Published in final edited form as:
*Environ Res*. 2019 April ; 171: 218–227. doi:10.1016/j.envres.2019.01.013.

# Ambient Ozone and Fine Particulate Matter Exposures and Autism Spectrum Disorder in Metropolitan Cincinnati, Ohio

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## Abstract

**Background:** Epidemiological studies report fairly consistent associations between various air pollution metrics and autism spectrum disorder (ASD), with some elevated risks reported for different prenatal and postnatal periods.

**Objectives:** To examine associations between ASD and ambient fine particulate matter ($PM_{2.5}$) and ozone concentrations during the prenatal period through the second year of life in a case-control study.

**Methods:** ASD cases (n=428) diagnosed at Cincinnati Children's Hospital Medical Center were frequency matched (15:1) to 6,420 controls from Ohio birth records. We assigned daily $PM_{2.5}$ and ozone estimates for 2005–2012 from US EPA's Fused Air Quality Surface Using Downscaling model to each participant for each day based on the mother's census tract of residence at birth. We calculated adjusted odds ratios (aORs) using logistic regression across continuous and categorical exposure window averages (trimesters, first and second postnatal years, and cumulative measure), adjusting for maternal- and birth-related confounders, both air pollutants, and multiple temporal exposure windows.

**Results:** We detected elevated aORs for $PM_{2.5}$ during the 2$^{nd}$ trimester, 1$^{st}$ year of life, and a cumulative period from pregnancy through the 2$^{nd}$ year (aOR ranges across categories: 1.41–1.44, 1.54–1.84, and 1.41–1.52 respectively), and for ozone in the 2$^{nd}$ year of life (aOR range across categories: 1.29–1.42). Per each change in IQR, we observed elevated aORs for ozone in the 3$^{rd}$

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trimester, 1st and 2nd years of life, and the cumulative period (aOR range: 1.19–1.27) and for $PM_{2.5}$ in the 2nd trimester, 1st year of life, and the cumulative period (aOR range: 1.11–1.17).

**Discussion:** We saw limited evidence of linear exposure-response relationships for ASD with increasing air pollution, but the elevated aORs detected for $PM_{2.5}$ in upper exposure categories and per IQR unit increases were similar in magnitude to those reported in previous studies, especially for postnatal exposures.

### Keywords

air pollution; environmental neurology; environmental psychology; environmental epidemiology

## INTRODUCTION

Autism spectrum disorder (ASD) is a heterogeneous neurodevelopmental condition characterized by impaired communication and social interaction and a pattern of repetitive behaviors or restricted interests. Approximately 1 in 59 children in the US have ASD, with the prevalence about 4.5 times higher for boys (1 in 38) than girls (1 in 152) (Biao et al. 2018). Recent studies suggest that the etiology of ASD likely involves a complex interplay of genetic and environmental factors. Twin studies have found ASD to have strong heritability (Lichtenstein et al. 2010; Ronald et al. 2006), though current estimates including the largest twin study to date (Sandin et al. 2014) suggest equally strong roles for genetic and environmental factors (Hallmeyer et al. 2011).

Epidemiologic studies have reported associations between ASD and several environmental exposures during the prenatal and early postnatal periods, with some of the strongest evidence for late pregnancy and early life exposures to air pollution (Raz et al. 2018; Ritz et al. 2018; Kalkbrenner et al. 2014; Talbott et al. 2015a; Volk et al. 2013). Of the fifteen epidemiological studies we identified on ASD and air pollution, all but two (Gong et al. 2017; Guxens et al. 2016) reported some positive associations, with some of the strongest evidence being for $PM_{2.5}$ during late pregnancy and the postnatal period (Morales-Suárez-Varela et al. 2017; Flores-Pajot et al. 2016; Becerra et al. 2013; Raz et al. 2015; Ritz et al. 2018; Talbot et al. 2015a; Volk et al. 2013). Other air pollutants including ozone, nitrogen dioxide, sulfur dioxide, and $PM_{10}$ have also been linked to ASD in some epidemiological studies (Morales-Suárez-Varela et al. 2017; Ritz et al. 2018; Raz et al. 2018), though fewer epidemiological and toxicological studies have examined these and the evidence is less consistent. Overall, the strongest associations for these pollutants have also been seen for early life exposures.

Despite fairly consistent evidence demonstrated for some air pollutants in ASD studies, it is not known how the timing or duration of pre- or post-natal environmental insults to the developing brain could contribute to the onset of ASD. Also, given that air pollution is a complex time-varying mixture of gases and particulates often occurring in correlated concentrations, it is important to assess health outcomes in relation to multiple air pollutants for different exposure windows that susceptible populations may encounter. We examined associations between ASD and ambient $PM_{2.5}$ and ozone levels during pre- and post-natal exposure periods in the greater Cincinnati, Ohio metropolitan area from 2005–2012, based

on cases born from 2006–2010 identified from a large regional ASD diagnostic and treatment center. We used daily air pollution estimates for census tracts that combine air monitoring data and atmospheric model data to create exposure averages during each trimester of pregnancy and the first two years of life. We adjusted for multiple exposure windows and both pollutants in the same models, which improved our ability to isolate specific associations.

## METHODS

### Outcome Data

For this case-control study, we identified ASD cases in the four-county metropolitan Cincinnati area in southwest Ohio (Butler, Clermont, Hamilton, and Warren counties) using two approaches. First, we queried the electronic medical records (EMR) of Cincinnati Children's Hospital Medical Center (CCHMC) to identify all patients with a diagnosis of ASD. We included all patients born from 2006–2010 with a 299 ICD-9 diagnostic code in their EMR that was recorded by CCHMC's Division of Developmental and Behavioral Pediatrics (DDBP). We also searched EMR records for other developmental disabilities cases diagnosed at DDBP (ICD-9 codes for attention deficit hyperactivity disorder (314.), general delay (315.), including global developmental delay, delays in motor skills or speech language, and aphasia and apraxia (784.)) to exclude them from the control selection process. Second, we employed natural language processing techniques to glean clinical concepts including diagnoses from free-text office visit notes at the DDBP. DDBP's Kelly O'Leary Center for Autism Spectrum Disorders has a rigorous and well-established standard-of-care ASD assessment process that involves the completion of comprehensive, multidisciplinary evaluations, including psychological testing (cognitive, adaptive skills, and behavioral assessments), speech/language evaluations, and an autism-specific assessment with the Autism Diagnostic Observation Schedule. As the only free-standing pediatric institution in the region that evaluates >1000 suspected ASD incident cases per year and provides medical care to >2500 ASD patients per year, CCHMC's EMR database is believed to cover most of the ASD cases diagnosed in the metropolitan area. CCHMC's DDBP patients represent a wide socioeconomic spectrum, with approximately 60% of patients on private insurance and 40% on public insurance, and patients from urban and rural areas from Ohio and neighboring Kentucky, Indiana, and West Virginia.

We matched the EMR data with vital records data provided by the Ohio Department of Health in order to identify the residence at delivery of mothers of ASD cases, to obtain demographic and health covariates, and to identify a comparison group of control pregnancies not affected by ASD. Due to a lack of overlapping unique identifiers between the datasets, EMR case records were linked to their corresponding birth records through a 20-digit string containing the child's eight-digit birth date, the mother's eight-digit birth date, and the child's four-digit birth weight in grams. Birth records that did not match a child with ASD or other developmental disability provided the control group. Fifteen birth record controls from the four-county area were frequency matched to each of 428 cases based on birth year for a total study population of n=6,848. We excluded multiple births, siblings who both had ASD diagnoses, and subjects with missing data on gestational age, birthweight, or

census tract of mother's residence at delivery. ASD cases and other developmental disabilities cases diagnosed at DDBP were also excluded from the pool of potential birth records controls. We conducted data cleaning efforts to identify errors or implausible values in the key covariate data reported on birth records. Additionally, duplicate case entries recorded in the ASD database were identified and deleted.

