EARLY LIFE EXPOSURE TO AIR POLLUTION AND AUTISM SPECTRUM DISORDER: SUSCEPTIBLE TIME WINDOWS AND POPULATIONS

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ABSTRACT

Laura McGuinn: Early Life Exposure to Air Pollution and Autism Spectrum Disorder: Susceptible Time Windows and Populations
(Under the direction of Julie Daniels)

Autism Spectrum Disorder (ASD) is a highly heterogeneous disease, with multiple underlying causes, including genetic and environmental factors. Early life air pollution exposure has been implicated as a risk factor for ASD, potentially working through an inflammatory pathway. Previous air pollution and ASD studies have been limited by several factors, including spatial and exposure variability, consideration of area level socioeconomic status, and evaluation of associations by ASD severity. To address these limitations, this dissertation assessed the association between pre- and postnatal air pollution exposure during critical windows of neurodevelopment using data from the Study to Explore Early Development, a population-based case-control study with six different sites located throughout the United States.

Specific Aim 1 assessed the association between particulate matter ≤2.5 μm in diameter ($PM_{2.5}$) and ozone exposure three months prior to pregnancy, over the entire pregnancy period, as well as more refined periods of exposure reflecting each trimester of pregnancy and the first year of life. Specific Aim 2 evaluated the modifying role of neighborhood deprivation on the association between pregnancy and first year of life roadway proximity and $PM_{2.5}$ exposure and ASD. For both of these aims, important confounders were considered and several sensitivity analyses were conducted to assess the robustness of the study findings. In Specific Aim 1, a

potential critical window of susceptibility was identified during the late prenatal and early postnatal period, including associations for $PM_{2.5}$ exposure during the first year of life [OR=1.26 per 1.6 $\mu g/m^3$ (95% CI: 1.02, 1.57)] and ozone exposure during the third trimester [OR=1.22 per 6.6 ppb (95% CI:1.05, 1.42)]. Associations varied by geographic location, potentially due to different exposure levels and composition of $PM_{2.5}$. In Specific Aim 2, the association between first year of life $PM_{2.5}$ exposure and ASD varied by neighborhood deprivation level, with the strongest observed association for those in the highest deprivation level. The findings from this study may aid in setting air pollution standards for regulatory action for susceptible populations and suggests that economically deprived regions may be of greatest risk.

To my mother, who has never stopped believing in me, and to my brother Stephen who taught me so much in such a short amount of time.

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LIST OF ABBREVIATIONS

ADI-R	Autism Diagnostic Interview-Revised
ADHD	Attention Deficit Hyperactivity Disorder
ADOS	Autism Diagnostic Observation Schedule
AOD	Aerosol Optical Depth
ASD	Autism spectrum disorder
AQS	Air quality system
A-TAC	Autism – Tics, AD/HD and other Comorbidities Inventory
CAPs	Criteria Air Pollutants
CA	California
CI	Confidence interval
CDC	Centers for Disease Control and Prevention
CHARGE	Childhood Autism Risks from Genetics and the Environment
CMAQ	Community Multiscale Air Quality system
CO	Carbon Monoxide
CO	Colorado
CRP	C-reactive protein
CSS	Core Symptom Severity
CSS	Calibrated Severity Score
DAG	Directed acyclic graph
DD	Developmental delay
DSM	Diagnostic and Statistical Manual of Mental Disorders
EDC	Endocrine disrupting chemicals

EMM	Effect Measure Modification
EPA	Environmental Protection Agency
FRM	Federal reference method
GA	Georgia
GEOS	Goddard Earth Observing System
GIS	Geographic Information System
HAPs	Hazardous Air Pollutants
HEI	Health Effects Institute
ID	Intellectual disability
IDW	Inverse distance weighting
IQ	Intelligence quotient
IRB	Institutional review board
LUR	Land use regression model
MD	Maryland
NAMS	National Air Monitoring Stations
NAAQS	National Ambient Air Quality Standard
NC	North Carolina
NDI	Neighborhood deprivation index
NO_2	Nitrogen dioxide
NOx	Nitrogen oxides
OR	Odds ratio
O3	Ground-level ozone
PA	Pennsylvania

PAH	Polycyclic aromatic hydrocarbon
$PM_{2.5}$	Fine particulate matter less than 2.5 micrometers
PM_{10}	Coarse particulate matter between 2.5 and 10 micrometers
POP	Population control
PCA	Principal components analysis
PTB	Preterm Birth
SCQ	Social Communication Questionnaire
SEED	Study to Explore Early Development
SES	Socioeconomic status
SGA	Small for gestational age
SLAMS	State and Local Air Monitoring Stations network
SO_2	Sulfur dioxide
TRAP	Traffic-related air pollution
U.S.	United States
VOCs	Volatile organic compounds

CHAPTER 1: INTRODUCTION AND SPECIFIC AIMS

Autism spectrum disorder (ASD) are a group of neurodevelopmental disorders marked by impairments in social interaction and communication, and repetitive behaviors. The Centers for Disease Control and Prevention (CDC) estimates that 1 in 68 children (1 in 42 boys and 1 in 189 girls) has been identified with ASD [1]. ASD is a highly heterogeneous disease, with multiple underlying causes, including genetic and environmental factors [2-4]. There is additionally strong evidence for a prenatal and early postnatal window of susceptibility for ASD risk [5].

Several epidemiologic studies have reported associations between prenatal and early postnatal air pollution exposure and ASD, however findings have differed by pollutant, developmental window, and geographic location. Particulate matter and ozone are among the most ubiquitous criteria air pollutants and have been shown to induce inflammation and oxidative stress [6-8], both of which have been implicated in the onset of ASD [9, 10]. Particulate matter $\leq$2.5 and $\leq$10 μm in diameter ($PM_{2.5}$ and PM_{10}) exposure during the prenatal [11-13] and postnatal [13, 14] developmental periods has been associated with ASD in studies in the United States (U.S.), with two studies suggesting the third trimester may be a potential critical window of susceptibility [12, 15], while one study found a cumulative effect from before birth through the second year of life. Few studies have assessed associations with ozone exposure, with only one previous study finding a positive association between early life ozone exposure and ASD [11].

Previous studies have been limited in geographic variability, which is important to consider because PM components vary spatially and there may be geographic differences in air

pollution and ASD associations due to the spatial variation of PM components. In addition, few previous studies have evaluated if the association between air pollution and ASD differed by ASD severity or more homogenous phenotypic subgroups of ASD. Investigating associations by ASD severity and refined phenotypic subgroups of ASD may provide insight as to which aspects of brain development may be most susceptible to air pollution. Finally, chronic stress from neighborhood deprivation may influence individual susceptibility, and this stress-borne susceptibility may shape response to air pollution. Few studies to date have specifically assessed the combined effect of social stressors and air pollution on ASD. This is significant because by studying the combined effect of these two exposures this study will be able to target susceptible subgroups that are particularly vulnerable to both social and environmental stressors.

The goal of this dissertation is to investigate the association between early life $PM_{2.5}$ and ozone exposure and ASD during critical periods of neurodevelopment including, three months before pregnancy, over the entire pregnancy period and trimester averages, as well as during the first year of life. Data for these analyses came from the Study to Explore Early Development (SEED), a multi-site study that aims to identify risk factors for ASD and other developmental disabilities. SEED study sites are located in North Carolina, Georgia, Maryland, Pennsylvania, Colorado, and California, thus providing adequate spatial variability for studying associations between air pollutants and autism. This dissertation aims to identify windows of susceptibility for air pollution and ASD associations and provide insight into the heterogeneity in associations from air pollution exposure. Limitations from previous studies are addressed by assessing the effect of air pollution by ASD severity and the potential modification by neighborhood deprivation on air pollution and ASD associations, which are both important in order to identify particularly susceptible subgroups.

Specific Aims

Specific Aim 1: **Investigate the association between early life $PM_{2.5}$ and ozone exposure and ASD.** In order to identify potential windows of susceptibility that may be most important for toxicant exposure, analyses will examine exposure three months prior to pregnancy, over the entire pregnancy period, as well as more refined periods of exposure reflecting each trimester of pregnancy and the first year of life.

> **Specific Aim 1a:** Determine if the association between air pollution and ASD varies by level of ASD severity.

Specific Aim 2: Determine whether neighborhood deprivation modifies the association between air pollution and ASD. This aim was accomplished by creating a neighborhood deprivation index to characterize neighborhood level social stressors. This aim assesses the combined effect of both environmental and social stressors in relation to ASD.

Hypotheses

Pre- and postnatal $PM_{2.5}$ and ozone exposure will be associated with ASD in the child, and this association will vary by developmental window. It is additionally hypothesized that associations will vary by level of severity and geographic location. Finally, this study hypothesizes that neighborhood deprivation will modify associations between air pollution and ASD, given the similar inflammatory pathways between the two stressors.

CHAPTER 2: BACKGROUND AND SIGNIFICANCE

2.1 Air Pollution Exposure

Air pollution is ubiquitous and is a widespread public health concern. Air pollutants are either primarily emitted from industrial processes, vehicle exhaust, combustion products, or dust, or are secondarily formed in the atmosphere through complex chemical reactions. There are two main classes of air pollutants including criteria air pollutants and hazardous air pollutants. Criteria air pollutants include fine particulate matter less than 2.5 micrometers ($PM_{2.5}$), coarse particulate matter less than 10 micrometers (PM_{10}), carbon monoxide (CO), ground-level ozone (O_3), sulfur dioxide (SO2), lead, and nitrogen dioxide (NO_2). Criteria air pollutants are in general more common and potent, and are regulated as part of the Clean Air Act [16].

The Clean Air Act of 1970 as amended in 1990 requires the United States (U.S.) Environmental Protection Agency (EPA) to set National Ambient Air Quality Standards (NAAQS) for criteria air pollutants. The EPA uses scientific data to propose revisions to the criteria air pollutant standards, particularly for susceptible populations [17]. Due to air pollution control polices, levels of these criteria air pollutants have been declining since the 1980's [18]. However, in that same time period there has been a dramatic increase in the number of vehicle miles traveled, number of vehicles purchased, and number of people moving to urban areas. Further, the health effects at lower levels of air pollution are still uncertain. The American Lung Association recently found that almost 44% of the nation live in areas where pollution levels are dangerous to breathe, therefore air pollution exposure is still of major public health concern, particularly for susceptible populations [19].

2.1.1 $PM_{2.5}$ and Ozone: Description, Sources, and Variability

$PM_{2.5}$ and ozone are the most common criteria air pollutants and have been found to cause the most adverse health effects at current ambient concentrations [20, 21]. These pollutants are not directly emitted from sources (primary pollutants) but are formed from complex chemical reactions (secondary pollutants) [22]. Table 2.1 includes a detailed description of $PM_{2.5}$ and ozone, including a description of sources, spatial and temporal variability, and the NAAQS primary standard for each pollutant.

Table 2.1 Description of $PM_{2.5}$ and ozone sources, variability, and NAAQS

Pollutant	Description	Components	Sources	Spatial and Temporal Variation	NAAQS Primary Standard
$PM_{2.5}$	Made up of tiny solid particles and liquid droplets suspended in the atmosphere	Sulfates, nitrates, carbon compounds and crustal materials	Formed when gases emitted from power plants, industries and vehicles react in the air	Higher in the summer for Eastern U.S. and during the winter for Western U.S. Higher in urban areas	12.0 ug/m^3 (Annual)
Ozone	Formed from chemical reactions between NOx and volatile organic compounds in the presence of sunlight	----	Precursor sources for ozone include industrial facilities, electric utilities, motor vehicle exhaust, gasoline vapors, and chemical solvents	Higher in the summer, in the presence of sunlight Higher outside of urban areas, and near mountain regions	0.075 ppm (8-hour)

Particulate Matter

Particulate matter is made up of tiny solid particles and liquid droplets suspended in the atmosphere. $PM_{2.5}$ includes fine particulates less than 2.5 micrometers in size and are primarily formed when gases react in the air [22]. $PM_{2.5}$ may pose a greater threat to human health than the larger particles because the finer particles can travel deep into the lungs and into the bloodstream [23]. Major components of $PM_{2.5}$ include sulfates, nitrates, carbon compounds and crustal materials [23]. Man-made sources of $PM_{2.5}$ include mobile source from motor vehicles, power

plants, wood burning, and industrial processes. Due to their smaller size, fine particulates can travel long distances and therefore may be found further away from their sources [22].

Sources of $PM_{2.5}$ vary throughout the United States. Fuel combustion is the major source for $PM_{2.5}$ in Pennsylvania, whereas there is a large contribution from fires and mobile sources in California [24]. $PM_{2.5}$ levels in Pennsylvania and Maryland are additionally greatly impacted by interstate transport of pollutants from sources in the Ohio River valley. Natural sources such as fires (which includes seasonal agricultural burning) and dust have a significant contribution to $PM_{2.5}$ levels throughout the country. Mobile source air pollution is additionally a major contributor to $PM_{2.5}$ for many urban areas throughout much of the country. Components of $PM_{2.5}$ additional vary spatially throughout the United States. Specifically, sulfates are often higher in the Eastern and Northeastern U.S. due to the high levels of SO_2 formed from power plants [25], whereas levels of nitrates are often higher on the west coast from NO_2 emitted from on-road mobile sources. Figure 2.1 shows the spatial variability for $PM_{2.5}$ throughout the United States.

In addition to regional variation, air pollution concentrations often vary within cities and between urban and rural areas [26]. $PM_{2.5}$ is considered a regional pollutant and is often relatively homogenous over large metropolitan areas, though several studies have found considerable within city variation with $PM_{2.5}$ [26, 27]. In general, $PM_{2.5}$ concentrations are often higher in urban areas because of the higher levels of mobile source air pollution. In the United States, sulfates from power plants are often higher in the East during the summer months, while nitrates from local mobile source pollution peak during the winter months across the U.S. [25]. Consequently, levels of $PM_{2.5}$ are higher in the East during the summer months and in the West during the winter months.

A) $PM_{2.5}$ B) Ozone

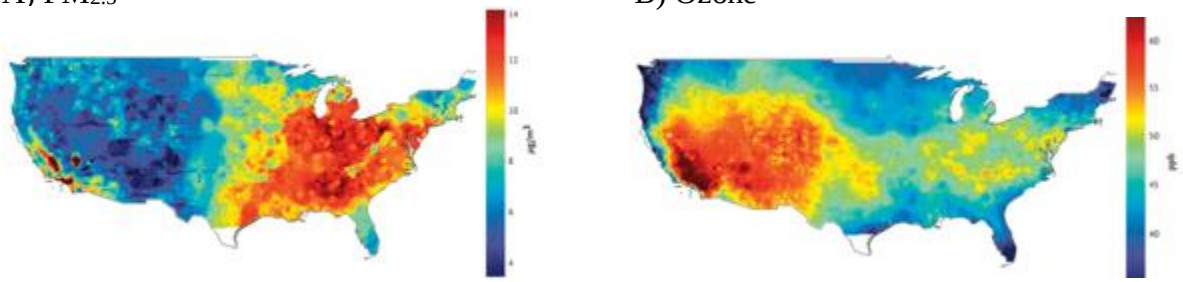


Figure 2.1 Spatial distribution of $PM_{2.5}$ and ozone concentrations across the Unites States [28]

Ground Level Ozone

Stratospheric ozone ("good ozone") occurs in the upper atmosphere and shield's the earth from the sun's ultraviolet rays. Ground level (tropospheric, or "bad ozone") ozone is a gaseous pollutant and is formed from chemical reactions between NOx and volatile organic compounds (VOCs) in the presence of sunlight [29]. Precursor sources for ozone include industrial facilities, electric utilities, motor vehicle exhaust, gasoline vapors, and chemical solvents [22]. Ground level ozone is the main component of smog.

Similar to $PM_{2.5,}$ ozone concentrations vary both spatially and temporally. Levels of ozone tend to be higher in the West and Southwest compared to the East, and higher in mountain regions (Figure 2.1). Elevated levels of nitrogen oxides in near roadway environments lead to lower ozone levels due to the scavenging effect of NO on ozone to create NO2 [29]. Therefore, ozone concentrations are often higher in rural or suburban areas, and lower in cities and along major roadways. Since sunlight is needed for the chemical reactions to take place in the formation of ozone, ozone concentrations peak during the summer months and are lowest in the winter months [25]. While levels of $PM_{2.5}$ and ozone often peak during the summer months, concentrations of primary local pollutants from mobile sources (such as CO and NOx) tend to be higher in the winter due to temperature inversions that limit dispersion of pollutants [30].

2.1.2 $PM_{2.5}$ and Ozone: Exposure Assessment Methods and Data Sources

A variety of methods exist to estimate individual air pollution exposure for epidemiological studies, ranging from proximity analyses to complex predictive models [31]. The choice of method depends on the epidemiologic study design, pollutant of interest, and available resources [32]. For the majority of these methods, a geographical information system (GIS) is used to assign an exposure estimate for a given time period based on the participants' residential, work, or school address. With the exception of personal biomonitoring, most exposure assessment methods capture area level exposures, and use the resulting area level estimates as a proxy for individual level exposure. This section describes a selection of available exposures assessment methods, and ends with a description of a hybrid modelling approach that attempts to combine data from several of the included methods.

Central Site Monitoring

Personal biomonitoring is often not feasible for large scale epidemiologic studies, and instead area level ambient concentrations are often used as proxies for individual exposure. Ambient air pollution data are measured by EPA, state, local, and tribal air pollution control agencies and are available for the major criteria air pollutants, including $PM_{2.5}$ and ozone. The most widely used monitored data come from the State and Local Air Monitoring Stations, a network of air monitoring systems that collect direct measurements of pollution levels daily for gaseous pollutants, and 1 in 3 or 1 in 6 days for particulate matter [33]. Since ozone is formed in the presence of sunlight, concentrations of ozone are often only measured from April-October [22]. Monitored data are publicly available for every US state and are relatively easy to use and access, though have limited spatial variation.

The most widely used method for linking participants to monitored air pollution data is to use a nearest neighbor approach, where each participant is linked to the nearest ambient air quality monitor, regardless of distance. Thus, for an area with only a single monitor, the same measured value is assigned to each person within that area. However, for areas with multiple monitors, spatial variation may be modeled and differing estimates assigned to locations within that area. This method is referred to as interpolation and the interpolation of air pollution data from monitoring sites to estimate pollutant concentrations at non-monitored sites is another commonly used air pollution assessment option. Examples of commonly used interpolation methods include kriging and inverse distance weighting [34, 35]. Interpolation methods are relatively simple and provide more spatial variability over monitored data alone. Monitored data can also be combined with other data sources in order to provide a more comprehensive individual air pollution measure.

Land Use Regression Models

Land use regression (LUR) models predict pollutant concentrations at a given site based on surrounding land use and traffic characteristics. Inputs into these models include major roads, traffic volume, meteorology, and land use [34]. The resulting models can then be applied to a large number of unsampled locations in the study area. LUR models are a more advanced exposure assessment method over the proximity and nearest monitor approaches, as they provide greater spatial variability, and are also relatively low cost. Some limitations of LUR models is that the quality of the models tend to depend on the density of the air quality monitors in the area [34]. Recently, studies have made use of data from land use regression models that incorporate data from chemical transport models and satellite data in order to address some of these spatial and temporal limitations.

Chemical Transport Models: GEOS-Chem Model

Chemical transport models combine input from a meteorological model and an emissions model with simulation of chemical and physical processes to describe pollutant transformation, transport and fate. GEOS-Chem is one type of chemical transport model, and combines meteorologic input from the Goddard Earth Observing System (GEOS) of the NASA Global Modeling and Assimilation Office [36]. GEOS-Chem models provide temporally resolved estimates of particle concentrations, in comparison to land use regression and proximity models that offer limited temporal variability [37]. $PM_{2.5}$ components can additionally be predicted using the GEOS-Chem model. Limitations of GEOS-Chem chemical transport models include poor correlation with monitored data and coarse spatial resolution [37]. These limitations can be addressed by combining GEOS-Chem predictions with monitored data and land use terms.

Satellite Remote Sensing Models

Aerosol optical depth (AOD) measurements may additionally aid in the prediction of pollutant concentrations [38-41]. Aerosols are tiny solid particles and liquid droplets suspended in the atmosphere that absorb and scatter incoming light, reducing visibility and increasing optical thickness [42]. An AOD is a measure of the amount of light the particle prevents from travelling through the atmosphere. An AOD of 0.1 indicates a clear sky, whereas an AOD of 1 indicates hazy conditions. Unlike chemical transport models, satellite data provide an estimate of spatial and temporal variability that is independent of meteorology. Still impacted by cloud cover, AOD data is processed to exclude grids with cloud cover.

Hybrid Models: Incorporation of Multiple Data Sources

There are several strengths and limitations to each exposure assessment option, which

may depend on the pollutant and geographic location of interest. Of increasing interest are the development of methods to incorporate multiple data sources in order to develop a more accurate exposure prediction. Hybrid models are one available option. These models combine personal or regional exposures with one of the previously mentioned air pollution exposure models, such as land use regression or proximity models [34]. Recent models have been developed that combine GEOS-Chem predictions, monitored data, land use terms, satellite data, and meteorological variables [37]. Epidemiological studies have used these models at both a 10 and 1km spatial resolution [39, 40, 43]. In sum, satellite- based hybrid models address several of the limitations of other air pollution exposure assessment options and attempt to predict a more accurate individual air pollution exposure measure.

2.1.3 Traffic-Related Air Pollution

Currently, 30-45% of North Americans live within 300-500m of a major road or highway [44]. A recent Health Effects Institute panel concluded that those living within this distance are most affected by the harmful effects of traffic-related air pollution (TRAP) [45]. Air pollution from traffic is composed of a diverse mixture of organic compounds, including particulate matter, which may have polycyclic aromatic hydrocarbons (PAHs) and oxidant metals adsorbed to the surface. PAHs are known to have estrogenic and endocrine disrupting properties and can potentially interfere with both reproductive and pubertal development. Epidemiological evidence links both short and long-term exposure to traffic related air pollutants to adverse health effects, particularly for vulnerable populations such as pregnant women and children [46-50].

Proximity based exposure assessment is a valid method for capturing long-term local variation in exposure to traffic-related air pollution. Proximity based traffic metrics are low cost and are valid alternatives to measured and modeled local traffic-related air pollution

concentrations [51]. In previous studies, distance to major road/freeway and distance weighted traffic density proved to be the best predictors of local traffic-related air pollution. In addition, studies have shown high correlations between proximity measures and concentrations of measured NOx [52].

2.1.4 Associations Between Air Pollution and Health Outcomes

Epidemiologic studies have reported associations between air pollution and several health outcomes, most significantly for cardiovascular and respiratory disease, as well as overall mortality [53, 54]. $PM_{2.5}$ is the most common air pollutant, can travel deep in to the lungs and potentially the bloodstream, and has consistently been associated with adverse health outcomes in previous studies [20, 21]. Ozone is a strong oxidizing agent and has additionally been associated with several health outcomes, most notably for respiratory and cardiovascular effects and mortality [29].

Of increasing interest is the impact of early life air pollution exposure on later life disease. Several previous studies have shown associations between air pollution exposure and birth outcomes using distance to major road [55-57] and measured and modeled levels of pollutants [55, 58-60]. Specifically, studies have shown associations between air pollution exposure during pregnancy and several adverse pregnancy outcomes including preterm birth [61-65], low birth weight [66-70], small for gestational age (SGA) [39, 71-73], and congenital malformations [74-77]. There is also evidence for an association between air pollution exposure and suboptimal maternal conditions such as obesity, diabetes, and preeclampsia [78-83]. Several studies have additionally shown associations between early life air pollution exposure and asthma [84-87] and obesity in children [88-90].

Early life air pollution exposure has previously seen to be associated with several

neurodevelopmental outcomes [91]. Specifically, there is compelling evidence for associations between both traffic related air pollution and measured and modeled levels of pollutants and several neurodevelopmental outcomes [41, 92]. One study found associations between early life black carbon exposure and memory domains in children, only among boys exposed to high levels of prenatal stress [93]. Another recent study found windows of susceptibility for air pollution exposure and lower IQ during 31-38 weeks of pregnancy and for memory domains in girls during 18-26 weeks of pregnancy [94]. Increasing research has focused on the association between early life air pollution exposure and autism spectrum disorder.

2.2 Autism Spectrum Disorder

2.2.1 Description and Prevalence

ASD are a group of neurodevelopmental disorders marked by impairments in social interaction and communication, and repetitive behaviors. The *Diagnostic and Statistical Manual of Mental Disorders (*4th edition, DSM-IV) criteria separated autistic disorder, Asperger's disorder, childhood disintegrative disorder, and pervasive developmental disorder, but with the new DSM-V criteria these disorders are now grouped into the autism spectrum disorder umbrella [95]. ASD symptoms vary widely, from mild to severe. Some individuals diagnosed with autism are verbal and highly intelligent, requiring minimal support; while others are nonverbal and unable to live independently. Individuals with ASD often have comorbid medical conditions including genetic disorders, gastrointestinal disorders [96-99], seizures [100], intellectual disabilities (ID), and several immune mediated conditions [101]. The etiology of ASD is poorly understood, however studies suggest contributions from both genetic and environmental factors [2, 3].

The CDC estimates that 1 in 68 children (1 in 42 boys and 1 in 189 girls) has been

identified with ASD [1]. This estimate, which was first reported in May of 2014, is about 30 percent higher than previous estimates reported in 2012. In general, prevalence of autism has continued to increase over the years. Part of this increase in prevalence may be due to increases in diagnostic practices and awareness. However, the increase in prevalence cannot be attributed to broadening of diagnostic criteria alone, and environmental factors are thought to play a role [2].

2.2.2 Diagnosis and Clinical Criteria for ASD

As there is no biomarker or medical test for ASD, physicians and psychologists rely on behavioral evaluation to diagnose children with ASD. Children with ASD can be reliably diagnosed at age 2 [102], however it has been reported by the CDC that diagnosis of ASD most commonly occurs after 4 years of age [1]. Although children can be screened for ASD as early as 18 months at physician check-ups, it is often the parents who notice the first signs. The Modified Checklist for Autism in Toddlers and the Communication and Symbolic Behavior Scales are common screening tools used in doctor's offices.

After the initial screening process, parents may be referred to specialists to complete more thorough diagnostic assessments to diagnose suspected ASD in children. The Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS) are considered gold standards for autism diagnosis. In addition, DSM-5 criteria may be used to make diagnoses. DSM-5 diagnostic criteria for ASD include: persistent deficits in social communication and social interaction and restricted, repetitive patterns of behavior, interests, or activities. The criteria requires that these symptoms be present early in life and cause clinically significant impairment [95].

2.2.3 Demographic Distribution of ASD

Prevalence of ASD varies within and between geographical areas, and there is geographical variability in ASD diagnoses throughout much of the U.S. [103, 104]. One study in North Carolina found that areas with lower ASD risk experienced the greatest increases in ASD over time, possibly due to education and outreach efforts [103]. Two other studies, from Utah and California, found geographic differences in ASD cases, though noted that most of the differences could be explained by maternal education and socioeconomic status [104, 105]. Assessing geographic differences in ASD prevalence is often difficult due to differences in data quality, diagnostic practices, and greater awareness in different geographic areas.

Autism spectrum disorder is almost 5 times more common in boys than girls, though the male to female ratio differs by severity [2]. Additionally, non-Hispanic white children are more likely to be identified as having ASD than African American or Hispanic children [1, 106]. One study found lower prevalence of ASD for children of black and Hispanic mothers, though these differences were attenuated after adjustment for SES factors [107]. Another study found disparities in diagnoses with black children showing lower rates of documented ASD, regardless of IQ [106]. This study additionally found that disparities in diagnoses for other ethnicities were only for those with an IQ lower than 70. Therefore, it is unclear whether there is an etiologic basis for these racial differences, or if the differences can be explained by increased awareness, differences in diagnostic practices, and access to healthcare.

SES and Neighborhood Deprivation

There is a complex relationship between individual and area level SES and ASD, with many previous studies showing inconsistent associations. A study from Denmark showed null results between familial SES and ASD, while a U.S. based study reported associations between

higher familial SES and ASD in children [108, 109]. In general, in U.S. based populations lower SES tends to be associated with under-diagnosis of ASD. Additionally, studies have shown living further than 20km from a medical school to be associated with under-diagnosis of ASD [110].

Findings on the association between neighborhood deprivation and ASD are additionally mixed. European-based studies have found associations between higher neighborhood deprivation and ASD [111, 112], while U.S. based studies have in general found associations between lower deprivation and ASD [113]. Delobel-Ayoub et al. [112] assessed associations between SES disparities and ASD in France and found a higher prevalence of ASD in areas of highest deprivation and for families in the highest unemployment and lowest education levels. Another study in Sweden reported an association between higher neighborhood deprivation (or lower area level SES) and ASD [111], whereas another U.S. based study found higher area level SES to be associated with increased ASD prevalence [113]. It is unclear if the differences for the U.S. studies can be explained by increased access to care in wealthier areas. Denmark and Sweden both have national health care systems, thus it is unlikely that there would be differential access to care across SES levels in these countries.

