference for both individual effects of each component of the mixture as well as the joint effect of the mixture (Pagalan et al., 2019). Methods to examine mixtures of exposures exist and using these

methods when examining the associations between environmental pollutants and health outcomes is critical to increasing the validity of these findings.

2.b.iii Physiology of the Relationship between Environmental Pollutants and ASD

The physiological relationship between environmental pollutants and ASD are unclear and may vary depending upon the pollutant. Furthermore, this relationship may change when examining a single exposure or a mixture of exposures, as the multiple exposures may interact with each other. Many pollutants, such as PFAS, phthalates, bisphenols and parabens, phenones, and air pollutants, can cross the placental barrier and be transported to the fetus (Shin et al., 2020; Lyall et al., 2018; Shin et al., 2019; Kalkbrenner et al., 2014; Barkoski et al., 2019).

The mechanism of action between each environmental pollutant may vary between pollutant family, and even within each pollutant family. Animal studies suggest that PFAS may adversely affect child's brain development (Shin et al., 2020). There is human evidence that PFAS disrupt thyroid hormone homeostasis of pregnant women. Deficiencies in thyroid hormones during pregnancy have been suggested to be associated with an increased risk of autistic behaviors (Barkoski et al., 2019). Phthalates, BPA, parabens, and phenones are suggested endocrine disrupters, and fetuses and infants are susceptible to hormonal disruption that can affect neurodevelopment (Shin et al., 2019; Hansen et al., 2021). Experimental studies in zebrafish have found that some phthalates are potentially neurotoxic (Kardas et al., 2015). Air pollution may impair placental function and decrease oxygen and nutrient transport which can negatively affect fetal development (Kardas et al., 2020).

Oxidative stress is another potential mechanism between environmental pollutants and ASD. Oxidative stress occurs when there is an imbalance in the production and removal of reactive oxygen species (ROS) in the body. The brain is a major metabolizer of oxygen, and consequently, is a sensitive target for the accumulation of ROS (Wnuk et al., 2018)). Additionally, accumulated ROS is a feature of ASD (Bennett et al., 2022). PFAS, phthalates, parabens, and particulate matter in air pollution have been associated with increased oxidative stress levels (Wielsoe et al., 2015; Erve et al., 2019; Samarasinghe et al., 2018; Deng et al., 2013; Pivovarciova et al., 2015). In an experimental study on rat brains, benzophenone-2 was found to reduce oxidative stress in the frontal cortex of rat brain (Wnuk et al., 2018).

Additionally, many environmental pollutants are associated with the alteration of genes associated with ASD. Among the 206 genes associated with ASD, pesticides, industrial pollutants, BPA, phthalates, PCBs, significantly targeted autism genes, enriching them (Keil et al., 2016) These findings, suggest that there may be mechanisms linking environmental pollutants and genes related to autism. Environmental pollutants have also been associated with DNA methylation (Leandro et al., 2006). DNA methylation is critical during neurodevelopment and when disrupted can have significant impacts neurodevelopment and cognitive function (Leandro et al., 2006).

There are other mechanisms that may explain the relationships between environmental pollutants and ASD. For example, phthalates are antiandrogenic and testosterone has been suggested to be associated with aggressive autistic behaviors (Genc et al., 2012) Air pollution may damage ion channels, or cause inflammation that is detrimental to the brain and may lead to neurological diseases (Duvekot et al.,2015).

2.b.iv Epidemiology of the Relationship between Environmental Pollutants and ASD

There are several epidemiologic studies that evaluated the relationship between environmental pollutants and autism or autistic traits. The studies varied by case definition, study design, study population, and specific exposures assessed. There was some heterogeneity between the results of the studies that examined the relationship between air pollution, in the form of particulate matter (F;pres-Pajot et al., 2016; Gong et al., 2017; Dutheil et al., 2021; Jo et al., 2019; Pagalan et al., 2019). Particulate matter (PM) is normally classified by size where $PM_{2.5}$ represents particulate matter that is 2.5 or less microns in diameter and PM_{10} represents particulate matter that is greater than 2.5 microns to 10 microns in diameter. These changes could be due to differences in study design, but also due to the composition of the air pollution examined. There were various cultural and geographic differences that could change the pollutant composition of air pollution. However, most of the results suggest that increasing $PM_{2.5}$ levels are associated with higher ASD diagnosis (Jo et al., 2019). Most of the studies found associations between the third trimester, and one-year post-natal exposure windows of $PM_{2.5}$ and ASD (Jo et al., 2019). Two studies that did not find significant associations did not consider trimester specific associations (Pagalan et al., 2019). Additionally, one of these studies focused only on PM_{10} and not $PM_{2.5}$ (Pagalan et al., 2019). The only study to consider stratifying results by child sex instead of adjusting for sex found a significant positive association between $PM_{2.5}$ and ASD among boys only (Jo et al., 2019).

There were limited studies for the investigation of the association between phthalates and autism. A case control study by Testa et al found that DEHP metabolites were significantly higher among children with autism compared to controls without autism

(Testa et al., 2012). Two studies suggest that increasing phthalates are associated with autistic traits (Testa et al., 2012; Miodovnik et al., 2011) some of these studies (Testa et al., 2012; Oulhote et al., 2020) also stratified these results and found significantly stronger associations between phthalates and autistic traits among boys only (Testa et al., 2012).

The results of studies examining the relationship between PFAS and ASD are mixed. Some studies have found positive associations between certain PFAS, but they were not significant (Shin et al., 2020; Shin et al., 2019). Other studies found significant inverse associations between PFOS (Lyall et al., 2018) and PFOA (Lyall et al., 2018; Kalkbrenner et al., 2014) with ASD. One study (Kalkbrenner et al., 2014) did use approaches to handle pollutant mixtures. These studies were also largely underpowered and could not examine sex specific differences with sufficient power.

There is limited evidence for evaluating the association between bisphenols and parabens and ASD. Most studies focused on bisphenol A (BPA). One study evaluated children's BPA levels after birth (Kardas et al., 2015). In this cross-sectional design, they found that children with ASD had significantly higher levels of BPA when compared to children without ASD. Two studies used mixtures approaches and did not find significant results when assessing the relationship between BPA and ASD (Kalkbrenner et al., 2014; Barkoski et al., 2019). There was one study that examined the relationship between BPA and ASD stratified by sex. They found significant positive associations between BPA and ASD among girls, and a similar, but insignificant, association among boys (Hansen et al., 2021).

Exposure Limitations of Current Research

One limitation of the current research is the lack of consideration of pollutant mixtures when analyzing the association between environmental pollutants and autism. A limited amount of the studies used statistical techniques for pollutant mixtures in their analyses.

Conflicting Evidence

Many of the within-family studies have conflicting evidence. This may be due to differences in exposure collection windows, parameterization of the exposure, and definition of the outcome. Additionally, some studies had sufficient sample size to stratify analysis by child sex while other stated that stratification was not appropriate due to the available sample size of the study.

2.c. Study Design and Methods

2.c.i Study Design

Using a prospective design, we propose to assess the relationship between environmental pollutants and autistic traits among women from the Growing Up in Singapore Towards healthy Outcomes (GUSTO) study (Soh et al., 2012). The exposure of interest is a mixture of environmental pollutants. These pollutants include: PFAS, phthalates, bisphenols and parabens, benzophenones, and air pollution. Pregnant women were initially enrolled at 7-11 weeks of pregnancy between June 2009 and September 2010 and were followed until the child's eight year of life. The outcome of interest are autistic traits.

2.c.ii Study Population

Between June 2009 and September 2010, the GUSTO study recruited 1450 pregnant Singapore citizens. These pregnant women contributed 1460 children for this

study. Participants were included if they attended first trimester antenatal ultrasound scan at the public maternity units at Kandang Kerbau (KK) Women's and Children's Hospital and National University Hospital in Singapore. Only women 18 years of age and above, women intending to deliver at the National University Hospital or the KK Women's Children's Hospital, women intending to reside in Singapore in the next five years, women willing to donate cord, cord blood, and placenta were included in this study. By the end of the 8th year of life follow-up, there were 1034 mothers and 1041 children available for analysis. Women with significant medical conditions, certain medications, such as psychotropic drugs and chemotherapy, women from mixed marriages, and women whose pregnancies end in miscarriages were excluded. There were 403 mothers and their children that did not have SRS scores and were excluded. This yielded a final analytical sample of 631 mothers and their children.

2.c.ii Study Population

The total study population for cycles from 1999-2016 has approximately 92,062 potential adult study participants aged 18-80. Informed consent was provided by each participant in accordance with section 308(d) of the Public Health Act (42 U.S.C. 242m). Participants were excluded if they do not have a recorded red blood cell folate level. Of the 50,588 individuals from the 2007-2016 NHANES cycles, we excluded individuals who lacked RBC folate measurements (n=9155). We additionally excluded all participants under the age of 18 (n=13,495). These exclusions left 27,938 individuals for our analysis.

2.c.iii Assessment of Environmental Pollutants

The pollutant families analyzed in the GUSTO cohort include: PFAS, phthalates, phenols and parabens, benzophenones, and air pollutants in the form of PM_{2.5}. PFAS, phthalates, phenols and parabens, and benzophenones were collected at maternal pregnancy week 26 and at birth via cord blood and their concentrations were measured in ng/mL. Non-fasting bloods samples were obtained from mothers upon arrival at the hospitals for delivery by standard venipuncture technique. The blood samples were collected in EDTA tubes, processed within 4 hours. Samples were centrifuged at 1600g for 10 min at 4 °C to obtain the plasma and stored at – 80 °C. Samples were thawed prior to analysis.

PFAS

The PFAS compounds analyzed include: pentafluorobenzoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorobutanesulfonic acid (PFBS), Perfluorohexanesulphonic acid (PFHxS), and Perfluorooctanesulfonic acid (PFOS).

Phthalates

The phthalate metabolites analyzed included: Mono-methyl phthalate (MMP), Mono-ethyl phthalate (MEP), Mono-n-butyl phthalate (MBP), Mono-2-ethylhexyl phthalate (MEHP), Mono-n-octyl phthalate (MNOP), Mono-(3-carboxypropyl) phthalate (MCPP), Mono-benzyl phthalate (MBzP), Mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), and Monocarboxyisononyl phthalate (MCINP).

Bisphenols and Parabens

The bisphenols and parabens analyzed include: Bisphenol A (BPA), Bisphenol F (BPF), Bisphenol S (BPS), methyl paraben, ethyl paraben, propyl paraben, and butyl paraben.

Benzophenones

The benzophenones analyzed include benzophenone and oxybenzone.

Air Pollution

Environmental air pollution data was provided by the National Environmental Agency (NEA) of Singapore. These data include 24-hour nation-wide average pollutant standards index (PSI), and fine particulate matter with a diameter less than 2.5 μm ($\text{PM}_{2.5}$) concentration in $\mu\text{g}/\text{m}^3$. $\text{PM}_{2.5}$ data was obtained as an average measure from eight stations: P03 Environment Building, P20 Bishan ITE, P28 Temasek Polytechnic, industrial stations—P17 Stagmont Camp, P31 Pandan Reservoir, suburban station—P24 Yishun ITE and roadside stations—P15 Ngee Ann Polytechnic and P32 Chin Swee. The daily $\text{PM}_{2.5}$ data were available throughout the study. Mean monthly $\text{PM}_{2.5}$ data were aggregated from the available $\text{PM}_{2.5}$ data available for each participant.

2.c.iv Assessment of Autistic Traits

The Social Responsiveness Scale-Second Edition (SRS-2) is a 65-item instrument used for reporting deficits in social behavior associated with ASD outlined by the Diagnostic and Statistical Manual of Mental Disorders (Bruni et al., 2014; Chan et al., 2017). The questionnaire takes approximately 15 to 20 minutes to complete. The SRS-2 was completed by parents. On the form, items are scored on a 4-point Likert-scale ranging from *not true* = 1, *sometimes true* = 2, *often true* = 3, to *almost always true* = 4. The results of the SRS-2 are computed and are standardized by sex and reported in the form of T-

Scores ($M=50$, $SD = 10$). There is a total SRS-2 T score as well as subscales: Social Awareness, Social Cognition, Social Communication, Social Motivation, and restricted Interests, and Repetitive Behavior. Overall T-scores of 76 or higher are considered severe suggesting that an individual has clinically significant deficits in social functioning that interfere with interactions with others. Scores that fall between 66 and 75 suggest some clinically significant social deficits. Scores that fall between 60 and 65 suggest mild to moderate deficiencies in social behavior. T-scores below 60 suggest that no social difficulties indicative of a possible ASD diagnosis.