## Exposure Data

We used census tract-level estimates for daily maximum eight-hour average ozone and 24-hour average $PM_{2.5}$ concentrations from the United States Environmental Protection Agency's (EPA's) Fused Air Quality Surface Using Downscaling (FAQSD) model (USEPA 2017). This model creates estimates for US census tracts by combining air pollutant monitoring data from the National Air Monitoring Stations/State and Local Air Monitoring Stations with 12 $km^2$ gridded output from the Community Multiscale Air Quality (CMAQ, version 4.6) atmospheric model (USEPA 2016). The FAQSD model combines the accuracy of monitoring data with the completeness of the CMAQ model data, as monitoring data may be sparsely collected in some areas. Census tracts vary in geographic size based on population density, with more densely populated tracts encompassing smaller areas.

## Exposure Assessment

Study participants were linked to daily estimates of 8-hour maximum ozone and 24-hour-average $PM_{2.5}$ for years 2005–2012 based on the census tract containing their mother's residential address at delivery, as listed on state birth records. EPA's FAQSD data for 2005–2008 are estimated for year 2000 census tracts, while data for 2009–2012 are estimated for year 2010 census tracts. In cases where tracts were divided or merged between the 2000 and 2010 censuses, we used geographic relationship files from the US Census Bureau (US Census Bureau 2017) to compare census tracts. To provide exposure estimates for study subjects with exposure periods overlapping 2008–2009, we linked tracts from 2000 and 2010 by using the geographically smaller tract accounting for the largest percentage of the geographically larger tracts. We compared census tract maps from the US Census Bureau (US Census Bureau 2015) to birth record addresses mapped in Google Maps to ensure that we assigned the correct tract for addresses that fell within a tract that had changed boundaries between the 2000 to 2010 censuses.

Averages of daily maximum eight-hour average ozone estimates and 24-hour-average $PM_{2.5}$ estimates were calculated across the $1^{st}$, $2^{nd}$, and $3^{rd}$ trimesters, the entire pregnancy period, the $1^{st}$ and $2^{nd}$ years after birth, and for a cumulative exposure index (CEI) based on pregnancy through the $2^{nd}$ year after birth. Trimester-specific exposure periods were calculated based on each child's estimated gestational ages and dates of birth (DOB) listed in birth records. Dates of conception (DOC) were estimated as [(DOB) - (gestational age in days) + 14]. The first trimester was assumed to begin with the DOC and ended 93 days later; the second trimester began 94 days after the DOC and ended 186 days after the DOC; third trimesters were calculated from 187 days after the DOC to the DOB.

## Statistical Analysis

SAS (version 9.4; SAS Institute, Inc., Cary, NC) was used for the statistical analysis. We used Spearman correlation coefficients to compare the different air pollution measures. We used ninety-five percent confidence intervals (95% CIs) to evaluate precision. We used unconditional multivariable logistic regression (Pearce 2016) to estimate adjusted odds ratios (aORs) and 95% CIs based on different exposure categories (sextile-based categories based on the distribution of exposures among the controls) for the two pollutants, as well as continuous exposures per IQR change to allow for better comparisons to other air pollution studies that commonly use this metric. To better contrast larger differences in exposure concentrations, we isolated the high and low exposure extremes and collapsed the middle four sextiles into one group (i.e. from the $17^{th}$ percentile to the $83^{rd}$ percentile). This approach provided better contrasts than more conventional categorical approaches such as quartiles or quintiles given the very narrow ranges in the middle categories, particularly for $PM_{2.5}$. This approach should also help reduce potential exposure misclassification that may occur from the use of implausibly narrow intermediate categorical ranges.

We assessed confounding for *a priori* selected covariates identified from the literature using a >10% change-in-estimate approach comparing bivariate (birth year and exposure) to trivariate models (birth year, exposure, and the $3^{rd}$ variable being assessed). Covariates obtained from birth records included child's sex, mother's and father's age at delivery, mother's and father's highest educational attainment, mother's and father's race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, other), mother's marital status at delivery (married or unmarried), maternal smoking during pregnancy (yes or no), mother's pre-pregnancy body mass index (BMI; underweight <18, healthy 18–24, overweight 25–30, obese >30), mother's birth country (U.S. or outside of U.S.), whether the mother received WIC (federal food and nutrition assistance; yes or no), insurance type (private, public, other), child's gestational age in weeks (preterm birth defined as <37 weeks), birth weight in grams (low birth weight defined as <2500g), season of conception (derived from month of conception; Dec-Feb=winter, Mar-May=spring, Jun-Aug=summer, Sep-Nov=fall), month of conception, parity, years since previous birth (1st birth, <2 years, 2–6 years, >6 years), number of prenatal care visits, and whether prenatal care began in the first trimester. We only included covariates in models that had less than 10% missing data (see Table 3 for percent of data missing for each variable by case status). We calculated aORs for ASD with ozone and $PM_{2.5}$ during each trimester of pregnancy, the first and second years of life, and for the CEI. Based on our confounding analysis, we included models with different adjustment sets to demonstrate the impact of confounding. We included the same set of five maternal and birth covariates (year of birth, mother's education, birth spacing, maternal pre-pregnancy BMI, and month of conception) in all models and focus the presentation of results primarily on the fully adjusted models, which also included adjustment for the other air pollutant and different exposure windows (except for CEI models). As a sensitivity analysis, we ran models restricted to 2006–2008 births to examine if results changed when using a subset expected to have more complete case ascertainment. We also ran models restricted to male cases and controls to examine possible differences by sex.

# RESULTS

## Air Pollution Distributions and Spearman Correlations

Trimester average exposure scores ranged from approximately 18–65 ppb for ozone and 4–22 μg/$m^3$ for $PM_{2.5}$ (Table 1). The mean CEI value was 39.2 ppb for ozone and 12.5 μg/$m^3$ for $PM_{2.5}$. IQR values varied across different averaging time windows, with trimester IQRs of approximately 16.0 ppb for ozone and 3.5 μg/$m^3$ for $PM_{2.5}$ (Table 4). Cut-points for exposure sextiles also varied for different exposure time periods. For example, 1st trimester cut-points for the highest sextiles were 48.6 ppb for ozone and 14.9 μg/$m^3$ for $PM_{2.5}$ (Table 4). We detected moderate to strong negative correlations between average ozone scores in the 1st and 3rd trimesters (r = −0.72), between ozone and $PM_{2.5}$ in the 2nd year of life (r = −0.64), between ozone and $PM_{2.5}$ in the 1st year of life (r = −0.56), between 1st trimester ozone and 3rd trimester $PM_{2.5}$ (r=−0.39), and between 3rd trimester ozone and 1st trimester $PM_{2.5}$ (r=−0.33). We detected moderate positive correlations between $PM_{2.5}$ in the 1st and 3rd trimesters (r=0.38), between ozone in the 1st and 2nd years of life (r=0.33), and between $PM_{2.5}$ in the 3rd trimester and 1st year (r=0.32) (Table 2).

## Study Characteristics

The cases (n=428) and controls (n=6420) were similar in most sociodemographic and health characteristics, except that cases were more likely than controls to be male (82% vs 50%) and to be the first child born to their mother (48% vs 39%) (Table 3). Cases were also less likely than controls to have missing paternal data (e.g. 13% vs 23% for paternal education) and less likely to be born between 2 and 6 years since their mother's previous birth (22% vs 32%). In the fully adjusted models, we included year of birth, mother's education, birth spacing, maternal pre-pregnancy BMI, month of conception, multiple pollutants, and multiple exposure time windows (except in the CEI models).

## Multivariable Regression Models

**$PM_{2.5}$ models—**Based on the fully adjusted models, we detected elevated aORs for the highest sextiles compared to the referent (i.e. the lowest exposure sextile) for $PM_{2.5}$ in the 2nd trimester (aOR: 1.41, 95% CI: 0.89, 2.24), 1st year (aOR: 1.54, 95% CI: 0.98, 2.40), and CEI (aOR: 1.52, 95% CI: 1.00, 2.31) (Table 4). A positive exposure-response relationship was detected across CEI $PM_{2.5}$ exposure categories. Similar results were detected for continuous $PM_{2.5}$ models per each IQR change in the 2nd trimester (aOR=1.13; 95% CI: 0.93, 1.38), 1st year (aOR=1.11; 95% CI: 1.00, 1.23), and CEI (aOR=1.17; 95% CI: 0.98, 1.40) metrics (Table 4). As presented in Supplemental Table 3, model results for the entire study population using all six exposure sextiles rather than the collapsed categories show similar general trends as our main results, though some intermediate exposure categories have very narrow concentration ranges which may increase the likelihood of exposure misclassification. Supplemental Table 4 presents similar patterns of model results using exposure quartiles, with an exposure-response relationship seen for first year of life $PM_{2.5}$.