2.2.4 Critical Windows of Neurodevelopment and Risk Factors for ASD

The brain is rapidly developing during the pregnancy period and early life, and there are several critical processes that occur during this timeframe, including neuron formation and migration, cell death, synapse formation, generation of glial cells, myelination, and synaptic pruning. Disruptions of these critical processes, potentially by environmental exposures, could interfere with normal brain development and potentially increase risk of ASD. In fact, though most ASD symptoms do not appear till around age two, studies have found exposures before,

during, and immediately after birth to be associated with an increased risk for autism [114]. Thus, these periods can be thought of as critical windows of susceptibility where exposures during these times may interfere with normal brain development [115, 116].

The etiology of ASD is poorly understood, however studies suggest contributions from both genetic and environmental factors. ASD is thought to be highly heritable, with original heritability estimates ranging up to 90% [117, 118]. Recent twin studies, however, have implicated shared environmental factors to play a larger role than previously suspected [119]. Genetic and environmental factors likely do not work in isolation, and it is likely a combination of genetic susceptibility and environmental insults.

There are few established risk factors of ASD. Among the few are advanced maternal and paternal age [120-122], family history, and male sex. Studies have also found an increasing risk for ASD with increasing difference in parental ages [123]. Previous studies have found associations between maternal conditions during pregnancy such as preeclampsia [124], maternal diabetes and obesity [125], and cesarean delivery [126] and ASD in offspring. Additionally, there is evidence for associations between adverse birth outcomes, including preterm birth, SGA, and low birth weight and ASD [127, 128]. There is also increasing evidence for an association between maternal infections during the pregnancy period and ASD, including evidence from both animal [129] and human studies [130, 131].

Environmental Exposures

Pre- and postnatal environmental exposures may play a role in the pathogenesis of autism [4, 132-134]. It is hypothesized that secondhand smoke exposure may play a role and increase risk of ASD, given that tobacco smoke contains many of the same chemicals found in air pollutants, though few studies have assessed this exposure specifically. Further, there is little

conclusive evidence for an association between maternal tobacco use and ASD [135-139]. Several studies have found associations between prenatal exposure to pesticides and ASD [140-147], most significantly for organochlorines and organophosphates. There is also suggestive evidence for an association between VOCs, such as methylene chloride, trichloroethylene, and styrene, and ASD [145, 148, 149]. VOCs are found in indoor and outdoor air and can be emitted from sources such as vehicle combustion, dry cleaners, and cigarette smoke. Findings for endocrine disrupting chemical (EDC) exposures such as polychlorinated biphenyls and bisphenol A and ASD are mixed [140, 141], though several studies have found associations between phthalate exposure and ASD [150-153]. However, recent results from the Childhood Autism Risks from Genetics and the Environment (CHARGE) study did not find an association between house dust levels of phthalates and ASD [154]. This, and several other recent ASD studies, assessed exposure after diagnosis of ASD, which does not reflect the most etiologically relevant period of exposure.

The variability in the outcome definition may contribute to the difficulty in consistently identifying risk factors for ASD. The DSM-4 criteria separated autistic disorder, from Asperger's disorder, childhood disintegrative disorder, and pervasive developmental disorder, but the current DSM-5 criteria groups each of these under the ASD umbrella. Therefore, studies using only the autistic disorder outcome could differ from those using the full ASD outcome. Additionally, several studies have assessed broad autistic traits, which would not fall under the ASD diagnosis.

2.3 Potential Biological Pathways for Air Pollutants to Contribute to ASD

Although it is possible for air pollution to have direct neurotoxic effects on the developing brain, the main hypothesized pathway linking air pollution exposure to autism is

through an inflammatory response (Figure 2.2) [9, 10, 155]. Air pollutants are established immune toxicants [6]. There is an extensive body of literature liking air pollution exposure to inflammation, oxidative stress, and production of pro-inflammatory cytokines [7, 8, 63]. There is additionally a growing body of literature linking prenatal air pollution exposure to maternal immune activation [156], and growing evidence for an association between maternal immune activation, early life immune dysregulation, and ASD in the child [157-160].

Inflammatory cytokines, produced from the mother in response to air pollution exposure, can cross the placenta, enter fetal circulation, and induce systemic inflammation and oxidative stress in the fetus [161]. Air pollution directly inhaled by the infant postnatally can additionally induce a systemic inflammatory response and perturb normal development of the immune system. Evidence supports that these pro-inflammatory cytokines may be able to reach the developing brain, cross the blood brain barrier, resulting in neuroinflammation, neuron damage/loss, microglia activation, and DNA damage [8].

Of emerging interest is the mediating role of microglia activation in prenatal and early postnatal toxicant exposure in relation to ASD [162]. Microglia play a critical role in normal brain development, and alterations in microglial development by in utero and early postnatal inflammation may alter synaptic pruning [163, 164], and disruption of normal brain development. Recent studies in mice have found associations between gestational exposure to ultrafine particles and microglial activation in male offspring [165], with one study suggesting a third trimester equivalent window of susceptibility in humans [166]. Another animal study found associations between gestational exposure to diesel exhaust particles, increased cytokine production, and altered trajectory of microglia, only in male mice [167].

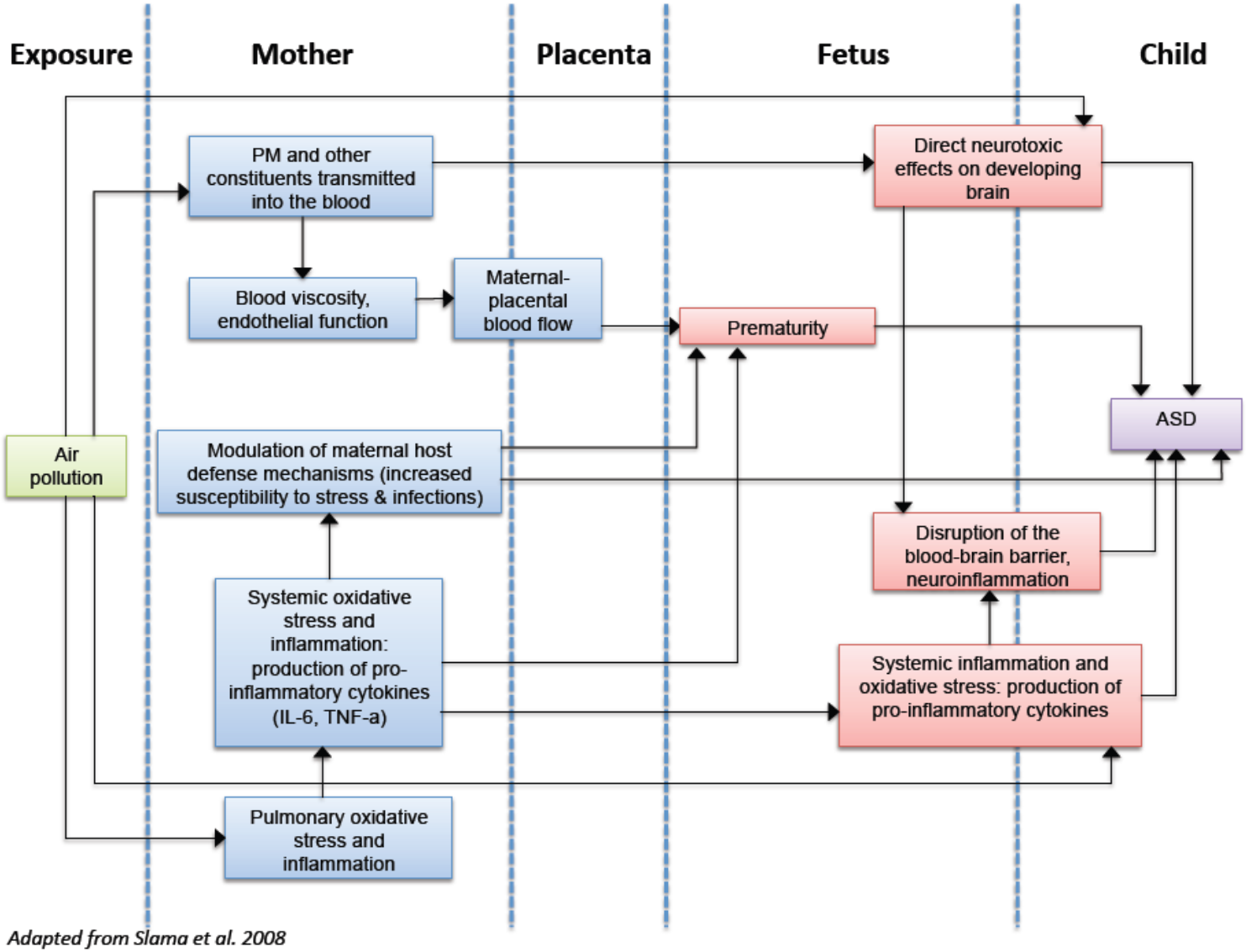


Figure 2.2 Mechanistic pathway for the association between air pollution exposure and autism

2.4 Previous Epidemiologic Research on Air Pollution and ASD

Since 2011, there have been ten studies conducted on the association between traffic related air pollution and/or criteria air pollutant exposure and ASD. These studies, discussed below, reported main effects of the association between air pollution exposure and ASD, and assessed exposures during critical windows of neurodevelopment. All of the previous U.S.-based studies have found positive associations, though studies differed in their exposure assessment, outcome ascertainment, and geographic variability.

Two California-based studies included participants from the CHARGE study and assessed associations using specific measures of traffic related air pollution and regional air pollutant measures. The first study by Volk et al. assessed the association between proximity of residence to freeways and major roadways and autism using 304 cases and 259 controls [168]. Although they made use of a relatively crude exposure measure, their findings showed strong associations between proximity to freeways and autistic disorder. Using data from the same study population, Volk et al. [13] found associations between modeled TRAP exposure, using a line-source air-quality model and measured levels of criteria air pollutants, from EPA's air quality system. Their findings were most significant for particulate matter and modeled TRAP. This same group additionally found interactions between the MET receptor tyrosine kinase CC genotype and air pollution exposure, particularly for NO_2 [169].

Becerra et al. assessed the association between criteria air pollutants and autistic disorder with a LUR model for NO and NO_2 and a nearest monitor approach for the other pollutants [11]. Limitations of the Becerra et al. study include limited geographic variability and use of a nearest monitor approach; strengths include its use of DSM criteria for autism ascertainment and data from 7594 cases. Kalkbrenner et al. used geostatistical interpolation methods to characterize

levels of PM_{10} for 979 cases from California and North Carolina and found stronger associations in the third trimester, which is a potential critical window of susceptibility for the air pollution exposure in relation to ASD [170]. Kalkbrenner's study was the first study to specifically focus on windows of susceptibility for air pollution exposure and ASD. Raz et al. assessed associations with $PM_{2.5}$ using data from the Nurses' Health Study and additionally observed stronger associations for exposure during the third trimester, most significantly when adjusting for the first and second trimester exposures [12]. Finally, the most recent U.S.-based air pollution and ASD study used data from six counties in Southwest Pennsylvania, with cases representing those who scored >15 on the social communication questionnaire (SCQ) or had a written documentation of an ASD diagnosis [14]. Findings from this study strong associations between $PM_{2.5}$ exposure during the pre-conception period through to the second year of life, though the study was limited by a small sample size and reduced geographic variability.

The first non-U.S. based study was conducted in Taiwan and assessed the association between air pollution and ASD 1-4 years before ASD diagnosis [171]. Since the exposure could have likely occurred after the development of ASD, this study was not included in the table of below. Gong et al. [172] conducted the first European-based study and assessed pre- and postnatal air pollution exposure from road traffic and risk of ASD in children from Stockholm. Results from this study showed no associations between air pollution exposure and ASD. Two other European-based studies additionally found null results for the association between NO_x and PM_{10} [173], and NO_2, NO_x, PM_{10}, and $PM_{2.5}$ [174]. Finally, a recent Israel-based study assessed the association between pre- and postnatal NO_2 exposure and found elevated odds of ASD during the first year of life, most significantly when adjusting for pregnancy exposures [175]. Table 2.2 provides a detailed review of the previous air pollution and ASD studies.

Table 2.2 Summary of previous epidemiological studies on early life exposure to air pollution and ASDx`

Author, Year	Location, year of births	Sample Size Case/Control	Study Design and Analytical Method	Outcome Ascertainment	Pollutants	Exposure Assessment	Confounders	Findings
Volk et al, 2011 [168]	California, 1997-2006	304/259	Case-control (part of the CHARGE study). Cases were recruited from the California Department of Developmental Services. Population controls were recruited from the sampling frame of birth files from the State of California.	ADOS + ADI-R Autistic disorder	Traffic-related air pollution	Distance to nearest major road/freeway	Child sex, race/ethnicity, education of parents, maternal age, smoking during pregnancy, SES factors	<309m of a freeway vs. >1419 - OR: 2.22 (1.16, 4.42)
Volk et al, 2013 [13]	California, 1997-2006	279/245	Case-control (part of the CHARGE study). Cases were recruited from the California Department of Developmental Services. Population controls were recruited from the sampling frame of birth files from the State of California.	ADOS + ADI-R Autistic disorder	NO2, ozone, PM_{10}, $PM_{2.5}$	CALINE line-source dispersion (TRAP), spatial interpolation (4 monitors) for NO2, ozone, PM_{10}, $PM_{2.5}$	Child sex, race/ethnicity, education of parents, maternal age, maternal smoking,	TRP during pregnancy – 1.98 (1.20, 3.31), 1st year of life – 3.10 (1.76, 5.57). NO2: 1.81 $PM2_{.5}$: 2.08 PM_{10}: 2.17
Becerra et al, 2013 [11]	California, 1995-2006	7594/75635	Case-control. Cases were recruited from the California Department of Developmental Services. Population controls were recruited from the sampling frame of birth files from the State of California.	DSM-IV-R Autistic disorder	CO, NO2, NO, Ozone, PM_{10}, $PM_{2.5}$	Closest monitor (1), LUR model for NO, NO2	Maternal age, education, race/ethnicity, maternal place of birth, type of birth, parity, insurance type, gestational weeks at birth,	Ozone: 1.12 $PM_{2.5}$: 1.15 LUR-NOx: 1.03 LUR-NO2: 1.09

Author, Year	Location, year of births	Sample Size Case/Control	Study Design and Analytical Method	Outcome Ascertainment	Pollutants	Exposure Assessment	Confounders	Findings
Kalkbrenner et al, 2014 [15]	California and North Carolina; 1994, 1996, 1998, 2000	979/14666	Case-control. Cases recruited from ADDM surveillance. Controls were defined as children in the surveillance system in North Carolina and California. In North Carolina a 15% random sample of births (94, 96, 98, and 00) was conducted and in California a 3% random sample of 1996 births.	DSM-IV-R criteria applied to developmental evaluations ASD	PM10	Bayesian Maximum Entropy (BME)	Year, state, maternal education and age, race/ethnicity, neighborhood level urbanization and median household income, nonparametric term for week of birth (seasonal trend)	3rd trimester PM_{10} – OR: 1.36
Gong et al, 2014 [172]	Sweden, 1992	109/3051	Cohort study (CATSS). 9 years old at outcome ascertainment.	A-TAC Autistic traits/ ASD	PM_{10}, NO_2	Dispersion model	Parity, gender, maternal age, maternal, smoking, SES	PM_{10} -1.01 NOx – 0.92
Raz et al, 2015 [12]	U.S., 1990-2002	245/1522	Nested case-control - cases and controls were recruited from the Nurses' Health Study.	Parental report (ADOS, SRS) ASD	PM2.5, PM10-2.5	Spatiotemporal models	Child sex, year and month of birth, maternal and paternal age at birth, and census income.	$PM_{2.5}$ during pregnancy – 1.63 $PM_{2.5}$ 3rd trimester – 1.42

Author, Year	Location, year of births	Sample Size Case/Control	Study Design and Analytical Method	Outcome Ascertainment	Pollutants	Exposure Assessment	Confounders	Findings
Guxens et al, 2015 [174]	Sweden, Netherlands, Italy, Spain; 1992-2008	541/10769	4 population-based birth cohorts (ESCAPE). Generation R (Netherlands), GASPII (Italy), INMA (Spain), CATSS (Sweden).	A-TAC CAST CBCL SRS Autistic traits	$PM_{2.5}$, PM_{10}, NO_2, NO	LUR Model	Maternal education, maternal smoking & BMI, country, sex, parity, and season	NO2 – 0.94
Talbott et al, 2015 [14]	Penn, USA; 2005-2009	217/226	Population based case control study	SCQ ASD	$PM_{2.5}$	LUR Model	Maternal age, education, race, smoking	$PM_{2.5}$ pre-pregnancy through 2nd year of life: 1.51
Gong et al, 2017 [173]	Stockholm, Sweden; 1993-2007	5136/18237	Case-control. Children with ASD National Patient Register, the Clinical Database for Child and Adolescent Psychiatry in Stockholm, the Habilitation Register, and the Stockholm Regional Health Care Data. Controls were randomly sampled from the same study area as cases.	DSM-IV criteria	PM10 and NOx	Dispersion models	Minimal model: calendar year of birth, municipality of birth, sex, month of birth	PM_{10}: 1.00 NOx: 1.02
Raz et al, 2017 [175]	Israel; 2005-2009	2098/ 54191	Cases were identified through the National Insurance Institute of Israel. Controls were a 20% random sample of the remaining children.	DSM-IV criteria	NO2	Dispersion models	Year of birth, month of birth, population group, paternal age, census poverty index	9 months post birth: 1.40

2.5 Methodological Limitations of Previous Studies

Air pollution exposure was consistently associated with ASD in each of the previous U.S.-based studies, most significantly for $PM_{2.5}$. However, there were inconsistencies with the magnitude of the association, as well as critical windows of susceptibility. There are several methodological issues to consider in air pollution and autism studies, some of which could help explain differences between previous study findings. The main issues include *1)* variations in the exposure assessment used, which may result in exposure misclassification, *2)* differences in the geographic locations of the studies which contributes to the air pollution mixtures to which the study populations are actually exposed, *3)* lack of adequate consideration of potential windows of exposure, *4)* differences in the diagnostic definitions of the outcome, *5)* failure to consider heterogeneity across phenotypic outcomes, and *6)* lack of consideration of potential effect modification by co-occurring air pollutants or social stressors.

Exposure Misclassification

There are three main sources of measurement error in epidemiological studies of air pollution: 1) using aggregate instead of individual data, 2) the difference in the community average personal exposure and the true ambient pollution level, and 3) the difference between measured and true ambient concentration level [176]. The first two types of measurement error are largely Berksonian in nature, meaning that part of the true exposure is measured. The second type is considered classical measurement error, where the exposure measurement includes the true exposure value plus noise. Classical measurement error is more of a concern, since Berksonian types of measurement error often do not induce large amounts of bias [176].

An example of the first type of measurement error is using exposure data from area level air quality monitors instead of personal exposure data, which is the standard approach in air

pollution epidemiology studies [176]. Both monitored and modeled air pollution estimates represent outdoor area level ambient concentrations and do not take into consideration indoor exposures or time spent away from home. Personal biomonitoring captures indoor exposures, but is often not feasible for large-scale epidemiological analyses and thus area level estimates are often used as proxies for individual level exposures. Exposure studies have found area level ambient air pollution measures to be fairly correlated with personal exposure levels. Two studies in particular assessed personal pollutant exposures, using personal exposure monitors affixed to backpacks, and corresponding ambient pollution levels ($PM_{2.5}$, O_3, NO_2, and SO_2) in subjects living in Baltimore and Boston [177, 178]. Findings from these studies showed strong correlations between personal and ambient concentrations, particularly for $PM_{2.5}$, with weaker correlations for the gaseous pollutants (O_3, NO_2, and SO_2). In addition, though the use of ambient concentrations as proxies for personal exposures may introduce error, it may actually help to reduce residual confounding from time varying activity patterns [110].

An example of the second type of measurement error is using aggregate monitored data that does not truly reflect the true ambient pollution level [176]. The use of modeled air pollution estimates may help to reduce measurement error by estimating a more accurate community average exposure. In addition to monitored data, several previous air pollution and ASD studies have used predictions from more advanced air pollution exposure assessment methods, including land use regression models [14, 15, 174].

Multi-Pollutant Models

The air we breathe is a mixture of pollutants and exposure is complex and has synergistic, additive, and antagonistic effects. Therefore, there is growing recognition that greater protection against the adverse health effects of air pollution could be achieved by focusing research and

policy not on individual pollutants, but by a multi-pollutant approach. However, few previous air pollution and ASD studies have considered multi-pollutant models. Becerra et al. assessed associations between multi-pollutant models and autistic disorder and found associations between both ozone and $PM_{2.5}$ and autism, only when models adjusted for both pollutants (ozone OR: 1.12, 95% CI: 1.06, 1.19; $PM_{2.5}$ OR: 1.15, 95% CI: 1.06, 1.24) [11]. Given the potential for pollutants to either confound or modify the effects of other pollutants, the effects from individual pollutants should not be assessed in isolation.

Geographic Variability

Previous studies have additionally been limited in their geographic variability. The majority of previous U.S.-based air pollution and ASD studies have been conducted in California. California contains some of the most heavily polluted areas in the country, thus is an optimal place to study the health effects from air pollutants due to the wide exposure variability. However, generalizability concerns arise when comparing results from studies of the same geographic area, and thus previous results may have been limited in their external validity. Further, constituents of PM vary widely across the country, with higher levels of nitrates on the west coast, and sulfates on the east coast [25]. Therefore, air pollution and autism associations may vary spatially due to the spatial variation in PM components. By studying populations from multiple geographic locations in the United States, this study will be able to assess the range of concentrations and mixtures of pollutants.

Residential Mobility

All of the reviewed air pollution and ASD studies have used residential address at birth to represent the entire pregnancy period and first year of life. Using only the residential address at birth assumes limited mobility during pregnancy. Thus, there is potential for exposure

misclassification by using incomplete residential information. However, several previous studies have shown little change in exposure assignment when using the birth address versus incorporating the complete residential history during pregnancy [179, 180], although one study did show somewhat greater exposure misclassification for the pregnancy period than for first year of life [181]. Further, if women in these studies spent more time away from home during the earlier pregnancy periods [182], then the modelled exposure estimates may not have been an accurate proxy for their actual exposure during these periods [110]. Therefore, it is possible that exposure misclassification for earlier pregnancy periods has attenuated effect estimates for these windows in previous studies.

Finally, most previous studies have found that residential mobility generally decreased with increasing age, parity, and socioeconomic status [179, 183]. Thus, older women of higher SES may be less mobile and consequently have lower exposure misclassification than younger women of lower SES. This is important to consider in air pollution and ASD studies given the complicated association with both advanced parental age and SES.

Windows of Susceptibility

The prenatal period and first year of life appear to be the most important developmental windows in relation to onset of ASD, though exact critical windows of susceptibility to exposure remain largely unknown. Three studies directly aimed to examine the research question with a specific focus on windows of susceptibility [12, 14, 15]. Two of these studies found stronger associations during the third trimester, only when adjusting for other trimesters of exposure [12, 15]. Trimester average exposures are correlated with exposures during the other trimesters. Thus, in order to isolate effects during a specific trimester, exposures during the other trimesters should also be included in the model [110]. This is important to consider when examining windows of

susceptibility as results could be misinterpreted if correlations with other windows of exposure are not considered. The other included study found stronger results for exposures averaged from preconception through to the second year of life, thus showing more of a cumulative effect [14]. It is likely that some pollutants, such as ozone, may have more acute effects during trimesters, whereas other pollutants, such as $PM_{2.5}$ may have more cumulative effects on risk of ASD.

Outcome Ascertainment and Phenotypic Subgroups

Several of the previous studies used different diagnostic definitions for the outcome. Guxens et al. [174] pooled study results from several European-based studies and assessed broader autistic traits, instead of requiring a documented ASD diagnosis, as was done in several of the U.S.-based studies. Each cohort in the Guxens et al. study used a different diagnostic test, and each particular measure classified autistic traits differently, thus making it difficult to compare findings across study sites. The authors noted that only a few cases in the study population would have actually been classified as having ASD, and that the cases did not represent the phenotypic extreme. It may be that air pollution could be related to ASD in general or more severe forms of ASD, but not broad autistic traits, as was seen in this study. Further, Gong et al. additionally found null results and also using a broad screening tool (A-TAC) [172]. One study that assessed the predictive ability of A-TAC found that of those screened positive for ASD using the A-TAC, less than half of them actually went on to be clinically diagnosed with ASD [184]. Thus, several of those included in the Gong et al. study most likely either did not have ASD or had broad autistic traits, as was the case in the other European-based study.

The DSM-4 criteria separated autistic disorder, Asperger's disorder, childhood disintegrative disorder, and pervasive developmental disorder, but with the new DSM-V criteria these disorders are now grouped into the autism spectrum disorder umbrella [95]. Autistic

disorder is considered the most severe of the autism spectrum disorders. Four of the previous air pollution and autism papers used autistic disorder cases [11, 13, 168, 169], while the remaining six included ASD cases or those with autistic traits [12, 14, 15, 171, 172, 174]. It is possible that more severe cases could have different response to air pollution than high-functioning autism cases. Additionally, it is possible that air pollution may be associated with ASD but not broad autistic traits, which may give insight into why the European studies found null results with their use of screening tools that assessed broad autistic traits.

ASD is a complex heterogeneous disease and symptoms vary widely. Previous air pollution and ASD studies have assessed associations in milder cases as in the European populations [172, 174] and potentially in more severe cases in other studies using the strict autistic disorder outcome definition [11, 13], but no studies have really assessed associations using phenotypic subgroups (ranging from mild to severe) in ASD cases of the same population. One previous study has assessed the effects of air pollution by phenotypic subgroups in ASD cases [15]. This one study found consistent results across phenotypic subgroups (co-occurring intellectual disability), though noted the potential for misclassification and small sample sizes. Therefore, further analyses are needed that include phenotypic subgroups in ASD cases in order to identify any potential heterogeneity of associations by severity subgroups.

Consideration of SES and Neighborhood Deprivation

There is an apparent spatial correlation with many environmental toxicants and social stressors, and in general both of these exposures tend to cluster in areas of low SES [185]. Specifically, individuals of lower SES are disproportionately exposed to higher levels of air pollution, from major highways and point sources, and social stressors, including violence and noise [186, 187]. Thus, individual SES and area level deprivation may have both confounding

and modifying effects on air pollution-health associations. Specifically for ASD, lower SES is positively associated with area level air pollution exposure, however individuals of low SES are often under diagnosed for ASD, thus is negatively associated with ASD ascertainment [186, 188, 189].

The role of SES may be different for U.S. and European-based air pollution and ASD studies. Guxens et al. [174] noted that the differences in study findings might be due to the fact that some of the U.S. studies may have had a diagnostic bias related to SES differences in access to care, and this may not have been an issue in the European cohorts. The children in the European cohorts were on average of lower SES, whereas many of the kids with autism in the U.S. studies were more likely to come from higher SES backgrounds. As noted, several of the populations in the European ASD studies came from areas with national health care systems, therefore access to care should not vary by SES in these study populations. Thus, it is important to take into consideration SES and access to care differences when assessing air pollution and ASD associations, particularly within the U.S.

In addition to confounding air pollution and ASD relationships, individual and area level SES may modify the association between air pollution and ASD. Neighborhood deprivation is one measure of area level SES and can contribute to overall chronic stress and increased susceptibility [190]. Social theories have described this relationship between social and environmental stressors with one in particular describing that stressors at the neighborhood level can contribute to individual chronic stress, chronic individual stress can influence individual susceptibility, and this stress-borne physiologic susceptibility may then shape response to environmental exposures [185, 191]. Chronic stress from neighborhood deprivation could influence individual susceptibility and shape maternal responses to air pollution exposure. This

susceptibility could impair the body's ability to maintain allostasis, compromise immune function, and indirectly increase risk of health outcomes in the mother or offspring [185, 192].

Figure 2.3 shows the proposed mechanistic pathway linking neighborhood deprivation, air pollution, and ASD. Repeated chronic stress from neighborhood deprivation may illicit a maternal inflammatory response and alter hypothalamic pituitary adrenal (HPA-axis) function, increasing levels of pro-inflammatory cytokines and subsequently influencing maternal and fetal susceptibility [185]. This inflammatory reaction can sensitize microglia in the mother and offspring, predisposing them to an overactive inflammatory response. Thus, when exposed to an environmental stressor, such as air pollution, the individual and offspring may be physiologically susceptible. The pro-inflammatory cytokines produced from this inflammatory response can reach the developing brain and induce neuroinflammation, increasing risk of ASD [8].