2.c.v Validity of the Assessment of Autistic Traits

The SRS-2 has been validated in multiple studies consisting of many demographics and is a preferred method of self-reported proxies for autism diagnosis (Bruni et al., 2014). Previous studies have demonstrated the validity and reliability of the SRS-2 for the assessment of autistic traits (Chan et al., 2017). The sensitivity of SRS-2 was 92% and specificity was 92% in terms of accurately identifying those who have been diagnosed with autism and those without autism.⁴⁹ Compared to a standardized diagnostic instruments, such as the autistic diagnostic observation schedule (ADOS) and the dimensional and diagnostic interview (3Di), the SRS-2 displayed high sensitivity and specificity (Duvekot et al., 2015). Additionally, analyzing continuous scores is more appropriate in the general population. By using continuous scores, we obtain more power, but more importantly, we can determine fine changes in SRS-2 scores that may not be clinically relevant but may be of substantial public health significance (Bellengir et al., 2002).

2.c.vi Covariate Assessment

Using a directed acyclic graph (DAG), we identified potential confounders from prior research that were associated with environmental pollutants and autistic traits. Self-reported questionnaire data were collected for child sex, maternal age, maternal ethnicity, maternal education, maternal smoking status at pregnancy week 26, family income, parity, and maternal pre-pregnancy body mass index. Additionally, plasma folate (ng/mL) data were collected at pregnancy week 26.

2.c.vii Data Analysis Plan

Mean participant blood concentrations of environmental pollutants were calculated as well as mean SRS-2 scores and subs core values. The geometric mean (GM), geometric standard deviation (GSD), interquartile range (IQR), and maximum values were calculated for environmental pollutants and non-geometric summary statistics were calculated for the SRS-2 scores. The percent distribution or mean value of each covariate and/or basic summary statistics were calculated where appropriate and included.

Spearman correlation between the natural log of each environmental pollutant and autistic traits were calculated using spearman correlation coefficients. Additionally, bivariate analyses between autistic traits and important study characteristics were performed. These analyses include a student's t test or a one-way analysis of variance (ANOVA) test depending on the study characteristic. If the comparison between autistic traits and a study characteristic includes a dichotomous variable, student's t test was used and if the variable is instead a variable with more than two levels, ANOVA was used.

The individual and cumulative relationship between multiple exposures and autistic traits were investigated using Bayesian Kernel Machine (BKMR). Because pollutant mixtures may have non-linear effects, the pollutants may be correlated with each other, and

there are often numerous pollutants to be considered in the mixture, BKMR is a great choice for examining the health effects of pollutant mixtures (Bobb et al., 2014; Coker et al., 2018; Keil et al., 2016; Bobb et al., 2015). Log-transformed concentrations of environmental pollutants will be standardized and included in a BKMR model; a method developed for investigating pollutant mixtures that flexibly models the joint effect of pollutants while accounting for covariates using a kernel function. The BKMR approach allows the visualization of the exposure-response association for each component of a mixture, while considering the correlation between the mixture components. This approach estimates the multivariable exposure-response function in a flexible way that allows for nonlinear and non-additive effects. Specifically, outcome scores will be included in the model as a smooth function equation (represented using a kernel function) of the exposure variables, adjusted for potential confounding factors: $Y_i = \mathbf{h}(\mathbf{z}_{i1}, \dots, \mathbf{z}_{iM}) + \mathbf{x}_i' \boldsymbol{\beta} + \epsilon_i$, where Y_i denotes the response for individual i , z_{im} is the m^{th} exposure variable, h denotes the unknown exposure-response function to be estimated, $\boldsymbol{\beta}$ represents the effect of the vector of covariates, and the residuals ϵ_i are assumed to be independent and identically normally distributed. Moreover, the hierarchical variable selection approach provides a principled manner of addressing multiple testing and multicollinearity. Natural log transformed environmental pollutants will be standardized and included in the model. Effect modification with child sex will be investigated by stratifying analyses by sex.

All statistical analyses were conducted using R version 3.5.2. (R Core Team 2018), and SAS version 9.4 (SAS Institute, Inc).

This study was approved by the Institutional Review Board (IRB) of the University of Massachusetts Amherst.

2.d. Results

2.d.i Characteristics of the Study Population

GUSTO participants in our sample gave birth to babies who were predominantly male (51.98%), were full term (93.98%), and were average for gestational age (72.11%) (Table 3). The GUSTO mothers were predominantly aged 25 to 35 years (63.54%), of Chinese ethnicity (56.26%), had a high school diploma or a technical diploma (37.24%), had never smoked (85.73%), had a household family income of \$2,000.00 to \$3,999.00 (30.27%), breast fed for less than 1 month (22.82%), had folate values greater than 6 ng/mL (80.03%), and were married (96.35%) (Table 3).

2.d.ii Levels of Autistic Traits

The mean total SRS-2 score was 54.86 (SD: 7.06). For the SRS-2 sub scores, the mean awareness score was 58.38 (SD:8.52), the mean cognition score was 55.91 (SD: 7.69), the mean communication score was 54.57 (SD: 7.48), the mean motivation score was 53.69 (SD: 7.87), the mean RRB score was 50.55 (SD: 7.09) and the mean SCI score was 55.91 (SD: 7.38) (Table 4). Total SRS-2 scores were higher among female children, children born early term, children that were large for gestational age (table 8). Additionally, Total SRS-2 scores were in children with mothers that were less than 25 years of age, had less than a high school degree, had ever smoked, had household monthly incomes between \$0.00 and \$1999.00, and had breastfed their child for less than one month (Table 4).

2.d.iii Levels of Environmental Pollutants

Geometric mean concentrations were 1.57 ng/mL for PFBA, 1.61 ng/mL for PFOA, 0.83 ng/mL for PFNA, 0.14 ng/mL for PFDA, 0.20 ng/mL for PFUnDA, 21.02 ng/mL for PFBS, 0.49 ng/mL for PFHxS, 1.11 ng/mL for PFOS, 0.53 ng/mL for MMP, 0.31 ng/mL

for MECCP, 0.62 ng/mL for MCINP, 0.69 ng/mL for MEP, 2.45 ng/mL for MBP, 9.29 ng/mL for MEHP, 0.08 ng/mL for BPS, 2.79 ng/mL for methyl paraben, 0.13 ng/mL for propyl paraben, 9.32 ng/mL for benzophenone, 2.13 ng/mL for oxybenzophenone, and 18.50 ng/mL for PM2.5 (Table 4).

2.d.iv Correlations between Environmental Pollutants

Environmental pollutants within the same family were moderately correlated (Figure 1). Correlation between pollutant families varied (Figure 4). PFNA was weakly positively associated with autistic traits and oxybenzophenone was weakly negatively associated with the SRS-2 awareness sub score (Figure 4).

2.d.v Results from BKMR

The single exposure differences in response (95% confidence interval) associated with a change in a particular exposure from its 25th to 75th percentile where all of the other pollutant mixture components are fixed to their 50th percentile after adjustment are reported in figure 5. In the overall BKMR analysis, 10 pollutants were significantly inversely associated with autistic traits. The pollutant families significantly inversely associated with autistic traits included: PFAS, phthalates, BPS/parabens, phenones, and air pollution. Additionally, a doubling of PFNA concentration was significantly positively associated with the SRS cognition sub score (1.888 95%CI: 0.75, 4.28) (Figure 5). There were no pollutant mixtures significantly associated with total SRS or SRS sub scores (Figure 6).

For the sex stratified analyses, MBP was significantly inversely associated with total SRS scores and each SRS sub score among females, and BPS was significantly inversely associated with total SRS scores and each SRS sub score among male children. Additionally, among males, a doubling of MEP was associated with a significant positive

increase in the SRS RRB sub score (1.115 95%CI: 0.23, 0.264) (Figure 5). Among males and females, there were no pollutant mixtures significantly associated with total SRS or SRS sub scores (Figure 6), but there were sex differences between a pollutant mixture of PFAS, phthalates, bisphenols and parabens, PM2.5, and benzophenones and its association with total SRS-2 scores, as well as the SRS-2 awareness and cognition sub scores (Table 5).

2.e Discussion

2.e.i Summary of Findings and Significance

In this cohort study, we found that many environmental pollutant families, including PFAS, phthalates, BPS/parabens, phenones, and air pollution were significantly inversely associated with autistic traits. Additionally, PFNA in the overall sample, and MEP among male children, were positively associated with autistic traits. The priority of certain individual pollutants did differ when the BKMR analyses were stratified by sex. For example, among female children, MBP was significantly inversely associated with total SRS as well as all SRS subscales. However, among males, BPS was significantly inversely association with total SRS as well as all SRS subscales, and oxybenzophenone was significantly inversely associated with total SRS and all SRS subscales except for the SRS sub score RRB. Additionally, MEP was significantly positively associated with the SRS sub score RRB among males, but inversely associated with the SRS sub score RRB among female children.

These findings add to the conflicting body of literature between environmental pollutants and autistic traits. There have been both significant positive and negative associations between PFAS and autistic traits. Similarly for air pollution. For phthalates

and BPS/parabens, the current research is pretty consistent with multiple reports of positive associations between phthalates and autistic traits. To our knowledge no study has examined the relationship between phenones and autistic traits.

2.e.ii Biologic Mechanisms

These results largely appear to be contradictory to our hypothesis that environmental pollutants are positively associated with autistic traits. The role of folate on the relationship between environmental pollutants and autistic traits could explain some of these findings. Folate, and its synthetic derivative folic acid, are critical components of neurodevelopment. Insufficient folate levels during pregnancy have been associated with neurodevelopment in both human and animal studies and increased risk for autism (Oulhote et al., 2020). However, excess folate during pregnancy has also been associated with increased risk for autism and hindered neurodevelopment (Wiens & DeSoto, 2017). To our knowledge, there are no studies examining the relationship between environmental pollutants and folate levels. However, our preliminary results from analysis on this topic suggests that many environmental pollutants are significantly inversely associated with folate. Previous research and our preliminary analyses suggest that when folate levels are low or adequate in a study population, environmental pollutants may appear to be positively associated with autistic traits. Conversely, if the study population has excessively high levels of folate during pregnancy, the environmental pollutants may appear to be beneficial to reducing risk of autistic traits.

The sex specific differences were largely noticed in the associations between phthalates, BPS/parabens, and oxybenzophenone. Phthalates and BPS are endocrine

disruptors and exert their effects on neurodevelopment differentially with respect to sex (Oulhote et al., 2020; Yang et al., 2021).

2.e.iii Strengths and Limitations

This study has several limitations. The exposures in this study were all represented as biomarkers in cord blood. Depending on the metabolization of these pollutants, this may be a source of exposure misclassification in this study. This is a particular concern for phthalates because they are rapidly metabolized and cumulative potential for phthalate metabolites are low (source). Although this is a limitation in our study, there have been comparison studies that suggest phthalate metabolites captured in urine are significantly positively correlated with metabolites captured in serum.

Our air pollution exposure is recorded as PM_{2.5} and is reported from daily area-level measurements as an individual level exposure. Although this may be a source of misclassification of this exposure, a recent commentary may suggest that, in the context of pregnancy, proxies for air pollution, rather than individual exposures may be preferred. This is because changes in individual behaviors and lifestyles can occur and may be more likely to occur during pregnancy. These changes may affect individual estimates of air pollution exposures but will less likely affect an area-level exposure used to approximate individual exposure (Weisskopf et al., 2015; Genuis et al., 2012). The potential for misclassification is acceptable when considering the advantages of cost and the added benefit to this specific study to avoid behavioral changes.

Although these analyses were adjusted for confounders, there may be residual confounding in this study. These pollutants have different patterns of confounding because of the varying routes of exposure between pollutant families. For example, PFAS can be

found in drinking water, and humans are typically exposed to phenones through the application of sunscreens. This difference in exposure routes may suggest that there are also differences in an appropriate adjustment set between pollutant families. Additionally, adding specific confounders may not be idea because the confounder may not be applicable to all exposure outcome relationships.

Additionally, although BKMR addresses issues with multiple comparisons about the many exposures that comprise the pollutant mixtures in this study, there were multiple outcomes assessed in this study as well. BKMR was not designed to consider the spurious significant associations associated with multiple comparisons due to multiple outcomes.

Strengths of this study include: the breadth of environmental pollutants available through the longitudinal GUSTO cohort, consideration of effect modification by sex, the availability and usage of a standardized and validate outcome measurement for autistic traits, and the adoption of modern statistical approaches to analyze both mixture and individual associations between environmental pollutants and autistic traits.

2.f Conclusion

Some pollutants were significantly negatively associated with autistic traits in this cohort. The results of our study further emphasize the inconsistency of results when analyzing the relationship between environmental pollutants and autistic traits. Further research is necessary to examine the interrelationships between environmental pollutants and autistic traits, with emphasis on the role folate plays in this relationship.

CHAPTER 3

Assessing the Role of Folate on Associations between Environmental Pollutants and Autistic Traits in Singaporean Children: Growing Up in Singapore Towards healthy Outcomes (GUSTO)

2.a Specific Aims and Hypotheses

Specific Aim 1: Determine whether plasma folate modifies the association between chemical mixtures and autistic traits.

Hypotheses: Circulating plasma folate levels play modifier role in the association between environmental chemicals and autistic traits.

