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# Prenatal phenol and paraben exposures in relation to child neurodevelopment including autism spectrum disorders in the MARBLES Study

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## Abstract

**Background:** Environmental phenols and parabens are endocrine disrupting chemicals (EDCs) with the potential to affect child neurodevelopment including autism spectrum disorders (ASD). Our aim was to assess whether exposure to environmental phenols and parabens during pregnancy was associated with an increased risk of clinical ASD or other nontypical development (non-TD).

**Methods:** This study included mother-child pairs (N=207) from the Markers of Autism Risks in Babies – Learning Early Signs (MARBLES) Cohort Study with urinary phenol and paraben metabolites analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) from repeated pregnancy urine samples. Because family recurrence risks in siblings are about 20%, MARBLES enrolls pregnant women who already had a child with ASD. Children were clinically assessed at 3 years of age and classified into 3 outcome categories: ASD, non-TD, or typically

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developing (TD). Single analyte analyses were conducted with trinomial logistic regression and weighted quantile sum (WQS) regression was used to test for mixture effects.

**Results:** Regression models were adjusted for pre-pregnancy body mass index, prenatal vitamin use (yes/no), homeowner status (yes/no), birth year, and child's sex. In single chemical analyses phenol exposures were not significantly associated with child's diagnosis. Mixture analyses using trinomial WQS regression showed a significantly increased risk of non-TD compared to TD (OR = 1.58, 95% CI: 1.56, 1.60) with overall greater prenatal phenol and paraben metabolites mixture. Results for ASD also showed an increased risk, but it was not significant.

**Discussion:** This is the first study to provide evidence that pregnancy environmental phenol exposures may increase the risk for non-TD in a high-risk population.

## Introduction

The latest prevalence estimates from the Centers for Disease Control and Prevention show autism spectrum disorders (ASD) now affects 1 in 59 children in the US and is 4.5 times more prevalent in boys compared to girls (Baio et al. 2018). The etiology of ASD is not understood; however, evidence from twin studies show that both genetic factors and pregnancy exposures play a role. Within the last decade the number of non-genetic factors linked to ASD has increased, providing useful clues, and a mechanistic understanding of non-genetic causes of ASD is beginning to develop (Becerra et al. 2013; Croen et al. 2011; Eskenazi et al. 2007; Hallmayer et al. 2011; Harrington et al. 2014; Krakowiak et al. 2012; Landrigan et al. 2012; Larsson et al. 2009; Eric M. Roberts et al. 2007; Sandin et al. 2014; Sandin et al. 2017; Schmidt et al. 2012; Shelton et al. 2014; Volk et al. 2013; Volk et al. 2014; Windham et al. 2006). Associations of ASD with environmental exposures such as air pollution, pesticides, nutrition, and medications have been emerging rapidly in recent years, (Becerra et al. 2013; Croen et al. 2011; Eskenazi et al. 2007; Harrington et al. 2014; Krakowiak et al. 2012; Landrigan et al. 2012; Larsson et al. 2009; Eric M. Roberts et al. 2007; Schmidt et al. 2012; Shelton et al. 2014; Volk et al. 2013; Volk et al. 2014; Windham et al. 2006) leading to increased recognition that pregnancy environmental exposures likely contribute, either on their own or in combination with genetic factors, to ASD risk (National Research Council 2000).

There is growing body of literature that implicates several classes of endocrine disrupting chemicals (EDC) as potential risk factors for ASD (Braun et al. 2014b; Miodovnik et al. 2011). Additionally, EDCs have exhibited sex-specific effects (Frye et al. 2012) and given the sex difference in ASD risk some researchers hypothesize that there may be a hormonal etiology for ASD (Baron-Cohen et al. 2005). One mechanism by which EDCs may increase the risk for ASD is through dysregulation of thyroid hormones during pregnancy, which are essential for brain development (Andersen et al. 2014; Braun 2012; Colborn 2004; Khan et al. 2014).

Environmental phenols and parabens are EDCs found in common household and personal care products (US Centers for Disease Control and Prevention 2017a, b). Exposure to these chemicals is widespread and they have been detected in pregnant women (Mortensen et al. 2014; Woodruff et al. 2011), adults, and children in the general US population (Centers for

Disease Control and Prevention 2017). Phenols can disrupt thyroid hormones and signaling pathways that are critical for development (Mustieles et al. 2015). In rodent studies, prenatal exposures to phenols have resulted in lasting effects on brain structure, function, and behavior (as reviewed by (Richter et al. 2007)). In epidemiology studies, phenol exposures have been associated with altered sex and thyroid hormones (Aker et al. 2018; Berger et al. 2018), and child behavioral outcomes (Braun et al. 2017; Harley et al. 2013; Perera et al. 2012; Philippat et al. 2017).