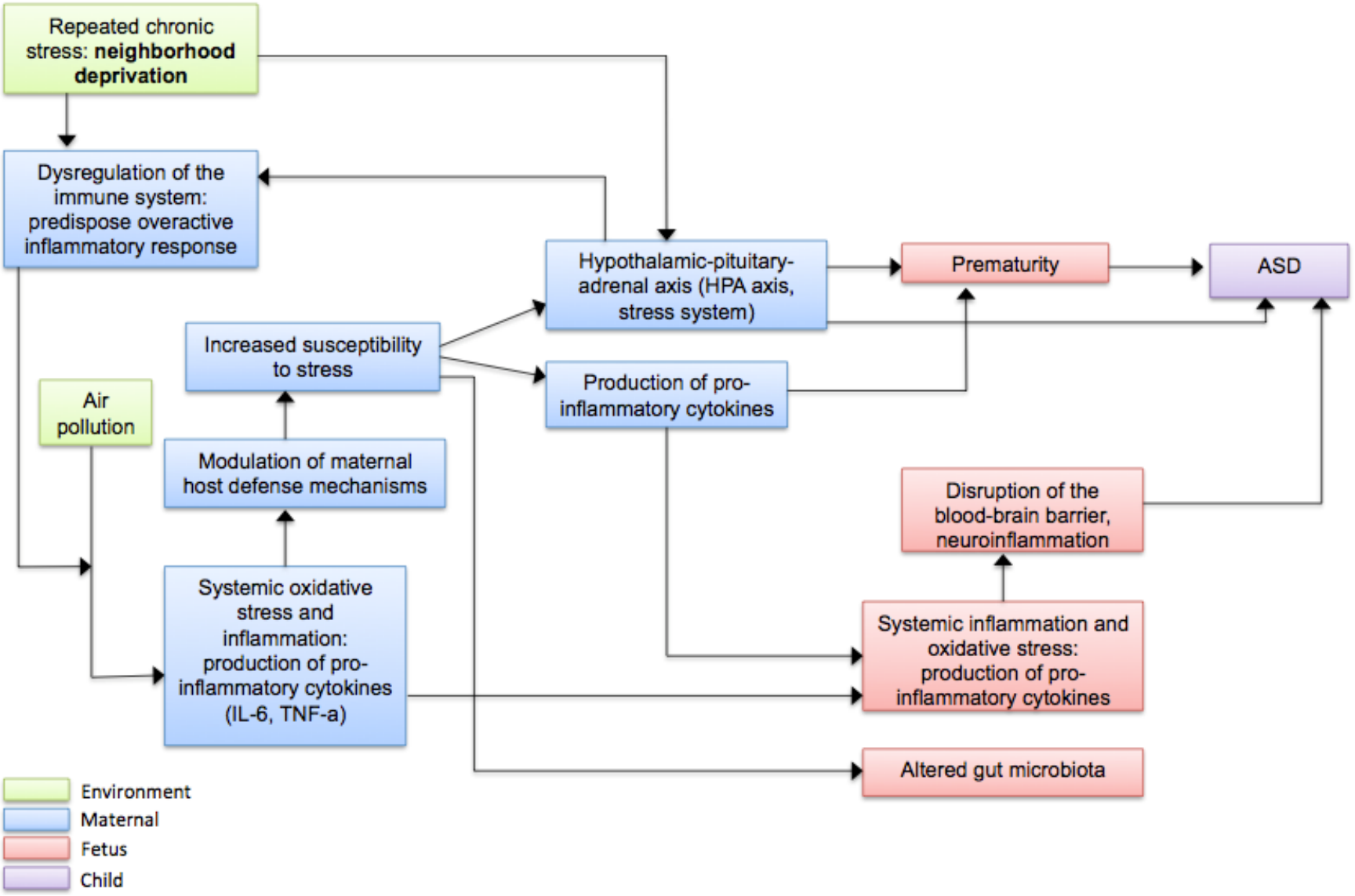


Figure 2.3 Mechanistic pathway leading neighborhood deprivation and air pollution to ASD in children

Few studies to date have examined whether neighborhood deprivation can modify air pollution and ASD associations, however previous epidemiological studies have shown that psychological stress and social disadvantage can modify air pollution-health associations in general [193-197]. One recent epidemiological study found that neighborhood SES modified the association between air pollution exposure and neural tube defects [198]. Another study used principal components analysis to reduce census data at the census tract level to a continuous neighborhood deprivation index [197]. Findings from their study showed modification of the nitrogen dioxide and birth weight association by neighborhood deprivation. Thus, a deprivation index developed from census variables may be a useful tool in order to characterize neighborhood deprivation [199].

One animal study found synergistic effects between maternal chronic stress and air pollution exposure on adverse mental health outcomes in the offspring [200]. Another recent study found increased levels of systemic inflammatory biomarkers (including CRP) only when the rats were exposed to both chronic stressors and air pollution together [201]. Results from these studies show that repeated chronic stress can therefore compromise immune function in mothers and make them, and their developing fetus, more susceptible to the effects of air pollution exposure.

2.6 Significance and Innovation

Significance

Studies demonstrate the substantial health loss across the lifespan for individuals with ASD [202]. There are also economic losses to society, with the lifetime economic costs of autism estimated to be around $3.2 million per individual [203]. This estimate will increase given the increasing prevalence of ASD from 2006 to present. In addition, studies indicate that children

with ASD have almost nine times the healthcare costs of other children eligible for Medicaid [204]. Thus, ASD can have implications not only for individuals with ASD and their families, but also for the society as a whole. Given the rising prevalence and impact, there is a need to identify risk factors for ASD. Air pollution is one ubiquitous and modifiable suspected risk factor. Identifying air pollution as a risk factor for ASD could aid in primary prevention and have substantial policy implications, given the need to set air pollution standards, particularly for susceptible populations.

Study Innovation

Previous air pollution and ASD studies have been limited in sample size, geographic variability, outcome ascertainment methods, assessment of windows of susceptibility, and heterogeneity of associations. This study addresses these limitations by using data from the Study to Explore Early Development, the largest study to date in the United States to help identify risk factors that may put children at risk for ASD and other developmental disabilities. SEED has six study sites located throughout the country, thus providing adequate spatial variability to assess the effects from air pollution exposure.

Multiple windows of susceptibility were assessed, modeled separately and together to isolate critical windows of exposure. There could be spatial variability in the effect of air pollution on the risk of autism, and this may be due to the spatial variation in $PM_{2.5}$ components. Finally, this study assesses the effect of air pollution across ASD severity groups and the modifying effect of neighborhood deprivation on the air pollution and ASD association, which are both important in order to identify particularly susceptible subgroups.

CHAPTER 3: METHODS

This study investigated the association between early life air pollution exposure and ASD using a well-characterized, diverse study population. Specific Aim 1 investigates the association between $PM_{2.5}$ and ozone exposure and ASD in the child. To identify potential windows of susceptibility that may be most important for toxicant exposure, this study examines exposure three months prior to pregnancy, over the entire pregnancy period, as well as more refined periods of exposure reflecting each trimester of pregnancy and the first year of life. Aim 1a assesses if the association between air pollution and ASD varies by level of severity. Specific Aim 2 of the dissertation investigates whether neighborhood level stressors modify the association between air pollution and ASD.

3.1 Study Design and Population

The Study to Explore Early Development is a multi-site case-control study that aims to identify risk factors for ASD and other developmental disabilities. SEED has wide spatial variability with site locations in California, Colorado, Georgia, North Carolina, Pennsylvania, and Maryland. These six SEED sites are part of the Centers for Autism and Developmental Disabilities Research and Epidemiology (CADDRE) network [205]. Individuals were eligible to participate in SEED if they were born in a study site catchment area during 2003-2006 and still resided there at 30-68 months of age. In additional to high spatial variability of participants, SEED also has diverse racial, ethnic, and socioeconomic distribution.

SEED includes children aged 2-5 from three different case and control groups: 1) children with clinically confirmed ASD (n=707), 2) children with a non-ASD developmental delay (DD n=995), and 3) general population controls (POP n=898). ASD cases were those children meeting study definitions for ASDs, including autistic disorder, pervasive developmental disorder-not otherwise specified, and Asperger syndrome. The current dissertation focuses on the ASD and POP groups.

3.2 Data Collection

Data were collected through birth certificates, medical records, caregiver interviews, mailed questionnaires, and in-person clinic and home visits. Maternal medical records were collected for the preconception, prenatal, and labor/ delivery periods. These records were collected in order to collect information on prenatal, perinatal, and postnatal medical information. Medical records of the child were additionally collected for the neonatal and pediatric periods. The following information was collected from the mailed questionnaire: paternal medical history, maternal medical history, autoimmune history, gastrointestinal information, paternal occupational history, services and treatment history, and early development information (ASD only). The following data was collected during the caregiver interview: biologic mother's full reproductive and pregnancy history and diagnosed developmental outcomes in all children; medical, therapeutic, obstetric and lifestyle characteristics of pregnancy (considered 3 months before conception through breastfeeding) with index child (including infertility treatments of either biological parent); maternal occupational history during index pregnancy [205]. All data collected are stored at the data coordinating center in Michigan.

3.3 Case Ascertainment and Definition

Both potential ASD and DD children were ascertained through multiple sources serving

or evaluating children with developmental problems, including: early intervention, special education, hospitals, clinics, and individual providers. Parents with children with ASD were additionally able to contact the study if the child had proper documentation of ASD or ASD-related diagnosis. The multisource ascertainment of ASD and DD cases reduces potential selection biases often seen in other studies that recruit cases from single facilities. Population controls were identified by randomly sampling state vital records of children born in the specified date range to mothers that resided in the study catchment area at the time of delivery. The goal for recruitment was to have at least a 1:1 ratio of ASD to POP children.

Families in the ASD, DD, and POP recruitment groups were sent a written invitation (in English and Spanish) to participate. Caregivers who were interested in participating returned a positive response card and received a follow-up telephone call to assess their eligibility. Nonresponders received a second invitation letter 2-6 weeks after the initial contact or a follow-up invitation call. After the initial eligibility check, the Social Communication Questionnaire (SCQ) was given to the primary caregiver in order to assess the behavior of their child and evaluate whether clinical diagnosis was needed to determine final ASD status of their child [206].

A positive SCQ screen was defined as a SCQ score $\geq$11 [207]. Any children who had a positive SCQ screen or previous ASD diagnosis or who were in services for ASD were placed in the ASD workflow group. The term workflow is used throughout to describe the process of case ascertainment. DD and POP with negative SCQ screens were assigned to the DD or POP workflow. If a clinician suspected a child in the DD and POP workflow to have ASD they were then moved to the ASD workflow. Figures A.1 and A.2 in Appendix A show the workflow for the ASD and POP groups. For the ASD and DD recruitment groups, 64% of the families did not

respond to the written invitation or invitation call. For the POP recruitment group, 68% of the families did not respond to the written invitation or invitation call.

Gold standard instruments were used to confirm ASD cases, including 1) the autism diagnostic observation schedule (ADOS) and the 2) the autism diagnostic interview-revisited (ADI-R). The ADOS is a standardized, semi-structured assessment of communication, social interaction, and play (or imaginative use of materials) [208]. The ADI-R is a standardized interview with the primary caregiver. The ADI-R obtains information about child's developmental and behavioral history (communication, social interaction, patterns of behavior) [209].

Final ASD classification was based off of meeting criteria on both ADOS and ADI-R, or from meeting ADOS criteria and one of three relaxed criteria for the ADI-R. The relaxed ADI-R criteria include: 1) the child met the cutoff score on the ADI-R social deficits domain and was within two points of the cutoff score of the communication deficits domain, 2) the child met the cutoff score on the ADI-R communication deficits domain and was within two points of the cutoff score of the social deficits domain, and 3) the child met the cutoff score on the ADI-R social deficits domain and had at least two points noted on the behavioral domain [210, 211]. The other classification groups were as follows: DD, POP, incomplete classification. Children in the incomplete classification group were excluded from analyses.

Intellectual Disability, ASD Severity, and Phenotypic Subgroups

All children participated in a general developmental assessment that included Mullen Scales of Early. Children who did not have an indication of possible ASD (negative SCQ screen, no previous ASD diagnosis and no ASD-specific service classification) received only this

general developmental assessment. Children with a Mullen Early Learning Composite Score of less than 70 were classified as having intellectual disability [212].

ASD severity was additionally assessed using the ADOS calibrated severity score (CSS). This score was calculated using the ADOS total score, ADOS language level, and age at evaluation. Recent studies have found this score to be the most representative of the core features of ASD in pre-school children [213]. The ten-point scale was dichotomized into low/moderate (scores 4-7) and more severe (scores of 8-10).

Phenotypic subgroups of ASD were developed using latent class analysis that considered behavioral and medical characteristics that, based on statistical modeling, segregate specific subgroups of ASD, as described in Wiggins et al. (2017b). The four subgroups include 1) *Mild language delay with cognitive rigidity* (average motor and nonverbal functioning, mild impairments in expressive language, increased cognitive rigidity, and unusual sensory responses), 2) *significant developmental delay with repetitive motor mannerisms* (significant developmental delays, including both delayed verbal and motor development, prominent repetitive monitor mannerisms, and unusual sensory responses), 3) *general developmental delay* (high-moderate social-communication difficulties, significant developmental delays, and unusual sensory responses), and 4) *mild language and motor delays with dysregulation* (average nonverbal functioning, mild impairments in language and motor skills, increased cognitive rigidity, and high rates of problem behaviors).

3.4 Exposure Assessment

Residential History and Geocoding

Residential address at birth was used to represent the entire pregnancy period and first year of life. Using only the residential address at birth assumes limited mobility during

pregnancy. Therefore, there is potential for exposure misclassification by using incomplete residential information. However, studies suggest that air pollution exposure assignment does not change greatly when using complete residential history during pregnancy, therefore using address at birth should not substantially bias results [179, 180].

Births and their corresponding pregnancy addresses were identified using electronic birth certificate data from the State of California, North Carolina, Georgia, Pennsylvania, Maryland, and Colorado. SEED births occurred between 2003 and 2006, therefore exposure data was collected from 2002 through 2007. Addresses at birth were geocoded using ArcGIS and latitude/ longitude coordinates for each participant were outputted [214]. Date of births and geocoded addresses for each site were stored on a secure server with limited access.

Exposure Sources

Distance to Nearest Major Road

Proximity to nearest major road/highway captures long-term local variation in exposure to traffic related air pollutants. Spira-Cohen et al. found significant linear associations between distance to roadway and elemental carbon concentrations from personal monitors, thus providing rationale for the use of roadway proximity metrics as an appropriate proxy for TRAP exposure [215]. This study used distance to major road/highway to capture the mixture of chemicals from traffic related air pollution. Road networks for the entire U.S. were obtained from ESRI StreetMap. U.S. major roads include U.S. and state highways, major streets, and other major thoroughfares within the United States. Local residential roads are not included in these analyses. Each participant's address at birth was matched to the nearest major road/highway using ArcGIS [214]. Specifically, using the road network shapefile for each study area and geocoded coordinates of participants, the near function in ArcMap was used to find the nearest major

road/highway to each participant. The resulting output file had a continuous distance metric for each participant.

$PM_{2.5}$ and Ozone Predictions

A thorough review of all available air pollution exposure assessment methods was conducted. This dissertation used data from a satellite-based hybrid model that combines GEOS-Chem predictions, monitored and satellite data, land use terms, and meteorological variables, as this was the optimal exposure assessment method identified from the review. GEOS-Chem is a global 3-D chemical transport model that simulates atmospheric chemistry. GEOS-Chem is driven by meteorologic input from the Goddard Earth Observing System of the NASA Global Modeling and Assimilation Office [36]. GEOS-Chem models provide temporally resolved estimates of particle concentrations, however the predictions are poorly correlated with monitored data, thus predictions were calibrated using monitored data [37]. Land use terms (percentage of urban areas, population density, road density, and elevation), meteorological variables (such as air temperature and precipitation), and satellite data were used to help calibrate GEOS-Chem outputs and to aid in downscaling to a smaller spatial resolution. Aerosol optical depth (AOD) is a measure of the amount of light the particle prevents from travelling through the atmosphere. AOD are monitored using the Moderate Resolution Imaging Spectroradiometer aboard NASA's Terra and Aqua Satellites.

Finally, neural network analysis was used to calibrate the various input sources and concentrations were predicted for 1x1km grid cells. Predictions are available at a daily temporal resolution and a 1x1 km spatial resolution.

Assigning Exposures to Pregnancy and Creating Exposure Windows

Participants were matched to the centroid of the nearest 1x1km grid cell based on their

residence at birth. Participants were also linked to the census tract they reside in in order to link individuals with 2000 census data. Table 3.2 shows the exposure periods of interest. Exposure estimates were created for several pregnancy periods, including the 1st, 2nd, and 3rd trimester, as well as 3 months before conception, entire pregnancy period and year post birth.

Table 3.1 Developmental windows of interest

Developmental Window	Exposure Length
Preconception	3 months prior to conception through 1 day before conception
Trimester 1	Weeks 1 through 12 of pregnancy
Trimester 2	Weeks 13 through 28 of pregnancy
Trimester 3	Weeks 29 through 40 of pregnancy
Entire pregnancy	Conception to date of birth
Year post birth	Date of birth + 365 days

Due to privacy concerns, the CDC randomly shifted each participant's date of birth by different increments of up to two weeks in either direction. Because of this shifting, this study did not use more refined windows of exposure, including weekly or monthly.

Neighborhood Deprivation Index

Both individual SES and neighborhood level deprivation may result in chronic maternal stress, which could influence individual susceptibility and shape responses to environmental stressors, such as air pollution. Neighborhood level deprivation could potentially influence individual stress, resulting in increased susceptibility, and ultimately have the potential to modify the association between air pollution and ASD. Specific Aim 2 of the dissertation focuses on this modifying potential of neighborhood deprivation on the air pollution and autism association.

Neighborhood level deprivation was characterized using a neighborhood deprivation index measure originally developed by Messer et al [199]. This neighborhood deprivation has previously been used to describe relationships between neighborhood deprivation and low birth

weight, SGA, and preterm birth [216-218]. Area level SES data was obtained from the U.S. Census at the census tract level and used to create a neighborhood level deprivation index. The census tract is generally well accepted to be suitable for neighborhood level analyses [219]. Data was obtained from the 2000 U.S. Census since SEED births occurred between 2003-2006.

Census variables are often correlated; thus, it is difficult to interpret results from multiple census variables. Thus, census data from all sites were pooled and principal components analysis (PCA) was used to develop a neighborhood deprivation index from eight variables from the following domains: education, employment, housing, occupation, and poverty. Table 3.2 shows the eight variables of interest and corresponding domains. PCA is a data reduction technique, with loadings representing the correlation between the components [199]. The first principal component was retained because it accounted for the largest proportion of the total variability in the component measures.

SES related variable values were weighted according to final factor loadings to create a continuous index score for each census tract. The index score was standardized by dividing the index by the square of the eigenvalue, resulting in a deprivation index with a mean of 0 and a standard deviation (SD) of 1. Higher values of the NDI indicate higher levels of neighborhood disadvantage. Census tracts of the SEED study areas were categorized as having high, moderate, or low deprivation based on tertile cutpoints of the continuous index. The deprivation index was linked to SEED participants based on the birth residence census tract.

Table 3.2 Neighborhood level U.S. Census SES indicators

Neighborhood level SES category	Census indicator
Education	• Percent males and females with less than a high school education
Employment	• Percent males and females unemployed
Housing	• Percent crowded
Occupation	• Percent males not in management and professional occupations
Poverty	• Percent households in poverty • Percent female headed households with dependent children • Percent households earning under $30,000 per year • Percent households on public assistance

3.5 Covariate Assessment

Figure 3.1 shows the directed acyclic graph (DAG) for the association between air pollution exposure and autism. Exposure to air pollution is in part determined by location of maternal residence. Socioeconomic factors are one of the drivers of choice of maternal residence and many of these socioeconomic factors are also associated with ASD. The main individual SES factors include maternal and paternal education, household income, marital status, and race/ethnicity. Diagnosis of ASD may vary by race/ethnicity [220] and there are racial differences in exposure to air pollution [187]. Maternal and paternal age are both associated with ASD in children and are related to residential location [123].

Maternal infections during pregnancy have previously seen to be associated with onset of ASD in children, and are partly driven by seasonal factors [130]. Additionally, previous studies have shown associations between both season of conception and birth and autism, with some citing higher risk of ASD for conception during winter months [221], while others have reported higher risk of ASD when born during the spring [222, 223]. Therefore, this study controlled for month of birth to control for seasonal trend. Air pollution levels and diagnoses of ASD may vary both spatially and temporally, thus both year and state of birth were included as covariates. Ambient pollution concentrations are serving as proxies for personal exposure concentrations. Thus, although time varying factors can influence personal exposure concentrations, it is unlikely that a time varying factor would impact ambient concentration levels [110]. Concentrations of other pollutants may influence both personal and ambient pollution levels. For instance, ozone may confound relationships between $PM_{2.5}$ and ASD, thus analyses will consider multi-pollutant models.

Body mass index has previously been seen to be associated with both air pollution

exposure and place of residence, though the exact causal nature is uncertain. Place of residence can influence maternal BMI, as living in an area with few walking paths could reduce exercise and increase obesity. Air pollution exposure can also initiate an inflammatory response and therefore additionally directly influence obesity. Maternal body mass index is also directly associated with ASD [125] and indirectly through other metabolic conditions such as diabetes. Therefore, maternal pre-pregnancy BMI is important to consider when assessing the air pollution-ASD association, though its role as a confounder, mediator, or modifier is uncertain. Child's sex is a strong predictor of autism, however is not associated with maternal residence or air pollution exposure. Air pollution exposure is associated with preterm birth and preterm birth is a strong risk factor for autism, therefore preterm birth will not be controlled for due to its potential mediating role in the association between air pollution and ASD.

The minimally sufficient adjustment set identified from the DAG includes: maternal age (continuous), maternal education (<bachelors, ≥bachelor's degree), race/ethnicity (non-Hispanic white, other), month of birth to account for seasonal trends (categorical), study state of birth (CA, CO, GA, MD, ND, PA), study year (2002-2003, 2004-2005), and maternal smoking (yes, no). Table 3.3 discusses the included covariates further, including their data source, rationale for inclusion, coding, and role in analyses.

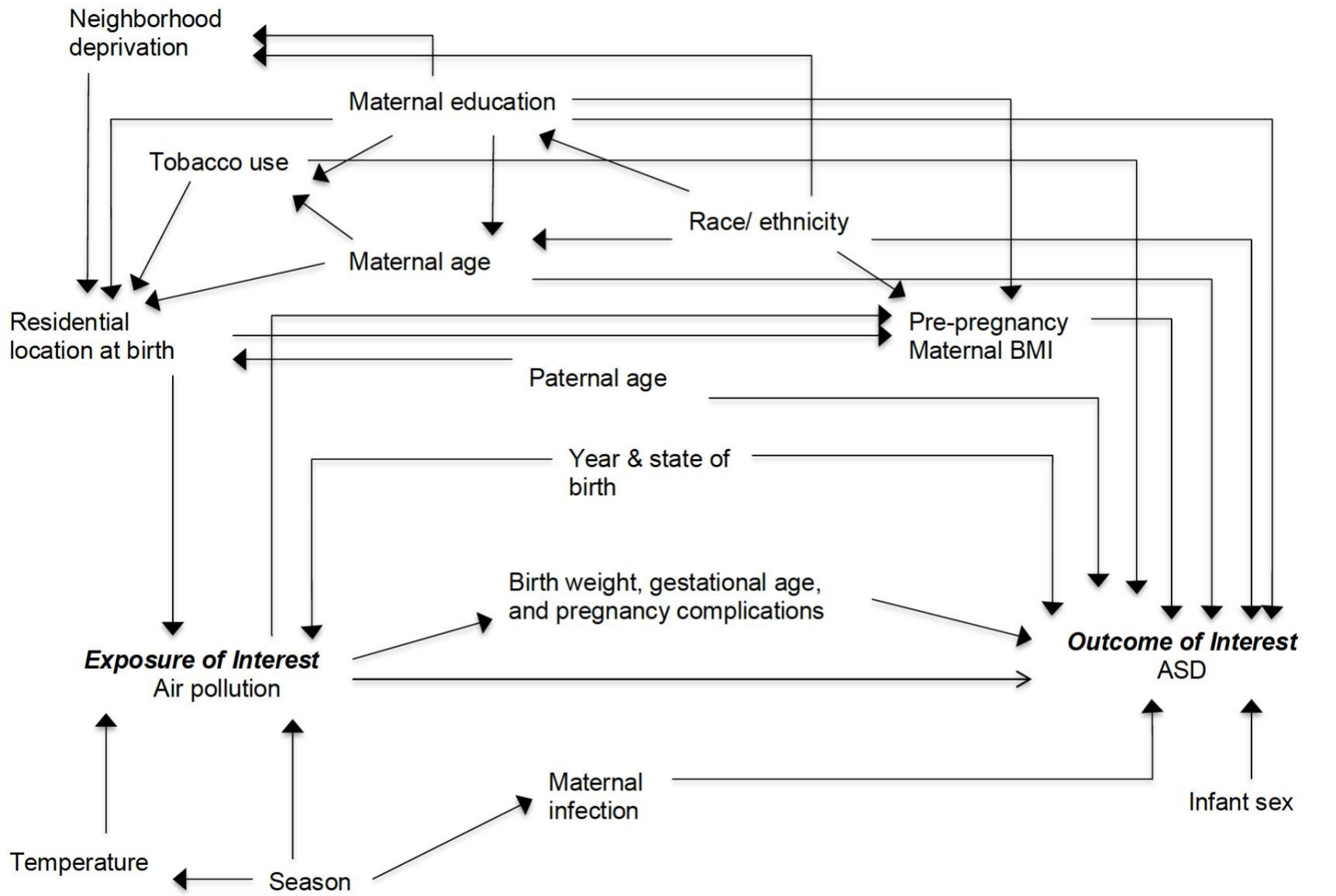


Figure 3.1 Directed Acyclic Graph of the relationship between air pollution exposure and ASD

Table 3.3 Confounders and effect modifiers of the relationship between air pollution exposure and ASD

Variable	Source	Rationale	Coding	Confounder/ modifier
Maternal age at delivery	Birth certificate, caregiver interview	Controlled for in previous AP-ASD studies, part of the minimally sufficient adjustment set from the DAG	Continuous	Confounder
Maternal education (proxy for SES)	Caregiver interview		<bachelors, ≥bachelor's degree	Confounder
Child's year of birth	Birth certificate		2002/2003 2004/2005	Confounder
Child's state of birth/ study site	Birth certificate		North Carolina California Georgia Pennsylvania Maryland Colorado	Confounder
Race/ethnicity	Birth certificate, caregiver interview		Non-Hispanic White, Other (Non-Hispanic Black, Hispanic, Asian, multiracial, and all others)	Confounder
Season of birth	Birth certificate	Associated with ASD, AP levels vary by season. Part of the minimally sufficient adjustment set	Month of birth (categorical)	Confounder
Maternal tobacco use during pregnancy	Caregiver interview	Controlled for in previous air pollution and ASD studies, part of minimal adjustment set	Yes/No	Confounder/ potential modifier
Neighborhood deprivation	Derived from geocoded address at birth (birth certificate), linked to census data	The association between air pollution and ASD may be modified by area level neighborhood deprivation	Continuous from PCA – categorized as high, medium, and low deprivation	Potential effect measure modifier

3.6 Data Analysis

Specific Aim 1 Analyses

Specific Aim 1 investigated the association between early $PM_{2.5}$ and ozone exposure and ASD. This aim examined exposure three months prior to pregnancy, over the entire pregnancy period, as well as more refined periods of exposure reflecting each trimester of pregnancy and the first year of life. Figure 3.2 shows the windows of exposure of interest, ranging from before conception through to one-year post birth.