2.b Background and Significance

2.b.i Physiology of the Relationship between Folate, Environmental Pollutants, and ASD

Although the mechanism by which folate protects against neurodevelopment is unclear, one proposed mechanism that has evidential support is that folate's role as a primary methyl donor helps to protect the proper methylation of DNA during neurodevelopment (Goodrich et al., 2017; Steegers et al., 2009; Oulhote et al., 2020).

Because PFAS (Xu et al., 2022), phthalates (Wang et al., 2021), bisphenols (Awada et al., 2019), parabens (Dutta et al., 2019), and benzophenones (Wnuk et al., 2022) have all been shown to decrease DNA methylation, this may explain a potential mechanism by which folate may ameliorate the hypomethylation of DNA by these pollutants by increasing methylation levels, providing aegis against neurodevelopmental insults.

2.b.iv Epidemiology of the Relationship between Folate, Environmental Pollutants, and ASD

There is little epidemiologic evidence investigating the effector modifier role of folate on the association between many environmental pollutants and ASD. Folic acid supplementation has been suggested to mitigate the associations between pesticide exposure and ASD (Schmidt et al., 2017), and reduce the risk of ASD diagnosis among women with high prenatal particulate matter exposure (Schmidt et al., 2018). Also, folic acid supplementation has been shown to attenuate the potential harmful effects of phthalates on social responsiveness (Oulhote et al., 2020). These few studies are limited to using self-reported folic acid supplementation instead of an internal folate measurement. To our knowledge, there are no studies that assess the role of folate in the associations between PFAS, bisphenols and parabens, and benzophenones and ASD.

2.c. Study Design and Methods

2.c.i Study Design

This study will use the results from chapter two. The environmental chemicals significantly associated with SRS-2 scores will be included in this study to determine if folate modifies or mediates the relationship. Plasma folate concentrations in ng/mL were captured at maternal pregnancy week 26. In addition to the exclusion criteria from chapter

two, participants will be excluded from analysis if they do not have recorded plasma folate concentrations.

2.c.ii Data Analysis Plan

Mean participant blood concentrations of environmental pollutants were calculated as well as mean SRS-2 scores and subs core values. The geometric mean (GM), geometric standard deviation (GSD), interquartile range (IQR), and maximum values were calculated for environmental pollutants and non-geometric summary statistics were calculated for the SRS-2 scores. The percent distribution or mean value of each covariate and/or basic summary statistics were calculated where appropriate and included.

Spearman correlation between the natural log of each environmental pollutant and autistic traits were calculated using spearman correlation coefficients. Additionally, bivariate analyses between autistic traits and important study characteristics were performed. These analyses include a student's t test or a one-way analysis of variance (ANOVA) test depending on the study characteristic. If the comparison between autistic traits and a study characteristic includes a dichotomous variable, student's t test was used and if the variable is instead a variable with more than two levels, ANOVA was used.

To investigate the effect modification role of folate in the association between an environmental pollutant mixture of phthalates, PFAS, bisphenols and parabens, and benzophenones, a Monte- Carlo-based quantile G-Computation was used. To estimate the variance of the g-computation, bootstrapping was used with 1,000 bootstrap iterations for each model. This method helps to ameliorate issues with multiple comparisons by focusing on mixtures, rather than individual pollutants (Keil et al., 2017). Additionally, quantile G-computation does not assume a linear relationship between exposures and outcomes and

does not require all mixture components to have the same direction of association. Quantile g computation was used to assess the role of folate on the associations between the family mixtures of phthalates, PFAS, bisphenols and parabens, and benzophenones and SRS-2 scores and sub-scores, as well as the associations between each mixture family and SRS-2 scores and sub-scores. The quantile g computation analyses were repeated using folate in the adjustment set. Additionally, we conducted sex-stratified analyses and compared the results from males and females by comparing the differences in exposures between male and female children divided by their standard errors and comparing to a standard normal distribution (Altman & Bland, 2003).

We conducted a sensitivity additional analysis to compare the distribution of covariates between the analytic sample and the 211 participants excluded because they had insufficient data for key variables of interest.

All statistical analyses were conducted using R version 3.5.2. (R Core Team 2018).

This study was approved by the Institutional Review Board (IRB) of the University of Massachusetts Amherst.

2.d. Results

2.d.i Characteristics of the Study Population

Children were predominantly born full term (93.6%) and were between the 10th and 90th percentiles of birthweight (71.7%) (Table 6). The GUSTO mothers were predominantly aged 25 to 35 years old (63.3%), had never smoked (84.3%), had a household income of \$2000.00 to \$3999.00 (35.2%), were nulliparous (41.2%), breastfed for less than 6 months (62.1%), had a BMI of less than 25 (43.1%), had sufficient folate (86.0%), and were married (95.5%) (Table 6).

2.d.ii Levels of Autistic Traits

The mean and standard deviation of SRS-2 scores and sub-scores are displayed in table 7. The mean total SRS-2 score was 55.5 (SD: 7.07) and the geometric mean of plasma folate was 12.9 ng/mL (SD: 2.0 ng/mL). Total SRS-2 scores were higher among female children and children that were born early term. Additionally, Total SRS-2 scores were higher in children with mothers that were less than 25 years of age, had less than a high school degree, had smoked at pregnancy week 26, had household monthly incomes between \$0.00 and \$1999.00, and had breastfed their child for less than six months (Table 7).

2.d.iii Levels of Environmental Pollutants and correlations

The geometric mean and geometric standard deviations for PFAS, phthalates, phenols and parabens, and benzophenones are displayed in table 7. Additionally, correlations between pollutants varied between -0.41 and 0.57 (Figure 7). The two pollutants with the largest negative correlation between them were benzophenone and PFBS. The two pollutants with the largest positive correlation between them were PFDA and PFOS. Plasma folate was significantly weakly negatively correlated with total SRS-2 scores. PFNA was significantly weakly positively associated with total SRS-2 scores (Figure 7).

2.d.v Results from QgComp

There were no chemical pollutant mixtures significantly associated with SRS-2 scores or subs-scores after adjustment with plasma folate as a confounder (Figure 8). Child sex modified the associations between the benzophenone mixture and total SRS-2 scores ($p=0.01$), the SRS-2 cognition sub-score ($p=0.04$), the SRS-2 motivation sub-score ($p=0.01$), and borderline modified associations between the benzophenone mixture and the SRS-2 awareness ($p=0.05$) and RRB ($p=0.07$) sub-scores.

The associations between environmental pollutants and SRS-2 scores and sub-scores at different percentiles of plasma folate are displayed in figure 9. Plasma folate modified the association between the phthalate mixture and the SRS-2 motivation sub-score ($p=0.01$). Among males, plasma folate modified the association between the phthalate mixture and the SRS-2 motivation sub-score ($p=0.04$). Among females, plasma folate modified the association between the benzophenone mixture and the SRS-2 cognition ($p=0.02$) and RRB ($p=0.08$) sub-scores. When plasma folate is fixed to its 25th percentile, a one quantile increase in the phthalate mixture increased the SRS-2 motivation sub-score by 2.12 (95%CI: 0.52 - 3.71) after adjustment. Among males, when plasma folate is fixed to its 75th percentile, a one quantile increase in the PFAS mixture increased the SRS-2 RRB sub-score by 2.18 (95%CI: 0.05 – 4.32) after adjustment. Among females, when plasma folate is fixed to its 25th percentile, a one quantile increase in the benzophenone mixture decreased SRS-2 cognition sub-score by -1.06 (95%CI: -2.10 – -0.02) after adjustment and this association was positive among males ($p < 0.01$). Among females, when plasma folate is fixed to its 25th percentile, a one quantile increase in the benzophenone mixture decreased the total SRS-2 score by 1.02 (95%CI: -1.95, -0.09) after adjustment, and this association was positive among males ($p < 0.01$). Among females, when plasma folate is fixed to its 25th percentile, a one quantile increase in the benzophenone mixture decreased the SRS-2 communication sub-score by -1.04 (95%CI: -2.01 – -0.07) after adjustment, and this association was positive among males ($p < 0.01$). Among females, when plasma folate is fixed to its 25th percentile, a one quantile increase in the benzophenone mixture decreased the SRS-2 RRB sub-score by -1.12 (95%CI: -2.12 – -0.12) after adjustment, among females, when plasma folate is fixed to its 75th percentile,

a one quantile increase in the benzophenone mixture decreased the total SRS-2 RRB sub-score by -0.23 (95%CI: -1.28 – -0.83) after adjustment.

2.e Discussion

2.e.i Summary of Findings and Significance

In this cohort study, we found that plasma folate modifies the associations between environmental pollutant mixtures of phthalates, PFAS, and benzophenones, and social responsiveness and this modification may depend on sex for benzophenones. As the percentile of plasma folate increased from its 25th to 75th percentiles the association between the phthalate mixture and the SRS-2 motivation sub-scores decreased. Among males, as the percentile of plasma folate increased from its 25th to 75th percentiles, the association between the phthalate mixture and the SRS-2 cognition sub-score decreased and this pattern was similar among females. Among females, as the percentile of plasma folate increased from its 25th to 75th percentiles, the direction of association between the benzophenone mixture and the SRS-2 cognition sub-score went from negative to positive and this pattern was not similar among males. Additionally, the directional trend of the association between the benzophenone mixture and SRS-2 scores and sub-scores was positive among female children and negative among male children.

One study examined the potential effect modification of folic acid supplementation in the association between individual phthalate metabolites and SRS-2 scores (Oulhote et al., 2020). Although our study used plasma folate instead of folic acid supplementation and focused on a phthalate mixture instead of individual phthalates, our results both suggest that folate and folic acid may modify the association between phthalates and the SRS-2 motivation sub-score.

2.e.ii Biologic Mechanisms

Although the mechanism by which folate protects neurodevelopment is unclear, one supported mechanism suggests that folate is a methyl donor that protects proper neurodevelopment (Schmidt et al., 2017; Oulhote et al., 2020). Because benzophenones, phthalates, and PFAS have been suggested to hypomethylate DNA, and this hypomethylation potentially leads to insults to neurodevelopment, folate may modify this relationship by providing adequate DNA methylation for neurodevelopment. We also found that the effect modification of folate on the association between phthalates and benzophenones and SRS-2 sub scores depended on child sex. Phthalates (Oulhote et al., 2020), and benzophenones (Kawamura et al., 2005) have antiandrogenic chemical properties, and their inhibition of male sex steroid hormones from binding to the appropriate receptors may help explain the sex-specific results observed in this study (table 8).

2.e.iii Strengths and Limitations

This study has several limitations. First, the exposures in this study were all captured as cord blood biomarkers. Phenols and parabens, benzophenones, and phthalates are rapidly metabolized and may the true exposure status of these chemical families may not be as accurately captured in cord blood compared to urine. Second, despite our use of quantile g computation to consider the effects of multiple comparisons in our study, we still had multiple outcomes. We are not aware of any mixture methods that consider multiple comparisons of both exposures and outcomes. Also, this study does not consider confounding by other nutrients that may protect neurodevelopment and are correlated with plasma folate were not considered for confounding in this study. Finally, we had to exclude

211 potential participants due to missing information on key variables of interest. We conducted a sensitivity analysis comparing key demographic variables as well as plasma folate and total SRS-2 scores between the analytic sample and those participants that were excluded and found no meaningful differences between the two groups (supplemental Table 4).

Strengths of this study include the novel design. This is the first, to our knowledge, study that assesses the role of plasma folate on the association between pollutant mixtures families of PFAS, phthalates, bisphenols and parabens, and benzophenones, and social responsiveness using quantile g computation stratified by child sex. These methods facilitated the examination of potential effect modification by folate, but also to examine the associations at increasing levels of plasma folate to begin to understand what type of influence folate might have on these associations instead of relying on the presence or absence of effect modification. This study also did not rely on self-reported folic acid supplementation, and instead an internal measurement of folate was used to determine maternal folate status. This internal measurement provides a more accurate measurement of bio-available folate by not relying on participant recall and considers multiple sources for folate rather than only folic acid supplementation. Additional strengths of this study include the longitudinal data on many environmental pollutants available through the GUSTO cohort. Additionally, the sex-stratified results provide additional information and avenues for future research to help explain the sex-specific difference in ASD diagnosis and utilizing a continuous outcome that is free from subjective cut-points enhances the generalizability of this study and enhances statistical power.

2.f Conclusion

This study enhances the current literature of ASD suggesting that folate is a potential effect modifier in the association between environmental pollutants, including mixtures of phthalates, benzophenones, and PFAS, and ASD.