**Ozone models—**Compared to the lowest sextile, we detected elevated aORs in the full models for 2nd year ozone (aOR range: 1.29–1.42) and reduced aORs for 1st trimester ozone (aOR range: 0.56–0.70) (Table 4). An exposure-response relationship was detected for the

entire pregnancy categorical data. Based on continuous models per change in IQR, fairly consistent aORs were detected for the CEI (aOR=1.19; 95% CI: 0.95, 1.49), 3rd trimester (aOR=1.19; 95% CI: 0.80, 1.77), the 1st year (aOR=1.22; 95% CI: 0.96, 1.54) and the 2nd year (aOR=1.27; 95% CI: 0.96, 1.69) metrics (Table 4). Supplemental Table 4 presents similar patterns of model results using exposure quartiles, with an exposure-response relationship seen for ozone CEI.

### Sensitivity Analyses

When restricting cases and controls to birth years 2006–2008 for more complete ASD ascertainment (children born earlier would have more time to receive a diagnosis before data were collected for the study), categorically analyzed aORs were predominately elevated for the same exposure metrics as when as 2006–2010 births were included, as shown in Supplemental Table 1. The strongest results were seen for $PM_{2.5}$ in the 2nd trimester (highest sextile aOR=1.52, 95% CI: 0.86, 2.70), 1st year of life (highest sextile aOR=1.84, 95% CI: 1.11, 3.05), and CEI (highest sextile aOR=1.57, 95% CI: 0.95, 2.59), and for ozone in the 1st year of life (highest sextile aOR=1.67, 95% CI: 0.98, 2.87), 2nd year of life (highest sextile aOR=1.40, 95% CI: 0.75, 2.67), and CEI (highest sextile aOR=1.40, 95% CI: 0.80, 2.46). We did not observe consistent exposure-response relationships in these multivariate models.

To ensure that the commonly used covariates sex and race did not affect our estimates, in addition to the confounding analysis we ran models adjusting for race and sex with the other covariates, individually and together. These statistical adjustments did not materially or consistently change aOR estimates, including for the highest aORs that were detected (results not shown). We also ran models restricted to male cases and controls only and observed a similar pattern of results as for the entire sample, shown in Supplemental Table 2. For $PM_{2.5}$, in addition to elevated aORs for the 2nd trimester (highest sextile aOR: 1.44, 95% CI: 0.86, 2.41), 1st year of life (highest sextile aOR=1.53, 95% CI: 0.92, 2.53), and CEI (highest sextile aOR=1.72, 95% CI: 1.08, 2.76), we observed elevated aORs for the 3rd trimester (highest sextile aOR=1.36, 95% CI: 0.82, 2.25).

## DISCUSSION

Across covariate combinations and in both categorical and continuous exposure models, we observed the strongest associations between ambient air pollution and ASD in the first two years of life, with mixed results for different trimesters of pregnancy. For ozone, aORs were elevated for categorical models in the 2nd postnatal year, and for continuous models in the 3rd trimester, 1st and 2nd postnatal years, and the CEI covering the prenatal and postnatal periods. For $PM_{2.5}$, aORs in both categorical and continuous exposure models were elevated during the 2nd trimester, 1st postnatal year, and the cumulative exposure period.

### Particulate Matter

We observed aORs of 1.41 (95% CI: 0.89, 2.24), 1.54 (95% CI: 0.98, 2.40) and 1.52 (95% CI: 1.00, 2.31) for the highest sextiles of $PM_{2.5}$ in the 2nd trimester (≥14.9 vs <10.1 $\mu g/m^3$), 1st year of life (≥13.8 vs <11.7 $\mu g/m^3$), and CEI measures (≥13.2 vs <11.8 $\mu g/m^3$), respectively. Our continuous exposure measure results based on IQR changes of 0.87 to 0.94

μg/$m^3$ for the 2nd trimester, 1st year, and CEI $PM_{2.5}$ metrics were smaller in magnitude (aOR range: 1.11–1.17) than the 1.37 to 1.51 reported in a case-control study from Pittsburgh, Pennsylvania (Talbott et al. 2015a) per 2.8 μg/$m^3$ change in IQR. These differences may partially reflect the relatively low variability in $PM_{2.5}$ exposures in our study. However, our IQR-based results for 2nd trimester, 1st year, and CEI $PM_{2.5}$ are similar in magnitude to the aOR of 1.15 (95% CI: 1.06, 1.24) for pregnancy average $PM_{2.5}$ (per 8.25 μg/$m^3$ IQR increase) exposures adjusted for ozone in a Los Angeles, California study (Becerra et al. 2013), and to results from a large Danish case-control study (Ritz et al. 2018) for $PM_{2.5}$ exposures in the nine months after birth (aOR=1.06, 95% CI: 1.01, 1.11). A California-wide case-control study (Volk et al. 2013) also detected their strongest results per 8.7 μg/$m^3$ IQR increase in $PM_{2.5}$ during the 1st year of life (aOR=2.12, 95% CI: 1.45, 3.10) and entire pregnancy (aOR=2.08, 95% CI: 1.93, 2.25), while trimester-specific estimates were smaller (aOR range: 1.22–1.48). While we did not observe an association with entire pregnancy average $PM_{2.5}$ exposures, our categorical $PM_{2.5}$ exposure results are similar in magnitude to trimester-specific (aOR range: 1.23–1.49) and entire pregnancy (aOR=1.50, 95% CI: 1.16, 1.94) results reported by Raz et al. (2015) for each 4.4 μg/$m^3$ increase in $PM_{2.5}$ exposures. Our exposure contrasts were narrower than this study, which likely resulted in smaller aORs for our continuous exposure metrics. Nonetheless, the findings across our study and the six prior studies examining ASD and $PM_{2.5}$ are fairly consistent, with the exception of one multi-cohort study (Guxens et al. 2016) that may be subject to increased misclassification of both disease and exposure in comparison to other studies.

### Ozone

We observed the highest aORs for ozone in categorical models in the 2nd year of life (≥41.5 vs <36.6 ppb), and in continuous IQR-based models for the 3rd trimester, 1st and 2nd years of life, and the CEI. Similar to our 3rd trimester ozone per-IQR-change based model with multi-window and multipollutant adjustment (per 16.5 ppb increase; aOR=1.19; 95% CI: 0.80, 1.77), Becerra et al. (2013) reported their strongest results for 3rd trimester ozone for each 11.5 ppb IQR increase (aOR=1.12; 95% CI: 1.06, 1.19 including adjustment for $PM_{2.5}$). Comparable results were detected in a California-wide case-control study (Volk et al. 2013) based on 1st year of life ozone IQR increases (aOR=1.15; 95% CI: 0.72, 1.86 per 16.1 ppb). We also detected our strongest IQR-based results for ozone in the postnatal period, with aORs of 1.22 (95% CI: 0.96, 1.54) and 1.27 (95% CI: 0.96, 1.69) for the 1st and 2nd years of life. A cohort study from Taiwan (Jung et al. 2013; Jung et al. 2018) reported an adjusted hazard ratio of 1.59 (95% CI: 1.42, 1.78) per 100 ppb increase in early childhood ozone exposures in the one to four years preceding diagnosis, as well as a positive exposure-response relationship for postnatal ozone quartiles (hazard ratio range: 1.72–5.88; referent <97 ppb). Though effect estimates vary somewhat in magnitude across studies of ozone and ASD, our elevated results for IQR-based and categorical ozone exposures in the 3rd trimester and the postnatal period are fairly consistent with three previous studies (Morales-Suárez-Varela et al. 2017; Flores-Pajot et al. 2016).