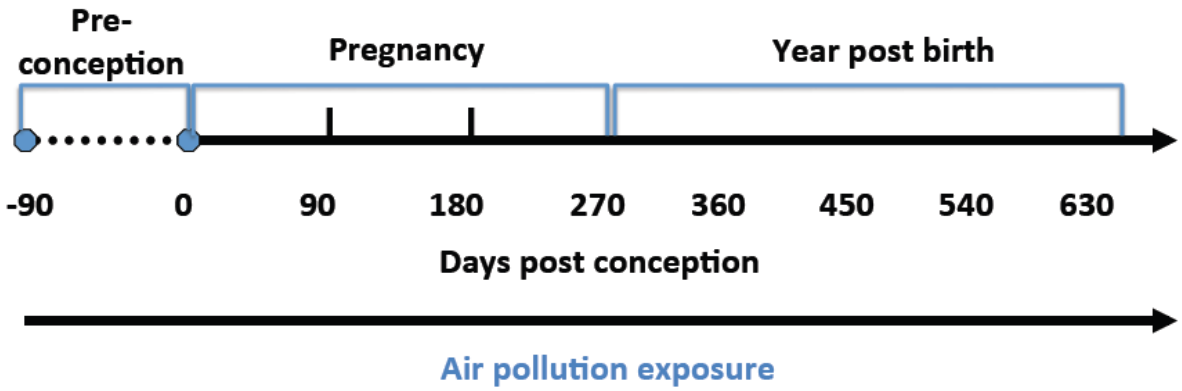


Figure 3.2 Windows of susceptibility for early life exposure to air pollution

Unconditional logistic regression was used to estimate odds ratios (OR) and corresponding 95% confidence intervals (CI) for the associations between air pollution and ASD, with the population group serving as the control group for all analyses [224]. Since the main hypotheses target associations with autism spectrum disorder specifically, analyses did not use data from the developmental delay group and instead focused on the ASD group compared to population controls.

Descriptive statistics were first reported for all variables. The minimum, maximum, median, 25^{th} and 75^{th} percentiles were reported for air pollution exposures and distributions were

compared across pollutants. Correlations were assessed for $PM_{2.5}$ and ozone for each of the included exposure windows.

Natural cubic splines were initially used to assess the linearity of the associations. $PM_{2.5}$ and ozone exposures were analyzed as continuous measures, as this provided a better fit than categorical coding, as indicated by the lower Akaike Information Criterion value. Effect estimates were rescaled to the interquartile range (IQR) value for the entire pregnancy period, averaged across study sites. This approach allows the comparison of effect estimates across pollutants, study sites, and developmental windows and ensures the unit increases are within the exposure variability of each study site.

Effect measure modification (EMM) by study site was assessed for each of the developmental windows in order to investigate possible heterogeneity in the $PM_{2.5}$ and ozone and ASD associations by geographic location. Likelihood ratio tests were conducted by comparing models with and without interaction terms for study site and an alpha of $p<0.10$ was used as a threshold for presence of modification on the multiplicative scale.

Sensitivity Analyses

Ozone may confound relationships between $PM_{2.5}$ and ASD [225], thus results were reported from multi-pollutant models for each developmental window by controlling for the other pollutant in each exposure model. Results were additionally reported for each developmental window mutually adjusted for the other windows in order to isolate potentially critical windows of susceptibility. Specifically, results are presented for each trimester mutually adjusted for the other trimesters; and for the preconception, pregnancy, and postnatal windows mutually adjusted for the other developmental windows. Finally, analyses evaluated if the associations between $PM_{2.5}$ and ozone and ASD varied by maternal tobacco use.

Specific Aim 1a: ASD Severity and Co-Occurring Intellectual Disability

Specific Aim 1a investigated if $PM_{2.5}$ and ozone and ASD associations differed for children with ASD depending on the presence of co-occurring intellectual disability. Next, this aim compared associations by ASD severity using a dichotomized ASD severity score (Wiggins et al. 2017a). The ten-point scale was dichotomized into low/moderate (scores 4-7) and more severe (scores of 8-10). Multinomial logistic regression was used to compare the four ASD phenotypic subgroups and the two severity subgroups to the population controls.

Specific Aim 2 Analyses

Specific Aim 2 evaluated whether neighborhood level social stressors modified the association between air pollution exposure and ASD. This aim was accomplished by creating a neighborhood deprivation index to characterize neighborhood level social stressors. The neighborhood deprivation index was categorized as tertiles with categories of high, moderate, and low deprivation. In addition to assessing the modifying effect of NDI, analyses assess the spatial distribution of the neighborhood deprivation index by study area and compare exposure distributions with the distributions from the air pollution exposure metrics.

Logistic regression was used to estimate odds ratios (OR) and corresponding 95% confidence intervals (CI) for the associations between roadway proximity, $PM_{2.5}$, and ASD, with the population group serving as the control group for all analyses. Results are first reported for the main effects of each of the exposures in relation to ASD. Distance to major roadway was dichotomized at the 10^{th} percentile level in controls (<45m vs. ≥45m). $PM_{2.5}$ exposure during pregnancy and first year of life was modelled continuously and also dichotomized at the National Ambient Air Quality Standard (NAAQS) level of 12.0 $\mu g/m^3$ (≥12.0 $\mu g/m^3$ vs. <12.0 $\mu g/m^3$).

Effect measure modification by neighborhood deprivation was first evaluated on the

multiplicative scale for continuous measures of $PM_{2.5}$ exposure, and categorized measures of distance to roadway (<45m vs. ≥45m) and $PM_{2.5}$ exposure (≥12.0 μg/m^3 vs. <12.0 μg/m^3). Departure from multiplicatively was evaluated by including an interaction term between the deprivation index and exposure metrics and compared models with and without interaction terms. Multiplicative interaction was assessed using the likelihood ratio test, with a significance level of 0.10.

EMM was additionally evaluated on the additive scale by constructing single-referent models for each of the categorized exposures and computed the relative excess risk due to interaction (RERI) for each exposure [226]. Corresponding 95% confidence intervals were calculated using the delta method [227].

Protection of Human Subjects

The study was initially approved by the IRB at each of the six SEED study sites. Once exposure linking is complete, all data were de-identified and all date of births and geocoded coordinates were stored separate from the covariate and outcome data. Before analyses are initiated, I will apply for IRB approval from UNC for non-human subjects research.

CHAPTER 4: EARLY-LIFE AIR POLLUTION EXPOSURE AND AUTISM SPECTRUM DISORDER: FINDINGS FROM THE STUDY TO EXPLORE EARLY DEVELOPMENT

4.1 Introduction

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders marked by impairments in social interaction and communication, and repetitive behaviors. The Centers for Disease Control and Prevention (CDC) estimates that 1 in 68 children (1 in 42 boys and 1 in 189 girls) has been identified with ASD [1]. The etiology of ASD is poorly understood, however studies suggest contributions from both genetic and environmental factors [2, 4, 5]. Recent studies have suggested the prenatal and early postnatal periods to be critical windows of susceptibility for ASD [116, 228].

Several epidemiologic studies have reported associations between prenatal and early postnatal air pollution exposure and ASD, however findings have differed by pollutant and developmental window [229]. Particulate matter and ozone are among the most ubiquitous criteria air pollutants and have been shown to induce inflammation and oxidative stress [6-8], both of which have been implicated in the development of ASD [9, 10]. Particulate matter $\leq$2.5 and $\leq$10 μm in diameter ($PM_{2.5}$ and PM_{10}) exposure during the prenatal [11-13] and postnatal [13, 14] developmental periods has been associated with ASD in studies in the United States (U.S.), with two studies suggesting the third trimester may be a potential critical window of susceptibility [12, 15]. In addition, one previous study found a positive association between prenatal ozone exposure and ASD [11]. However, few studies to date have

assessed associations between air pollution and ASD across different geographic locations using uniform exposure and outcome assessment methods.

To address these limitations, this analysis investigated the association between early life exposure to $PM_{2.5}$ and ozone in association with ASD across varied periods of exposure using data from the U.S.-based Study to Explore Early Development (SEED). Associations were assessed for exposures during critical periods of neurodevelopment, including the period three months before pregnancy, over the entire pregnancy period and by trimester, as well as during the first year of life. This analysis additionally investigated whether the association with $PM_{2.5}$ and ozone differed for ASD with or without a co-occurring intellectual disability, by ASD severity, and by SEED study site.

4.2 Methods

4.2.1 Study Population

The Study to Explore Early Development is a multi-site case-control study that aims to identify risk factors for ASD and other developmental disabilities [205]. The catchment area for the first phase of SEED includes study sites in California, Colorado, Georgia, Maryland, North Carolina, and Pennsylvania. Individuals were eligible to participate in the first phase of SEED if they were born in a study catchment area between September 1, 2003 and August 31, 2006 and resided there at 30-68 months of age. Participation in this analysis was restricted to children who were confirmed to have ASD or sampled as a control from the community and to those whose biological mothers were available to report on their pregnancy experience in English or Spanish.

Children with possible ASD were ascertained through multiple sources serving or evaluating children with developmental problems, including: early intervention programs, special education programs, clinics, and individual providers. Population controls were identified

by randomly sampling state birth records of children born in the specified date range to mothers that resided in the study catchment area at the time of delivery. Analyses focused on the comparison of children with a final classification of ASD versus typically developing population controls. Analyses excluded siblings and those who did not complete a clinic visit. Participants were additionally excluded if they did not have a geocoded address or were missing data on key covariates.

Institutional review boards at each study site and at the Centers for Disease Control and Prevention approved the SEED study. Informed consent was obtained from all enrolled participants.

4.2.2 Outcome Ascertainment

Upon telephone enrollment, all children, regardless of how they were initially identified, were screened for autism symptoms using the Social Communication Questionnaire (SCQ), which was administered to their parent or other primary caregiver [206]. Any children who screened positive (SCQ score ≥11) or reported a previous ASD diagnosis received a comprehensive developmental assessment to determine final ASD classification. This assessment included gold standard instruments to assess autism symptoms, 1) the Autism Diagnostic Observation Schedule (ADOS) [208], 2) the Autism Diagnostic Interview-Revised (ADI-R) and the Vineland Adaptive Behavioral Scales [209, 211]. Final ASD case classification was based on the results from the ADOS and ADI-R. ASD severity was additionally assessed using the ADOS calibrated severity score (CSS). This score was calculated using the ADOS total score, ADOS language level, and age at evaluation [213]. The ten-point scale was dichotomized into low/moderate (scores 4-7) and more severe (scores of 8-10).

All children participated in a general developmental assessment that included Mullen

Scales of Early. Children who did not have an indication of possible ASD (negative SCQ screen, no previous ASD diagnosis and no ASD-specific service classification) received only this general developmental assessment. Children with a Mullen Early Learning Composite Score of less than 70 were classified as having intellectual disability [212].

4.2.3 Exposure Assessment

Exposure to air pollution was determined using data from satellite-based models linked to address at birth. Study participants' dates of births and addresses at birth were identified using birth certificates. Addresses at birth were geocoded in ArcGIS using the ESRI Maps street database. Geocoding match rates ranged from 95-100% across study sites. To ensure participant's privacy, all dates, including dates of birth, were randomly shifted by 0-14 days in either direction. For a given participant, the shift was the same for all dates in order to maintain the relation among dates. Start date of pregnancy (time of conception) was calculated by subtracting the child's gestational age from their date of birth. Analyses used the clinical estimate of gestational age reported on birth certificates.

Average $PM_{2.5}$ and ozone concentration estimates were derived at a daily temporal resolution and a 1x1 km spatial resolution using an exposure prediction model for the study exposure period years (2002-2007). This prediction model has been thoroughly described elsewhere [37] and has previously been used in a study of air pollution and mortality in the U.S. [28]. Briefly, the hybrid prediction model incorporated satellite-based aerosol optical depth measurements, simulated outputs from a chemical transport model (GEOS-chem), monitored data, land use terms, and meteorological variables. GEOS-Chem is a global 3-D chemical transport model that simulates atmospheric chemistry, and is driven by meteorologic input from the Goddard Earth Observing System (GEOS) of the NASA Global Modeling and Assimilation

Office [36]. GEOS-Chem models provide temporally resolved estimates of particle concentrations, however the predictions are poorly correlated with monitored data, thus the predictions were calibrated using monitored data. Land use terms (percentage of urban areas, population density, road density, and elevation), meteorological variables (such as air temperature and precipitation), and satellite data were used to help calibrate GEOS-Chem outputs and to aid in downscaling to a smaller spatial resolution. This previously developed hybrid model used a neural network to calibrate all the predictors to monitored $PM_{2.5}$ and ozone and was trained and validated with ten-fold cross-validation.

Participants were matched to the centroid of the nearest 1x1km grid cell based on their residence at birth. Daily $PM_{2.5}$ and ozone concentrations were averaged for several periods, including the 1st (weeks 1-13 of pregnancy), 2nd (weeks 14-26), and 3rd trimesters (weeks 27 to birth); preconception (3 months prior to conception), entire pregnancy period, and year post birth.

4.2.4 Covariates

Information to assess potential confounders was obtained from the caregiver interview, medical records, and birth certificates. A directed acyclic graph (DAG) was used to identify the covariate adjustment set to be included in the model that would result in the least biased estimate. The final adjustment set consisted of the following variables: maternal age (continuous), maternal race/ethnicity (non-Hispanic-white, other race/ethnicity), maternal education (<bachelor's degree, ≥bachelor's degree), maternal smoking (any smoking three months before conception or during pregnancy), and year of birth (2003-2004, 2005-2006). Season of birth is related to the levels of air pollution and has been linked with ASD in some previous studies. Therefore, analyses additionally included an indicator variable for month of

birth to control for seasonal trend. Air pollution exposure has been associated with preterm birth [230] and preterm birth is a strong risk factor for ASD [231], thus preterm birth was not controlled for due to its potential mediating role in the association between air pollution and ASD. All models included an indicator for study site (California, Colorado, Georgia, Maryland, North Carolina, Pennsylvania).

4.2.5 Statistical Analyses

Logistic regression was used to estimate adjusted odds ratios (OR) and corresponding 95% confidence intervals (CI) for the associations between $PM_{2.5}$ and ozone and ASD, with the population group serving as the control group for all analyses. $PM_{2.5}$ and ozone exposures were analyzed as continuous measures because continuous fit better than categorical coding, per the lower Akaike Information Criterion value. Effect estimates are scaled to the the interquartile range (IQR) value for the entire pregnancy period, averaged across study sites. This approach allows the comparison of effect estimates across pollutants, study sites, and developmental windows.

Effect measure modification by study site was additionally assessed for each of the developmental windows in order to assess possible heterogeneity in the $PM_{2.5}$ and ozone and ASD associations by geographic location. Likelihood ratio tests were conducted by comparing models with and without interaction terms for study site and used a $p<0.10$ as a threshold for presence of modification on the multiplicative scale.

Analyses additionally investigated if $PM_{2.5}$ and ozone and ASD associations differed for children with ASD depending on the presence of co-occurring intellectual disability. Next, analyses compared associations by ASD severity using a dichotomized ASD severity score (Wiggins et al. 2017a). Multinomial logistic regression was used to compare the two severity

subgroups to the population controls.

Several sensitivity analyses were conducted. Ozone may confound relationships between $PM_{2.5}$ and ASD [225], thus results were reported from multi-pollutant models for each developmental window by controlling for the other pollutant in each exposure model. Results were additionally reported for each developmental window mutually adjusted for the other windows in order to isolate potentially critical windows of susceptibility. Specifically, results are presented for each trimester mutually adjusted for the other trimesters; and for the preconception, pregnancy, and postnatal windows mutually adjusted for the other developmental windows. Finally, analyses evaluated if the associations between $PM_{2.5}$ and ozone and ASD varied by maternal smoking during pregnancy.

4.3 Results

The final sample consisted of 1,529 mother-child pairs with complete outcome classification information based on a completed developmental assessment and geocoded address data available. This included 674 cases of children with ASD and 855 population controls. Compared to the population controls, children with ASD were more likely to be boys, born preterm, and born to non-white mothers and mothers with lower level of education (Table 4.1). These differences were consistent across SEED study sites (see Table B.1). Mothers in the excluded subset (n=396) were more likely to be non-Hispanic white and have lower education, compared to those in the included sample (data not shown).

$PM_{2.5}$ and ozone levels varied both spatially and temporally within and across the SEED study areas (Figures 4.1 and 4.2). During the study years, $PM_{2.5}$ peaked during the summer months for the Eastern study areas and during the winter months for the California study site. There was less temporal variation in $PM_{2.5}$ in the Colorado study area. In contrast, ozone

concentrations peaked in the summer months for all of the study areas.

The overall $PM_{2.5}$ average for the pregnancy period was 12.7 $\mu g/m^3$ (range: 4.9 – 18.6 $\mu g/m^3$) among all controls (Table 4.2), which varied considerably across study sites, ranging from 9.0 $\mu g/m^3$ in CO to 15.5 $\mu g/m^3$ in GA. The overall ozone average for the pregnancy period was 37.1 ppb, which was lowest in California (29.5 ppb) and highest in Colorado (39.8 ppb). Mean $PM_{2.5}$ levels were strongly correlated across several of the developmental periods (see Table B.2), particularly for pregnancy and first year of life (correlation of 0.92). $PM_{2.5}$ and ozone levels were often inversely correlated between the first and third trimester: $PM_{2.5}$ was inversely correlated in California (-0.64), Georgia (-0.65), and North Carolina (-0.71); and ozone was inversely correlated in Colorado (-0.78), Georgia (-0.86), Maryland (-0.85), North Carolina (-0.89), and Pennsylvania (-0.86).

$PM_{2.5}$ exposure during the first year of life was associated with increased odds of ASD, with an adjusted odds ratio of 1.26 (95% CI: 1.02, 1.57) per 1.6 $\mu g/m^3$ increase in $PM_{2.5}$ (Table 5.3; also see Table B.3 for unadjusted results). $PM_{2.5}$ or ozone exposure prior to conception and during pregnancy were not generally associated with ASD; however, odds ratios were elevated for exposure during the third trimester for $PM_{2.5}$ [OR=1.06 per 1.6 $\mu g/m^3$ (95% CI:0.98, 1.14)] and ozone [OR=1.22 per 6.6 ppb (95% CI:1.05, 1.42)]. Results were similar comparing multi-pollutant to single pollutant models, although odds ratios were generally attenuated in multipollutant models (see Table B.4).

When adjusting one time period for another, by including pregnancy and first year of life $PM_{2.5}$ averages in the same model, the magnitude of the OR was more pronounced for the first year of life $PM_{2.5}$ exposure [OR: 1.33 (95% CI: 1.04, 1.70)], but the OR attenuated for the pregnancy period. The elevated OR for third trimester ozone [OR=1.27 (95% CI: 1.08, 1.49)]

exposure remained when including the other trimester averages in the same model (Table B.5).

Although the association between $PM_{2.5}$ exposure and ASD did not differ significantly by maternal tobacco use during pregnancy, the OR for third trimester $PM_{2.5}$ exposure among smokers was elevated [OR=1.17 (95% CI: 1.01, 1.35)] compared to non-smokers [OR=1.04 (95% CI: 0.97, 1.12)] (p=0.11) (see Table B.6). In addition, the OR for second trimester ozone exposure among smokers was elevated [OR=1.15 (95% CI: 0.94, 1.41)] compared to non-smokers [OR=0.93 (95% CI: 0.82, 1.06)] (p=0.03)

Associations for $PM_{2.5}$ exposure during the first year of life did not vary considerably across study sites (Figure 4.3; also see Table B.7 for numeric data). However, the association between $PM_{2.5}$ and ASD differed by study site for the first (p=0.05) and third (p=0.03) trimesters, as well entire pregnancy exposures (p=0.07). First trimester and pregnancy-wide $PM_{2.5}$ concentrations were positively associated with ASD for the Georgia and Pennsylvania study sites, but were inverse or null for the other study sites. Third trimester $PM_{2.5}$ exposure was associated with ASD for the California, North Carolina, and Pennsylvania study sites. The association between third trimester ozone exposure and ASD did not vary across sites (Figure 4.4), however associations with ozone exposure varied across study site for the preconception period (p=0.02), first trimester (p=0.06), and first year of life (p<0.001).

When exploring whether associations differed for severe versus mild ASD symptoms, the magnitude of the associations differed by the exposure period. Ozone [OR: 1.30 per 6.6 ppb (95% CI 1.06, 1.58)] exposure during the third trimester was associated with increased odds of more severe ASD symptoms (Table 4.4), but no other differences were noted for ozone. However, $PM_{2.5}$ exposure during the first year of life was associated with low/moderate ASD symptom severity [OR: 1.39 per $PM_{2.5}$ IQR (95% CI: 1.08, 1.80)]. Associations did not differ by

co-occurring intellectual disability status for ozone or $PM_{2.5}$ exposure (see Table B.8).

Table 4.1 Characteristics of the Study to Explore Early Development population by case-control status

Characteristic	ASD Cases (*n*=674)	Controls (*n*=855)
Child sex		
Male	551 (82)	453 (53)
Female	123 (18)	402 (47)
Birth Year		
2003-2004	273 (41)	393 (46)
2004-2005	401 (59)	462 (54)
Maternal race/ethnicity		
Non-Hispanic White	378 (56)	611 (71)
Other[a]	296 (44)	244 (29)
Maternal education		
<Bachelor's	331 (49)	289 (34)
≥Bachelor's	343 (51)	566 (66)
Maternal age at birth (years)		
<35	486 (72)	589 (69)
≥35	188 (28)	266 (31)
Maternal Smoking		
Yes	112 (17)	79 (9)
No	562 (83)	776 (91)
Preterm		
Yes	111 (16)	82 (10)
No	563 (84)	773 (90)
SEED Study Site		
California	96 (14)	134 (16)
Colorado	139 (21)	185 (22)
Georgia	130 (19)	160 (19)
Maryland	107 (16)	126 (15)
North Carolina	100 (15)	146 (17)
Pennsylvania	102 (15)	104 (12)
$PM_{2.5}$ ($\mu g/m^3$) (mean ± SD)		
Pregnancy	12.8 ± 2.7	12.7 (2.6)
First year of life	12.7 ± 2.5	12.5 (2.5)
Ozone (ppb) (mean ± SD)		
Pregnancy	36.8 ± 5.7	37.0 ± 5.7
First year of life	37.8 ± 4.4	38.1 ± 4.3

Note: Numbers are N (%) or mean ± SD. Abbreviations: ASD, Autism Spectrum Disorder; $PM_{2.5}$, particulate matter <2.5 µm; SEED, Study to Explore Early Development; SD, standard deviation.

[a]Includes Asian, Hispanic, multiracial, and all others.

Table 4.2 $PM_{2.5}$ and ozone exposure distribution by SEED study site and developmental windows, among controls only

	All sites Mean (IQR)	**California** Mean (IQR)	**Colorado** Mean (IQR)	**Georgia** Mean (IQR)	**Maryland** Mean (IQR)	**North Carolina** Mean (IQR)	**Pennsylvania** Mean (IQR)
Ozone (ppb)							
Preconception	37.7 (18.8)	31.6 (9.4)	39.8 (20.8)	37.5 (21.5)	37.5 (21.3)	40.3 (13.5)	39.1 (17.9)
Entire pregnancy	37.1 (8.5)	29.5 (4.5)	39.8 (7.9)	39.3 (7.5)	36.8 (7.8)	39.7 (4.4)	35.3 (7.5)
First trimester	36.1 (21.2)	28.8 (13.1)	39.8 (21.3)	36.9 (25.1)	34.2 (16.6)	40.2 (21.7)	34.7 (25.4)
Second trimester	37.2 (20.0)	29.1 (13.9)	40.6 (18.6)	40.5 (18.8)	36.2 (21.9)	40.0 (14.3)	33.7 (17.2)
Third trimester	38.2 (20.8)	30.7 (11.7)	39.2 (21.3)	41.1 (21.9)	40.3 (14.1)	38.6 (21.6)	38.0 (24.1)
First year of life	38.2 (5.0)	31.1 (3.2)	40.0 (5.3)	40.0 (3.4)	38.2 (3.5)	40.5 (2.6)	38.3 (3.3)
$PM_{2.5}$ ($\mu g/m^3$)							
Preconception	12.7 (5.0)	10.7 (5.2)	9.1 (2.2)	15.8 (4.8)	15.3 (3.9)	12.9 (3.3)	13.3 (3.2)
Entire pregnancy	12.7 (3.9)	11.6 (1.8)	9.0 (1.6)	15.5 (1.7)	14.8 (1.8)	13.0 (1.5)	12.9 (1.1)
First trimester	12.6 (5.2)	11.9 (7.7)	9.0 (2.3)	15.0 (3.0)	14.3 (2.5)	13.3 (4.8)	13.0 (3.7)
Second trimester	12.7 (4.7)	12.1 (7.9)	8.9 (2.3)	15.6 (4.8)	14.7 (2.4)	13.0 (3.9)	12.6 (2.1)
Third trimester	12.7 (5.6)	10.8 (5.8)	9.1 (2.3)	16.1 (4.8)	15.5 (5.0)	12.9 (4.8)	13.1 (2.7)
First year of life	12.5 (3.9)	11.3 (1.1)	8.7 (1.5)	15.5 (0.8)	14.5 (1.6)	13.1 (0.9)	13.0 (0.8)

Abbreviations: IQR, interquartile range; $PM_{2.5}$, particulate matter <2.5 μm; SEED, Study to Explore Early Development.

Table 4.3 Adjusted[a] odds ratios and 95% confidence interval for early life exposure to $PM_{2.5}$ and ozone in association with ASD, among SEED participants

	Ozone [OR (95%CI)]	$PM_{2.5}$ [OR (95%CI)]
Preconception	0.92 (0.82, 1.05)	1.04 (0.97, 1.12)
Entire pregnancy	1.06 (0.85, 1.32)	1.02 (0.88, 1.19)
First Trimester	0.94 (0.83, 1.07)	0.98 (0.91, 1.05)
Second Trimester	0.96 (0.85, 1.09)	0.97 (0.91, 1.04)
Third Trimester	1.22 (1.05, 1.42)	1.06 (0.98, 1.14)
First year of life	0.79 (0.60, 1.04)	1.26 (1.02, 1.57)

Results are reported per 1.6-$\mu g/m^3$ increase in $PM_{2.5}$ and 6.6-ppb increase in ozone. Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; OR, odds ratio; $PM_{2.5}$, particulate matter <2.5 µm.
[a]Models are adjusted for study site, maternal age, maternal education, maternal race/ethnicity, maternal smoking, month of birth, and year of birth.

Table 4.4 Adjusted[a] odds ratios and 95% confidence intervals for the associations between $PM_{2.5}$ and ozone exposure and ASD severity

	Low/moderate Severity [OR (95%CI)] (*n*=401)	High Severity [OR (95%CI)] (*n*=272)
Ozone		
Preconception	0.93 (0.81, 1.08)	0.91 (0.77, 1.07)
Entire pregnancy	0.99 (0.77, 1.28)	1.18 (0.88, 1.59)
First Trimester	0.91 (0.78, 1.07)	0.98 (0.83, 1.17)
Second Trimester	0.95 (0.82, 1.10)	0.98 (0.83, 1.17)
Third Trimester	1.18 (0.99, 1.40)	1.30 (1.06, 1.58)
First year of life	0.77 (0.55, 1.06)	0.82 (0.57, 1.18)
$PM_{2.5}$		
Preconception	1.02 (0.94, 1.11)	1.07 (0.98, 1.18)
Entire pregnancy	1.10 (0.92, 1.32)	0.92 (0.76, 1.12)
First Trimester	1.04 (0.96, 1.13)	0.90 (0.82, 1.00)
Second Trimester	0.97 (0.90, 1.06)	0.97 (0.88, 1.06)
Third Trimester	1.04 (0.95, 1.13)	1.08 (0.98, 1.19)
First year of life	1.39 (1.08, 1.80)	1.10 (0.82, 1.47)

Results are reported per 1.6-µg/m^3 increase in $PM_{2.5}$ and 6.6-ppb increase in ozone. Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; OR, odds ratio; $PM_{2.5}$, particulate matter <2.5 µm.

[a]Models are adjusted for study site, maternal age, maternal education, maternal race/ethnicity, maternal smoking, month of birth, and year of birth.