Studies on the effects of pregnancy paraben exposures on child development are limited but much of this research has focused on thyroid function. Pregnancy phenol and paraben exposures have been associated with lower thyroid-stimulating hormone (TSH) and free thyroxine (T4) in pregnant women (Berger et al. 2018). Pregnancy paraben and phenol exposures have been shown to alter reproductive and thyroid hormone levels in pregnant women (Aker et al. 2016; Aker et al. 2018). Maternal thyroid hormones play an important role in early brain development by influencing processes such as neuronal cell myelination, migration, and signaling (Bernal and Nunez 1995; Bernal 2005).

Given widespread exposures and impacts on thyroid hormones, phenols may contribute to developmental delays including ASD risk; however, epidemiology studies are limited. Research on environmental phenol exposures and child neurodevelopment has largely focused on bisphenol A (BPA). Exposure to BPA has been associated with child behavioral problems and attention-deficit/hyperactivity disorder (ADHD); however, results are inconsistent, as reported in a recent review of EDCs effects on child neurodevelopment (Braun 2017). In one study, pregnancy urinary BPA concentrations were associated with externalizing behaviors in girls at age 2 years but not among all children in the study (Braun et al. 2009). Pregnancy triclosan exposures, another type of phenol, have been associated with lower child cognitive scores at 8 years (Jackson-Browne et al. 2018). Triclosan was used in personal care products for its antimicrobial properties until the US Food and Drug Administration banned its use in consumer wash products in 2017 (Food and Drug Administration (FDA) 2016), but it can still be found in toothpaste. Associations between pregnancy phenol exposures and ASD symptoms in early childhood have not been reported but pregnancy exposures to other EDCs such as brominated flame retardants, phthalates, and organochlorine pesticides have been associated with ASD or ASD symptoms (Braun et al. 2014b; Miodovnik et al. 2011; E. M. Roberts et al. 2007; Shin et al. 2018).

Past studies have investigated effects of individual parabens or phenols but more than one of these compounds are often found in a single product. Additionally, phenols such as BPA, bisphenol S (BPS) and bisphenol F (BPF) act on similar pathways (Rochester and Bolden 2015). Given the common exposure routes and similar mechanisms we aimed to assess the combined effects of exposure to paraben and phenol mixtures during pregnancy. The aims of this study were to determine whether exposure to individual environmental phenols, specifically BPA, BPS, BPF, triclosan (TCS), triclocarban (TCC), and parabens or exposure to the mixture of these compounds during pregnancy was associated with an increased risk of clinical ASD or other non-typical development (non-TD) in the Markers of Autism Risks in Babies – Learning Early Signs (MARBLES) prospective cohort study.

## Methods

### Study population

The present study included 207 participants enrolled in the MARBLES Study from 2007 – 2014 with pregnancy environmental phenol and paraben metabolite concentrations and child neurodevelopmental assessments. The MARBLES Study is an enriched-risk longitudinal cohort in northern California as described in Hertz-Picciotto et al. (Hertz-Picciotto et al. 2018). Participating mothers had a previous child with ASD; therefore they were at higher risk (18 – 24%) for delivering another infant who would develop ASD (Hertz-Picciotto et al. 2018; Ozonoff et al. 2011). Two women did not have a previous child with ASD but were included because they were at higher risk for having a child with ASD (eg. identical twin had a child with ASD). Families were recruited from lists of children receiving services for ASD obtained through the California Department of Developmental Services, from other studies at the University of California, Davis Medical Investigation of Neurodevelopmental Disorders (MIND) Institute, other referrals and self-referrals. Study participants were enrolled prior to or during pregnancy. Mothers were followed through pregnancy, and infants from birth to 3 years. Inclusion criteria for the full study were: (i) mother or father had one or more child(ren) with ASD and/or the gestating younger child had an older half-sibling, or an equivalent or closer blood relative with ASD; (ii) mother was at least 18 years of age or older; (iii) mother was already pregnant or planning a pregnancy, and biologically able to become pregnant; (iv) mother lived within 2 hours of the Davis/Sacramento region at time of enrollment. The University of California, Davis (UCD) institutional review board approved the MARBLES Study and informed consent was obtained from each participant.