We also saw some evidence of inverse associations between first trimester ozone estimates and ASD. We are not aware of any evidence that would suggest that these are biologically plausible findings, including a previous meta-analysis (Flores-Pajot et al. 2016) of first

trimester ozone exposures that reported no association (OR=1.00; 95% CI: 0.98, 1.02) based on two studies. Thus, these may be due to chance alone or alternative explanations including positive confounding by an inversely correlated pollutant that is also an ASD risk factor. One possibility of the latter is nitrogen dioxide ($NO_2$), a marker of traffic-related air pollution that is scavenged in the formation of ground-level ozone. Although we did not have data to examine it, $NO_2$ appears unlikely to be a strong confounder of this relationship since only one ASD study reported elevated associations for first trimester $NO_2$ measures (Volk et al. 2013). Another study also showed that ozone results were robust to additional adjustment for NOx in joint air pollutant models (Jung et al. 2013).

### Case Ascertainment

Case ascertainment is an important strength of our study, as CCHMC's DDBP is the primary diagnostic and treatment center in our study area for childhood neurodevelopmental disorders. A documented ASD diagnosis in the CCHMC EMR database is based on consistent diagnostic criteria, as recommended in a recent systematic review of the ASD-air pollution literature (Morales-Suárez-Varela et al. 2017). Additionally, the database likely includes most of the ASD cases in the study area. Compared to the corresponding ICD-9 code in the encounter diagnosis list, we found nearly perfect agreement in our manual review of approximately 100 clinical notes (Connolly et al. 2016). As part of our study design, we also excluded children diagnosed with non-ASD developmental disabilities at CCHMC from our control pool. It is possible that some children in our study area were diagnosed elsewhere or had not been diagnosed at the time data were compiled, though these scenarios are more likely for the non-ASD developmental disabilities cases than for the ASD cases. Additionally, while ASD diagnosis rates tend to be higher among groups with better access to healthcare (Liptak et al. 2008), we did not observe large differences between cases and controls by socioeconomic status including type of primary source of payment for delivery that was reported (Table 3).

Some cases might have been missed if they were too young to have been diagnosed when our dataset was compiled. Although some ASD cases can be diagnosed as early as two years of age, the median age at diagnosis varied for the three subtypes of ASD previously diagnosed under the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (used prior to 2013): 3.8 years for autistic disorder, 4.1 years for pervasive developmental disorder not otherwise specified, and 6.2 years for Asperger disorder (Christensen et al. 2016). Thus, the cases from later years of our study period may be more likely to represent children with certain ASD presentations, since these children are more likely to be diagnosed at a younger age, potentially resulting in some under-ascertainment for the later years of our study period. We observed similar patterns in the results of our sensitivity analysis restricted to 2006–2008 births compared to the full dataset, with the most consistent elevated results for $PM_{2.5}$ in the 2$^{nd}$ trimester (aOR range: 1.43–1.52), 1$^{st}$ year of life (aOR range: 1.84–2.00), and CEI (aOR range: 1.39–1.57), and for ozone in the 2$^{nd}$ year of life (aOR range: 1.33–1.42) and the highest sextile in the 1$^{st}$ year (aOR=1.67, 95% CI: 0.98, 2.87). These results suggest that there was sufficient time for cases to be detected across the study period. The consistency in diagnostic criteria in using a single hospital database and

our high case ascertainment are strengths in our study that likely reduce misclassification of disease and improve our ability to detect associations for ASD.

## Confounding

We identified eight confounders based on our change-in-estimate confounding analysis. Confounding by factors related to socioeconomic status (SES) is of particular concern in autism and air pollution studies, as SES indicators (e.g., income, education, healthcare access) can influence both the ascertainment and potential causes of ASD, and air pollution exposures have been shown to vary with SES (Weisskopf et al. 2015). Except for parental education, which was a confounder identified in 11 of the 14 previous studies on ASD and air pollution, we saw no evidence of confounding by SES or related sociodemographic characteristics. Though residual confounding by SES is possible in our study given the complex pathways by which SES can relate to both ASD and air pollution, our confounding analysis as well as the relative homogeneity of examining a single metropolitan area are strengths that help reduce the likelihood of confounding. This homogeneity should also increase the generalizability of study results, as our study population was comparable with national statistics (ADDMN 2014), with the exception of not detecting any differences in ASD status by race or ethnicity (Table 3). Adjusting for race in our models did not materially or consistently change aOR estimates, including for the most elevated estimates (data not shown). Additionally, while male sex is an established risk factor for ASD, sex was not associated with air pollution estimates in our study and adjusting for sex in models did not change aOR estimates. Models restricted to males produced similar patterns of aORs, with some results more highly elevated for $PM_{2.5}$, in particular for the 3$^{rd}$ trimester. The extensive data available from birth records allowed us to assess numerous ASD risk factors identified in the literature (e.g., Gardener et al. 2009), though, as with most observational studies, it is possible that residual confounding remains from unknown risk factors that may be associated with both ASD and air pollution.

We examined seasonality as a potential confounder, since ozone levels vary seasonally due to chemical reactions with solar ultraviolet radiation, with peaks occurring in summer in the northern hemisphere. Seasonality in ASD risk has been reported in some previous studies (Zerbo et al. 2011; Lee et al. 2008), with various temporal associations hypothesized in relation to exposures such as pesticides (Eskenazi et al. 2007), maternal allergies (Croen et al. 2005), and influenza infection (Deykin and MacMahon 1979). Month of conception, our marker of seasonality, was identified as a confounder and led to average changes in aORs of −10%, −14%, and +14% for the first, second, and third trimester ozone estimates, respectively. Three previous studies on ASD and air pollution also adjusted for seasonality to control for confounding using birth week (Kalkbrenner et al. 2015) and birth month windows (Raz et al. 2015; Raz et al. 2018). Additionally, the associations between ASD and pre- and post-natal air pollution estimates for time periods of one year or longer (e.g., the CEI) observed in our study and three previous studies (Talbott et al. 2015a; Volk et al. 2013; Jung et al. 2013) would be less influenced by time-varying confounding. Although the underlying etiology for an association between ASD and season is unknown, our inclusion of month of conception should help control for confounding by seasonality.

Exposure scores in our data represent a wide temporal range of averaging windows that we would not necessarily expect to have particular correlation patterns, though we did detect moderate to strong negative correlations between some metrics, and moderate positive correlations between others. In the few comparisons we could make to other studies of ASD and air pollution that included correlation data for exposure scores, the direction (positive or negative) of correlations in our data was generally in agreement (Becerra et al. 2013; Volk et al. 2013; Kalkbrenner et al. 2015). Variations in the magnitude of exposure score correlations between our study and others may be related to regional differences (i.e. seasonal effects in California compared to in the eastern US) in climate and geography, or differences in particular years due to changes in emissions.

Air pollution is a complex mixture of gases containing metals, semivolatile and volatile organic compounds, and particulates that vary in size and composition. It remains unclear whether specific components or whole mixture combinations are most relevant to ASD etiology. Of the 15 previous epidemiological studies on ASD and air pollution that examined various individual pollutants, all but two (Gong et al. 2017; Guxens et al. 2016) reported associations for some individual pollutants. Including our study, six out of seven studies observed elevated results for $PM_{2.5}$, which may be more etiologically relevant for health impacts compared to coarser measures (e.g. $PM_{10}$). Although the chemical composition of PM varies across different geographical areas with different sources and can contain a combination of organic and inorganic pollutants and biological materials, the five previous studies to examine ASD with PM chemical composition reported elevated results for various metals, solvents, and other volatile organic compounds (Talbott et al. 2015b; Kalkbrenner et al. 2010; Roberts et al. 2013; von Ehrenstein et al. 2014; Windham et al. 2006). Additionally, five of seven studies observed elevated results for $NO_2$ (Raz et al. 2018; Ritz et al. 2018; Volk et al. 2013; Becerra et al. 2013; Jung et al. 2013), which is highly correlated ($r=0.7–0.9$) with $PM_{2.5}$ in some urban areas (Carlsten et al. 2011; Gehring et al. 2010; Oftedal et al. 2009), and often negatively correlated with ozone (Sillman et al. 1990). Relative to individual contaminants, Volk et al. (2013) observed strong associations with ASD for traffic-related air pollution, a summary measure representing a mixture of pollutants which may be more indicative of the effects of multiple interacting pollutants. Our joint pollutant model results showed that simultaneous adjustment for ozone and $PM_{2.5}$ had minimal impact on our effect estimates (all aORs were impacted by ≤14%). These small differences likely result from weak correlations detected between ozone and particulate matter in our study. So, while other pollutants such as $NO_2$ could potentially confound these associations, they would have to be strongly correlated with both ASD and either $PM_{2.5}$ or ozone levels to fully explain the results detected in our study. Lastly, our adjustment for $PM_{2.5}$ in the ozone models should control for some of the potential confounding by $NO_2$ if they spatially and temporally co-occur. Though we focused on two pollutants with evidence of associations with ASD reported in previous studies, future research would benefit from examining a broader range of pollutants and further elucidation of potential interactive effects.