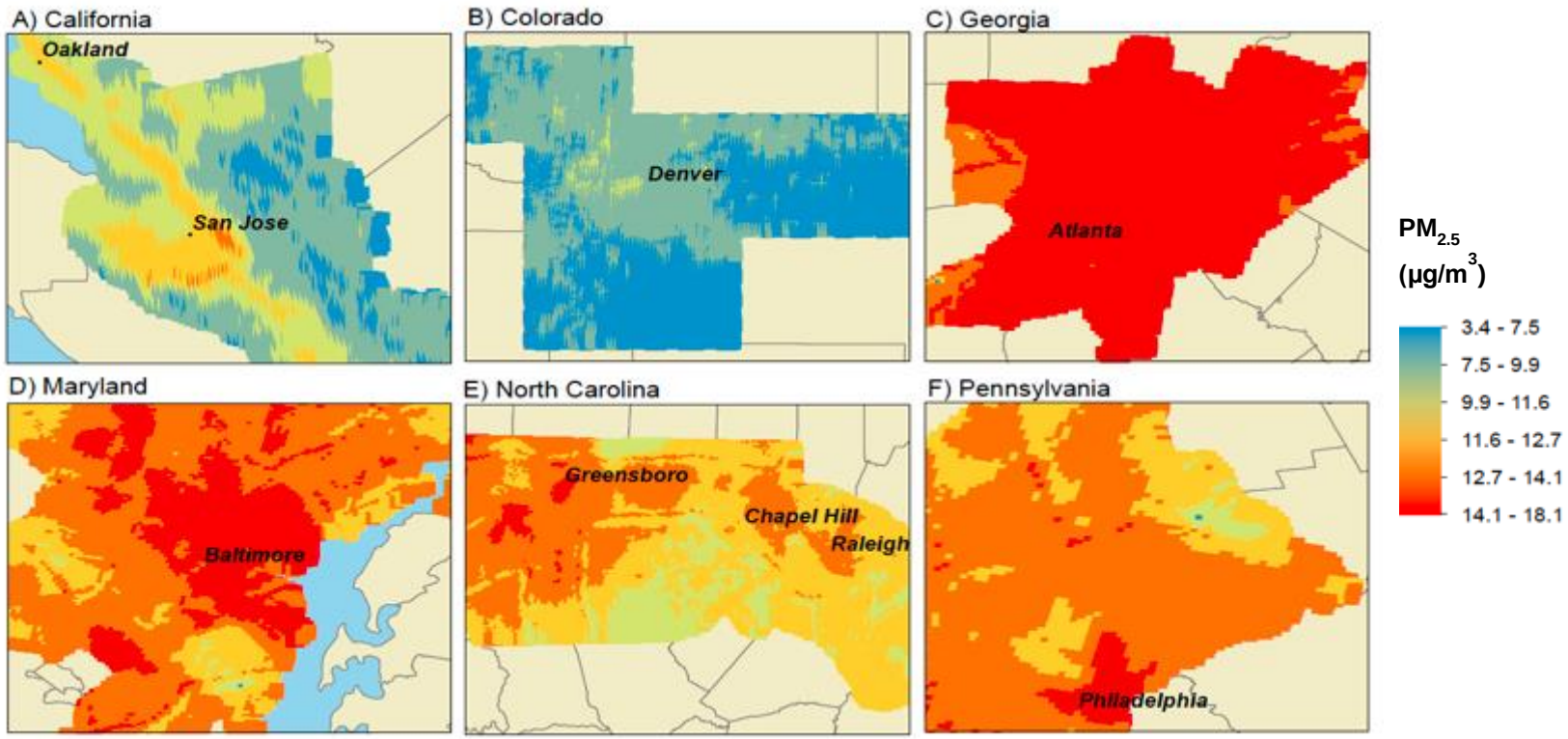
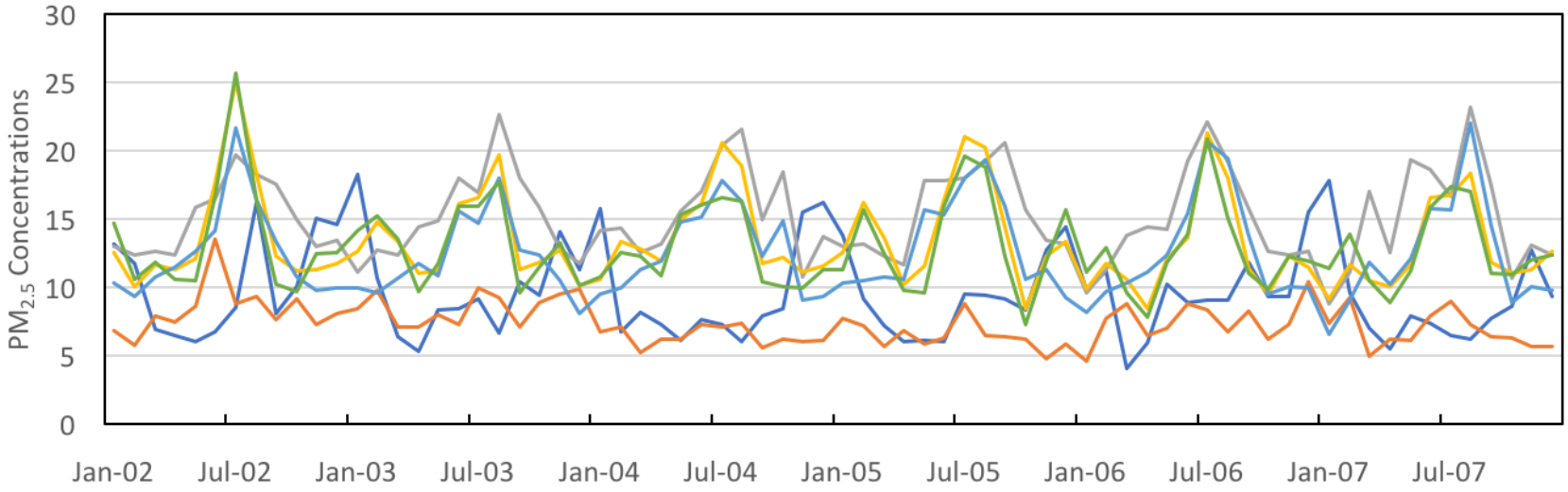


Figure 4.1 $PM_{2.5}$ (µg/m^3) concentrations averaged for the SEED study years (2002-2007) by SEED study site

A) $PM_{2.5}$ ($\mu g/m^3$)



B) Ozone (ppb)

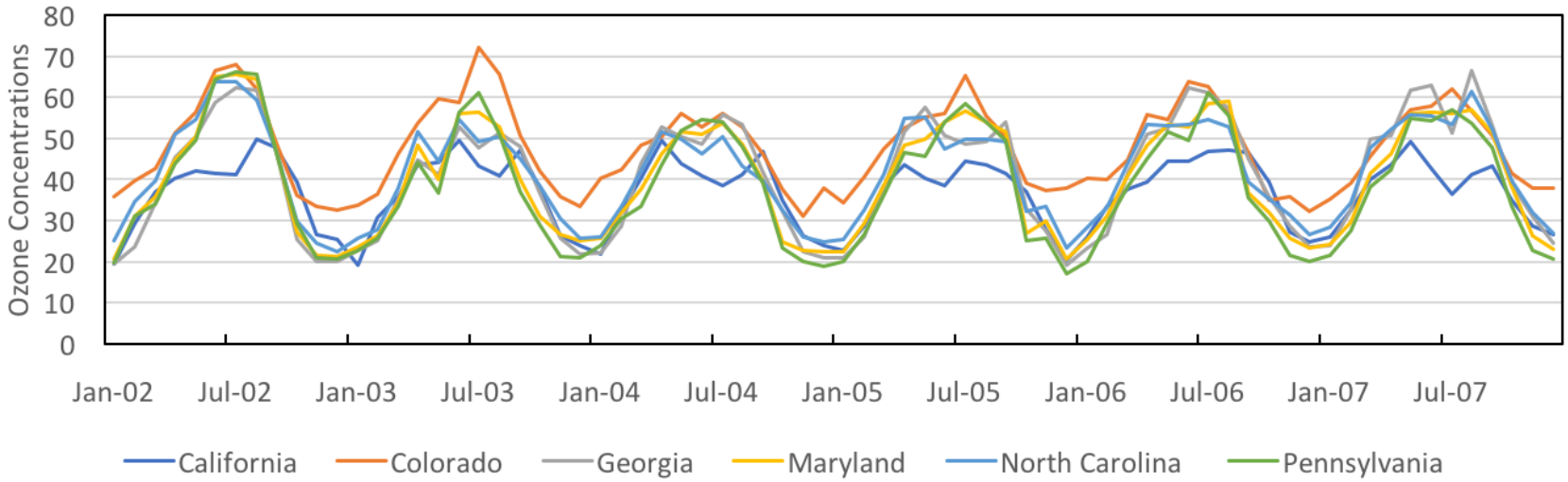


Figure 4.2 Monthly $PM_{2.5}$ and ozone concentrations from 2002-2007, by SEED study site

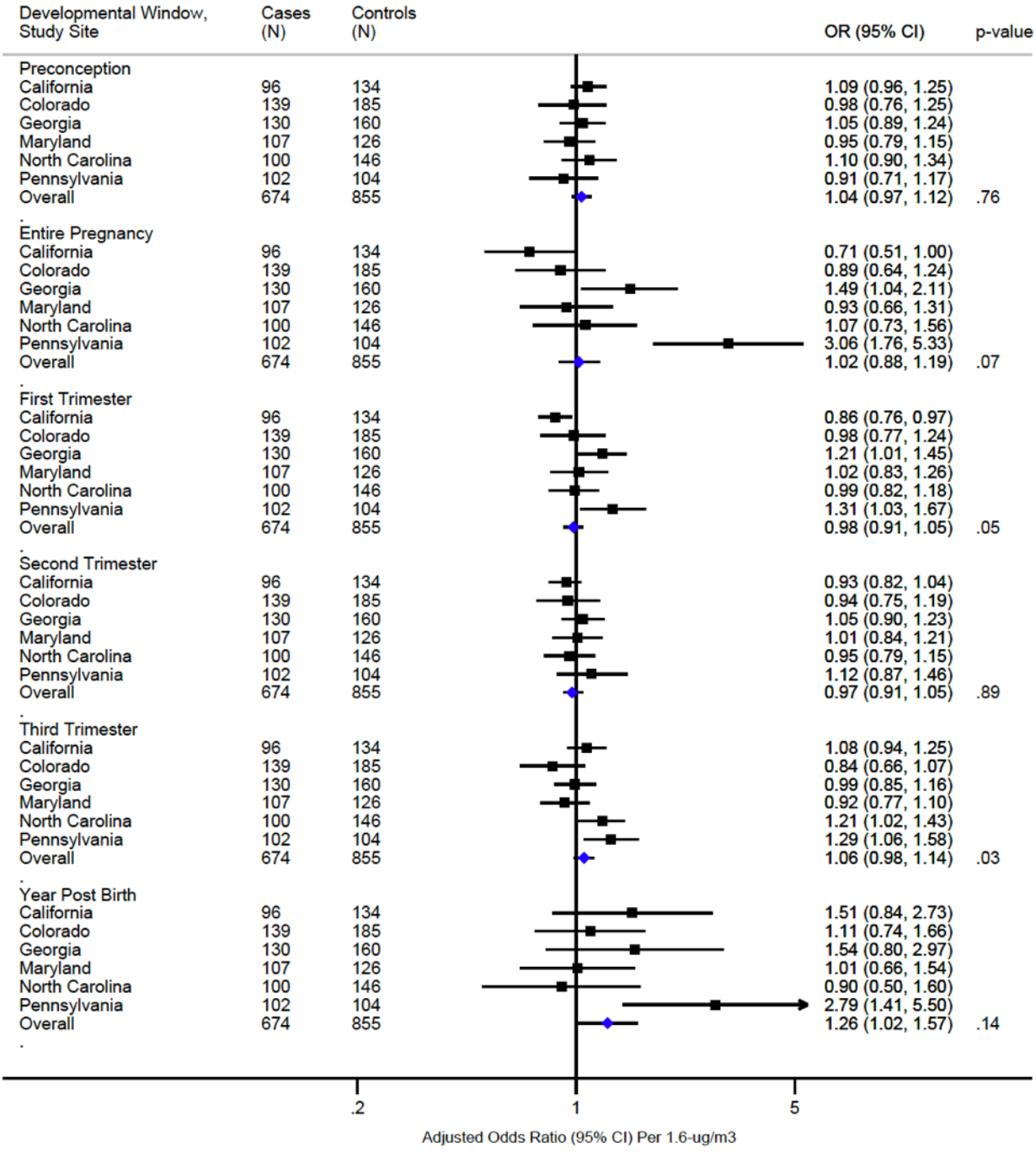


Figure 4.3 Site-specific and overall adjusted odds ratios and 95% confidence intervals for the associations between $PM_{2.5}$ and ASD, by developmental window

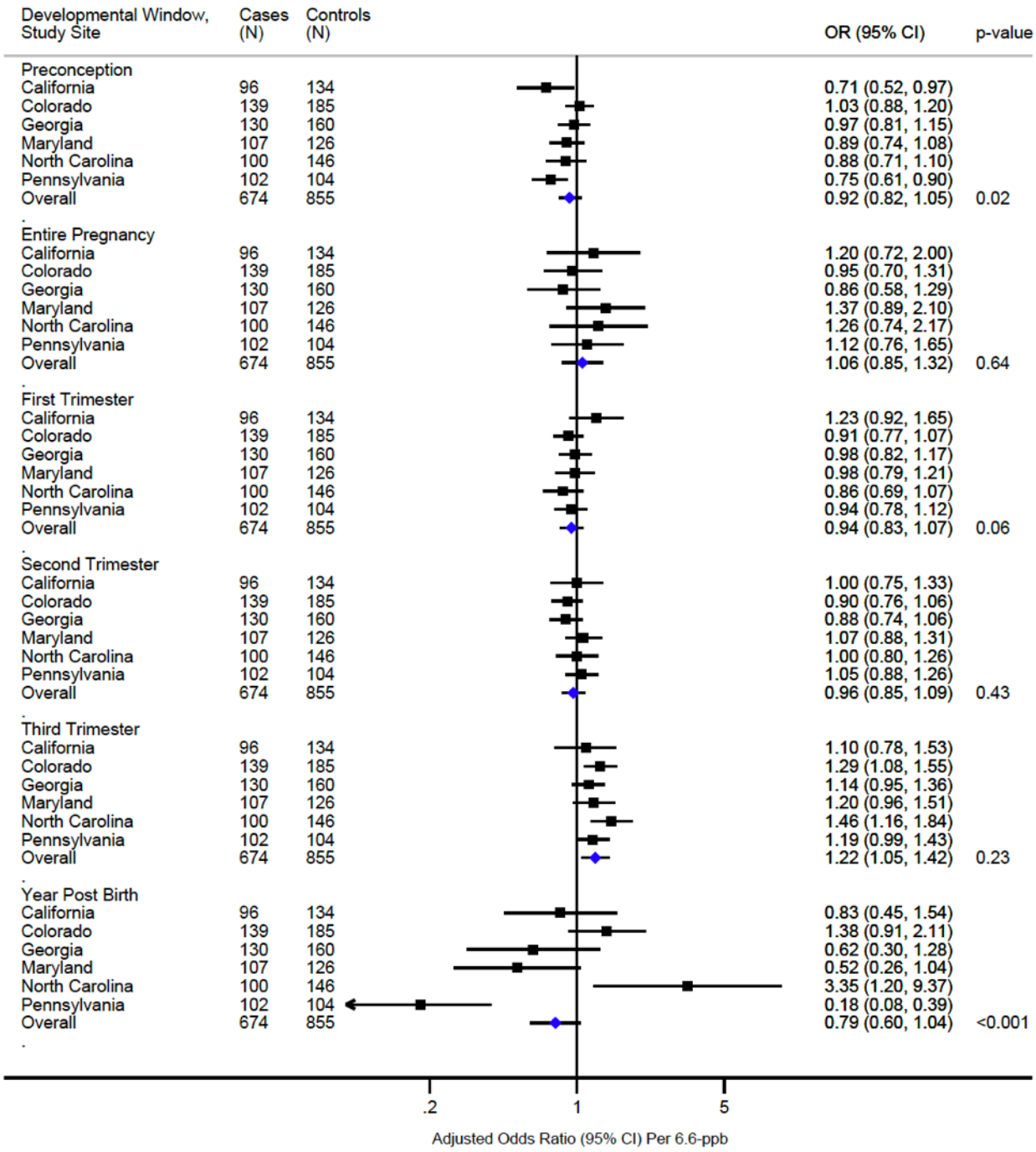


Figure 4.4 Site-specific and overall adjusted odds ratios and 95% confidence intervals for the associations between ozone and ASD, by developmental window

4.4 Discussion

In this U.S.-based multisite case-control study, $PM_{2.5}$ exposure during the first year of life was associated with increased risk of ASD. The trimester-specific analyses also revealed a potential third trimester as a potentially critical window of susceptibility for ozone, and to some extent $PM_{2.5}$, exposure. These findings held in models mutually adjusted for the other developmental windows, as well as multi-pollutant models. Associations for third trimester ozone and first year of life $PM_{2.5}$ exposure were fairly consistent across study sites, though results varied across sites for several of the other developmental windows. A growing number of studies have noted associations with exposures during the third trimester or early postnatal period. This may reflect a period of development where the brain structures are formed, but neuronal maturity (myelination and synaptic density) is rapidly increasing.

There are several plausible mechanistic pathways for late prenatal and early postnatal air pollution to contribute to the development of ASD. Synapse formation and neurotransmitter receptor formation both occur during the third trimester of development [232]. Environmental exposures during this critical window could disrupt these processes and interfere with normal brain development. Of emerging interest is the mediating role of microglia activation in the association between early life toxicant exposure and ASD [162]. Alterations in microglial development by late prenatal and early postnatal inflammation may alter synaptic pruning [163, 164], resulting in altered neuronal connectivity, and disruption of normal brain development. Recent studies in mice have found associations between gestational exposure to ultrafine and diesel exhaust particles and altered trajectory of microglia in male offspring [165, 167], with one study suggesting a third trimester equivalent window of susceptibility in humans [166].

The finding that $PM_{2.5}$ exposure specifically when during the first year of life is

associated with ASD risk is consistent with recent U.S.-based studies in California [13] and Pennsylvania [14], despite some differences in exposure levels (see Table B.9 for comparison of results). The observation that the first year of life may be a specifically susceptible window for exposure during development is consistent with a recent study based in Israel that found associations with postnatal NO_2 exposure, specifically when also adjusting for pregnancy averages [175]. There were additionally slight increases in risk of ASD for $PM_{2.5}$ exposure during the third trimester, though results were imprecise; but that finding is consistent with two recent U.S.-based studies of $PM_{2.5}$ [12] and PM_{10} [15] exposure in relation to ASD.

Ozone exposure in the third trimester was more pronounced in models mutually adjusted for exposures in the first and second trimester, and for those in the high ASD severity group. In a previous California-based study, Becerra et al. [11] found associations between ozone exposure during the entire pregnancy and ASD in single and multi-pollutant models additionally adjusted for $PM_{2.5}$ exposure. In general, several epidemiologic studies have found associations with short and intermediate ozone exposure, particularly during the warm season, with fewer identifying adverse health effects from long-term ozone exposure [29].

Previous air pollution and ASD studies have differed in geographic location, exposure assessment, outcome ascertainment, and control for confounding, thus challenging comparison of results across studies [4, 229]. This study additionally assessed the association between $PM_{2.5}$ and ozone and ASD using similar exposure assessment and outcome ascertainment methods across study sites from different geographic regions in the U.S. Associations for third trimester ozone and first year of life $PM_{2.5}$ exposure did not vary significantly across different geographic locations in this study. This finding is in line with the consistency of findings in the literature for these developmental windows (Flores-Pajot et al. 2016). However, there was heterogeneity in

site-specific findings for several of the other developmental windows. Geographic variation in study findings might be explained by exposure variability. Exposure levels varied between sites, with the highest $PM_{2.5}$ levels in Georgia, and the lowest in Colorado and California. In addition, on the East coast $PM_{2.5}$ is dominated by sulfates from power plants and other regional sources; in contrast, nitrates make up the largest composition of $PM_{2.5}$ on the west coast [25]. Thus, the regional differences in particulate mass composition and exposure levels could contribute to differences in the estimated associations between air pollution and ASD from different geographic regions described in the literature, and across study sites in the current analysis.

The stronger signal of association during the late prenatal and early postnatal developmental windows could also reflect more accurate exposure measures during these windows. This study assigned exposure from just before pregnancy to one year after birth based on only the residential address at birth, which assumes limited mobility during pregnancy and the infant's first year of life. Many previous studies have shown little change in exposure assignment when using the birth address versus incorporating the complete residential history during pregnancy [179, 180], although one study did show somewhat greater exposure misclassification for the pregnancy period than for first year of life [181]. Further, if women in this study spent more time away from home during the earlier pregnancy periods [182], then the modelled exposure estimates may not have been an accurate proxy for their actual exposure during these periods [110]. Therefore, it is possible that exposure misclassification for earlier pregnancy periods has attenuated effect estimates for these windows.

This study was unable to explore shorter windows of exposure because of procedures introduced to ensure privacy of participants. The date of birth of SEED participants were shifted by up to two weeks in either direction; this shift in date of birth did not impact longer exposure

average periods (trimester, year post birth, etc.), based on the sensitivity analyses in the one site for which both actual and shifted data were available (data not shown), but prevented us from confidently studying shorter exposure periods (such as weeks).

Confounding is another potential source of bias in this analysis. Although various markers of socioeconomic status were adjusted for in these analyses, there is still the potential for residual confounding by SES or place of residence. Study site was controlled for in all analyses. Adjustment for site produced estimates from models that accounted for the multi-site study design, which also provides for further adjusts for any residual confounding due to between-site variability in air pollution exposure. To further explore between-site variability we generated study-specific effect estimates. The estimate for the year post birth exposure period increased when adjusting for study site, but estimates for shorter exposure averages did not. Longer exposure averages rely more on spatial contrasts in exposure and are therefore potentially more sensitive to confounding by unmeasured characteristics related to site [233].

Finally, ozone shows strong seasonal trends in many areas of the country, with the highest concentrations during the summer months. In addition, several previous studies have found associations between season of birth and ASD [222, 223]. Therefore, month of birth was adjusted for in all analyses to account for seasonal trends related to both exposure and ASD. Previous studies have additionally found that season of birth is related to SES and other environmental pollutants have seasonally varying patterns [161].

This study has several strengths. First, it is the first to assess associations between $PM_{2.5}$ and ozone and ASD using a state-of-the-art model that incorporates satellite-based data at a fine spatial resolution, as well as land-use terms, meteorological variables, and data from a chemical transport model. Land-use regression models have adequate spatial resolution, however often

have poor temporal resolution since the land-use terms are usually time-invariant [37]. The use of satellite data helps to improve temporal resolution in the resulting exposure predictions, and allows the inclusion of rural locations further away from monitors, thus limiting the need to exclude participants located further away from a monitor.

SEED also used gold standard outcome assessment tools, including ADOS and ADI-R, therefore reducing any potential outcome misclassification. This allowed the current study to investigate the heterogeneity of ASD and to distinguish associations by core ASD symptom severity. In addition, SEED study participants were drawn from a variety of settings serving children with disabilities across six different study sites located in the western, central, and eastern U.S. This ensures greater representation of ASD than previous clinically oriented studies. The geographic variability also provides adequate exposure variability to assess health-related associations from exposure to $PM_{2.5}$ and ozone. Finally, SEED provided rich covariate data, allowing thorough adjustment for potential confounding.

In summary, this U.S.-based, multi-center study provides evidence for a positive association between early life exposure to air pollution and ASD, and identifies a potential late prenatal and early postnatal period as a critical window of susceptibility. Future research is warranted to confirm these findings regarding the adverse impact of $PM_{2.5}$ and ozone exposure on ASD development, to explore the influence of variable PM composition by geographic region, and to further explore possible variability by ASD severity.

CHAPTER 5: AIR POLLUTION, NEIGHBORHOOD DEPRIVATION, AND AUTISM IN THE STUDY TO EXPLORE EARLY DEVELOPMNENT

5.1 Introduction

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders marked by impairments in social interaction and communication, and repetitive behaviors. ASD is a highly heterogeneous disease, with multiple underlying causes, including genetic and environmental factors. Additionally, strong evidence exists for a prenatal and early postnatal window of susceptibility for ASD risk [115, 116, 228]. Several epidemiologic studies have reported associations between prenatal and early postnatal air pollution exposure and ASD [229], specifically for particulate matter <2.5 μm in diameter ($PM_{2.5}$) [11, 12] and measures of traffic-related air pollution [13]. Early life air pollution exposure may increase risk of ASD through an inflammatory response pathway impacting brain development [159].

Maternal stress has also been hypothesized to alter inflammatory response [234] and has been associated with ASD in a few previous studies [235-237]. Factors at the neighborhood level, such as crime and poverty, have been implicated as social stressors in previous studies [238, 239], with one study finding associations between neighborhood level stressors and cortisol reactivity among women [240]. Environmental toxicants and social stressors are often spatially correlated, and both exposures often cluster in more deprived areas [185-187]. Given this relationship, individual and area level socioeconomic status may confound the association between air pollution and ASD, but air pollution and area level SES may also have synergistic effects on disease development, working through a shared inflammatory pathway.

Neighborhood deprivation is a multi-component measure of area level SES [190] that has been used in previous epidemiological studies to evaluate the impact of stressors at the neighborhood level on air pollution and health associations [197, 198]. There are several plausible pathways for these two exposures to contribute to health outcomes. Morello-Frosch and Shenassa [191] theorized that stressors at the neighborhood level can contribute to individual chronic stress, which can influence individual susceptibility, and this stress-induced susceptibility can shape response to environmental exposures. Using this framework, this study hypothesized that chronic stress from neighborhood deprivation could influence individual susceptibility by impairing the body's ability to maintain allostasis leading to compromised immune function, and ultimately shaping maternal and infant responses to air pollution exposure [185, 192].

The goal of the current study was to investigate the modifying role of neighborhood deprivation on the association between prenatal and postnatal roadway proximity and $PM_{2.5}$ exposure and ASD using data from the Study to Explore Early Development (SEED).

5.2 Methods

5.2.1 Study Population

The Study to Explore Early Development is a multi-site case-control investigation that aims to identify risk factors for ASD and other developmental disabilities in children [205]. The SEED catchment area includes geographically diverse sites across the U.S., including California, Colorado, Georgia, North Carolina, Pennsylvania, and Maryland (Table C.1). Individuals were eligible to participate in SEED if they were born in a study catchment area between September 1, 2003 and August 31, 2006 and still resided there at 30-68 months of age [205], and lived with an English (all sites) or Spanish (California and Colorado sites) speaking caregiver. Children with

possible ASD were ascertained through multiple sources serving or evaluating children with developmental problems. Population controls were identified from a random sample of state birth records within a site's catchment area. Institutional review boards at each study site and at the Centers for Disease Control and Prevention approved the SEED study. Informed consent was obtained from all enrolled participants.

5.2.2 Outcome Ascertainment

The primary caregiver was initially administered the Social Communication Questionnaire (SCQ), in order to screen for potential autism symptoms in their child [206]. Any child who had a positive SCQ screen (considered as SCQ score ≥11, previously described [207]) or previous ASD diagnosis received a comprehensive developmental assessment to determine final ASD classification. Children were administered the Autism Diagnostic Observation Schedule [208] and their caregivers were administered the Autism Diagnostic Interview-Revised [209, 211]. Final ASD case classification was based on the results from the Autism Diagnostic Observation Schedule and Autism Diagnostic Interview-Revised [211]. Children who did not have an indication of possible ASD (negative SCQ screen, no previous ASD diagnosis and no ASD-specific service classification) received a general developmental assessment only.

5.2.3 Exposure Assessment

Study participants' dates of birth and residential addresses at birth were obtained from electronic birth certificates. Addresses at birth were geocoded in ArcGIS using the ESRI Maps street database. Geocoding match rates ranged from 95-100% for each study site.

Start date of pregnancy was calculated by subtracting the clinical estimate of the child's gestational age, recorded on the birth certificate, from the child's date of birth. To ensure the privacy of all participants, all dates related to the date of births were randomly shifted, in a

manner maintaining the relationship between dates, by up to two weeks in either direction.

Roadway proximity was used to capture the mixture of chemicals from traffic-related air pollution. Road networks for the entire U.S. were obtained from ESRI StreetMap. U.S. major roads include U.S. and state highways, major streets, and other major thoroughfares within the United States. Local residential roads are not included in this assessment. Each participant's geocoded address at birth was matched to the nearest major road/highway using ArcGIS, to calculate an individual distance measure (in meters) [214]. Distance to major roadway was dichotomized at the 10^{th} percentile level in controls (<45m vs. ≥45m).

This study used a previously developed exposure prediction model to characterize $PM_{2.5}$ exposure for the study period years (2002-2007), which has been previously described in detail [37]. Briefly, the hybrid prediction model incorporated satellite-based aerosol optical depth measurements, simulated outputs from a chemical transport model, monitored data, land use terms, and meteorological variables. The model used a neural network to calibrate all the predictors to monitored $PM_{2.5}$ and was trained and validated with ten-fold cross-validation. Predictions were available at a daily temporal resolution and a 1x1 km spatial resolution. Participants were matched to the centroid of the nearest grid cell based on their residence at birth. Exposure averages were created for the entire pregnancy period and the year post birth. $PM_{2.5}$ exposure during pregnancy and first year of life was modelled continuously and also dichotomized at the $PM_{2.5}$ National Ambient Air Quality Standard (NAAQS) level of 12.0 $\mu g/m^3$ (≥12.0 $\mu g/m^3$ vs. <12.0 $\mu g/m^3$).