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Preface

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Chapter One

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Chapter Three

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Appendix A

Tables

Covariate	N	GM	GSD	Maximum	p
Overall RBC Folate	27938	469.49	1.53	3125.8	N/A
Sex					<0.001
Male	13599	457.01	1.52	3126	
Female	14339	481.63	1.55	2980	
Age					<0.001
18 to 34	8022	409.75	1.45	2290	N/A
35 to 64	13650	465.58	1.51	2980	<0.001
65 and Older	6266	569.11	1.59	3125.8	<0.001
Race					<0.001
Mexican American	4441	1.45	1.45	2931.6	N/A
Other Hispanic	3012	1.47	1.47	2013.2	0.918
Non-Hispanic White	11605	1.54	1.54	2984.5	<0.001
Non-Hispanic Black	5768	1.53	1.53	3125.8	<0.001
Other	3112	1.5	1.5	2750	0.861
Education					<0.001
Less than 9th Grade	3107	445.83	1.53	2653.4	N/A
9th to 11th Grade	4094	449.17	1.55	2755	0.921
High School Graduate/ GED	6309	464.86	1.56	2984.5	<0.001
Some College or AA	8074	472.86	1.54	3125.8	<0.001
College Graduate or Above	6254	496.98	1.49	2980	<0.001
Missing	100	427.55	1.51	2931	0.984
Smoking Status					<0.001
Has not Smoked in Last 30 Days	22200	486.6	1.53	3125.8	N/A
Smoked in Last 30 Days	5729	409.06	1.52	2675.5	0.54
Missing	9	429.9	1.37	657.8	0.999
FPR					<0.001
Lowest Quartile	6335	433.45	1.53	2675.5	N/A
2nd Quartile	6349	469.31	1.53	2970	<0.001
3rd Quartile	6365	482.42	1.53	3125.8	<0.001
Highest Quartile	6401	502.31	1.51	2980	<0.001
Missing	2488	451.49	1.56	2931.6	<0.001
Urinary Creatinine					<0.001
Lowest Quartile	6376	499.22	1.54	2984.5	N/A
2nd Quartile	6372	483.61	1.54	3125.8	<0.001
3rd Quartile	6473	457.88	1.52	2075.1	<0.001
Highest Quartile	6469	432.75	1.51	2980	<0.001
Missing	2248	492.76	1.58	2970	0.999
Cycle					0.251
2007-2008	5600	486.45	1.56	2984.5	N/A
2009-2010	6019	452.31	1.53	3125.8	<0.001
2011-2012	5269	442.98	1.53	2420	<0.001
2013-2014	5636	486.43	1.54	2980	0.88
2015-2016	5414	481.06	1.51	2970	0.03
HEI					<0.001
Lowest Quartile	6534	431.91	1.53	2653.4	N/A
2nd Quartile	6533	454.42	1.53	2970	<0.001
3rd Quartile	6534	480.78	1.54	2984.5	<0.001
Highest Quartile	6534	519.2	1.51	2980	<0.001
Missing	1803	455.44	1.53	3125.8	<0.001

Table 1. Concentrations of red blood cell (RBC) folate per covariate among adult NHANES participants from 2007-2016. Abbreviations: geometric mean (GM); geometric standard deviation (GSD); family to income poverty ratio (FPR); healthy eating index (HEI); not applicable (N/A).

Variable	n	% < LOD	GM	GSD	IQR	Maximum
Mono(carboxyisooctyl) phthalate (MCOP) (ng/mL)	9012	1.22%	11.34	4.17	23.98	1813.1
Mono-2-ethyl-5-carboxypentyl phthalate (MECPP) (ng/mL)	9012	0.23%	15.19	3.25	22.9	15828
Mono-n-butyl phthalate (MBP) (ng/mL)	9012	2.24%	11.83	3.44	19.94	25863
Mono-(3-carboxypropyl) phthalate (MCPP) (ng/mL)	9012	9.55%	2.1	3.67	3.7	1588.7
Mono-ethyl phthalate (MEP) (ng/mL)	9012	0.13%	61.78	4.94	153.48	31660
Mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) (ng/mL)	9012	0.67%	9.68	3.51	15.9	9326.1
Mono-(2-ethyl)-hexyl phthalate (MEHP) (ng/mL)	9012	33.60%	1.61	3.1	2.63	1252.7
Mono-isobutyl phthalate (MiBP) (ng/mL)	9012	2.00%	7.49	3.12	12	627
Mono-isononyl phthalate (MNP) (ng/mL)	9012	67.63%	1.05	2.74	0.61	875.4
Mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) (ng/mL)	9012	0.95%	5.96	3.4	9.45	6079.9
Mono-benzyl phthalate (MBzP) (ng/mL)	9012	2.66%	4.88	3.72	9.8	450.22
Cadmium (µg/L)	22401	15.71%	0.36	2.24	0.38	9.3
Lead (µg/dL)	22401	0.29%	1.2	2.01	1.14	61.29
Manganese (µg/L)	10787	0.00%	9.38	1.43	4.31	62.51
Mercury (µg/L)	22401	9.85%	0.9	2.69	1.22	85.7
Selenium (µg/L)	10787	0.00%	192.83	1.14	29.97	734.8
Arsenic, total (ng/mL)	9170	0.87%	8.35	3.17	12.4	1269
Perfluorodecanoic acid (PFDeA) (ng/mL)	8963	16.82%	0.23	2.25	0.26	51.3
Perfluorohexane sulfonic acid (PFHxS) (ng/mL)	8963	1.91%	1.39	2.61	1.79	81.6
2-(N-methylperfluorooctanesulfonamido)acetic acid (Me-PFOSA-AcOH) (ng/mL)	8963	53.33%	0.16	2.52	0.23	12.2
Perfluorononanoic acid (PFNA) (ng/mL)	8963	1.53%	0.91	2.11	0.88	80.77
Perfluoroundecanoic acid (PFUA) (ng/mL)	8963	59.88%	0.15	2.27	0.13	77.4
Perfluorooctanoic acid (PFOA) (ng/mL)	8961	0.22%	2.34	2.14	2.4	104
Perfluorooctane sulfonic acid (PFOS)(ng/mL)	8961	0.39%	7.49	2.61	9.59	1403
Bisphenol A (BPA) (ng/mL)	9013	0.65%	1.53	3.03	2.4	965
Triclosan (ng/mL)	9013	7.51%	10.93	7.05	37.47	9572
Butyl Paraben (ng/mL)	9013	0.18%	0.25	5.14	0.33	1150
Methyl Paraben (ng/mL)	9013	2.49%	59.09	6.03	213.8	15964.1
Propyl Paraben (ng/mL)	9013	0.18%	7.33	9.78	43.7	6399.6
One hydroxynaphthalene (ng/L)	7194	0.06%	2235.54	4.68	5480	35920000
Two hydroxynaphthalene (ng/L)	7221	0.00%	4474.3	3.2	8318.1	368500
Three hydroxyfluorene (ng/L)	7256	1.86%	104.31	3.97	208.15	8784
Two hydroxyfluorene (ng/L)	7267	0.00%	261.61	3.43	470.5	21271.3
Three hydroxyphenanthrene (ng/L)	5439	1.84%	79.04	2.8	118.8	6683
One hydroxyphenanthrene (ng/L)	7280	0.38%	123.95	2.56	164.73	7475
Two hydroxyphenanthrene (ng/L)	5417	1.14%	68.29	2.55	90	4540.8
One hydroxypyrene (ng/L)	7253	7.33%	118.89	2.84	176.3	8685.6
Nine hydroxyfluorene (ng/L)	5448	0.00%	304.7	3	490.23	65656.6
Four phenanthrene (ng/L)	1704	25.23%	21.81	2.47	29	951
Cotinine (ng/mL)	27646	27.41%	0.31	47.5	8.72	1820

Table 2. Concentrations of red blood cell (RBC) folate and environmental pollutants among adult NHANES participants from 2007-2016. Abbreviations: geometric mean (GM); geometric standard deviation (GSD); interquartile range (IQR); limit of detection (LOD). Values below the limit of detection were replaced by the limit of detection divided by the square root of 2.

Covariate	N	Mean	Standard Deviation (SD)	Maximum	p
Total SRS 2 score					
Child Sex					<0.001
Female	211	56.2	6.9	79	
Male	219	54.94	7.17	73	
Maternal Age					0.15
Less than 25	78	56.96	8.08	78	N/A
25 to 35	272	55.21	6.97	79	0.13
35 and Older	80	55.39	6.19	70	0.34
Maternal Ethnicity					0.37
Chinese	205	55.14	6.78	72	N/A
Malaysian	148	56.04	7.17	79	0.47
Other	77	55.74	7.61	78	0.8
Maternal Education					<0.001
< High School Education	151	57.97	6.48	78	N/A
High School Education/ Technical School	169	54.73	7.07	79	<0.001
College Degree or Higher	107	73.56	6.94	77	<0.001
Missing	3	52.33	9.74	62	0.49
Maternal Smoking Status					0.15
Never	358	55.23	6.94	79	N/A
Ever	64	57.23	7.61	73	0.13
Missing	8	57.13	6.53	66	0.91
Household Monthly Income					<0.001
0 - 1999 USD	72	60.21	7.79	79	N/A
2000 - 3999 USD	152	55.53	6.32	72	<0.001
4000 - 5999 USD	98	53.68	6.32	69	<0.001
> 6000 USD	83	53.17	6.72	70	<0.001
Missing	22	57.27	6.17	69	0.36
Parity					0.42
Nulliparous	177	54.9661	6.59	72	N/A
Parous	139	55.58993	7.38	77	0.84
Multiparous	114	56.44737	7.35	79	0.35
Breast Feeding Status					0.02
Less than 6 Months	269	56.12	7.05	79	N/A
6 Months or More	140	54.22	6.88	69	0.03
Missing	21	57.33	7.37	78	0.73
BMI at Pregnancy week 26					0.09
<25	181	54.57	6.69	73	N/A
25 to 30	146	56.17	7.22	77	0.17
30+	85	56.6	7.58	79	0.13
Missing	18	55.67	6.11	68	0.92
Gestational Age at Birth					<0.001
Early Term	28	58.46	8.07	78	
Term	402	55.36	6.95	79	
Birthweight for GA					0.77
AGA	308	55.71	7.03	78	N/A
LGA	74	55.26	6.98	79	0.87
SGA	48	55.06	7.49	72	0.83
Folate					0.27
Deficient	59	55.95	6.09	73	N/A
Sufficient	361	55.4	7.23	79	0.85
Missing	18	58.9	5.88	68	0.44
Marital Status					0.94
Married	410	55.56	7.12	79	N/A
Not Married	12	55.08	6.16	64	0.96
Missing	8	56.25	6.07	62	0.93

Table 3. Total SRS-2 scores per covariate among adult GUSTO participants. Abbreviations: Social Responsiveness Scale (SRS); standard deviation (SD); United States Dollars (USD); Body Mass Index (BMI); Gestational Age (GA); Average (A), Large (L), and Small (S) for Gestational Age (A/L/SGA) not applicable (N/A).

Pollutant	n	% Below Limit of Detection (LOD)	Mean / Geometric Mean (GM)	Mean / Geometric Standard Deviation (GSD)	Interquartile Range (IQR)	Max
SRS Awareness	631	N/A	58.99	8.08	11	79
SRS Cognition	631	N/A	56.57	7.62	11	80
SRS Communication	631	N/A	55.34	7.53	10	76
SRS Motivation	631	N/A	54.04	7.86	12	89
RRB (Restrictive and Repetitive Behaviors)	631	N/A	51.15	7.35	8	87
SRS Total Score	631	N/A	55.56	7.06	10	79
PFBA	430	0.14%	1.58	1.68	0.82	50.43
PFOA	430	0.00%	1.61	1.87	1.47	10.24
PFNA	430	0.00%	0.83	1.68	0.6	4.77
PFDA	430	4.34%	0.13	1.88	0.08	1.37
PFUnDA	430	3.82%	0.2	2.07	0.14	2.55
PFBS	430	0.00%	21.02	1.74	16.04	86.85
PFHxS	430	0.89%	0.49	1.84	0.39	3.24
PFOS	430	0.13%	1.11	1.87	0.78	9.23
MMP	430	32.91%	0.52	2.42	0.41	4.35
MECCP	430	16.45%	0.32	3.14	0.39	5.41
MCINP	430	34.31%	0.64	7.69	2.71	30.43
MEP	430	28.83%	0.67	3.58	0.9	28.96
MBP	430	0.00%	2.45	1.59	1.47	12.85
MEHP	430	0.00%	9.29	1.72	5.6	70.31
BPS	430	44.26%	0.08	2.62	0.07	3.53
Methyl_Paraben	430	14.53%	2.91	4.1	7	98.45
Propyl_Paraben	430	35.46%	0.13	3.45	0.21	7.45
Benzophenone	430	1.15%	9.3	5.06	26.49	1159.42
Oxybenzophenone	430	0.00%	2.13	1.37	0.93	9.51
Air Pollution	631	0.00%	18.51	1.08	2.32	21.12

Table 4. Levels of SRS-2 scores and sub-scores and environmental pollutants (ng/mL) among GUSTO participants. Abbreviations: geometric mean (GM); geometric standard deviation (GSD); interquartile range (IQR); limit of detection (LOD).