We adjusted for multiple temporal exposure windows in all models except for the CEI to examine specific periods of etiological relevance and to help control confounding across the time windows examined (Weisskopf et al. 2015). After adjusting for the nine months before

and after pregnancy, Raz et al. (2015) observed a small increase (aOR range: 1.50 to 1.63) in the entire pregnancy for their $PM_{2.5}$ estimate. In contrast, this adjustment in the pre- and post-pregnancy periods led to null results compared to single window regression models (from 1.32 and 1.29 to 0.83 and 0.96). In a separate study, Raz et al. (2018) mutually adjusted for exposures during pregnancy and the first nine months after birth, which led to a lower OR for pregnancy (from 1.08 to 0.77) and higher OR for the first nine postnatal months (from 1.09 to 1.40). Our $PM_{2.5}$ results increased 7% on average for the 2$^{nd}$ trimester following multi-window adjustment but remained largely unchanged for the other exposure windows. Another study of ASD and $PM_{10}$ (Kalkbrenner et al. 2015) adjusted for multiple three-month-long windows in all models from three months before pregnancy to the 4$^{th}$ quarter of the 1$^{st}$ year after pregnancy. They only observed consistently elevated results for the 3$^{rd}$ trimester, with the largest increase of 16% (aOR=1.38; 95% CI: 1.03, 1.84) following additional adjustment for 1$^{st}$ trimester exposures. Mutual adjustment for different temporal exposure windows likely improved both our ability to control for confounding and enhanced the temporal specificity of our results (Weisskopf et al. 2015), though differences in multi-window adjustment approaches between studies make direct comparisons less interpretable. Future studies with improved exposure assessment data should consider distributed lag models that could use daily air pollution data to further examine more specific temporal windows of vulnerability and that may be better able to address the potential for confounding by seasonality (Wilson et al. 2017).

### Exposure Measurement

A primary limitation in many epidemiological studies of air pollution is the lack of individual-level exposure data, which can lead to measurement error and exposure misclassification. Our air pollution estimates were developed from the EPA FAQSD model, which combines modeled atmospheric chemistry data from the CMAQ model with measured data from air monitoring stations to create daily estimates for census tracts. These exposure estimates provided better spatial resolution for urban areas, where denser populations result in geographically smaller tracts. Based on normalized median bias estimates, the overall CMAQ model predictions for ozone appear to be within 5% of the observed measures reported during January 2006 (a winter month in the northern hemisphere) and August 2006 (a summer month) in the eastern U.S (Appel et al. 2010). A similar comparison for $PM_{2.5}$ showed that overall CMAQ model predictions appear to be within 10% and 30% of observations reported during January 2006 and August 2006, respectively, in the eastern U.S. These are likely over-estimates of the uncertainty, as Appel et al. (2010) did not make these comparisons using a downscaler, a post-processing step that the FAQSD model applies to the CMAQ outputs. The downscaler uses mathematical models to adjust spatial and temporal model estimates based on observational measures, thereby improving model predictions (Berrocal et al. 2010). A study comparing the impact of ozone on birth weight using both CMAQ and FAQSD models found that results using FAQSD had less variability, with data more closely resembling actual monitoring station measurements (Warren et al. 2013). Therefore, while our analyses did not account for variability in modeled air pollutant estimates, these data represent the best publicly available spatially modeled data and should accurately reflect the ambient levels near the study population’s residences.

The use of ambient data to estimate individual-level exposures is a study limitation given the lack of data on intra- and inter-individual variability in activity patterns of study participants. For example, Americans spend 93% of their time indoors (Klepeis et al. 2001), and both indoor sources and infiltration of outdoor sources vary for ozone and $PM_{2.5}$. Although there can be indoor ozone sources, for most buildings indoor ozone is largely transported from outside (Weschler 2000). Daily inhalation intakes of indoor ozone typically account for 25–60% of total ozone exposure (Weschler 2006). Infiltration of outdoor ozone to indoor environments is greatly reduced by central air conditioning, which is typically more common in newer buildings. Therefore, our ambient ozone estimates might better approximate exposures for residents of older buildings more common in Cincinnati's central neighborhoods, compared to newer, suburban buildings. Indoor concentrations of $PM_{2.5}$, in contrast, vary widely depending on indoor sources (Wallace 1996). Indoor PM levels may be affected by air conditioning, which will generally decrease outdoor PM infiltration (Bell et al. 2009), though there is evidence that microbial PM (e.g., mold spores, airborne bacteria) could increase in humid conditions where air conditioning filters can provide suitable growing environments (Moritz et al. 2001). A study of six Cincinnati households in 2004 found indoor-to-outdoor ratios for $PM_{2.5}$ concentrations ranging from 0.5–2.9 in spring and 0.7–4.7 in fall, with ratios >1 (concentrations higher indoors than outdoors) due to indoor smoking, cooking, and cleaning (Martuzevicius et al. 2008). In our study population, 25% of mothers reported ever smoking before pregnancy and 19% reported ever smoking during pregnancy, though these variables were not identified as confounders. Though our smoking data may be underreported, previous research has indicated general agreement between cotinine levels and self-reported cigarette smoking during pregnancy from birth records data (Searles Nielson et al. 2014). Additionally, we did not have information on post-natal maternal smoking habits or whether others smoked in the primary residence during or after pregnancy. Due to the wider variety of factors affecting indoor $PM_{2.5}$ concentrations compared to ozone, there may be more potential measurement error in our $PM_{2.5}$ estimates. Time-activity studies have found that pregnant women tend to spend more time at home as pregnancies progress (Nethery et al. 2009, Clarke et al. 2005), which may result in more accurate third trimester estimates compared to the first or second trimester measures. Generally, exposure mismeasurement due to lack of data on intra-individual mobility would be expected to be non-differential and largely bias measures of association towards the null (Setton et al. 2011).

Another exposure assessment limitation is that our ambient pollutant concentration estimates for census tracts were based on residential addresses at birth and do not account for residential mobility. A review article of 14 environmental epidemiology studies with residential mobility data found that 9–32% of pregnant women moved residences, with the median distance moved <10 km (6.2 miles), most during the second trimester (Bell and Belanger 2012). The two previous epidemiological studies of ASD and air pollution that measured mobility indicated that the majority of women did not change residences during pregnancy or during the post-natal period. Talbott et al. (2015a) reported that 85% of subjects did not move during their pregnancy and reported their strongest results for exposure metrics furthest removed from when addresses were recorded at birth (i.e. CEI and the 2$^{nd}$ year of life). In an analysis of pre- and post-pregnancy addresses, Raz et al. (2013)

reported that 65% of cases and controls did not move during pregnancy or the 9 months afterward. They also found very little difference in aORs for $PM_{2.5}$ quartiles between non-movers (aOR=2.06, 95% CI: 1.17, 3.63) and those with exposure estimates based on the first known post-pregnancy address (aOR=1.99, 95% CI: 1.27, 3.10). These data on mobility suggest that given the various sources of exposure assessment uncertainty, this source of measurement error may be minimal. Although the lack of individual-level exposure data and low variability in intermediate exposure categories limited the ability to examine exposure-response relationships, our results are fairly comparable to several previous studies.