5.2.4 Neighborhood Deprivation

Neighborhood level deprivation was characterized using a neighborhood deprivation index (NDI) measure developed by Messer et al [199]. This index has previously been used to

describe relationships between neighborhood deprivation and several pregnancy outcomes, including low birth weight, small for gestational age, and preterm birth [216-218]. To create the index, eight area-level SES-related parameters were obtained from the 2000 U.S. Census at the census tract level: percentage of males and females with less than a high school education; percentage of males and females unemployed; percentage of households defined as crowded (housing units with more than one occupant per room); percentage of males that are not in management and professional occupations; percentage of households in poverty; percentage of female headed households with dependent children; percentage of households earning less than $30,000 per year; and percentage of households on public assistance (see Table C.2 for a detailed description of these measures).

To create the weighted NDI, tract-level data from all six study sites were pooled and the data reduction technique principal components analysis was used; to represent the correlation between the components, the eight area-level SES parameters were used as the loadings [241]. The first principal component was retained because it accounted for the largest proportion of the total variability in the component measures. SES related variable values were weighted according to final factor loadings to create a continuous index score for each census tract. The index score was standardized by dividing the index by the square of the eigenvalue, resulting in a deprivation index with a mean of zero and a standard deviation (SD) of one. Higher values of the NDI indicate higher levels of neighborhood disadvantage. Census tracts of the SEED study areas were categorized as having high, moderate, or low deprivation based on tertile cutpoints of the continuous index. The neighborhood deprivation index was linked to SEED participants based on their residential census tract at birth.

5.2.5 Confounders

Information to assess potential confounders was obtained from a caregiver interview, medical records, and birth certificates. A directed acyclic graph was used to identify the covariate set to be included in the model that would result in the least biased estimate. The final adjustment set consisted of the following variables: study site, year of birth, month of birth, maternal age (continuous), maternal race/ethnicity (non-Hispanic-white, other race/ethnicity), maternal education (<bachelor's degree, >bachelor's degree), and maternal smoking (any smoking three months before conception or during pregnancy).

5.2.6 Statistical Analyses

Multivariable logistic regression was used to estimate odds ratios (OR) and corresponding 95% confidence intervals (CI) for the associations between roadway proximity, $PM_{2.5}$, and ASD, with the population group serving as the control group for all analyses. Results are first reported for the main associations of each of the exposures in relation to ASD.

Effect measure modification (EMM) by neighborhood deprivation was first evaluated on the multiplicative scale for continuous measures of $PM_{2.5}$ exposure and categorized measures of distance to roadway (<45m vs. ≥45m) and $PM_{2.5}$ exposure (≥12.0 $\mu g/m^3$ vs. <12.0 $\mu g/m^3$). Departure from multiplicity was assessed by including an interaction term between the deprivation index and exposure metrics and compared models with and without interaction terms. Multiplicative interaction was assessed using the likelihood ratio test (LRT), with a significance level of 0.10. EMM on the additive scale was additionally assessed by constructing single-referent models for each of the categorized exposures and computed the relative excess risk due to interaction (RERI) for each exposure [226]. Corresponding 95% confidence intervals were calculated using the delta method [227]. The RERI measure indicates whether there is

positive, negative, or no interaction on the additive scale.

5.3 Results

Case-control characteristics of the SEED study population, stratified by neighborhood deprivation level, are presented in Table 5.1. Overall, compared to controls, children with ASD were more likely to be boys, born preterm, and born to non-white, lower educated mothers. In this study population, 187 cases (28%) and 159 controls (19%) were categorized as residing at birth in a census tract with the highest deprivation tertile. Controls in the high deprivation group were more likely to be non-white, and born to lower educated, lower income mothers who used tobacco during pregnancy than those in the lowest deprivation. $PM_{2.5}$ levels also differed by neighborhood deprivation level. $PM_{2.5}$ averages in controls during the pregancy period were 13.3 $\mu g/m^3$ in the highest deprivation group and 12.6 $\mu g/m^3$ and 12.4 $\mu g/m^3$ in the moderate and low deprivation groups, respectively. Those in the highest deprivation group were additionally more likely to live closer to a major road/highway.

Figure 5.1 shows the spatial distribution of the deprivation measures for the census tracts of each of the six study catchment areas. In general, more deprived census tracts tended to cluster near major cities and more populated areas. There was moderate variability in the census indicators by study catchment area (Table 5.2). Participants from the Colorado and North Carolina study sites tended to live in census tracts of higher SES compared to those from the Georgia and Pennsylvania sites. For example, SEED participants from the Pennsylvania study site resided in census tracts with a greater percentage of households in poverty (9.9%), compared to those from the Colorado study site (4.9%). Continuous deprivation index levels of SEED participants also varied by study site (Table 5.2). Mean neighborhood deprivation of study participants varied by site, with a lower mean neighborhood deprivation index for Colorado

participants (mean: -0.35, range: -1.3 to 2.1) and higher deprivation for participants from the Pennsylvania study site (mean: 0.14, range: -1.2 to 4.3).

Residence at birth within 45m of a major road was associated with childhood ASD (OR=1.21, 95% CI: 0.88, 1.68) (Table 5.3). Childhood ASD was also associated with $PM_{2.5}$ exposure in the first year of life when measured on a continuous scale (OR=2.08 per 5-$\mu g/m^3$, 95% CI: 1.05, 4.10) and when considered as a dichotomous variable (OR=1.46, 95% CI: 0.86, 2.46 for $PM_{2.5}$ levels >12.0 $\mu g/m^3$ in the first year of life compared to $\leq$12.0 $\mu g/m^3$).

There was significant modification by neighborhood deprivation for the association between $PM_{2.5}$ during the first year of life and ASD on the additive (RERI: 0.81, 95% CI: -0.88, 2.47) and multiplicative (p=0.08) scales when $PM_{2.5}$ was dichotomized at 12.0 $\mu g/m^3$ (Table 5.4). The association between $PM_{2.5}$ exposure and ASD was strongest in regions of high deprivation (OR=2.42, 95% CI: 1.20, 4.86), but not in moderate (OR=1.21, 95% CI: 0.67, 2.17) or low (OR=1.46, 95% CI: 0.80, 2.65) deprivation regions. Although there was no evidence of modification by neighborhood deprivation for the association between roadway proximity and ASD, there was some heterogeneity in this association by deprivation level. The association for living within 45m of a major road was strongest for those in the moderate deprivation group (OR=1.65, 95% CI: 0.95, 2.86), compared to the low and high groups (Table 5.4). Modification by neighborhood deprivation was not observed on the multiplicative scale when using continuous measures of $PM_{2.5}$ exposure (Table C.3).

Table 5.1 Distribution of participant characteristics [*n* (%)] in the Study to Explore Early Development by case-control status and neighborhood deprivation level

Characteristic	Low deprivation		Moderate deprivation		High deprivation	
	ASD Case	Control	ASD Case	Control	ASD Case	Control
Total	252 (37)	427 (50)	235 (35)	269 (31)	187 (28)	159 (19)
Sex						
Male	203 (81)	228 (53)	194 (83)	150 (56)	154 (82)	75 (47)
Female	49 (19)	199 (47)	41 (17)	119 (44)	33 (18)	84 (53)
Maternal race/ethnicity						
NH White	190 (75)	364 (85)	142 (60)	182 (68)	46 (25)	65 (41)
Other	62 (25)	63 (15)	93 (40)	87 (32)	141 (75)	94 (59)
Maternal education						
<Bachelor's	76 (30)	84 (20)	112 (48)	105 (39)	143 (76)	100 (63)
≥Bachelor's	176 (70)	343 (80)	123 (52)	164 (61)	44 (24)	59 (37)
Maternal income						
<$50,000	38 (15)	46 (11)	94 (41)	74 (28)	123 (72)	94 (61)
≥$50,000	210 (85)	369 (89)	133 (59)	191 (72)	49 (28)	60 (39)
Maternal age at delivery						
<35 years	165 (65)	262 (61)	177 (75)	194 (72)	144 (77)	133 (84)
≥35	87 (35)	165 (39)	58 (25)	75 (28)	43 (23)	26 (16)
Maternal smoking						
Yes	24 (10)	27 (6)	47 (20)	25 (9)	41 (22)	27 (17)
No	228 (90)	400 (94)	188 (80)	244 (91)	146 (78)	132 (83)
Preterm						
Yes	37 (15)	39 (9)	37 (16)	21 (8)	37 (20)	22 (14)
No	215 (85)	388 (91)	198 (84)	248 (92)	150 (80)	137 (86)
SEED Study Site						
California	27 (11)	58 (14)	44 (19)	51 (19)	25 (13)	25 (16)
Colorado	65 (26)	99 (23)	46 (20)	52 (19)	28 (15)	34 (21)
Georgia	53 (21)	73 (17)	34 (14)	46 (17)	43 (23)	41 (26)
Maryland	39 (15)	70 (16)	46 (20)	41 (15)	22 (12)	15 (9)
North Carolina	38 (15)	65 (15)	43 (18)	55 (20)	19 (10)	26 (16)
Pennsylvania	30 (12)	62 (15)	22 (9)	24 (9)	50 (27)	18 (11)
$PM_{2.5}$ pregnancy mean (SD)	12.4 (2.9)	12.4 (2.7)	12.5 (2.5)	12.6 (2.4)	13.5 (2.5)	13.3 (2.6)
Distance to road (m)	404 (420)	458 (595)	386 (556)	423 (564)	264 (284)	247 (338)

Abbreviations: ASD, Autism Spectrum Disorder; $PM_{2.5}$, particulate matter <2.5 μm; SEED, Study to Explore Early Development; SD, standard deviation

Table 5.2 Mean (SD) of U.S. census indicators for census tracts of each SEED study catchment area

	CA	CO	GA	MD	NC	PA
% Poverty	5.7 (5.6)	4.9 (5.7)	7.2 (9.1)	5.7 (7.3)	5.5 (6.0)	9.9 (12.7)
% Public assistance	3.3 (3.1)	1.9 (1.9)	2.2 (3.5)	2.2 (3.4)	1.7 (1.9)	4.9 (6.9)
% Unemployment	2.9 (1.7)	2.5 (1.6)	3.4 (2.5)	2.6 (2.1)	2.5 (2.3)	4.1 (3.5)
% <HS education	15.8 (11.1)	12.5 (12.9)	13.6 (11.1)	15.3 (11.3)	13.2 (9.5)	18.3 (13.9)
% Crowding	12.9 (10.6)	4.8 (6.0)	5.3 (5.1)	2.1 (1.9)	2.8 (3.5)	3.1 (3.6)
% Income <30K	18.7 (11.2)	20.7 (14.5)	23.6 (15.4)	25.1 (16.0)	25.8 (13.7)	31.5 (20.2)
% Female headed household	6.0 (3.3)	6.2 (3.0)	8.2 (6.6)	7.3 (5.2)	6.4 (4.2)	8.1 (6.9)
% Management occupation	75.0 (10.5)	79.6 (9.2)	78.5 (10.0)	75.6 (11.7)	77.7 (9.7)	81.3 (9.9)
Neighborhood deprivation index	-0.20 (0.7)	-0.35 (0.7)	-0.18 (0.9)	-0.30 (0.8)	-0.34 (0.7)	0.14 (1.3)

%Poverty: Percentage of males and females with less than a high school education; *%Public assistance*: Percentage of households on public assistance; *%Unemployment*: Percentage of males and females unemployed; *%<HS education*: Percentage of males and females with less than a high school education; %Crowding: Percentage of housing units with more than one occupant per room; *%Income <30K*: Percentage of households earning less than $30,000 per year; *%Female headed household*: Percentage of female headed households with dependent children; *%Management occupation*: Percentage of males not in management and professional occupations.

Table 5.3 Adjusted[a] odds ratios and 95% confidence intervals for the association between proximity to roadway, $PM_{2.5}$ exposure, and ASD

	ASD (N)	Controls (N)	Odds ratio (95% CI)
Distance to major road			
>45m	582	767	1.00 (ref)
≤45m	92	88	1.21 (0.88, 1.68)
Pregnancy $PM_{2.5}$			
5-$\mu g/m^3$ increase	674	855	1.08 (0.67, 1.72)
≤12.0 $\mu g/m^3$	238	309	1.00 (ref)
>12.0 $\mu g/m^3$	436	546	0.83 (0.57, 1.20)
First year of life $PM_{2.5}$			
5-$\mu g/m^3$ increase	674	855	2.08 (1.05, 4.10)
≤12.0 $\mu g/m^3$	226	318	1.00 (ref)
>12.0 $\mu g/m^3$	448	537	1.46 (0.86, 2.46)
Neighborhood deprivation			
Low	252	427	1.00 (ref)
Moderate	235	269	1.24 (0.97, 1.59)
High	187	159	1.26 (0.92, 1.73)

Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; OR, odds ratio; $PM_{2.5}$, particulate matter <2.5 µm

[a]All models are adjusted for study site, year of birth, month of birth, maternal education, maternal race/ethnicity, maternal age, and maternal smoking

Table 5.4 Effect measure modification by neighborhood deprivation for the association between proximity to roadway and $PM_{2.5}$ exposure in relation to ASD

NDI Levels	Exposure	ASD (N)	Controls (N)	Single referent ORs (95% CI)	RERI (95% CI)	Stratified ORs (95% CI)	LRT p-value
	Distance to major road						
Low	>45m	230	397	1.00 (ref)		1.00 (ref)	
	≤45m	22	30	1.19 (0.66, 2.14)		1.18 (0.66, 2.14)	
Moderate	>45m	198	242	1.18 (0.91, 1.54)		1.00 (ref)	
	≤45m	37	27	1.94 (1.13, 3.36)	0.57 (-0.41, 1.19)	1.65 (0.95, 2.86)	
High	>45m	154	128	1.32 (0.94, 1.83)		1.00 (ref)	
	≤45m	33	31	1.13 (0.64, 1.99)	-0.38 (-1.10, 0.52)	0.86 (0.49, 1.51)	0.27
	Pregnancy $PM_{2.5}$						
Low	≤12.0 $\mu g/m^3$	99	165	1.00 (ref)		1.00 (ref)	
	>12.0 $\mu g/m^3$	153	262	0.81 (0.52, 1.26)		0.81 (0.52, 1.26)	
Moderate	≤12.0 $\mu g/m^3$	92	96	1.33 (0.89, 1.98)		1.00 (ref)	
	>12.0 $\mu g/m^3$	143	173	0.96 (0.61, 1.52)	-0.18 (-0.68, 0.32)	0.73 (0.45, 1.16)	
High	≤12.0 $\mu g/m^3$	47	48	1.06 (0.64, 1.77)		1.00 (ref)	
	>12.0 $\mu g/m^3$	140	111	1.12 (0.68, 1.84)	0.28 (-0.23, 0.73)	1.05 (0.58, 1.90)	0.50
	First year of life $PM_{2.5}$						
Low	≤12.0 $\mu g/m^3$	89	160	1.00 (ref)		1.00 (ref)	
	>12.0 $\mu g/m^3$	163	267	1.46 (0.80, 2.65)		1.46 (0.80, 2.65)	
Moderate	≤12.0 $\mu g/m^3$	91	98	1.40 (0.93, 2.10)		1.00 (ref)	
	>12.0 $\mu g/m^3$	144	171	1.69 (0.93, 3.07)	-0.17 (-1.71, 1.37)	1.21 (0.67, 2.17)	
High	≤12.0 $\mu g/m^3$	46	60	0.90 (0.54, 1.49)		1.00 (ref)	
	>12.0 $\mu g/m^3$	141	99	2.17 (1.14, 4.15)	0.81 (-0.88, 2.47)	2.42 (1.20, 4.86)	0.08

Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; LRT, likelihood ratio test; NDI, neighborhood deprivation index; OR, odds ratio; $PM_{2.5}$, particulate matter <2.5 μm; RERI, relative excess risk due to interaction. All models are adjusted for study site, year of birth, month of birth, maternal education, maternal race/ethnicity, maternal age, and maternal smoking.

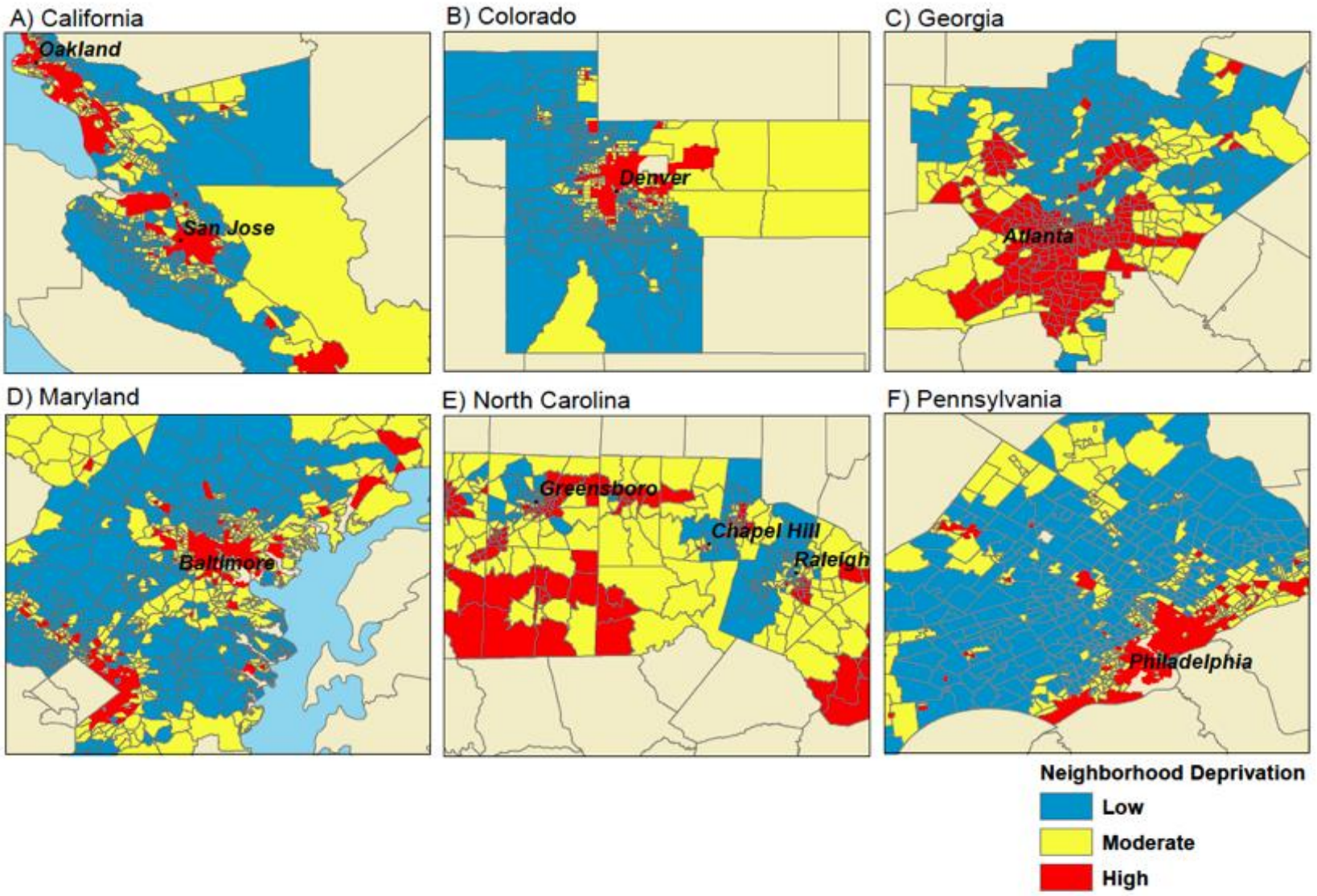


Figure 5.1 Neighborhood deprivation distribution by SEED study site

5.4 Discussion

This study observed modification by neighborhood deprivation for the association between $PM_{2.5}$ exposure during the first year of life and ASD, with the strongest association observed for the joint effect between high neighborhood deprivation and $PM_{2.5}$ levels above 12.0 $\mu g/m^3$. This study was the first U.S.-based study to address the combined effect of neighborhood deprivation and air pollution on risk of ASD. One previous study in Sweden assessed the modifying role of neighborhood deprivation on air pollution in association with ASD and found none [173]. The role of neighborhood deprivation likely differs for the U.S. and Sweden, given the overall differences in access to health care and childcare between the two countries. Previous U.S.-based epidemiological studies have shown that psychological stress and social disadvantage can modify air pollution and health associations [193-197], with several showing synergistic effects of air pollution and social disadvantage in relation to pregnancy outcomes [197, 198].

Distance to major roadway was used as a marker of the mixture of chemicals from traffic-related air pollution. The cutoff for living in close proximity to a major road was based on the distribution in the controls (closest 10%), which is similar to the distribution in a previous study of roadway proximity and ASD [168]. Although mothers in the most deprived census tracts of this study were more likely to live in close proximity to a major road, this study found elevated odds of ASD in relation to roadway proximity only for those in the moderate deprivation group. Thus, modification by neighborhood deprivation differed for exposure to roadway proximity and exposure to $PM_{2.5}$ in the first year of life. The distance to roadway measure is a proxy for local traffic particles [34], whereas $PM_{2.5}$ represents both local and regional transported particles [242], thus differences in the results may be due to the difference in the two exposures.

Neighborhood deprivation may impact health outcomes in multiple ways. First, living in a deprived area may limit access to resources (health care, parks and other places for physical activity). Alternatively, stressors at the neighborhood level could contribute to individual chronic stress. The hypotheses of this study were based on of the second pathway, although it is plausible that living in a more deprived neighborhood could reduce access to health care, with particular implications for ascertainment of ASD. Neighborhood deprivation has been implicated as a social stressor in previous studies [238, 239], with one study finding associations between neighborhood deprivation and cortisol reactivity among women [240]. There are several theories relating the combined effects of social and environmental stressors to health outcomes. One in particular (22) theorizes that stressors at the neighborhood level can contribute to individual chronic stress, which can influence individual susceptibility, and this stress-induced susceptibility can shape response to environmental exposures. Using this framework, this study hypothesized that chronic stress from neighborhood deprivation could influence individual susceptibility and ultimately shape maternal and infant responses to air pollution exposure.

A synergistic effect of air pollution and maternal stress in relation to disease development is biologically plausible given their potentially shared inflammatory pathway. Recent animal studies have investigated the combined effect of maternal stress and air pollution exposure on health outcomes in offspring. Findings in mice showed a combined effect of maternal stress during pregnancy and air pollution exposure on neuroinflammation, microglia activation, and neurobehavioral outcomes in offspring [200]. These findings led to the theory that early life maternal stress can induce an inflammatory reaction, sensitize microglia in the offspring, and make individuals more vulnerable to subsequent challenges, such as air pollution exposure [159]. In relation to the development of ASD, alterations in microglial development by early postnatal

inflammation may alter synaptic pruning [163, 164], resulting in altered neuronal connectivity, and disruption of normal brain development.

Like many other air pollution epidemiologic studies, the modeled air pollution estimates used in this study represent outdoor area level ambient concentrations and do not take into consideration indoor exposures or time spent away from home. Further, exposure assessment and linkage with census tract data was based solely on the residential address at birth, which assumes limited mobility during pregnancy and the year after delivery; however, previous studies have shown little change in exposure assignment when using the birth address versus the complete residential history during pregnancy [179, 180], although one study did show somewhat greater exposure misclassification for the pregnancy period than for first year of life [181].

This study used information from the U.S. Census to construct a weighted area-level deprivation index, and made no direct measurement of neighborhood physical and social environments. This measure of area level neighborhood deprivation was used as a proxy for inequalities to resources and maternal stress, but this study made no direct measure of self-reported stress during pregnancy or early life. By using this measure this study makes the assumption that those living in more deprived areas would potentially have higher levels of chronic stress, however social control and other individual characteristics may modify this relationship [239].

Another potential limitation is the selectivity of the SEED sample. A number of families of potentially eligible children did not respond to the SEED invitation letter. One SEED site was able to assess characteristics of responders and non-responders – their findings showed that maternal education, age, and race/ethnicity were associated with non-response. Analyses adjusted for all three of these variables in the statistical models in order to address this limitation.

Despite these limitations, this study has several strengths. The Clean Air Act required the U.S. Environmental Protection Agency to set National Ambient Air Quality Standards for criteria air pollutants, including $PM_{2.5}$. The primary standard of 12.0 $\mu g/m^3$ was set to "protect public health, including the health of sensitive populations such as asthmatics, children, and the elderly" [243]. Therefore, this study chose this cut-point for the categorized version of $PM_{2.5}$. This study additionally assessed associations with continuous measures of $PM_{2.5}$ for comparability with other studies. The cut-point for living in close proximity to a major road is similar to that of a previous air pollution and ASD study [168], and other proximity to roadway studies [244, 245].

The current study is the first U.S.-based study to assess the modifying role of neighborhood deprivation on the association between air pollution and ASD. This study assessed modification on the both the additive and multiplicative scale using a validated measure of neighborhood deprivation. This study additionally used rigorous case-classification based on gold standard outcome ascertainment tools. Finally, this study used both roadway proximity and satellite-based modelled $PM_{2.5}$ estimates, in order to capture both local near roadway and background $PM_{2.5}$ exposure.

In summary, this study observed suggestive evidence of a stronger association between $PM_{2.5}$ exposure in the first year of life and ASD for those living in more deprived areas. Additional research in this area of the combined effects of environmental and social stressors is warranted in order to help identify susceptible subgroups that are particularly vulnerable to both of these stressors. Future studies should consider associations using direct measurements of the neighborhood environment, as well as individual measures of perceived stress or biologic measures.

CHAPTER 6: SUMMARY OF FINDINGS

The goal of this dissertation was to investigate the association between pre- and postnatal $PM_{2.5}$ and ozone exposure and ASD during critical windows of susceptibility. In order to accomplish this goal, this dissertation used data from the Study to Explore Early Development, a multi-site case-control study with six different sites throughout the United States with differing exposure levels and variability. This study additionally investigated the modifying role of neighborhood deprivation on the association between early life air pollution exposure and ASD. This study made use of gold standard outcome ascertainment tools, exposure data from a state of the art air pollution model, and extensive covariate information.

6.1 Early Life Air Pollution Exposure and ASD

Specific Aim 1 assessed associations between $PM_{2.5}$ and ozone and ASD during critical windows of neurodevelopment, including exposure three months before pregnancy, over the entire pregnancy period and trimester averages, as well as during the first year of life. Overall, these analyses identified two potential windows of susceptibility to environmental insults in relation to ASD. First, $PM_{2.5}$ exposure during the first year of life was associated with increased risk of ASD, consistent with a few recent U.S.-based studies [13] [14]. This finding held in multipollutant models and models mutually adjusted for pregnancy exposure averages. $PM_{2.5}$ exposure during the third trimester was additionally associated with ASD, though results were weak and imprecise. This study did not observe an association between $PM_{2.5}$ exposure during pregnancy and ASD. Several U.S.-based studies have found associations with $PM_{2.5}$

exposure during pregnancy and ASD [11-14], however the findings have been null or inconsistent for studies in Europe [173, 246].

Third trimester ozone exposure was associated with an increased risk of ASD in this study population. There was additionally an association with ozone exposure during the entire pregnancy period, however this result was only present in models mutually adjusted for the first year of life exposure average. In a previous California-based study, Becerra et al. [11] found associations between ozone exposure during pregnancy and ASD, in single and multi-pollutant models additionally adjusted for $PM_{2.5}$ exposure. $PM_{2.5}$ and ozone exposure averages were not highly correlated in this study population and thus results were similar in single and multi-pollutant models.

Associations for third trimester ozone and first year of life $PM_{2.5}$ exposure were consistent across different geographic locations in this study. However, results varied across study site for several of the other developmental windows. There are a few potential reasons for this geographic variation in study findings. First, exposure levels varied between sites, with the highest $PM_{2.5}$ levels in Georgia, and the lowest in Colorado and California. In addition, on the East coast $PM_{2.5}$ is dominated by sulfates from power plants and other regional sources; in contrast, nitrates make up the largest composition of $PM_{2.5}$ on the west coast [25]. Thus, the regional differences in particulate mass composition and exposure levels could contribute to differences in the estimated associations between air pollution and ASD from different geographic regions described in the literature, and across study sites in this study population.