Chemical	Overall Estimate (95%CI)	Male Estimate (95%CI)	Female Estimate (95%CI)	 Difference 	p - Difference
Total SRS	0.488 (-1.44,2.42)	1.847 (-1.42,5.11)	-2.363 (-5.73,1.01)	4.21	0.079*
Awareness	-1.707 (-4.45,1.04)	0.705 (-3.36,4.77)	-5.325 (-9.42,-1.24)	6.03	0.040**
Cognition	0.711 (-1.97,3.39)	2.868 (-1.29,7.03)	-1.937 (-5.77,1.90)	4.81	0.096*
Communication	0.408 (-1.76,2.58)	1.802 (-1.87,5.47)	-1.508 (-4.80,1.78)	3.31	0.188
Motivation	-0.012 (-2.46,2.44)	2.333 (-1.59,6.26)	-1.966 (-5.45,1.52)	4.3	0.108
RRB	0.587 (-1.88,3.05)	1.864 (-1.94,5.66)	-0.361 (-4.16,3.43)	2.22	0.417
SCI	-0.035 (-1.95,1.88)	2.003 (-1.26,5.26)	-2.463 (-6.18,1.25)	4.47	0.076*

Table 5. Test for effect modification of sex on the association between a pollutant mixture of PFAS, phthalates, bisphenols, and parabens, PM2.5, and benzophenones and SRS-2 scores and sub-scores in the GUSTO cohort. Estimates represent the SRS-2 score and sub-scores difference associated with an IQR difference in the pollutant mixture.

Covariate	N	Mean	Standard Deviation (SD)	p
Total SRS 2 Score	420	55.48	7.07	N/A
Child Sex				<0.001
Female	206	56.15	6.92	
Male	214	54.84	7.17	
Maternal Age				0.174
Less than 25	75	56.85	8.17	N/A
25 to 35	266	55.13	6.96	0.15
35 and Older	79	55.35	6.22	0.387
Maternal Ethnicity				0.464
Chinese	199	55.14	6.84	N/A
Malaysian	145	55.86	7.12	0.62
Other	76	55.64	7.62	0.858
Maternal Education				<0.001
< High School Education	147	57.95	6.52	N/A
High School Education/ Technical School	164	54.59	7.03	<0.001
College Degree or Higher	106	53.52	6.96	<0.001
Missing	3	52.33	8.74	0.496
Maternal Smoking Status				0.16
Never	354	55.16	6.92	N/A
Ever	63	57.14	7.64	0.142
Missing	3	58.67	10.21	0.903
Household Monthly Income				<0.001
0 - 1999 USD	72	60.24	7.89	N/A
2000 - 3999 USD	148	55.37	6.25	<0.001
4000 - 5999 USD	96	53.7	6.38	<0.001
> 6000 USD	82	53.11	6.74	<0.001
Missing	22	57.27	6.17	0.362
Parity				0.193
Nulliparous	173	54.81	6.53	N/A
Parous	137	55.63	7.42	0.569
Multiparous	110	56.35	7.42	0.172
Breast Feeding Status				0.017
Less than 6 Months	261	56.05	7.07	N/A
6 Months or More	138	54.13	6.88	0.027
Missing	21	57.33	7.37	0.7
BMI at Pregnancy week 26				0.129
<25	181	54.57	6.69	N/A
25 to 30	144	56.04	7.18	0.243
30+	82	56.51	7.63	0.165
Missing	13	55.46	6.79	0.971
Gestational Age at Birth				<0.001
Early Term	27	58.59	8.19	
Term	393	55.27	6.95	
Birthweight for GA				0.614
AGA	301	55.67	7.07	N/A
LGA	73	55.26	7.02	0.898
SGA	46	54.6	7.3	0.612
Folate				0.584
Deficient	59	55.95	6.09	
Sufficient	361	55.4	7.23	
Marital Status				0.949
Married	401	54.47	7.12	N/A
Not Married	11	55.27	6.42	0.995
Missing	8	56.25	6.07	0.949

Table 6. Total SRS-2 scores per covariate among adult GUSTO participants. Abbreviations: Social Responsiveness Scale (SRS); standard deviation (SD); United States Dollars (USD); Body Mass Index (BMI); Gestational Age (GA); Average (A), Large (L), and Small (S) for Gestational Age (A/L/S/GA) not applicable (N/A).

Variable	N	% Below Limit of Detection (LOD)	Mean / Geometric Mean (GM)	Mean / Geometric Standard Deviation (GSD)	Interquartile Range (IQR)	Maximum
SRS Awareness	420	N/A	58.9	8.08	11	79
SRS Cognition	420	N/A	56.47	7.62	9.25	80
SRS Communication	420	N/A	55.24	7.53	10	76
SRS Motivation	420	N/A	54.05	7.92	12	89
RRB (Restrictive and Repetitive Behaviors)	420	N/A	51.11	7.38	8	87
SCI (Social Communication and Interaction)	420	N/A	56.53	7.3	10	77
SRS Total Score	420	N/A	55.48	7.07	10	79
PFBA (ng/mL)	420	1.25%	1.58	1.68	0.8	50.43
PFOA (ng/mL)	420	0.00%	1.59	1.86	1.46	8.99
PFNA (ng/mL)	420	0.00%	0.82	1.67	0.6	4.77
PFDA (ng/mL)	420	4.08%	0.13	1.88	0.08	1.37
PFUnDA (ng/mL)	420	2.82%	0.2	2.09	0.13	2.55
PFBS (ng/mL)	420	0.00%	20.9	1.72	15.84	86.85
PFHxS (ng/mL)	420	0.94%	0.49	1.84	0.38	3.24
PFOs (ng/mL)	420	0.00%	1.11	1.87	0.77	9.23
MMP (ng/mL)	420	33.86%	0.51	2.44	0.41	4.35
MECCP (ng/mL)	420	15.05%	0.32	3.11	0.39	5.41
MCINP (ng/mL)	420	31.35%	0.64	7.6	2.7	30.43
MEP (ng/mL)	420	28.21%	0.67	3.61	0.9	28.96
MBP (ng/mL)	420	0.00%	2.45	1.58	1.47	10.57
MEHP (ng/mL)	420	0.00%	9.29	1.72	5.61	70.31
BPS (ng/mL)	420	43.89%	0.08	2.63	0.07	3.53
Methyl Paraben (ng/mL)	420	15.67%	2.92	4.09	6.87	98.45
Propyl Paraben (ng/mL)	420	39.50%	0.13	3.48	0.21	7.45
Benzophenone (ng/mL)	420	1.25%	9.32	5.12	26.97	1159.42
Oxybenzophenone (ng/mL)	420	0.00%	2.12	1.36	0.91	9.51

Table 7. Levels of SRS-2 scores and sub scores and environmental chemicals (ng/mL) among GUSTO participants. Abbreviations: geometric mean (GM); geometric standard deviation (GSD); interquartile range (IQR); limit of detection (LOD)

Pollutant Family	SRS-2 Outcome	Male Estimate	Male Standard Error	Female Estimate	Female Standard Error	P Interaction
PFAS	Total SRS	-0.06	0.71	0.78	0.9	0.46
PFAS	Awareness	-1.3	0.87	-0.23	1.14	0.46
PFAS	Cognition	0.69	0.89	1.44	0.9	0.55
PFAS	Motivation	-0.17	0.76	0.25	0.92	0.73
PFAS	Communication	-0.15	0.91	0.52	0.95	0.61
PFAS	RRB	0.37	0.71	1.12	1.01	0.54
Phthalates	Total SRS	0.7	1.04	0.53	0.85	0.9
Phthalates	Awareness	-0.42	1.06	-0.88	0.95	0.75
Phthalates	Cognition	0.3	1.14	0.44	0.9	0.92
Phthalates	Motivation	0.74	1.05	0.73	0.88	0.99
Phthalates	Communication	0.75	1.13	1.2	0.96	0.76
Phthalates	RRB	1.41	1.09	0.4	1.03	0.5
Phenol and Parabens	Total SRS	0.35	0.69	-0.37	0.69	0.46
Phenol and Parabens	Awareness	0.71	0.81	-0.77	0.83	0.2
Phenol and Parabens	Cognition	0.66	0.82	0.54	0.74	0.92
Phenol and Parabens	Motivation	0.8	0.67	-0.4	0.78	0.24
Phenol and Parabens	Communication	-0.65	0.81	-1	0.81	0.76
Phenol and Parabens	RRB	-0.16	0.79	-0.25	0.82	0.94
Benzophenones	Total SRS	-0.75	0.43	0.77	0.46	0.01*
Benzophenones	Awareness	-0.65	0.51	0.75	0.51	0.05
Benzophenones	Cognition	-0.55	0.52	0.87	0.46	0.04*
Benzophenones	Motivation	-0.83	0.43	0.81	0.49	0.01*
Benzophenones	Communication	-0.57	0.55	0.53	0.51	0.14
Benzophenones	RRB	-0.73	0.41	0.5	0.53	0.07

Table 8. Test for effect modification of sex on the association between a pollutant family mixture and SRS-2 scores and sub scores in the GUSTO cohort.