## Biological Plausibility

The actual mechanisms related to ASD onset are unknown. Hypotheses include epigenetic changes to patterns of DNA methylation in the developing brain (Keil and Lein 2016) and immune alterations leading to neurologic inflammation (Noriega and Savelkoul 2013). Several studies have reported relationships between DNA methylation variations influencing immune responses and air pollution exposures, including for $PM_{2.5}$ (Panni et al. 2016) and ozone (Fry et al. 2014). Toxicology studies in rodents have examined ASD-like characteristics following exposures to air pollution, particularly $PM_{2.5}$. For example, a study in mice exposed to $PM_{2.5}$ throughout early neurodevelopment found ASD-like decreased sociability, with more extensive impacts among males (Church et al. 2018). Another study of mice exposed to $PM_{2.5}$ in the early postnatal period reported behavioral features consistent with ASD including poor communication and social interaction, as well as differences in gene expression and markers of inflammation in the brain (Li et al. 2018). Though there is less research examining ozone as a risk factor specifically for ASD, there is evidence for the neuron-damaging potential of ozone-induced oxidative stress in rodent experiments, with documented effects on motor activity (Pereyra-Muñoz et al. 2006) and social recognition memory (Guevara-Guzmán et al. 2009).

Genetic studies have not identified causal mechanisms underlying a large majority of ASD cases, and there is not complete ASD concordance among monozygotic twins, thus there is a general consensus that genetic susceptibilities and environmental stressors are both involved in ASD development (Schwartzer et al. 2013). For example, one study of twin pairs with ASD reported that shared environmental factors could explain about 55% of the variability in behavioral deficits, a higher figure than previously estimated (Hallmayer et al. 2011). In an attempt to reduce genetic influences in our ambient air pollution study, we excluded multiple births from cases and controls, and excluded siblings who both had ASD from our cases. While most studies on environmental contributions to ASD have focused on in utero exposures, some toxicological evidence supports the biological plausibility of ASD development from exposures including particulate matter in the late prenatal and very early postnatal period, when considerable neurodevelopment occurs (Church et al. 2018, Chang et al. 2018, Allen et al. 2014, Wagner et al. 2006). Although the lack of established biologically-based critical exposure windows is a key source of uncertainty, the use of trimester- or year-long averaging timeframes for assessing possible health effects of air pollution allows for direct comparison to other studies using these common averaging windows. Animal studies or other experimental evidence can provide biological plausibility

to the epidemiological findings and can help narrow the specific critical windows of interest and the combination of pollutants that are examined in future studies.

### Future Directions

Our use of daily air pollution estimates allowed us to examine specific time windows for each study subject. By averaging estimates across longer time periods, however, we may miss the impact of peak exposures, which have not been examined in previous studies. A previous study by Kalkbrenner et al. (2015) examined two-week averages while Raz et al. (2018) examined one-week averages, which may be more relevant to elucidate specific critical windows of exposure and potential biological mechanisms. In addition to peak exposures and critical windows, future studies of ASD and air pollution should examine specific ASD phenotypes in relation to different air pollution components. Lastly, collection of indoor air monitoring and personal measurements for various air pollutants, which can be more time-consuming and expensive, would also help provide more accurate estimates of exposure and potential risk.

Our study adds to the epidemiologic evidence demonstrating elevated associations between ambient air pollution and ASD, with our highest results observed for the early postnatal period and late pregnancy. Our study benefited from the use of daily estimates for two common pollutants, high expected case ascertainment, consistent diagnostic criteria, and the ability to adjust for relevant potential confounders, co-exposures, and multiple critical exposure windows. Future epidemiological research should focus on further identifying specific developmental time periods of susceptibility for a broader range of co-pollutants, and on examining the impact of cumulative versus peak exposures. Future studies would also benefit from tracking residential mobility during critical exposure periods and by including samples sizes sufficient to detect associations small in magnitude (Flores-Pajot et al. 2016). Additional mechanistic studies are needed to inform the biological plausibility of postnatal exposures contributing to ASD, and on the mechanisms of environmental contributions to ASD more generally.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## FUNDING

JA Kaufman was supported by cooperative agreement no. X3-83555301 from the U.S. EPA and the Association of Schools and Programs of Public Health (ASPPH). The views expressed in this manuscript are those of the authors and do not necessarily reflect the views or policies of the U.S. EPA or the ASPPH.

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## Highlights

- Highest results for ASD with $PM_{2.5}$ were for exposures in 1st year of life.
- Highest results for ASD with ozone were for exposures in 2nd year of life.
- Stronger postnatal results in agreement with most prior ASD-air pollution studies.

**Table 1:**

Ozone and $PM_{2.5}$ Exposure Distributions for All Study Participants

| Exposure Metrics | N | Mean | Std Dev | Min | Max | IQR |
|---|---|---|---|---|---|---|
| Ozone (ppb)[a] | | | | | | |
| Trimester 1 | 6848 | 39.3 | 9.6 | 18.5 | 65.3 | 15.5 |
| Trimester 2 | 6848 | 38.8 | 9.6 | 18.5 | 65.4 | 15.7 |
| Trimester 3 | 6787 | 39.9 | 10.1 | 17.5 | 65.2 | 16.5 |
| Year 1 of life | 6848 | 39.1 | 2.5 | 32.8 | 48.5 | 3.8 |
| Year 2 of life | 6848 | 39.1 | 2.3 | 32.8 | 45.4 | 3.7 |
| Cumulative exposure index (CEI), pregnancy through year 2 | 6848 | 39.2 | 2.0 | 33.1 | 45.6 | 2.8 |
| PM2.5 ($\mu g/m^3$)[b] | | | | | | |
| Trimester 1 $PM_{2.5}$ average | 6848 | 12.3 | 2.5 | 3.9 | 21.8 | 3.4 |
| Trimester 2 | 6848 | 12.3 | 2.4 | 4.2 | 20.7 | 3.3 |
| Trimester 3 | 6787 | 12.3 | 2.6 | 3.9 | 24.4 | 3.7 |
| Year 1 of life | 6848 | 12.6 | 1.3 | 5.3 | 17.5 | 0.9 |
| Year 2 of life | 6848 | 12.5 | 1.2 | 8.7 | 17.4 | 0.9 |
| Cumulative exposure index (CEI), pregnancy through year 2 | 6848 | 12.5 | 0.7 | 7.2 | 14.8 | 0.9 |

IQR=Interquartile Range

[a] Averages of daily 8-hour maximum ozone values

[b] Averages of 24-hour-average $PM_{2.5}$ levels

**Table 2:**

Spearman Correlations across Ozone and $PM_{2.5}$ Exposure Window Averages

| | **Ozone Tri1** | **Ozone Tri2** | **Ozone Tri3** | **$PM_{2.5}$ Tri1** | **$PM_{2.5}$ Tri2** | **$PM_{2.5}$ Tri3** | **Ozone Yr1** | **Ozone Yr2** | **$PM_{2.5}$ Yr1** |
|---|---|---|---|---|---|---|---|---|---|
| Oz Tri2 | 0.07 | | | | | | | | |
| Oz Tri3 | −0.72 | 0.22 | | | | | | | |
| $PM_{2.5}$ Tri1 | 0.09 | −0.19 | −0.33 | | | | | | |
| $PM_{2.5}$ Tri2 | −0.08 | 0.14 | −0.10 | 0.32 | | | | | |
| $PM_{2.5}$ Tri3 | −0.39 | −0.08 | 0.15 | 0.38 | 0.23 | | | | |
| Oz Yr1 | 0.05 | 0.13 | 0.20 | −0.03 | −0.13 | −0.24 | | | |
| Oz Yr2 | −0.14 | 0.08 | 0.09 | −0.03 | 0.04 | 0.09 | 0.33 | | |
| $PM_{2.5}$ Yr1 | −0.02 | 0.04 | −0.10 | −0.03 | 0.12 | 0.32 | −0.56 | −0.23 | |
| $PM_{2.5}$ Yr2 | 0.15 | 0.08 | −0.02 | −0.10 | −0.19 | −0.21 | −0.27 | −0.64 | 0.12 |