Specific Aim 1 included several sensitivity analyses. First, analyses assessed the independent effects of both ozone and $PM_{2.5}$ in multipollutant models, by including both pollutants in the same model for each developmental window. In general, the results were similar

to single pollutant models, though effect estimates were often attenuated. Next, in order to isolate potential critical windows of susceptibility, associations were assessed in models mutually adjusted for the other developmental windows. For trimester-specific mutually adjusted models, there were stronger associations for third trimester ozone exposure, but not for the other trimesters. Additionally, results varied by pollutant and developmental window when including both first year of life and pregnancy averages in the same model. For ozone exposure there were stronger associations for the pregnancy period when adjusting for first year of life exposures, while for $PM_{2.5}$ the strength of the association during the first year of life increased when adjusting for pregnancy averages. Interpretation of these results is somewhat complicated by the high correlations between the pregnancy and first year of life exposure averages.

Tobacco smoke contains many of the same chemicals found in air pollutants, thus may have synergistic effects with air pollution to increase risk of ASD. Specific Aim 1 explored this hypothesis. Findings from this analysis showed suggestive modification for third trimester $PM_{2.5}$ exposure and second trimester ozone exposure, with elevated ORs for these windows only in women who used tobacco during pregnancy. These findings provide preliminary evidence for a combined effect of maternal tobacco use and air pollution exposure on risk of ASD and suggest a need for future studies to examine the effects of multiple toxicants together.

Few studies have considered the heterogeneity of ASD, but Specific Aim 1a examined several methods of grouping children with ASD, including by co-occurring intellectual disability and ASD severity. Overall, associations did not vary between those with and without an intellectual disability, consistent with one previous study [15]. The ADOS Core Symptom Severity was used to reflect the degree of impairment specific to ASD core severity symptoms. Associations differed by ASD core symptom severity, but were inconsistent across

developmental window and pollutant. One previous study assessed associations with air pollution exposure and cognitive and adaptive function and ASD severity [247]. Findings from their study showed associations between air pollution exposure and decreased language, communication, adaptive abilities, and fine motor skills, but not with ASD severity. Associations were additionally assessed in phenotypic subgroups of ASD cases (see Table D.1 in Appendix D). In the phenotypic subgroup analyses, there were slightly stronger associations for the dysregulation subtype of ASD, distinguished by mild language and motor delays. This class additionally had higher rates of co-occurring conditions, including anxiety/depression and sleep disorders. While this reveals some insight regarding which aspects of brain development may be susceptible to air pollution, the phenotypic class analyses were based on limited sample size, thus results should be interpreted with caution, and therefore were not included in the main results in Chapter 4.

The temporal and spatial complexity of air pollution exposures presents several methodological challenges in epidemiological studies. Study site was controlled for in all analyses. There was an increase in estimate for the year post birth exposure period when adjusting for study site. Results from trimester exposure models were less sensitive to adjustment for study site, however were affected by adjustment for month of birth to control for seasonal trends. Thus, spatial and temporal confounding differed by length of exposure period – the longer exposure periods were more sensitive to spatial confounding, while shorter exposure periods were in general more sensitive to temporal confounding.

Taken together, the results from Specific Aim 1 suggest a late prenatal and early postnatal potential critical window of susceptibility. In addition to the findings in relation to autism, previous studies have noted associations with air pollution exposure during these

developmental windows and several other adverse early life health outcomes. Previous epidemiologic studies have found associations between third trimester ozone exposure and several reproductive outcomes including IUGR [248] and low birth weight [249]. Using a distributed lag model approach, Rosa et al. [250] found associations between $PM_{2.5}$ during the late prenatal period (35-40 weeks) and lower mitochondrial DNA (mtDNA) content in cord blood. Given that mtDNA is a marker of cumulative oxidative stress, this study gives insight into the mechanisms by which air pollution exposure may influence several early life outcomes, including ASD. Finally, studies have revealed associations between air pollution exposure during the first year of life and lung function [251, 252] allergies [253], and asthma [254, 255] in children. The immune system may therefore be particularly vulnerable to insults from environmental toxicants during this developmental window.

Although it is possible for air pollution to have direct neurotoxic effects on the developing brain, the main hypothesized pathway linking air pollution exposure to autism is through an inflammatory response. Air pollutants are established immune toxicants [6], and have been shown to induce inflammation and oxidative stress [9, 10, 155]. Maternal and infant air pollution exposure can result in systemic oxidative stress, inflammation, and production of pro-inflammatory cytokines (IL-6, TNF-a) [7, 8, 63]. These pro-inflammatory cytokines may be able to reach the developing brain, cross the blood brain barrier, resulting in neuroinflammation, neuron damage/loss, microglia activation, and DNA damage [8]. Studies have found higher levels of these cytokines in children with ASD [160].

Of emerging interest is the mediating role of microglia activation in prenatal and early postnatal toxicant exposure in relation to ASD [162]. Microglia are the resident immune cells

of the central nervous system and play a key role in pruning of developing synapses [164, 256]. Alterations in microglial development by in utero and early postnatal inflammation may alter synaptic pruning [163, 164], and disruption of normal brain development. Recent studies in mice have found associations between gestational exposure to ultrafine particles and microglial activation in male offspring [165], with one study suggesting a third trimester equivalent window of susceptibility in humans [166]. Another animal study found associations between gestational exposure to diesel exhaust particles, increased cytokine production, and altered trajectory of microglia, only in male mice [167].

In summary, the results from this study provide evidence for a positive association between early life exposure to air pollution and ASD, and identifies a potential late prenatal and early postnatal critical window of susceptibility.

6.2 Air Pollution, Neighborhood Deprivation, and ASD

Specific Aim 2 investigated the potential modifying role of neighborhood deprivation on the association between distance to nearest major roadway and $PM_{2.5}$ exposure and ASD. Findings from these analyses showed modification by neighborhood deprivation for the association between $PM_{2.5}$ exposure during the first year of life and ASD among children, with the strongest association observed for the joint effect between high neighborhood deprivation and $PM_{2.5}$ levels above 12.0 $\mu g/m^3$. Although one previous study in Sweden assessed the modifying role of neighborhood deprivation on air pollution and ASD association [173], this was the first U.S.-based study, and first to assess associations on both the additive and multiplicative scale. Previous U.S.-based epidemiological studies have shown that psychological stress and social disadvantage can modify air pollution and health associations [193-197], with several

showing synergistic effects of air pollution and neighborhood disadvantage in relation to pregnancy outcomes [197, 198].

This aim used distance to major roadway as a marker of the mixture of chemicals from traffic-related air pollution. The cutoff for living in close proximity to a major road was based on the distribution in the controls (closest 10%), which is similar to the distribution in a previous study of roadway proximity and ASD [168]. Although mothers in the most deprived census tracts of this study were more likely to live in close proximity to a major road, this study found elevated odds of ASD in relation to roadway proximity for those in the moderate deprivation group. Thus, the modification by neighborhood deprivation findings were slightly different for roadway proximity and $PM_{2.5}$ exposure in the first year of life. The distance to roadway measure is a proxy for local traffic particles, whereas $PM_{2.5}$ represents both local and regional transported particles, thus some differences in the results between the two exposures is to be expected.

Findings from Specific Aim 2 additionally have environmental justice implications. In these analyses, controls in the highest deprivation category were more likely to be non-white, and born to lower educated, lower income mothers who used tobacco. Additionally, $PM_{2.5}$ averages in controls during the pregnancy period were 13.3 µg/m3 in the highest deprivation group and 12.6 µg/m3 and 12.4 µg/m3 in the moderate and low deprivation groups, respectively. Those in the highest deprivation group were additionally more likely to live closer to a major road/highway. These findings are consistent with several previous epidemiologic studies that have found higher air pollution levels in more deprived areas [187].

Neighborhood deprivation has been implicated as a social stressor in previous studies [238, 239], with one study finding associations between neighborhood deprivation and cortisol reactivity among women [240]. There are several theories relating the combined effects of social

and environmental stressors to health outcomes. One in particular [191] theorizes that stressors at the neighborhood level can contribute to individual chronic stress, which can influence individual susceptibility, and this stress-induced susceptibility can shape response to environmental exposures. Using this framework, this study hypothesized that chronic stress from neighborhood deprivation could influence individual susceptibility, which could impair the body's ability to maintain allostasis, compromise immune function, and ultimately shape maternal and infant responses to air pollution exposure.

The association between a synergistic effect of air pollution and maternal stress in relation to disease development is biologically plausible given their potentially shared inflammatory pathway. Findings from one recent study in mice showed a combined effect of maternal stress during pregnancy and air pollution exposure on neuroinflammation, microglia activation, and neurobehavioral outcomes in offspring [200]. Findings from their study led to the theory that early life maternal stress can induce an inflammatory reaction, sensitize microglia in the offspring, and make individuals more vulnerable to subsequent challenges, such as air pollution exposure [159]. In relation to the development of ASD, alterations in microglial development by early postnatal inflammation may alter synaptic pruning [163, 164], resulting in altered neuronal connectivity, and disruption of normal brain development.

In conclusion, this study found modification by neighborhood deprivation for the association between $PM_{2.5}$ exposure during the first year of life and ASD, with the strongest associations seen for the joint effect between high neighborhood deprivation and $PM_{2.5}$ levels above the NAAQS level of 12.0 $\mu g/m^3$. Future research is needed to specifically address the combined effect of air pollution and maternal exposure to social stressors and to clarify the complex mechanisms mediating social and environmental stressors in relation to ASD.

6.3 Strengths and Limitations

Study Limitations

As in similar studies of air pollution and ASD, it is important to consider the potential impact of exposure misclassification when interpreting results. This study used data from satellite-based air pollution models to characterize $PM_{2.5}$ and ozone exposure. Using ambient air quality data instead of personal exposure data could result in measurement error. However, this source of measurement error is Berksonian in nature, thus does not induce large amounts of bias, but could increase standard errors of the effect estimates [176]. Using an inaccurate community average exposure could potentially bias estimates, however the use of modeled air pollution data should help to reduce this potential source of measurement error [176].

Although exposures were assigned from just before pregnancy to one year after birth, exposure assessment was based on only the residential address at birth. This assumes limited mobility during pregnancy; however, studies have shown little change in exposure assignment when incorporating the complete residential history during pregnancy [179, 180]. Though one study showed somewhat greater exposure misclassification for the pregnancy period than for first year of life [181]. The residential address at birth was additionally used to assign census data measures. By doing this, this study assumes that women have a constant neighborhood deprivation level from start of pregnancy through the first year of life. One study found that although women of low SES tend to move more often, they tend to stay in the same neighborhood deprivation level, while women of higher SES tend to move to more deprived areas.

On the East coast $PM_{2.5}$ is dominated by sulfates from power plants and regional sources, while nitrates make up the largest composition of $PM_{2.5}$ on the west coast [25]. Therefore, some

of the discrepancy in findings by study site could be due to different effects of $PM_{2.5}$ components on risk of ASD. This study did not have information on $PM_{2.5}$ components in the current study but this is of interest in future studies. Assessing associations with $PM_{2.5}$ components would likely give insight into the variation in study findings by geographic location.

Due to privacy concerns, the CDC shifted each participant's date of birth by up to two weeks in either direction. The shifting of date of births precluded this study from assessing associations with shorter windows of exposure, including weekly and monthly. The exact date of births were available for the NC SEED study site, thus sensitivity analyses were conducted using the NC site's exact date of births and compared the exposure distribution and results when using the shifted date of births. In general, mean $PM_{2.5}$ levels were similar for each of the longer developmental windows (trimesters, pregnancy, etc.) when using the shifted and true date of births (see Tables D.2 and D.3 in Appendix D), however differed when using weekly averages. For example, the mean individual differences between weekly $PM_{2.5}$ exposure when using the true vs. the shifted DOB was 2.2-2.7 $\mu g/m^3$, with a maximum difference of 9.3-16.9 $\mu g/m^3$. Correlations between true and shifted date of birth exposure averages ranged from 0.61 to 0.76 for weekly averages, 0.94-0.97 for monthly averages, and 0.99-1.00 for trimester and pregnancy averages. This study additionally found similar effect estimates for the shifted date of birth results, though many were attenuated to the null. In sum, the amount of exposure misclassification increased as length of exposure average was reduced - i.e. weekly exposure averages showed the most amount of misclassification (Figure D.1 in Appendix D), pregnancy averages showed the least. Therefore, although exact dates are preferred in most analyses, these sensitivity analyses show minimal exposure misclassification for longer exposure averages.

Another potential limitation includes the low/modest response rates in SEED. Poor

response rates could lead to selection bias since there could potentially be differences in the types of people that do and do not agree to participate in SEED, which could in turn threaten both internal and external validity. Selection bias may additionally occur when controls are not representative of the source population from where cases arise. The controls in SEED are of slightly higher SES than the cases and the general population, as has been seen in several other case-control studies [257]. One SEED site was able to assess characteristics of responders and non-responders – their findings showed that maternal education, age, and race/ethnicity were associated with non-response. All three of these variables were adjusted for in this study.

The use of data from multiple sites throughout the country increased the sample size and statistical power for the main pooled analyses. However, there may have been limited power to detect an association in the site-specific and subgroup analyses because of the reduced sample size in each site and subgroup. SEED II recently enrolled ASD and population groups with comparable sizes to SEED I. Pooling both sets of data in future analyses will increase the sample size and power for the site-specific and subgroup analyses.

Confounding is another potential source of bias in this analysis. Although maternal education was adjusted for as a marker of socioeconomic status, there is still the potential for residual confounding by SES or place of residence. Study site was controlled for in all analyses, therefore limiting potential confounding by characteristics related to study site. There was an increase in estimate for the year post birth exposure period when adjusting for study site, but not for the shorter exposure averages. Longer exposure averages rely more on spatial contrasts in exposure and are therefore potentially more sensitive to confounding by unmeasured characteristics related to site.

Ozone shows strong seasonal trends in many areas of the country, with the highest

concentrations during the summer months. In addition, several previous studies have found associations between season of birth and ASD [222, 223]. Therefore, this study adjusted for month of birth in all analyses to account for seasonal trends related to both exposure and ASD. Previous studies have additionally found that season of birth is related to SES and other environmental pollutants have seasonally varying patterns [161].

Finally, this study used census data to construct an area level deprivation index, and made no direct measurement of neighborhood physical and social environments. This measure of area level neighborhood deprivation was used in this study as a proxy for inequalities to resources and maternal stress, but made no direct measure of self-reported stress during pregnancy or early life. By using this measure this study makes the assumption that those living in more deprived areas would potentially have higher levels of chronic stress, however social control and other individual characteristics may modify this relationship [239]. However, living in a deprived area limits access to care and healthy foods, physical activity, and increases an individual's exposure to other environmental toxicants that concentrate in lower SES areas. Thus, this measure captures a broad sense of deprivation.

Study Strengths

Despite these limitations, this study has several strengths. SEED study participants come from six different study sites located in the western, central, and eastern U.S., thus providing adequate spatial variability to assess the health effects from air pollution exposure. The multisite component of the SEED study ensures inclusion of diverse populations and generalizability of SEED results compared to studies from just one localized geographic area. In addition to the generalizability of results, use of multiple sites allows for potential exploration of differences in air pollution mixtures by geographic location, particularly for western versus eastern locations. SEED also has considerable temporal variability with SEED births spanning 2003-2006. Finally,

this study assessed associations with air pollution exposure during multiple windows of susceptibility, modeled separately and together to isolate critical windows of exposure.

SEED uses gold standard outcome ascertainment tools, including ADOS and ADI-R, therefore reducing any potential outcome misclassification. The ADOS and ADI-R instruments are administered by trained personal and more accurately capture disease status, over parental report measures often used in other studies. SEED also collects data on phenotypic subgroups and autism severity measures, thus allowing stratification by disease subgroups, which few previous air pollution and ASD studies have done. Further, SEED uses uniform study protocol across sites thus ensuring uniform eligibility criteria and data collection protocols. This in turn ensures high intrasite and intersite developmental assessment reliability and case confirmation. The only other multisite air pollution and ASD study used multiple outcome ascertainment methods and did not have one standard protocol across sites. SEED additionally uses multisource case ascertainment, thus attempting to capture the full range of children with ASD (from mild to severe), and reducing bias that could arise from limiting study families to those seen at a single facility.

This study was the first to assess associations between $PM_{2.5}$ and ozone and ASD using a state of the art model that incorporates satellite-based data at a fine spatial resolution, as well as land-use terms, meteorological variables, and data from a chemical transport model. Land-use regression models have adequate spatial resolution, however often have poor temporal resolution since the land-use terms are usually time-invariant [37]. The use of satellite data helps to improve temporal resolution in the resulting exposure predictions, and allows the inclusion of rural locations further away from monitors, thus limiting the need to exclude participants located further away from a monitor.

This was the first study in the U.S. to assess whether neighborhood deprivation modifies the association between early life exposure to air pollution and ASD. Becerra et al. [11] stratified by individual level SES, but few to date have evaluated whether area level deprivation modifies the association between air pollution and ASD. The combination of neighborhood deprivation and air pollution is important to consider because of their similar spatial distribution and potential for both to impact health through inflammatory pathway. This study showed that air pollution and social stress may have synergistic effects, resulting in a greater impact on the onset of ASD in children.

Finally, this study has considerable data to measure and account for important covariates and better represent the causal model. Few previous air pollution-ASD studies of this size have been able to incorporate such extensive covariate information to reduce the potential for confounding.

6.4 Public Health Implications and Future Research

This dissertation provides evidence of an association between air pollution exposure during a critical window of susceptibility and ASD. This observed association was stronger in the presence of neighborhood deprivation. Due to the ubiquitous nature of air pollution, even small effect sizes can have significant public health impacts, especially when sensitive populations are affected. The Clean Air Act mandates that air quality standards be set to protect susceptible populations, such as pregnant women, infants, and children. Thus, the results from this study may aid in setting air pollution standards for regulatory action for these susceptible populations and suggests that economically deprived regions may be of greatest risk.

Findings from this study varied by window of susceptibility and geographic location. Additional research is needed to further clarify windows of susceptibility for toxicant exposure

in relation to ASD, potentially by using more refined windows of exposure, including weekly exposure averages during pregnancy. Use of the distributed lag model may help to identify a potential critical window of susceptibility while taking into account the correlated nature of the developmental windows. In addition, some of the discrepancy in findings by study site could be due to different effects of $PM_{2.5}$ components on risk of ASD. Further research is needed to specifically address the association between individual $PM_{2.5}$ components and ASD.

Animal studies are emerging that shed light on the causal mechanisms between air pollution exposure and neurodevelopmental outcomes. However, further research is needed to understand the mechanistic pathways linking air pollution exposure and ASD, and specifically to address the combined effects of multiple risk factors, including social and environmental toxicants.

The exact etiology of ASD is poorly understood, however it is likely a combination of multiple risk factors, including genetic and environmental factors. At the time of this study, limited genetic data was available on the child and mother. Future analyses using SEED data will take into consideration genetic susceptibility when investigating the association between early life air pollution exposure and neurodevelopmental outcomes.

This study assessed associations between air pollutant exposure and both ASD severity and phenotypic subgroups of ASD cases. Results from this study showed suggestive evidence of differences by severity and subgroup, however the small sample size of each subgroup precluded this study from making any meaningful inferences. Additional work is needed in this area, in order to evaluate if associations vary by level of severity and subgroups.

Finally, this study found modification of the air pollution and ASD association by neighborhood deprivation. Area level deprivation was characterized using a neighborhood

deprivation index measure at the census tract level. Measures of perceived stress during pregnancy may more adequately capture stress experienced by the mother during pregnancy. Additional measures of perceived stress may help to clarify associations between maternal stress and ASD, and the modifying role of maternal stress on air pollution and ASD associations.

6.5 Conclusions

Given the rising prevalence and impact on individuals and their families, there is a need to identify risk factors for ASD. This study investigated the association between early life air pollution exposure and ASD using windows of exposure before, during, and after pregnancy. SEED participants came from six different states across the country, thus providing adequate spatial variability for studying the health effects of air pollutants. The magnitude of the association differed by geographic location and window of susceptibility. This study additionally investigated whether individual and neighborhood level stressors modify the association between air pollution and ASD. The results from this study identified a late prenatal and early postnatal potential window of susceptibility for the association between air pollution and ASD, revealed potentially susceptible subgroup populations, and allowed comparison of effects for different geographical areas of the U.S. Future research is warranted to confirm the findings from this study regarding the adverse impact of $PM_{2.5}$ and ozone exposure on ASD development, and to explore possible variations by additional measures of ASD severity, as well as PM composition.

APPENDIX A: FIGURES FOR CHAPTER 3

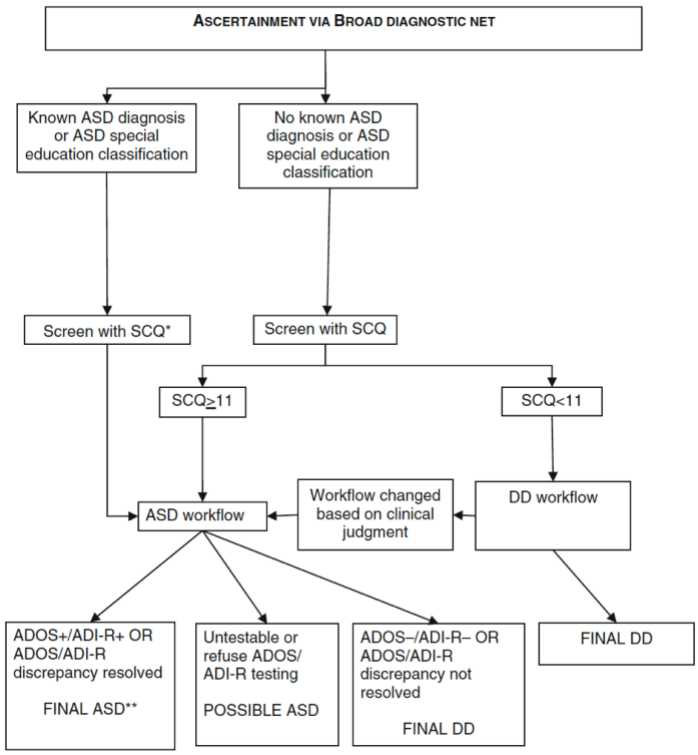


Figure A.1 Autism spectrum disorder diagnostic workflow, from Schendel et al. [205]

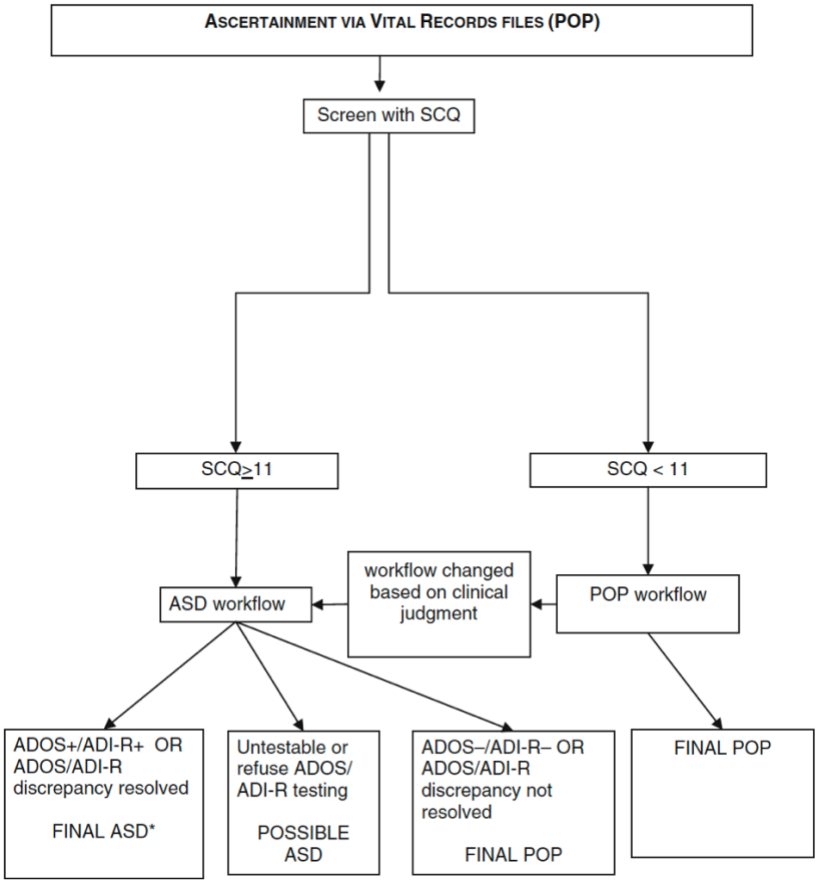


Figure A.2 Population control diagnostic workflow, from Schendel et al. [205]

APPENDIX B: SUPPLEMENTARY TABLES FOR CHAPTER 4

Table B.1 Characteristics of the Study to Explore Early Development study population by case-control status and study site

	California		Colorado		Georgia		Maryland		North Carolina		Pennsylvania	
Characteristic	ASD (*n*=96)	Controls (*n*=134)	ASD (*n*=139)	Controls (*n*=185)	ASD (*n*=130)	Controls (*n*=160)	ASD (*n*=107)	Controls (*n*=126)	ASD (*n*=100)	Controls (*n*=146)	ASD (*n*=102)	Controls (*n*=104)
Sex												
Male	72 (75)	76 (57)	124 (89)	103 (56)	104 (80)	79 (49)	86 (80)	62 (49)	81 (81)	68 (47)	84 (82)	65 (63)
Female	24 (25)	58 (43)	15 (11)	82 (44)	26 (20)	81 (51)	21 (20)	64 (51)	19 (19)	78 (53)	18 (18)	39 (38)
Birth Year												
2003-2004	42 (44)	38 (28)	52 (37)	106 (57)	59 (45)	70 (44)	57 (53)	70 (56)	33 (33)	79 (54)	30 (29)	30 (29)
2005-2006	54 (56)	96 (72)	87 (63)	79 (43)	71 (55)	90 (56)	50 (47)	56 (44)	67 (67)	67 (46)	72 (71)	74 (71)
Maternal race												
White	30 (31)	68 (51)	98 (71)	141 (76)	53 (40)	85 (53)	79 (74)	107 (85)	66 (66)	123 (84)	52 (51)	87 (84)
Other[a]	66 (69)	66 (49)	41 (30)	44 (24)	77 (60)	75 (47)	28 (26)	19 (15)	34 (34)	23 (16)	50 (49)	17 (16)
Maternal education												
<Bachelor's	50 (52)	49 (37)	85 (61)	70 (38)	64 (49)	66 (41)	39 (31)	53 (50)	33 (33)	42 (29)	46 (45)	23 (22)
≥Bachelor's	46 (48)	85 (63)	54 (39)	115 (62)	66 (51)	94 (59)	87 (69)	54 (50)	67 (67)	104 (71)	56 (55)	81 (78)
Maternal age at delivery (years)												
<35	66 (69)	83 (62)	103 (74)	134 (72)	91 (70)	115 (72)	82 (77)	85 (67)	70 (70)	107 (73)	74 (73)	65 (63)
≥35	30 (31)	51 (38)	36 (26)	51 (28)	39 (30)	45 (28)	25 (24)	41 (33)	30 (30)	39 (27)	28 (27)	39 (38)
Maternal smoking												
Yes	10 (10)	7 (5)	29 (21)	20 (11)	20 (15)	15 (9)	23 (22)	13 (10)	14 (14)	14 (10)	16 (16)	10 (10)
No	86 (90)	127 (95)	110 (79)	165 (89)	110 (85)	145 (91)	84 (78)	113 (90)	86 (86)	132 (90)	86 (84)	94 (90)
Preterm birth												
Yes	10 (10)	14 (10)	24 (17)	19 (10)	26 (20)	21 (13)	8 (7)	11 (9)	26 (26)	7 (5)	17 (17)	10 (10)
No	86 (90)	120 (90)	115 (83)	166 (90)	104 (80)	139 (87)	99 (93)	115 (91)	74 (74)	139 (95)	85 (83)	94 (90)
$PM_{2.5}$ (μg/m³) (mean ± SD)												
Pregnancy	11.3±1.4	11.6±1.4	8.9±1.1	9.0±1.2	15.8±1.3	15.5±1.1	14.7±1.4	14.8±1.3	13.1±1.3	13.0±1.0	13.5±0.9	12.9±0.9
First year of life	11.4±0.7	11.3±0.7	8.8±0.9	8.7±1.0	15.6±0.6	15.5±0.6	14.5±1.0	14.5±1.1	13.1±0.7	13.1±0.7	13.5±0.9	13.0±0.6
Ozone (ppb) (mean ± SD)												
Pregnancy	29.7±3.8	29.5±3.6	38.8±5.2	39.8±5.3	38.8±4.5	39.3±4.5	37.3±4.5	36.8±4.6	40.0±4.1	39.7±3.0	35.2±5.6	35.3±4.9
First year of life	30.6±2.8	31.1±2.9	40.5±3.4	40.0±4.0	39.7±2.3	40.0±2.2	37.4±2.8	38.2±2.5	41.2±1.9	40.5±1.7	36.0±2.8	38.3±2.6

Abbreviations: ASD, autism spectrum disorder; $PM_{2.5}$, particulate matter <2.5 μm; SD, standard deviation.
[a]Includes African American, Asian, Hispanic, multiracial, and all others.