Chemical	Imputed			Complete Case		
	Overall	Males	Females	Overall	Males	Females
	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)	Estimate (95% CI)
MCOP	0.004 (-0.003,0.011)	0.004 (-0.002,0.011)	0.001 (-0.005,0.008)	0.007 (-0.004,0.019)	0.011 (-0.005,0.027)	0.004 (-0.013,0.02)
MECPP	0.004 (-0.003,0.011)	0.005 (-0.008,0.018)	0.003 (-0.012,0.017)	0.019 (0.003,0.035)	0.023 (0.001,0.045)	0.014 (-0.01,0.038)
MBP	-0.002 (-0.015,0.012)	0.002 (-0.008,0.011)	-0.008 (-0.018,0.002)	0.001 (-0.015,0.018)	0.007 (-0.016,0.031)	-0.005 (-0.028,0.019)
MCPP	0.003 (-0.003,0.009)	0.006 (-0.003,0.015)	0 (-0.008,0.009)	0.011 (-0.002,0.024)	0.017 (-0.001,0.035)	0.005 (-0.014,0.024)
MEP	0.001 (-0.004,0.005)	0.003 (-0.007,0.013)	-0.002 (-0.008,0.003)	-0.007 (-0.017,0.003)	-0.004 (-0.018,0.01)	-0.011 (-0.026,0.004)
MEHHP	0.003 (-0.003,0.008)	0.006 (-0.005,0.016)	-0.001 (-0.008,0.006)	0.012 (-0.003,0.027)	0.018 (-0.003,0.038)	0.005 (-0.017,0.027)
MEHP	0 (-0.007,0.007)	0.001 (-0.006,0.008)	-0.007 (-0.019,0.004)	-0.006 (-0.021,0.008)	-0.004 (-0.023,0.015)	-0.01 (-0.031,0.011)
MiBP	-0.001 (-0.007,0.005)	0.001 (-0.014,0.015)	-0.007 (-0.016,0.002)	-0.015 (-0.033,0.002)	-0.006 (-0.03,0.019)	-0.026 (-0.052,-0.001)
MNP	0.002 (-0.004,0.007)	0.001 (-0.007,0.008)	-0.006 (-0.015,0.002)	-0.005 (-0.019,0.01)	0 (-0.02,0.02)	-0.01 (-0.032,0.012)
MEOHP	0.003 (-0.004,0.009)	0.007 (0.0,0.015)	-0.001 (-0.011,0.009)	0.014 (-0.002,0.03)	0.021 (0.0,0.042)	0.005 (-0.018,0.029)
MBzP	0 (-0.007,0.007)	0.002 (-0.006,0.01)	-0.006 (-0.015,0.002)	0.016 (0.002,0.031)	0.022 (0.001,0.042)	0.01 (-0.011,0.031)
Cadmium	-0.054 (-0.06,-0.047)	-0.05 (-0.059,-0.041)	-0.059 (-0.069,-0.048)	-0.02 (-0.035,-0.005)	-0.026 (-0.046,-0.006)	-0.012 (-0.035,0.01)
Lead	-0.071 (-0.081,-0.061)	-0.061 (-0.072,-0.049)	-0.081 (-0.094,-0.069)	-0.08 (-0.096,-0.063)	-0.067 (-0.089,-0.045)	-0.094 (-0.118,-0.07)
Manganese	0.03 (0.012,0.047)	0.012 (-0.02,0.044)	0.053 (0.018,0.088)	0.078 (0.04,0.116)	0.039 (-0.016,0.093)	0.111 (0.057,0.164)
Mercury	-0.013 (-0.019,-0.007)	-0.014 (-0.022,-0.006)	-0.011 (-0.02,-0.003)	-0.035 (-0.045,-0.025)	-0.035 (-0.048,-0.022)	-0.035 (-0.05,-0.02)
Selenium	0.059 (0.011,0.107)	0.109 (0.038,0.181)	0.004 (-0.061,0.069)	0.148 (0.047,0.248)	0.282 (0.141,0.423)	0.029 (-0.115,0.173)
Arsenic	-0.006 (-0.011,-0.001)	-0.009 (-0.017,-0.001)	-0.002 (-0.016,0.012)	-0.025 (-0.037,-0.012)	-0.032 (-0.05,-0.015)	-0.018 (-0.035,0)
PFDeA	-0.028 (-0.035,-0.021)	-0.027 (-0.036,-0.017)	-0.03 (-0.041,-0.018)	-0.096 (-0.115,-0.077)	-0.096 (-0.123,-0.069)	-0.096 (-0.124,-0.069)
PFHxS	-0.008 (-0.016,-0.001)	-0.008 (-0.021,0.006)	-0.008 (-0.019,0.003)	-0.009 (-0.026,0.008)	-0.001 (-0.025,0.024)	-0.016 (-0.039,0.008)
Me-PFOA-AcOH	-0.013 (-0.02,-0.006)	-0.004 (-0.015,0.007)	-0.012 (-0.028,0.004)	-0.001 (-0.018,0.017)	0.009 (-0.015,0.033)	-0.011 (-0.036,0.015)
PFNA	-0.029 (-0.037,-0.021)	-0.028 (-0.05,-0.005)	-0.041 (-0.052,-0.029)	-0.106 (-0.128,-0.085)	-0.092 (-0.123,-0.06)	-0.119 (-0.149,-0.09)
PFUA	-0.034 (-0.042,-0.025)	-0.032 (-0.045,-0.02)	-0.031 (-0.044,-0.018)	-0.092 (-0.112,-0.072)	-0.09 (-0.117,-0.063)	-0.094 (-0.123,-0.065)
PFOA	-0.018 (-0.028,-0.008)	-0.012 (-0.029,0.005)	-0.023 (-0.036,-0.01)	-0.024 (-0.047,-0.002)	-0.008 (-0.041,0.026)	-0.037 (-0.068,-0.006)
PFOS	-0.024 (-0.032,-0.016)	-0.02 (-0.029,-0.01)	-0.028 (-0.037,-0.019)	-0.073 (-0.091,-0.055)	-0.06 (-0.086,-0.034)	-0.084 (-0.11,-0.059)
BPA	0 (-0.01,0.01)	0.003 (-0.007,0.013)	-0.002 (-0.014,0.01)	0.006 (-0.01,0.022)	0.014 (-0.008,0.036)	-0.003 (-0.026,0.02)
Triclosan	0.003 (-0.003,0.009)	0.002 (-0.004,0.009)	0.003 (-0.003,0.008)	0.003 (-0.005,0.011)	0.004 (-0.007,0.015)	0.002 (-0.009,0.013)
Butyl Paraben	0.001 (-0.002,0.003)	-0.001 (-0.007,0.006)	0.001 (-0.007,0.01)	0.003 (-0.007,0.013)	0 (-0.018,0.018)	0.004 (-0.008,0.016)
Methyl Paraben	0.005 (0.001,0.009)	0.004 (-0.001,0.009)	0.004 (-0.005,0.013)	-0.001 (-0.01,0.008)	-0.002 (-0.014,0.01)	0.001 (-0.013,0.014)
Propyl Paraben	0.003 (0.0,0.006)	0.003 (-0.002,0.008)	0.002 (-0.003,0.006)	-0.002 (-0.009,0.005)	-0.004 (-0.014,0.005)	0.001 (-0.01,0.011)
One hydroxynaphthalene	-0.022 (-0.026,-0.018)	-0.021 (-0.028,-0.014)	-0.022 (-0.027,-0.017)	-0.008 (-0.021,0.005)	-0.008 (-0.027,0.011)	-0.007 (-0.026,0.012)
Two hydroxynaphthalene	-0.029 (-0.036,-0.022)	-0.033 (-0.045,-0.021)	-0.03 (-0.039,-0.022)	-0.023 (-0.043,-0.003)	-0.039 (-0.067,-0.012)	-0.008 (-0.037,0.022)
Three hydroxyfluorene	-0.038 (-0.043,-0.034)	-0.036 (-0.042,-0.03)	-0.04 (-0.047,-0.034)	-0.024 (-0.044,-0.004)	-0.039 (-0.065,-0.012)	-0.005 (-0.036,0.026)
Two hydroxyfluorene	-0.041 (-0.047,-0.035)	-0.039 (-0.046,-0.032)	-0.042 (-0.053,-0.031)	-0.013 (-0.035,0.01)	-0.03 (-0.06,-0.001)	0.009 (-0.025,0.044)
Three hydroxyphenanthrene	-0.036 (-0.043,-0.03)	-0.03 (-0.04,-0.019)	-0.04 (-0.049,-0.031)	-0.011 (-0.037,0.016)	-0.017 (-0.051,0.018)	-0.003 (-0.044,0.037)
One hydroxyphenanthrene	-0.022 (-0.033,-0.01)	-0.022 (-0.033,-0.01)	-0.023 (-0.034,-0.011)	0.017 (-0.006,0.041)	0.01 (-0.022,0.041)	0.025 (-0.01,0.06)
Two hydroxyphenanthrene	-0.033 (-0.044,-0.023)	-0.033 (-0.042,-0.023)	-0.035 (-0.046,-0.024)	0.007 (-0.02,0.035)	-0.004 (-0.041,0.033)	0.019 (-0.023,0.06)
One hydroxyprene	-0.037 (-0.044,-0.029)	-0.032 (-0.047,-0.017)	-0.062 (-0.087,-0.037)	-0.014 (-0.036,0.007)	-0.019 (-0.047,0.008)	-0.009 (-0.043,0.024)
Nine hydroxyfluorene	-0.031 (-0.038,-0.024)	-0.026 (-0.037,-0.016)	-0.03 (-0.039,-0.021)	0.008 (-0.015,0.032)	0.004 (-0.028,0.036)	0.011 (-0.025,0.046)
Four phenanthrene	-0.025 (-0.032,-0.019)	-0.023 (-0.034,-0.012)	-0.029 (-0.04,-0.017)	0.017 (-0.03,0.064)	0.011 (-0.051,0.072)	0.019 (-0.053,0.091)
Cotinine	-0.017 (-0.018,-0.016)	-0.015 (-0.016,-0.013)	-0.019 (-0.021,-0.017)	-0.012 (-0.015,-0.01)	-0.01 (-0.013,-0.007)	-0.014 (-0.018,-0.011)

Supplemental Table 1. Comparison between the results from the exposome wide association study (ExWAS) using the imputed dataset and the complete case analysis among NHANES adults, males, and females. Adjusted for age, race, family to income poverty ratio, education, urinary creatinine, NHANES cycle, and diet, and sex where appropriate. Statistically significant values are highlighted in red.

	Imputed			Complete Case		
	Estimate	Lower CI	Upper CI	Estimate	Lower CI	Upper CI
	Whole Mixture			Whole Mixture		
Overall	-0.121	-0.14	-0.103	-0.048	-0.146	0.049
Boys	-0.083	-0.112	-0.055	-0.036	-4.134	4.062
Girls	-0.011	-0.029	0.008	-0.1	-0.256	0.056
	Phthalates			Phthalates		
Overall	0.004	-0.006	0.014	0.003	-0.013	0.019
Boys	0.018	0.004	0.031	0.029	0.007	0.052
Girls	-0.011	-0.025	0.004	-0.024	-0.05	0.001
	Metals			Metals		
Overall	-0.095	-0.114	-0.076	-0.07	-0.095	-0.044
Boys	-0.074	-0.088	-0.061	-0.049	-0.083	-0.015
Girls	-0.102	-0.117	-0.086	-0.08	-0.117	-0.042
	PFAS			PFAS		
Overall	-0.064	-0.074	-0.054	-0.085	-0.101	-0.07
Boys	-0.053	-0.067	-0.04	-0.071	-0.093	-0.049
Girls	-0.07	-0.084	-0.056	-0.093	-0.116	-0.071
	Bisphenols & Parabens			Bisphenols & Parabens		
Overall	0.011	0.001	0.021	0.023	0.006	0.04
Boys	0.011	-0.007	0.03	0.029	-0.007	0.065
Girls	0.006	-0.007	0.019	0.009	-0.013	0.032
	PAH			PAH		
Overall	-0.016	-0.027	-0.006	-0.013	-0.045	0.02
Boys	-0.02	-0.035	-0.006	-0.016	-0.057	0.025
Girls	-0.018	-0.033	-0.003	-0.005	-0.064	0.054

Supplemental Table 2. Results from the quantile g computation (qGcomp) analysis of adults from the NHANES from 2007-2016. The overall mixture association from quantile g-computation is interpreted as the effect on the outcome of increasing every exposure by one quantile. Adjusted for age, race, family to income poverty ratio, education, NHANES cycle, smoking status, and diet. Additional adjustments for sex in the overall analyses, and

urinary creatinine in models with pollutants collected in urine. Statistically significant values are highlighted in red.

Chemical	ExWAS			qGComp			
	n (Overall)	n (Males)	n (Females)	n (Overall)	n (Males)	n (Females)	
Phthalates	MCOP	7618	3736	3882	8164	4008	4156
	MECPP	7618	3736	3882			
	MBP	7618	3736	3882			
	MCPP	7618	3736	3882			
	MEP	7618	3736	3882			
	MEHHP	7618	3736	3882			
	MEHP	7618	3736	3882			
	MiBP	7618	3736	3882			
	MNP	7618	3736	3882			
	MEOHP	7618	3736	3882			
	MBzP	7618	3736	3882			
Metals	Cadmium	18986	9371	9615	4759	2360	2399
	Cotinine	23635	11581	12054			
	Lead	18986	9371	9615			
	Manganese	8927	4410	4517			
	Mercury	18986	9371	9615			
	Selenium	8927	4410	4517			
	Arsenic	7787	3889	3898			
PFAS	PFDeA	7575	3657	3918	7964	3855	4109
	PFHxS	7575	3657	3918			
	Me-PFOA	7575	3657	3918			
	PFNA	7575	3657	3918			
	PFUA	7575	3657	3918			
	PFOA	7575	3657	3918			
	PFOS	7575	3657	3918			
BPA/Parab	BPA	7618	3736	3882	8164	4008	4156
	Triclosan	7618	3736	3882			
	Butyl Paral	7618	3736	3882			
	Methyl Par	7618	3736	3882			
	Propyl Par	7618	3736	3882			
PAHs	One hydrox	6113	3072	3041	1531	778	753
	Two hydrox	6131	3074	3057			
	Three hydr	6163	3092	3071			
	Two hydrox	6172	3096	3076			
	Three hydr	4641	2340	2301			
	One hydrox	6182	3100	3082			
	Two hydrox	4621	2330	2291			
	One hydrox	6156	3086	3070			
	Nine hydro	4649	2344	2305			
	Four phena	1391	710	681			

Supplemental Table 3. Sample sizes for the complete case analyses. Abbreviations: exposome wide association study (ExWAS); quantile g computation (qGComp).

Covariate	Analytic Sample		Missing Sample		P
	N	Mean	N	Mean	
Total SRS 2 Score	420	55.48	211	53.63	<0.001
Maternal Age	420	30.07	211	31.99	<0.001
BMI at Pregnancy week 26	407	26.49	200	26.24	<0.001
Gestational Age at Birth	420	38.79	211	38.72	<0.001
Child Sex					0.5188
Female	206		97		
Male	214		114		
Maternal Ethnicity					<0.001
Chinese	199		156		
Malaysian	145		34		
Other	76		21		
Maternal Education					<0.001
< High School Education	147		50		
High School Education/ Technical School	164		71		
College Degree or Higher	106		87		
Missing	3		3		
Maternal Smoking Status					<0.001
Never	354		187		
Ever	63		17		
Missing	3		7		
Household Monthly Income					<0.001
0 - 1999 USD	72		17		
2000 - 3999 USD	148		43		
4000 - 5999 USD	96		50		
> 6000 USD	82		89		
Missing	22		12		
Parity					0.06
Nulliparous	173		115		
Parous	137		56		
Multiparous	110		40		
Breast Feeding Status					0.016
Less than 6 Months	261		109		
6 Months or More	138		95		
Missing	21		7		
Birthweight for GA					0.941
AGA	301		154		
LGA	73		35		
SGA	46		22		
Marital Status					0.069
Married	401		207		
Not Married	11		1		
Missing	8		3		

Supplemental Table 4. Comparison between important covariates among the analytic sample and participants excluded. Statistical significance was assessed using a two-sided two-sample student's t test for continuous variables, fisher's exact test for categorical variables with categories with a sample size less than 5, and a X^2 test of independence for other categorical variables.