Tri=trimester; Yr=year; $PM_{2.5}$=fine particulate matter

**Table 3:**

Characteristics of Study Participants by Case Status

| | Study Population, n (%) | ASD Cases, n (%) | Controls, n (%) |
|---|---|---|---|
| **Total Births** | 6848 (100) | 428 (6.3) | 6420 (93.7) |
| **Child's sex** | | | |
| Male | 3563 (52.0) | 350 (81.8) | 3213 (50.0) |
| Female | 3285 (48.0) | 78 (18.2) | 3207 (50.0) |
| **Mother's age at delivery** | | | |
| <20 | 709 (10.4) | 26 (6.1) | 683 (10.6) |
| 20–24 | 1672 (24.4) | 96 (22.4) | 1576 (24.5) |
| 25–29 | 2001 (29.2) | 131 (30.6) | 1870 (29.1) |
| 30–34 | 1542 (22.5) | 92 (21.5) | 1450 (22.6) |
| 35–39 | 772 (11.3) | 68 (15.9) | 704 (11.0) |
| >40 | 152 (2.2) | 15 (3.5) | 137 (2.1) |
| **Mother's education level** | | | |
| Did not complete high school | 1165 (17.0) | 43 (10.0) | 1122 (17.5) |
| High school diploma or GED | 1554 (22.7) | 105 (24.5) | 1449 (22.6) |
| Some college | 1498 (21.9) | 100 (23.4) | 1398 (21.8) |
| Associates degree | 505 (7.4) | 57 (13.3) | 448 (7.0) |
| Bachelors degree | 1347 (19.7) | 80 (18.6) | 1267 (19.7) |
| Masters or professional degree | 712 (10.4) | 39 (9.1) | 673 (10.5) |
| Missing | 67 (1.0) | 4 (0.9) | 63 (1.0) |
| **Mother's race** | | | |
| White | 5104 (74.5) | 310 (72.4) | 4794 (74.7) |
| Black | 1458 (21.3) | 98 (22.9) | 1360 (21.2) |
| Other | 230 (3.4) | 16 (3.7) | 214 (3.3) |
| Missing | 56 (0.8) | 4 (0.9) | 52 (0.8) |
| **Mother of Hispanic origin** | | | |
| Yes | 326 (4.8) | 15 (3.5) | 311 (4.8) |
| No | 6462 (94.4) | 412 (96.3) | 6050 (94.2) |
| Missing | 60 (0.9) | 1 (0.2) | 59 (0.9) |
| **Mother's marital status** | | | |
| Married | 3895 (56.9) | 268 (62.6) | 3627 (56.5) |
| Unmarried | 2953 (43.1) | 160 (37.4) | 2793 (43.5) |
| **Primary source of payment for delivery** | | | |
| Private | 3821 (55.8) | 260 (60.7) | 3561 (55.5) |
| Public | 2407 (35.1) | 141 (32.9) | 2266 (35.3) |
| Other | 454 (6.6) | 20 (4.7) | 434 (6.8) |
| Missing | 166 (2.4) | 7 (1.6) | 159 (2.5) |
| **Trimester prenatal care started** | | | |

| | Study Population, n (%) | ASD Cases, n (%) | Controls, n (%) |
|---|---|---|---|
| 1st | 3159 (46.1) | 200 (46.7) | 2959 (46.1) |
| After 1st | 1086 (15.9) | 62 (14.5) | 1024 (16.0) |
| Missing | 2603 (38.0) | 166 (38.8) | 2437 (38.0) |
| **Number of prenatal care visits** | | | |
| 0 – 6 | 955 (13.9) | 52 (12.1) | 903 (14.1) |
| 7 – 10 | 1491 (21.8) | 89 (20.8) | 1402 (21.8) |
| 11 – 13 | 1280 (18.7) | 77 (18.0) | 1203 (18.7) |
| 14 – 60 | 938 (13.7) | 56 (13.1) | 882 (13.7) |
| Missing | 2184 (31.9) | 154 (36.0) | 2030 (31.6) |
| **Number of cigarettes mother reported smoking during pregnancy** | | | |
| 0 | 5572 (81.4) | 353 (82.5) | 5219 (81.3) |
| 1–20 | 463 (6.8) | 29 (6.8) | 434 (6.8) |
| 21–40 | 450 (6.6) | 29 (6.8) | 421 (6.6) |
| >40 | 363 (5.3) | 17 (4.0) | 346 (5.4) |
| **Number of previous births** | | | |
| 0 | 2735 (39.9) | 204 (47.7) | 2531 (39.4) |
| 1 | 2193 (32.0) | 132 (30.8) | 2061 (32.1) |
| 2 | 1098 (16.0) | 63 (14.7) | 1035 (16.1) |
| 3 or more | 775 (11.3) | 25 (5.8) | 750 (11.7) |
| Missing | 47 (0.7) | 4 (0.9) | 43 (0.7) |
| **Maternal prepregnancy Body Mass Index** | | | |
| <18.5 (under weight) | 280 (4.1) | 15 (3.5) | 265 (4.1) |
| 18.5–25 (healthy weight) | 3205 (46.8) | 177 (41.4) | 3028 (47.2) |
| >25–30 (overweight) | 1591 (23.2) | 101 (23.6) | 1490 (23.2) |
| >30 (obese) | 1409 (20.6) | 118 (27.6) | 1291 (20.1) |
| Missing | 363 (5.3) | 17 (4.0) | 346 (5.4) |
| **Time since previous delivery** | | | |
| No previous births | 2713 (39.6) | 201 (47.0) | 2512 (39.1) |
| <2 years | 1025 (15.0) | 61 (14.3) | 964 (15.0) |
| 2–6 years | 2152 (31.4) | 92 (21.5) | 2060 (32.1) |
| >6 years | 549 (8.0) | 47 (11.0) | 502 (7.8) |
| Missing | 409 (6.0) | 27 (6.3) | 382 (5.6) |
| **Gestational length** | | | |
| 17 – 36 weeks | 757 (11.1) | 62 (14.5) | 695 (10.8) |
| 37 – 47 weeks | 6091 (88.9) | 366 (85.5) | 5725 (89.2) |
| **Birth weight in grams** | | | |
| 115 – 2496 (low, <2500 g) | 483 (7.1) | 37 (8.6) | 446 (6.9) |
| 2500 – 5510 (normal, ≥2500 g) | 6365 (92.9) | 391 (91.4) | 5974 (93.1) |
| **Month of conception** | | | |

| | Study Population, n (%) | ASD Cases, n (%) | Controls, n (%) |
|---|---|---|---|
| January | 595 (8.7) | 36 (8.4) | 559 (8.7) |
| February | 507 (7.4) | 35 (8.2) | 472 (7.4) |
| March | 550 (8.0) | 36 (8.4) | 514 (8.0) |
| April | 478 (7.0) | 29 (6.8) | 449 (7.0) |
| May | 582 (8.5) | 28 (6.5) | 554 (8.6) |
| June | 587 (8.6) | 35 (8.2) | 552 (8.6) |
| July | 609 (8.9) | 43 (10.0) | 566 (8.8) |
| August | 571 (8.3) | 34 (7.9) | 537 (8.4) |
| September | 526 (7.7) | 34 (7.9) | 492 (7.7) |
| October | 622 (9.1) | 37 (8.6) | 585 (9.1) |
| November | 598 (8.7) | 43 (10.0) | 555 (8.6) |
| December | 623 (9.1) | 38 (8.9) | 585 (9.1) |