Table B.2 Spearman correlation coefficients for $PM_{2.5}$ and ozone estimates averaged across developmental windows, among SEED participants

	$PM_{2.5}$ Exposure						Ozone Exposure					
	First trimester	Second trimester	Third trimester	Pre-conception	All Pregnancy	1st year	First trimester	Second trimester	Third trimester	Pre-conception	All Pregnancy	1st year
$PM_{2.5}$												
First trimester	1.0											
Second trimester	0.38	1.0										
Third trimester	0.20	0.43	1.0									
Preconception	0.42	0.21	0.41	1.0								
All pregnancy	0.70	0.81	0.72	0.47	1.0							
1st year	0.67	0.67	0.70	0.70	0.92	1.0						
Ozone												
First trimester	0.14	0.17	-0.18	-0.15	0.06	-0.03	1.0					
Second trimester	-0.10	0.15	0.25	-0.12	0.13	0.07	0.10	1.0				
Third trimester	-0.17	-0.14	0.25	0.20	-0.03	0.05	-0.67	0.15	1.0			
Preconception	0.09	-0.23	-0.22	0.17	-0.15	-0.08	0.10	-0.64	0.09	1.0		
All pregnancy	-0.07	0.13	0.21	-0.06	0.12	0.06	0.33	0.83	0.29	-0.29	1.0	
1st year	-0.03	-0.07	0.04	0.05	-0.03	-0.02	0.26	0.31	0.37	0.36	0.64	1.0

Table B.3 Odds ratios and 95% confidence intervals for the associations between early life air pollution and ASD, comparing crude and adjusted models

	Model 1[a] [OR (95%CI)]	Model 2[b] [OR (95%CI)]	Model 3[c] [OR (95%CI)]	Model 4[d] [OR (95%CI)]
Ozone (ppb)				
Preconception	0.97 (0.91, 1.02)	0.99 (0.93, 1.06)	0.98 (0.92, 1.05)	0.92 (0.82, 1.05)
Entire pregnancy	0.96 (0.85, 1.08)	1.05 (0.93, 1.20)	1.03 (0.88, 1.21)	1.06 (0.85, 1.32)
First Trimester	0.99 (0.94, 1.05)	1.02 (0.96, 1.08)	1.01 (0.95, 1.08)	0.94 (0.83, 1.07)
Second Trimester	0.97 (0.92, 1.03)	0.99 (0.93, 1.06)	0.98 (0.92, 1.05)	0.96 (0.85, 1.09)
Third Trimester	1.00 (0.95, 1.06)	1.02 (0.96, 1.08)	1.01 (0.95, 1.07)	1.22 (1.05, 1.42)
First year of life	0.89 (0.76, 1.03)	0.98 (0.83, 1.16)	0.80 (0.61, 1.05)	0.79 (0.60, 1.04)
$PM_{2.5}$ ($\mu g/m^3$)				
Preconception	1.04 (0.99, 1.09)	1.03 (0.99, 1.08)	1.03 (0.97, 1.11)	1.04 (0.97, 1.12)
Entire pregnancy	1.03 (0.97, 1.09)	1.03 (0.96, 1.09)	1.01 (0.88, 1.16)	1.02 (0.88, 1.19)
First Trimester	1.01 (0.96, 1.06)	1.00 (0.95, 1.05)	0.98 (0.91, 1.04)	0.98 (0.91, 1.05)
Second Trimester	1.00 (0.96, 1.05)	1.00 (0.96, 1.05)	0.99 (0.93, 1.06)	0.97 (0.91, 1.04)
Third Trimester	1.03 (0.98, 1.07)	1.03 (0.98, 1.08)	1.03 (0.96, 1.09)	1.06 (0.98, 1.14)
First year of life	1.05 (0.99, 1.12)	1.05 (0.98, 1.12)	1.24 (1.00, 1.53)	1.26 (1.02, 1.57)

Results are reported per 1.6-$\mu g/m^3$ increase in $PM_{2.5}$ and 6.6-ppb increase in ozone.
[a]Model 1: crude model.
[b]Model 2: adjusted for maternal age, maternal education, maternal race/ethnicity, maternal smoking, year of birth.
[c]Model 3: model 2 + adjustment for study site.
[d]Model 4: model 3 + adjustment for month of birth.

Table B.4 Associations between $PM_{2.5}$ and ozone exposure and ASD, among SEED participants. Results are shown for single and multiple pollutant models

	Ozone		$PM_{2.5}$	
Developmental window	Single pollutant [OR (95%CI)]	+ Adjustment for $PM_{2.5}$ [OR (95%CI)]	Single pollutant [OR (95%CI)]	+ Adjustment for ozone [OR (95%CI)]
Preconception	0.92 (0.82, 1.05)	0.90 (0.79, 1.03)	1.04 (0.97, 1.12)	1.06 (0.98, 1.14)
Entire pregnancy	1.06 (0.85, 1.32)	1.06 (0.85, 1.32)	1.02 (0.88, 1.19)	1.03 (0.88, 1.19)
First Trimester	0.94 (0.83, 1.07)	0.95 (0.83, 1.08)	0.98 (0.91, 1.05)	0.99 (0.92, 1.06)
Second Trimester	0.96 (0.85, 1.09)	0.97 (0.85, 1.11)	0.97 (0.91, 1.04)	0.98 (0.91, 1.05)
Third Trimester	1.22 (1.05, 1.42)	1.20 (1.03, 1.40)	1.06 (0.98, 1.14)	1.03 (0.96, 1.11)
First year of life	0.79 (0.60, 1.04)	0.84 (0.63, 1.12)	1.26 (1.02, 1.57)	1.22 (0.98, 1.53)

Results are reported per 1.6-$\mu g/m^3$ increase in $PM_{2.5}$ and 6.6-ppb increase in ozone. Models are adjusted for maternal age, maternal education, maternal race/ethnicity, maternal smoking, study site, month of birth, and year of birth.

Table B.5 Adjusted[a] associations between $PM_{2.5}$ and ozone and ASD, for single and mutual window adjustment models, among SEED participants

	Ozone [OR (95%CI)]	$PM_{2.5}$ [OR (95%CI)]
First Trimester		
Main model	0.94 (0.83, 1.07)	0.98 (0.91, 1.05)
+Adj preconception	0.95 (0.83, 1.09)	0.98 (0.91, 1.05)
+Adj 2nd, 3rd trimesters	0.98 (0.85, 1.13)	1.01 (0.93, 1.09)
Second Trimester		
Main model	0.96 (0.85, 1.09)	0.97 (0.91, 1.04)
+Adj preconception	0.93 (0.81, 1.07)	0.99 (0.91, 1.07)
+Adj 1st, 3rd trimesters	0.89 (0.77, 1.02)	0.96 (0.90, 1.03)
Third Trimester		
Main model	1.22 (1.05, 1.42)	1.06 (0.98, 1.14)
+Adj preconception	1.22 (1.05, 1.42)	1.06 (0.98, 1.14)
+Adj 1st, 2nd trimesters	1.27 (1.08, 1.49)	1.06 (0.98, 1.16)
Preconception		
Main model	0.92 (0.82, 1.05)	1.04 (0.97, 1.12)
+Adj pregnancy	0.92 (0.81, 1.04)	1.05 (0.97, 1.14)
+Adj year post	0.96 (0.84, 1.10)	1.02 (0.95, 1.10)
Entire pregnancy		
Main model	1.06 (0.85, 1.32)	1.02 (0.88, 1.19)
+Adj preconception	1.07 (0.86, 1.33)	1.07 (0.91, 1.25)
+Adj year post	1.35 (1.01, 1.80)	0.93 (0.79, 1.11)
First year of life		
Main model	0.79 (0.60, 1.04)	1.26 (1.02, 1.57)
+Adj preconception	0.82 (0.61, 1.11)	1.24 (1.00, 1.55)
+Adj pregnancy	0.62 (0.44, 0.89)	1.33 (1.04, 1.70)

Results are reported per 1.6-$\mu g/m^3$ increase in $PM_{2.5}$ and 6.6-ppb increase in ozone.
[a]All models are adjusted for maternal age, maternal education, maternal race/ethnicity, maternal smoking, study site, month of birth, and year of birth.

Table B.6 Adjusted odds ratios and 95% confidence intervals for the associations between $PM_{2.5}$ and ozone and ASD, stratified by maternal tobacco use

	Ozone			**$PM_{2.5}$**		
Developmental window	**Tobacco use** OR (95% CI)	**No tobacco use** OR (95% CI)	***p*-int**	**Tobacco use** OR (95% CI)	**No tobacco use** OR (95% CI)	***p*-int**
Entire pregnancy	1.37 (0.92, 2.03)	1.02 (0.81, 1.28)	0.13	1.10 (0.87, 1.39)	1.02 (0.87, 1.18)	0.43
First Trimester	0.90 (0.73, 1.11)	0.95 (0.83, 1.08)	0.59	0.89 (0.76, 1.04)	0.99 (0.92, 1.07)	0.18
Second Trimester	1.15 (0.94, 1.41)	0.93 (0.82, 1.06)	0.03	1.08 (0.93, 1.26)	0.96 (0.89, 1.03)	0.12
Third Trimester	1.29 (1.05, 1.58)	1.21 (1.04, 1.40)	0.42	1.17 (1.01,1.35)	1.04 (0.97, 1.12)	0.11

Results are reported per 1.6-$\mu g/m^3$ increase in $PM_{2.5}$ and 6.6-ppb increase in ozone.

Table B.7 Adjusted[a] odds ratios and 95% confidence intervals for the associations between $PM_{2.5}$ and ozone and ASD, stratified by SEED study site

	California (Cases=96, controls=134)	**Colorado** (Cases=139, controls=185)	**Georgia** (Cases=130, controls=160)	**Maryland** (Cases=107, controls=126)	**North Carolina** (Cases=100, controls=146)	**Pennsylvania** (Cases=102, controls=104)	***p-int***
Ozone							
Preconception	0.71 (0.52, 0.97)	1.03 (0.88, 1.20)	0.97 (0.81, 1.15)	0.89 (0.74, 1.08)	0.88 (0.71, 1.10)	0.75 (0.61, 0.90)	0.02
Trimester 1	1.23 (0.92, 1.65)	0.91 (0.77, 1.07)	0.98 (0.82, 1.17)	0.98 (0.79, 1.21)	0.86 (0.69, 1.07)	0.94 (0.78, 1.12)	0.06
Trimester 2	1.00 (0.75, 1.33)	0.90 (0.76, 1.06)	0.88 (0.74, 1.06)	1.07 (0.88, 1.31)	1.00 (0.80, 1.26)	1.05 (0.88, 1.26)	0.43
Trimester 3	1.10 (0.78, 1.53)	1.29 (1.08, 1.55)	1.14 (0.95, 1.36)	1.20 (0.96, 1.51)	1.46 (1.16, 1.84)	1.19 (0.99, 1.43)	0.23
Entire pregnancy	1.20 (0.72, 2.00)	0.95 (0.70, 1.31)	0.86 (0.58, 1.29)	1.37 (0.89, 2.10)	1.26 (0.74, 2.17)	1.12 (0.76, 1.65)	0.64
First year of life	0.83 (0.45, 1.54)	1.38 (0.91, 2.11)	0.62 (0.30, 1.28)	0.52 (0.26, 1.04)	3.35 (1.20, 9.37)	0.18 (0.08, 0.39)	<0.001
$PM_{2.5}$							
Preconception	1.09 (0.96, 1.25)	0.98 (0.76, 1.25)	1.05 (0.89, 1.24)	0.95 (0.79, 1.15)	1.10 (0.90, 1.34)	0.91 (0.71, 1.17)	0.76
Trimester 1	0.86 (0.76, 0.97)	0.98 (0.77, 1.24)	1.21 (1.01, 1.45)	1.02 (0.83, 1.26)	0.99 (0.82, 1.18)	1.31 (1.03, 1.67)	0.05
Trimester 2	0.93 (0.82, 1.04)	0.94 (0.75, 1.19)	1.05 (0.90, 1.23)	1.01 (0.84, 1.21)	0.95 (0.79, 1.15)	1.12 (0.87, 1.46)	0.89
Trimester 3	1.08 (0.94, 1.25)	0.84 (0.66, 1.07)	0.99 (0.85, 1.16)	0.92 (0.77, 1.10)	1.21 (1.02, 1.43)	1.29 (1.06, 1.58)	0.03
Entire pregnancy	0.71 (0.51, 1.00)	0.89 (0.64, 1.24)	1.49 (1.04, 2.11)	0.93 (0.66, 1.31)	1.07 (0.73, 1.56)	3.06 (1.76, 5.33)	0.07
First year of life	1.51 (0.84, 2.73)	1.11 (0.74, 1.66)	1.54 (0.80, 2.97)	1.01 (0.66, 1.54)	0.90 (0.50, 1.60)	2.79 (1.41, 5.50)	0.14

Results are reported per 1.6-$\mu g/m^3$ increase in $PM_{2.5}$ and 6.6-ppb increase in ozone. Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; OR, odds ratio; $PM_{2.5}$, particulate matter <2.5 µm.

[a]Models are adjusted for maternal age, maternal education, maternal race/ethnicity, maternal smoking, month of birth, and year of birth.

Table B.8 Adjusted[a] associations between early life $PM_{2.5}$ and ozone exposure and ASD, for those with and without a co-occurring intellectual disability, among SEED participants

	Ozone		$PM_{2.5}$	
Developmental window	ASD with ID (*n*=414) [OR (95%CI)]	ASD without ID (*n*=251) [OR (95%CI)]	ASD with ID (*n*=414) [OR (95%CI)]	ASD without ID (*n*=251) [OR (95%CI)]
Preconception	0.96 (0.82, 1.11)	0.85 (0.72, 1.01)	1.06 (0.97, 1.15)	1.01 (0.91, 1.12)
Entire pregnancy	1.02 (0.78, 1.33)	1.19 (0.89, 1.61)	1.01 (0.85, 1.20)	1.07 (0.86, 1.33)
First Trimester	0.94 (0.80, 1.10)	0.94 (0.78, 1.13)	0.97 (0.90, 1.06)	0.99 (0.90, 1.09)
Second Trimester	0.90 (0.77, 1.05)	1.12 (0.94, 1.34)	0.96 (0.89, 1.04)	0.99 (0.90, 1.09)
Third Trimester	1.24 (1.04, 1.49)	1.24 (1.02, 1.52)	1.07 (0.98, 1.16)	1.06 (0.96, 1.17)
First year of life	0.79 (0.57, 1.10)	0.77 (0.53, 1.12)	1.25 (0.97, 1.62)	1.29 (0.96, 1.75)

Results are reported per 1.6-$\mu g/m^3$ increase in $PM_{2.5}$ and 6.6-ppb increase in ozone. Abbreviations: ASD, Autism spectrum disorder; CI, confidence interval; ID, intellectual disability; OR, odds ratio; $PM_{2.5}$, particulate matter <2.5 µm.

[a]Models are adjusted for study site, maternal age, maternal education, maternal race/ethnicity, maternal smoking, month of birth, and year of birth.

Table B.9 Comparison of SEED results to those from previous $PM_{2.5}$, ozone, and ASD studies. Results are reported per 10-ppb increases for ozone and 5-ug/m^3 for $PM_{2.5}$

	Cases/ Controls (*n*)	Ozone [OR (95%CI)]	$PM_{2.5}$ [OR (95%CI)]
First Trimester			
SEED	674/855	0.91 (0.75, 1.11)	0.94 (0.75, 1.18)
Becerra et al. [11]	7,603/75,782	1.00 (0.97, 1.02)	1.04 (0.99, 1.09)
Raz et al. [12]	245/1,522	---	1.07 (0.81, 1.40)
Talbott et al. [14]	217/226	---	1.13 (0.84, 1.48)
Volk et al. [13]	279/245	1.05 (0.91, 1.20)	1.12 (0.97, 1.28)
Second Trimester			
SEED	674/855	0.94 (0.78, 1.14)	0.92 (0.74, 1.14)
Becerra et al. [11]	7,603/75,782	1.02 (1.00, 1.04)	1.02 (0.98, 1.06)
Raz et al. [12]	245/1,522	---	1.00 (0.74, 1.35)
Talbott et al. [14]	217/226	---	1.07 (0.81, 1.42)
Volk et al. [13]	279/245	1.02 (0.89, 1.17)	1.25 (1.21, 1.30)
Third Trimester			
SEED	674/855	1.35 (1.08, 1.70)	1.18 (0.95, 1.48)
Becerra et al. [11]	7,603/75,782	1.03 (1.01, 1.05)	1.03 (0.99, 1.09)
Raz et al. [12]	245/1,522	---	1.49 (1.10, 2.02)
Talbott et al. [14]	217/226	---	1.07 (0.80, 1.46)
Volk et al. [13]	279/245	1.01 (0.89, 1.15)	1.21 (1.06, 1.39)
Entire pregnancy			
SEED	674/855	1.09 (0.78, 1.52)	1.08 (0.67, 1.72)
Guxens et al.[a] [174]	541/8,079	---	0.71 (0.37, 1.37)
Becerra et al. [11]	7,603/75,782	1.05 (1.01, 1.10)	1.07 (1.00, 1.16)
Raz et al. [12]	245/1,522	---	1.74 (1.09, 2.73)
Talbott et al. [14]	217/226	---	1.38 (0.80, 2.36)
Volk et al. [13]	279/245	1.05 (0.84, 1.31)	1.52 (1.46, 1.59)
First year of life			
SEED	674/855	0.70 (0.46, 1.06)	2.08 (1.05, 4.10)
Talbott et al. [14]	217/226	---	1.74 (0.91, 3.30)
Volk et al. [13]	279/245	1.09 (0.81, 1.47)	1.54 (1.24, 1.92)

Abbreviation: ASD, autism spectrum disorder; CI, confidence interval; OR, odds ratio; $PM_{2.5}$, particulate matter <2.5 µm.

[a]Cohort study, numbers represent those within borderline/clinical range/ total

APPENDIX C: SUPPLEMENTARY TABLES FOR CHAPTER 5

Table C.1 SEED study site catchment areas

Study Site	Counties
California San Francisco Bay Area	Alameda, Santa Clara
Colorado Denver	Denver, Adams, Arapahoe, Jefferson, Broomfield, Douglas, Boulder
Georgia Metro Atlanta	Metropolitan Atlanta (Clayton, Cobb, DeKalb, Fulton, Gwinett)
Maryland NE MD	Baltimore City, Anne Arundel, Baltimore, Carroll, Cecil, Harford, Howard, Montgomery, Prince George
North Carolina Central NC	Alamance, Chatham, Davidson, Durham, Forsyth, Guilford, Johnston, Orange, Randolph, Wake
Pennsylvania Philadelphia	Bucks, Chester, Delaware, Montgomery, Philadelphia

Table C.2 Description of included neighborhood level census indicators, from the 2000 US Census

Neighborhood-level SES domain	Census indicator	Description
Education	%<HS education	Percentage of males and females with less than a high school education
Employment	%Unemployment	Percentage of males and females unemployed
Housing	%Crowding	Percentage of housing units with more than one occupant per room
Occupation	%Management	Percentage of males not in management and professional occupations
Poverty	%Poverty	Percentage of households in poverty
	%Female HH	Percentage of female headed households with dependent children
	%Income <30K	Percentage of households earning less than $30,000 per year
	%Public assistance	Percentage of households on public assistance

Table C.3 Adjusted[a] odds ratios and 95% confidence intervals for continuous[b] $PM_{2.5}$ exposure and ASD, by neighborhood deprivation level

Exposure	ASD (N)	Controls (N)	Stratified ORs (95% CI)	LRT p-value
Pregnancy $PM_{2.5}$				
Low deprivation	252	427	0.98 (0.58, 1.66)	
Moderate deprivation	235	269	0.97 (0.55, 1.71)	
High deprivation	187	159	1.16 (0.63, 2.16)	0.79
First year of life $PM_{2.5}$				
Low deprivation	252	427	1.83 (0.90, 3.70)	
Moderate deprivation	235	269	1.88 (0.83, 4.25)	
High deprivation	187	159	2.45 (1.08, 5.56)	0.57

Abbreviations: ASD, autism spectrum disorder; CI, confidence interval; LRT, likelihood ratio test; OR, odds ratio; $PM_{2.5}$, particulate matter <2.5 µm

[a] Models are adjusted for study site, year of birth, maternal education, maternal race/ethnicity, maternal age, and maternal smoking

[b] Results are reported per 5-µg/m^3 increases

APPENDIX D: TABLES AND FIGURES FOR CHAPTER 6

Table D.1 Adjusted[a] odds ratios and 95% confidence intervals evaluating the associations between early life $PM_{2.5}$ and ozone exposure and latent class membership[b], with population controls as the reference

Developmental windows	Class 1 (*n*=196)	Class 2 (*n*=184)	Class 3 (*n*=228)	Class 4 (*n*=82)
Ozone				
Preconception	0.98 (0.81, 1.19)	0.92 (0.76, 1.13)	0.93 (0.78, 1.12)	0.78 (0.59, 1.03)
Entire pregnancy	1.30 (0.94, 1.81)	0.90 (0.63, 1.29)	1.00 (0.73, 1.37)	1.10 (0.67, 1.81)
First Trimester	1.00 (0.82, 1.22)	0.93 (0.75, 1.15)	0.93 (0.77, 1.12)	0.85 (0.63, 1.14)
Second Trimester	1.09 (0.90, 1.32)	0.79 (0.65, 0.97)	0.96 (0.80, 1.15)	1.09 (0.82, 1.44)
Third Trimester	1.27 (1.02, 1.58)	1.18 (0.93, 1.49)	1.17 (0.94, 1.45)	1.48 (1.06, 2.08)
First year of life	0.80 (0.53, 1.20)	0.84 (0.53, 1.33)	0.73 (0.49, 1.08)	0.91 (0.49, 1.70)
$PM_{2.5}$				
Preconception	1.06 (0.95, 1.18)	1.02 (0.91, 1.15)	1.05 (0.95, 1.17)	0.98 (0.82, 1.18)
Entire pregnancy	0.99 (0.79, 1.23)	1.01 (0.79, 1.29)	1.02 (0.83, 1.27)	1.11 (0.76, 1.60)
First Trimester	0.97 (0.87, 1.09)	1.03 (0.91, 1.16)	0.96 (0.87, 1.07)	0.94 (0.78, 1.13)
Second Trimester	0.95 (0.86, 1.05)	0.96 (0.85, 1.08)	1.00 (0.90, 1.10)	0.99 (0.84, 1.18)
Third Trimester	1.05 (0.94, 1.18)	0.99 (0.88, 1.12)	1.08 (0.97, 1.19)	1.14 (0.97, 1.34)
First year of life	1.30 (0.94, 1.81)	1.37 (0.96, 1.95)	1.06 (0.78, 1.45)	1.60 (0.96, 2.66)

Results are reported per IQR-unit increases.

[a]Models are adjusted for study site, maternal age, maternal education, maternal race/ethnicity, maternal smoking, month of birth, and year of birth.

[b]Class 1 includes mild language delay with cognitive rigidity; Class 2: Significant developmental delay with repetitive motor behaviors; Class 3: Significant developmental delay; and Class 4: Mild language and motor delays with dysregulation.

Table D.2 Comparison of $PM_{2.5}$ exposure levels between true and shifted date of births, by case-control status and developmental window

	Cases		Controls	
Window of Exposure	True DOB	Shifted DOB	True DOB	Shifted DOB
Trimester 1	12.95 ± 2.7	12.97 ± 2.7	13.29 ± 2.6	13.27 ± 2.6
Trimester 2	12.81 ± 2.6	12.80 ± 2.5	12.98 ± 2.3	13.00 ± 2.3
Trimester 3	13.71 ± 3.1	13.69 ± 3.0	12.91 ± 2.8	12.85 ± 2.8
Preconception	13.27 ± 2.4	13.29 ± 2.4	12.88 ± 2.2	12.94 ± 2.2
Pregnancy	13.08 ± 1.3	13.08 ± 1.3	13.07 ± 1.0	13.05 ± 1.0
Year post birth	13.09 ± 0.7	13.09 ± 0.7	13.08 ± 0.7	13.08 ± 0.7

Table D.3 Comparison of mean and maximum individual differences and correlations for true and shifted date of births, by developmental window

	Mean individual difference ($\mu g/m^3$ $PM_{2.5}$)	Max individual difference ($\mu g/m^3$ $PM_{2.5}$)	Correlation (r)
Trimester 1	0.21	1.38	0.99
Trimester 2	0.24	1.01	0.99
Trimester 3	0.24	1.36	0.99
Preconception	0.25	1.24	0.99
Pregnancy	0.09	0.54	0.99
Post-birth	0.04	0.27	1.00

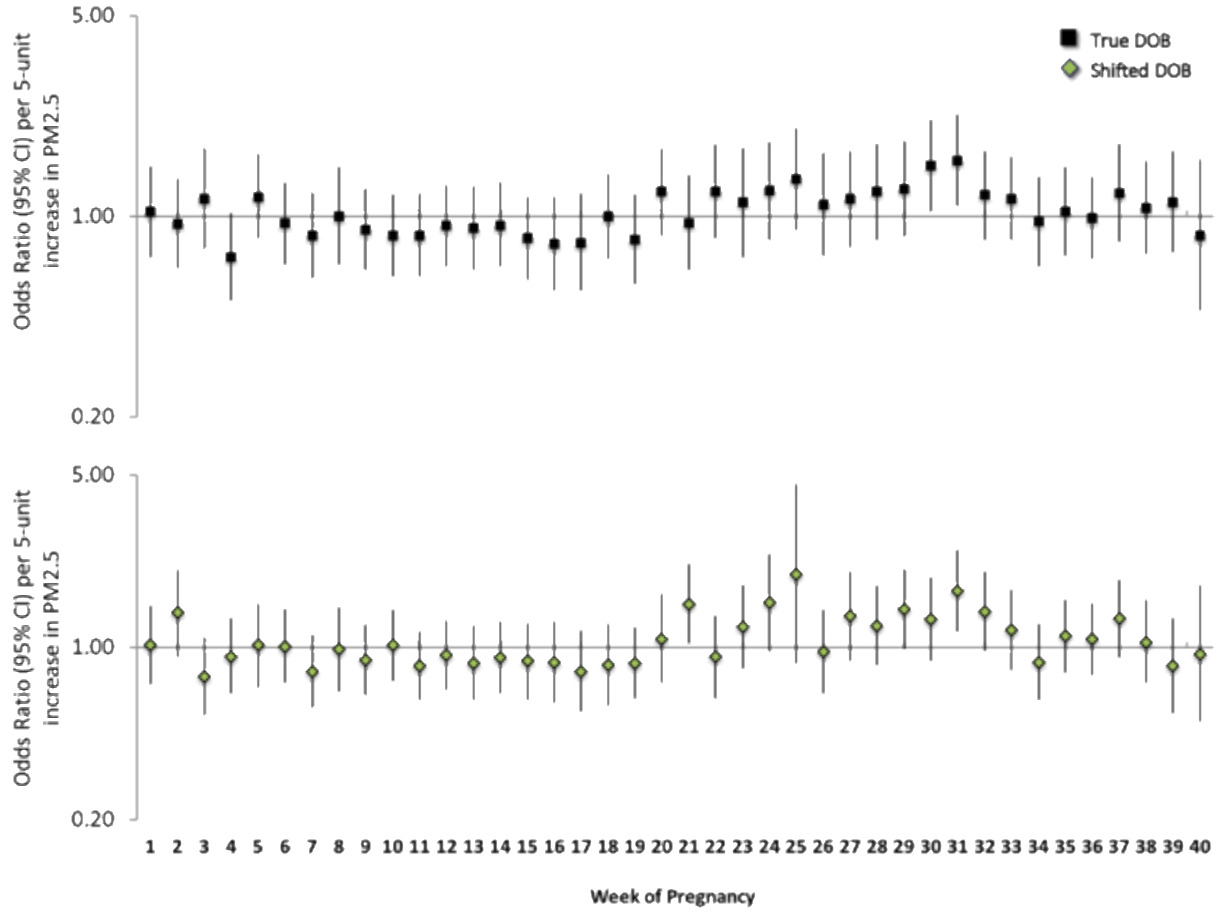


Figure D.1 Comparison of results for weekly $PM_{2.5}$ exposure during pregnancy and ASD when using the true and shifted date of birth for the NC SEED study site

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