Appendix B

Figures

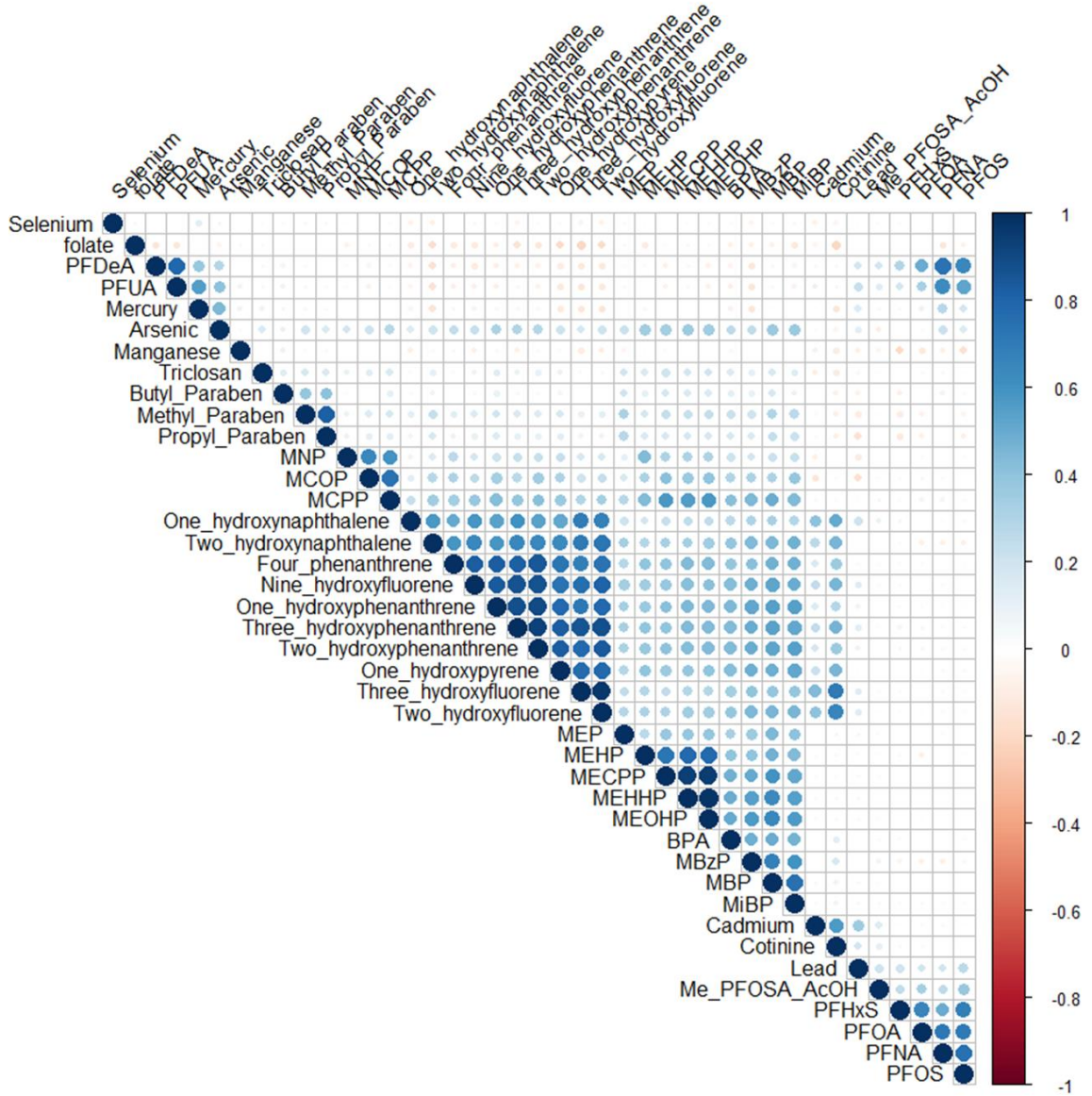


Figure 1. Pearson Correlations between environmental pollutants and folate. Red circles indicate a negative correlation and blue indicates a positive correlation.

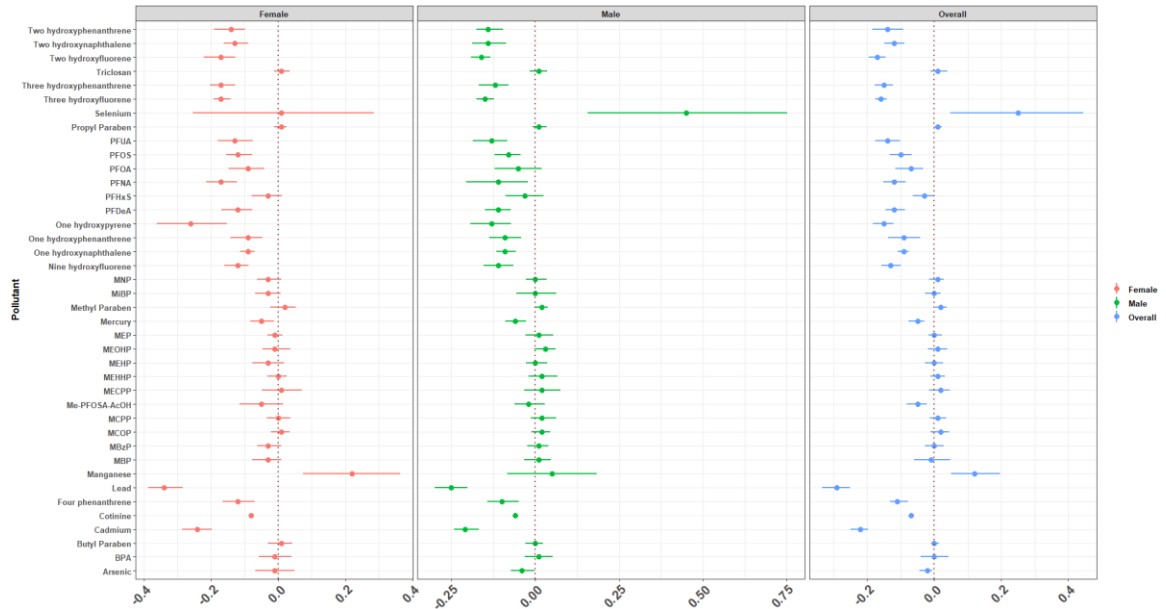


Figure 2. Results from the exposome wide association study (ExWAS) analysis from the NHANES from 2007-2016. Adjusted for age, race, sex, family to income poverty ratio, education, urinary creatinine, NHANES cycle, and diet in the overall analysis. Sex was not used for adjustment in the stratified analyses for males and females. Estimates represent the association between a 10-unit change in exposure and percent change in RBC folate. n = 27,938 for the overall analysis, 13,599 for males and 14,339 for females.

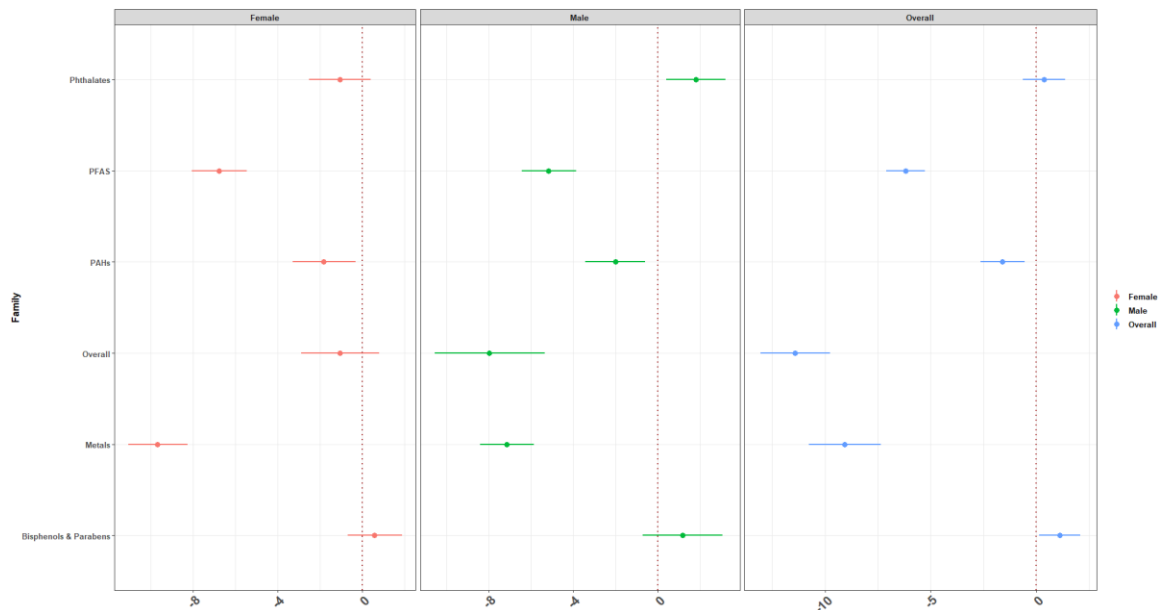


Figure 3. Results from the quantile g computation (qGcomp) analysis of adults from the NHANES from 2007-2016. The overall mixture association from quantile g-computation is interpreted as the effect on the outcome of increasing every exposure by one quantile. Adjusted for age, race, family to income poverty ratio, education, NHANES cycle, smoking status, and diet. Additional adjustments for sex in the overall analyses, and urinary creatinine in models with pollutants collected in urine. Estimates represent the association between a one quantile increase in exposure family and percent change in RBC folate. n = 27,938 in overall analyses and 13,599 in analyses among male participants and 14,339 in analyses among female participants

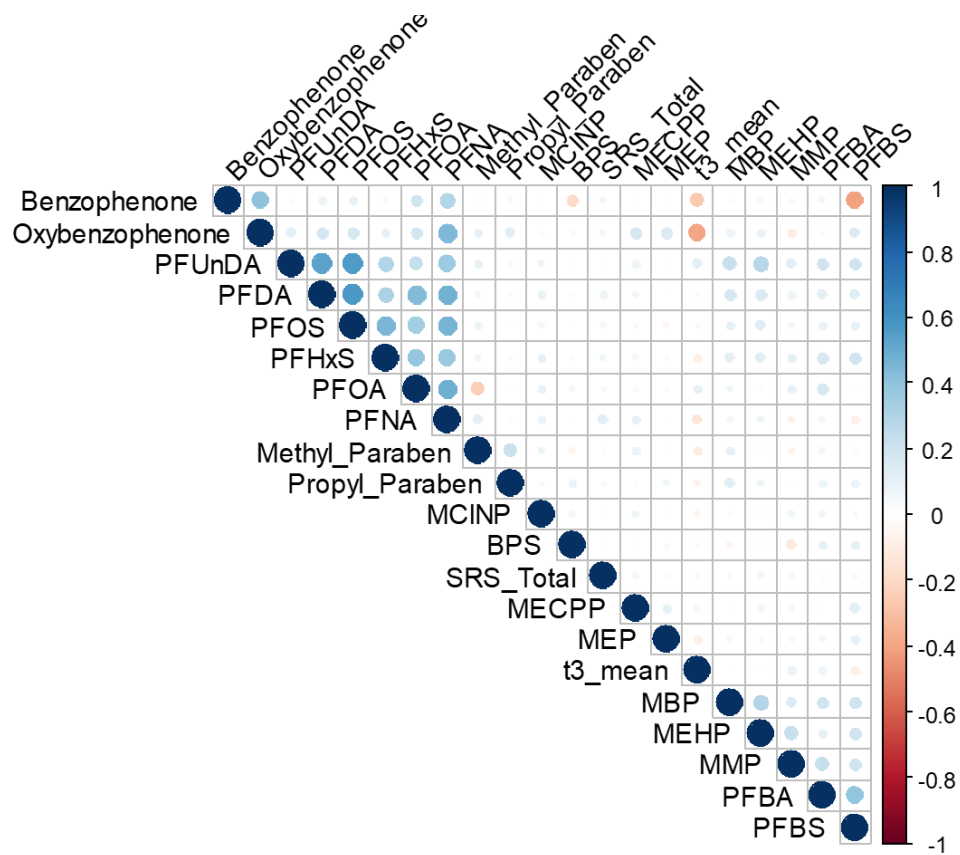


Figure 4. Correlations between environmental pollutants and SRS-2 scores and subscores.