**Table 4:**

Multivariable Regression Models for Categorical Exposures and Per IQR Increases in Ozone and $PM_{2.5}$

| Exposure categories[a] | Cases / Controls | Model 1[b] | Model 2[c] |
|---|---|---|---|
| | | aOR (95% CI) | aOR (95% CI) |
| $PM_{2.5}$ 1st trimester (μg/m$^3$) | | | |
| 3.9 – <10.1 (referent) | 64/932 | 1.00 | 1.00 |
| 10.1 – <14.9 | 256/3819 | 0.98 (0.72, 1.32) | 0.93 (0.67, 1.30) |
| 14.9 – 21.8 | 65/941 | 1.06 (0.72, 1.56) | 0.99 (0.66, 1.51) |
| Per IQR increase (3.39) | -- | 1.03 (0.86, 1.23) | 1.05 (0.86, 1.28) |
| $PM_{2.5}$ 2nd trimester (μg/m$^3$) | | | |
| 4.2 – <10.1 (referent) | 50/923 | 1.00 | 1.00 |
| 10.1 – <14.9 | 269/3810 | 1.36 (0.98, 1.89) | 1.44 (1.01, 2.05) |
| 14.9 – 20.7 | 66/959 | 1.30 (0.85, 1.99) | 1.41 (0.89, 2.24) |
| Per IQR increase (3.34) | -- | 1.08 (0.91, 1.29) | 1.13 (0.93, 1.38) |
| $PM_{2.5}$ 3rd trimester (μg/m$^3$) | | | |
| 3.9 – <9.9 (referent) | 62/936 | 1.00 | 1.00 |
| 9.9 – <15.1 | 254/3763 | 0.99 (0.73, 1.35) | 0.98 (0.70, 1.37) |
| 15.1 – 24.4 | 65/951 | 1.05 (0.70, 1.58) | 1.04 (0.67, 1.61) |
| Per IQR increase (3.72) | -- | 0.98 (0.81, 1.20) | 0.96 (0.77, 1.20) |
| $PM_{2.5}$ entire pregnancy (μg/m$^3$) | | | |
| 5.3 – <10.8 | 64/944 | 1.00 | 1.00 |
| 10.8 – <14.0 | 252/3800 | 0.95 (0.69, 1.30) | 0.87 (0.63, 1.21) |
| 14.0 – 18.3 | 69/948 | 1.07 (0.75, 1.53) | 0.99 (0.67, 1.46) |
| Per IQR increase (2.02) | -- | 1.01 (0.98, 1.04) | 1.01 (0.97, 1.04) |
| $PM_{2.5}$ 1st year of life (μg/m$^3$) | | | |
| 5.2 – <11.7 (referent) | 44/946 | 1.00 | 1.00 |
| 11.7 – <13.8 | 277/3807 | 1.67 (1.17, 2.38) | 1.84 (1.26, 2.69) |
| 13.8 – 17.5 | 64/939 | 1.40 (0.94, 2.09) | 1.54 (0.98, 2.40) |
| Per IQR increase (0.90) | -- | 1.07 (0.98, 1.17) | 1.11 (1.00, 1.23) |
| $PM_{2.5}$ 2nd year of life (μg/m$^3$) | | | |
| 8.7 – <11.5 (referent) | 63/991 | 1.00 | 1.00 |
| 11.5 – <13.2 | 268/3835 | 1.10 (0.82, 1.48) | 1.10 (0.81, 1.50) |
| 13.2 – 17.4 | 54/866 | 0.97 (0.61, 1.55) | 1.13 (0.67, 1.94) |
| Per IQR increase (0.87) | -- | 0.98 (0.88, 1.09) | 1.07 (0.93, 1.23) |
| $PM_{2.5}$, CEI (μg/m$^3$) | | | |
| 7.2 – <11.8 (referent) | 51/982 | 1.00 | 1.00 |
| 11.8 – <13.2 | 267/3798 | 1.37 (1.00, 1.86) | 1.41 (1.02, 1.95) |
| 13.2 – 14.8 | 67/912 | 1.45 (0.98, 2.15) | 1.52 (1.00, 2.31) |

| Exposure categories[a] | Cases / Controls | Model 1[b] | Model 2[c] |
|---|---|---|---|
| | | aOR (95% CI) | aOR (95% CI) |
| Per IQR increase (0.94) | -- | 1.10 (0.94, 1.28) | 1.17 (0.98, 1.40) |
| Ozone 1st trimester (ppb) | | | |
| 18.5 – <27.8 (referent) | 76/970 | 1.00 | 1.00 |
| 27.8 – <48.6 | 259/3825 | 0.76 (0.52, 1.10) | 0.70 (0.47, 1.04) |
| 48.6 – 65.3 | 50/897 | 0.59 (0.35, 1.00) | 0.56 (0.33, 0.96) |
| Per IQR increase (15.54) | -- | 0.87 (0.61, 1.25) | 0.87 (0.60, 1.26) |
| Ozone 2nd trimester (ppb) | | | |
| 18.5 – <28.1 (referent) | 70/964 | 1.00 | 1.00 |
| 28.1 – <49.2 | 252/3780 | 0.93 (0.64, 1.36) | 0.96 (0.65, 1.42) |
| 49.2 – 65.4 | 63/948 | 0.94 (0.57, 1.55) | 0.92 (0.54, 1.55) |
| Per IQR increase (15.66) | -- | 0.92 (0.62, 1.35) | 0.85 (0.56, 1.29) |
| Ozone 3rd trimester (ppb) | | | |
| 17.5 – <27.6 (referent) | 62/935 | 1.00 | 1.00 |
| 27.6 – <49.7 | 246/3784 | 0.94 (0.65, 1.37) | 0.92 (0.62, 1.35) |
| 49.7 – 65.2 | 73/931 | 1.16 (0.71, 1.88) | 1.17 (0.71, 1.93) |
| Per IQR increase (16.50) | -- | 1.22 (0.82, 1.80) | 1.19 (0.80, 1.77) |
| Ozone entire pregnancy (ppb) | | | |
| 23.7 – <34.3 (referent) | 62/967 | 1.00 | 1.00 |
| 34.3 – <44.6 | 259/3784 | 1.16 (0.83, 1.63) | 1.23 (0.86, 1.75) |
| 44.6 – 59.4 | 64/941 | 1.18 (0.74, 1.86) | 1.28 (0.78, 2.10) |
| Per IQR increase (7.39) | -- | 1.00 (0.99, 1.00) | 1.00 (0.99, 1.01) |
| Ozone 1st year of life (ppb) | | | |
| 32.8 – <36.5 (referent) | 66/924 | 1.00 | 1.00 |
| 36.5 – <41.6 | 251/3818 | 0.94 (0.70, 1.25) | 0.95 (0.69, 1.31) |
| 41.6 – 48.5 | 68/950 | 1.04 (0.72, 1.49) | 1.19 (0.79, 1.81) |
| Per IQR increase (3.83) | -- | 1.05 (0.87, 1.27) | 1.22 (0.96, 1.54) |
| Ozone 2nd year of life (ppb) | | | |
| 32.8 – <36.6 (referent) | 49/887 | 1.00 | 1.00 |
| 36.6 – <41.5 | 273/3836 | 1.41 (1.00, 1.99) | 1.42 (0.98, 2.05) |
| 41.5 – 45.4 | 63/969 | 1.28 (0.84, 1.96) | 1.29 (0.81, 2.05) |
| Per IQR increase (3.66) | -- | 1.19 (0.95, 1.48) | 1.27 (0.96, 1.69) |
| Ozone CEI (ppb) | | | |
| 33.1 – <37.3 (referent) | 67/927 | 1.00 | 1.00 |
| 37.3 – <41.4 | 252/3789 | 0.95 (0.71, 1.28) | 1.02 (0.76, 1.37) |
| 41.4 – 45.6 | 66/976 | 0.99 (0.66, 1.47) | 1.16 (0.76, 1.78) |
| Per IQR increase (2.79) | -- | 1.07 (0.88, 1.29) | 1.19 (0.95, 1.49) |

CEI=Cumulative Exposure Index (pregnancy through 2nd year of life)

[a]Sextiles with middle 2–5 categories collapsed into one group.

[b] Model 1 adjusted for year of birth, mother's education, birth spacing, maternal pre-pregnancy body mass index, month of conception.

[c] Model 2 adjusted for year of birth, mother's education, birth spacing, maternal pre-pregnancy body mass index, and month of conception, multi-window (except for CEI models), and multi-pollutant. Multi-window models: 1st trimester models adjusted for 3rd trimester average exposure; 2nd trimester models adjusted for 3rd trimester average exposure; 3rd trimester models adjusted for 1st trimester average exposure; 1st year of life models adjusted for pregnancy average exposure; 2nd year of life models adjusted for pregnancy average exposure. Multi-pollutant models: ozone models adjusted for $PM_{2.5}$ average exposures for the same time period; $PM_{2.5}$ models adjusted for ozone average exposure during the same time period.