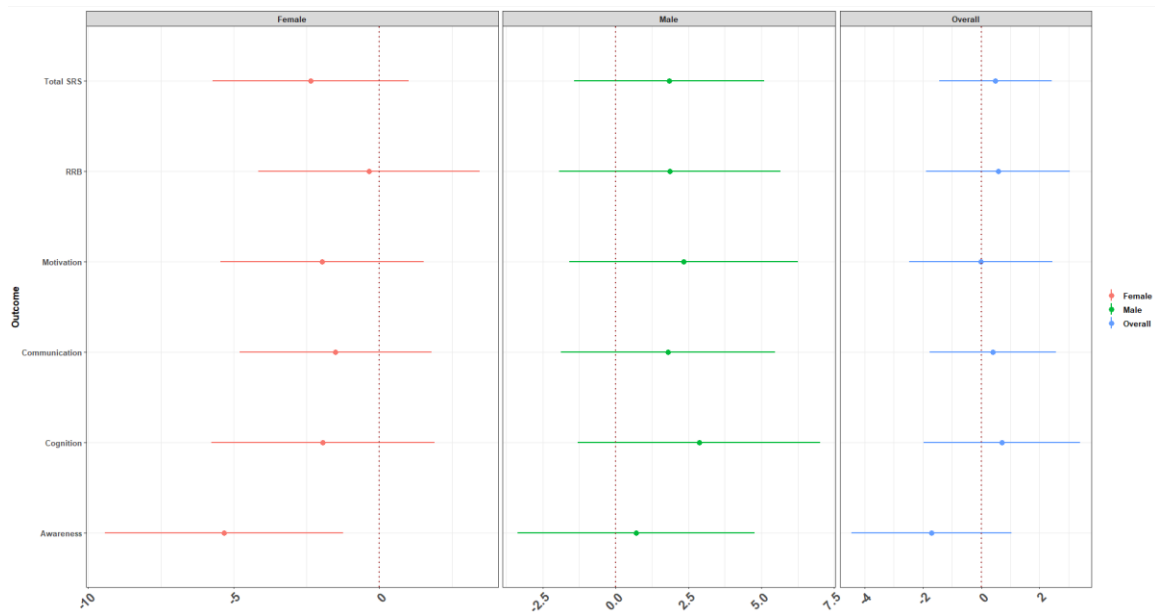


Figure 5. Pollutant mixture difference in SRS-2 scores and sub-scores and 95% CI associated with an interquartile range (IQR) difference in a pollutant mixture, consisting of PFAS, phthalates, phenols and parabens, benzophenones, and third trimester PM2.5, after adjustment.

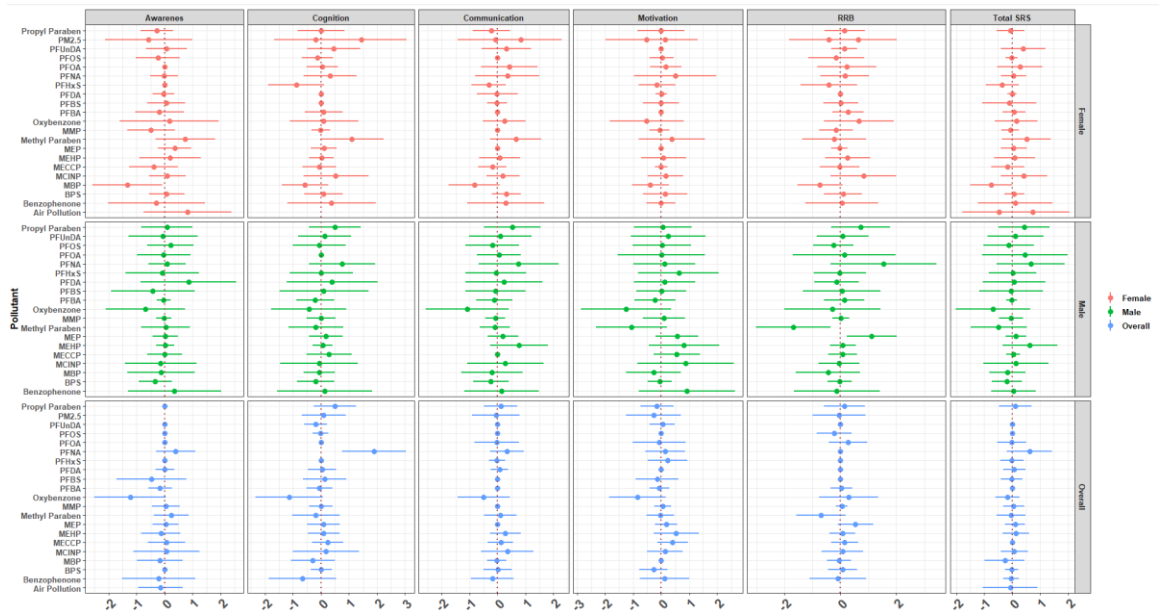


Figure 6. Single exposure differences in response (95% CI) associated with an IQR difference for each pollutant, where all of the other pollutant mixture components are fixed to their 50th percentile, and SRS-2 scores and sub-scores, after adjustment.

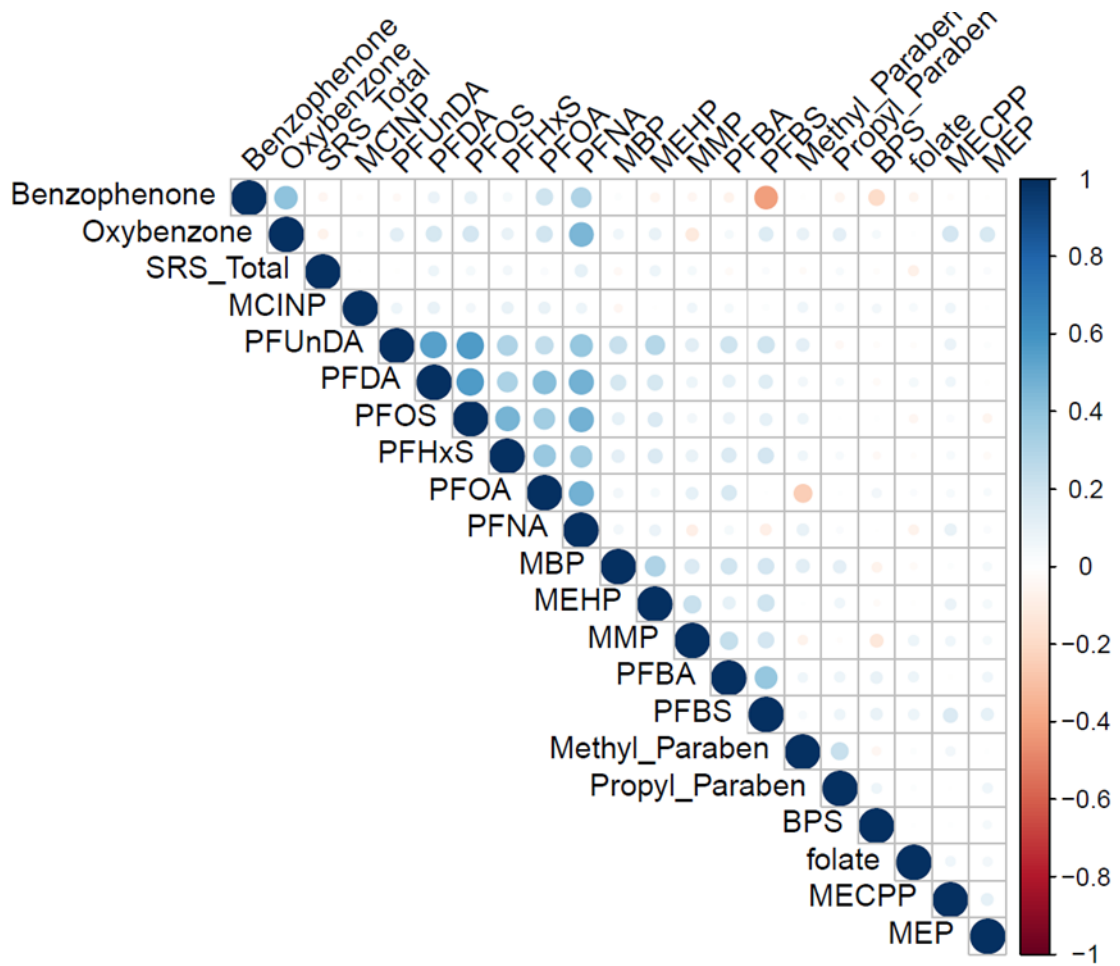


Figure 7. Pearson correlations between environmental pollutants and SRS-2 scores and plasma folate.

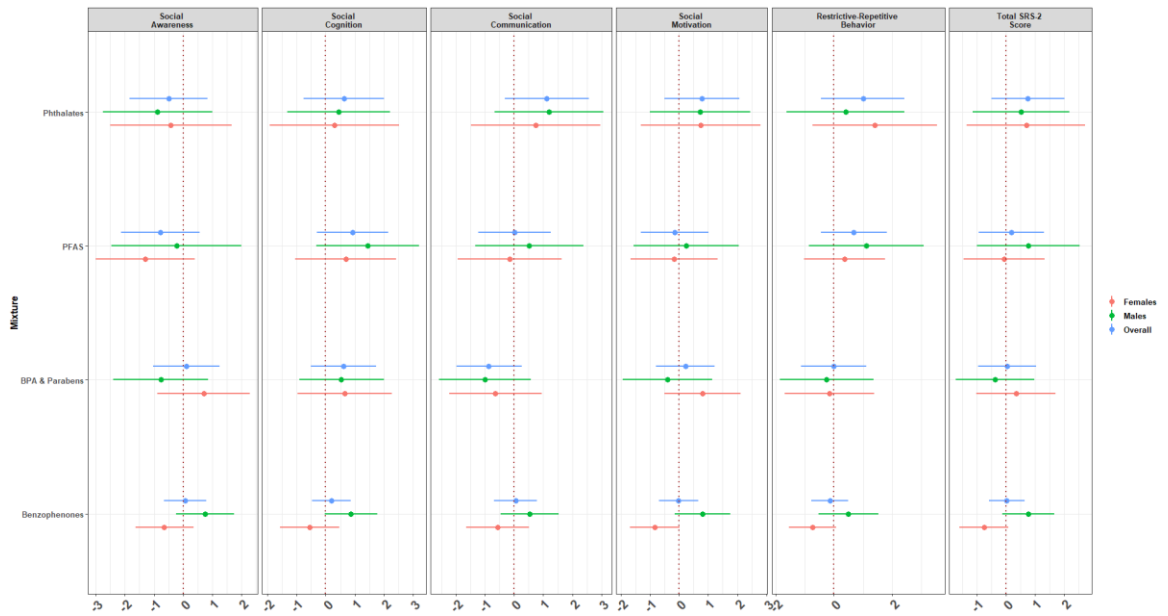


Figure 8. Results from the quantile g computation with plasma folate as a confounder. Estimates represent a point change in SRS-2 scores and sub-scores from a one quantile increase in the pollutant mixture after adjustment in males, females, and the overall sample of GUSTO participants.

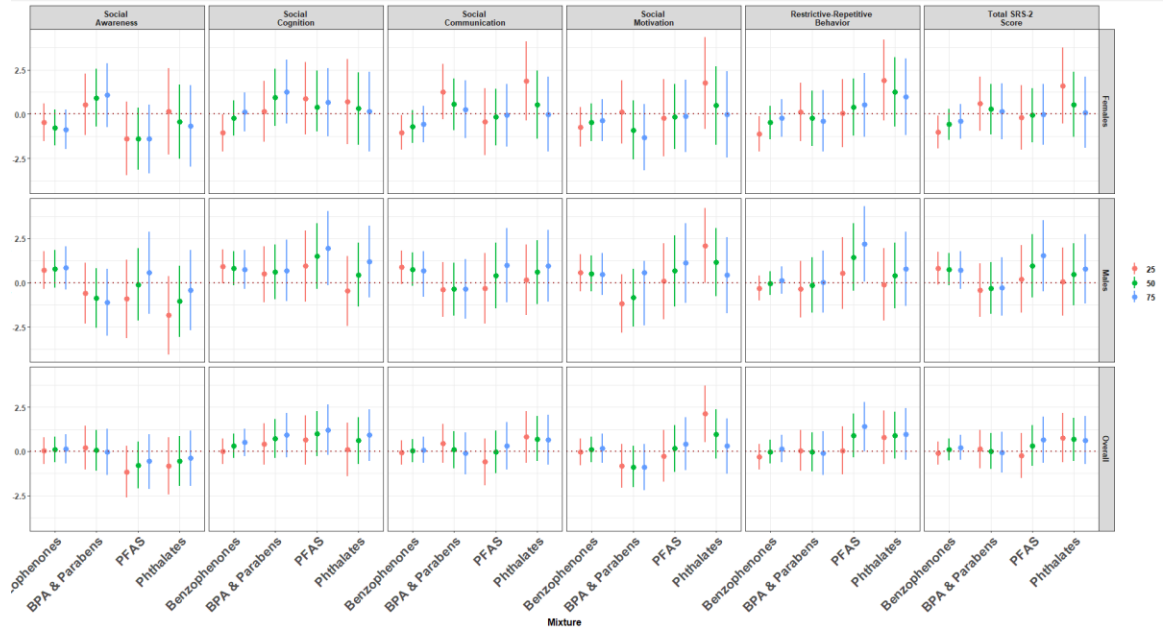


Figure 9. Results from the quantile g computation with plasma folate as a potential effect modifier. Estimates represent a point change in SRS-2 scores and sub-scores from a one quantile increase in the pollutant mixture when plasma folate is fixed at its 25th (red), 50th (green), and 75th (blue) percentile after adjustment in males, females, and the overall sample of GUSTO participants.