### Child neurodevelopmental assessment

Expert clinicians assessed children at the UC Davis MIND Institute. During the child's 3-year visit, expert clinicians evaluated children on the Autism Diagnostic Observation Scale (ADOS), the gold-standard diagnostic tool (Lord et al. 2008); and the Mullen Scales of Early Learning (MSEL), a normed referenced cognitive measure. The MSEL includes a composite score which combines four subscales (visual reception, fine motor skills, receptive language, and expressive language) (Mullen 1995). Three categories of children's outcomes at 3 years were defined using an algorithmic approach (Ozonoff et al. 2014), which took into account scores on the ADOS and MSEL. The first category was ASD, defined by a child scoring at or above the ASD cutoff on the ADOS, and meeting DSM-5 criteria for ASD. The second outcome category, non-typical development (non-TD), required that the child did not meet DSM-5 criteria for ASD, and had two or more MSEL subscale scores ≥ 1.5 standard deviation (SD) below the mean, and/or had one or more MSEL subscale score ≥ 2 SD below the mean, and/or had an ADOS ≤ 3 points below the ASD cutoff. The third outcome was typically developing (TD), defined as a child that did not meet DSM-5 criteria for ASD classification, based on clinical best judgment of two clinicians, and who scored above 2 SD below the mean on all MSEL subscales, no more than one MSEL subset ≥ 1.5 SD below the mean; and an ADOS > 3 points below the ASD cutoff.

## Urine sample collection

In the present study we focused on 2nd and 3rd trimester exposures because, on average, participants completed their first study visit early in the 2nd trimester. In this sample 67% of women collected 2nd trimester samples and 93% collected 3rd trimester samples. During each trimester of pregnancy, participants were instructed to collect three spot urine samples (one week apart) and one 24-hour urine sample. Urine samples were stored in home freezers and then collected during home visits and transported to UCD where they were then stored in −80° C freezers.

For analytical efficiency and cost-effectiveness, we pooled samples for participants with three or more samples in a trimester as previously described (Barkoski et al. 2018). Gestational age was calculated using the date of the mothers' last menstrual period (LMP) and, when available, pregnancy ultrasound information from medical records. The 1st trimester included gestational ages from LMP through 13 weeks, the 2nd trimester included gestational ages 14 – 27 weeks, and the 3rd trimester included gestational ages 28 weeks to birth. For each trimester the first sample was analyzed individually and the remaining samples were pooled. For participants with 1 – 2 samples in a trimester, each sample was analyzed individually. The vast majority of women had more than two samples within a trimester and their urine samples pooled.

A total of 1,063 primary samples were collected from the 2nd and 3rd trimesters. After pooling samples we had 700 urine samples, representing 220 mother-child pairs, analyzed for environmental phenol and paraben concentrations. For the current analysis, 660 samples were included from 207 participants who had complete covariate data and complete data on biomarker concentrations above 50% detect.

Specific gravity (SG) was measured from urine samples with a handheld refractometer (Atago Urine Specific Gravity Refractometer, PAL 10-S) at UCD. Distilled water was used to calibrate between each measurement. Then, urine samples were shipped overnight on dry ice to the Laboratory of Exposure Assessment and Development for Environmental Research (LEADER), Rollins School of Public Health, Emory University for paraben and phenol metabolite analyses.

## Assessment of phenols and parabens

Urine samples were randomized prior to analysis to reduce the amount of analytic bias introduced into the data. A 1-mL aliquot of urine was spiked with isotopically labeled analogues of the target phenols ((BPA), (BPS), (BPF), (TCC), TCS)) and parabens (butyl paraben (BuPB), ethyl paraben (ETPB), methyl paraben (MEPB), propyl paraben (PRPB)) then was subjected to an enzyme hydrolysis to liberate glucuronide-bound conjugates. The hydrolysate was extracted using an ABS Elut-NEXUS solid phase extraction cartridge and then eluted with acetonitrile and ethyl acetate, respectively. The extract was concentrated to dryness and reconstituted with BPA-free, Milli-Q water prior to analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS) using two separate injections and acquisition methods (Table S1). The BPA-free water was used in all laboratory methods. Analyte concentrations were calculated using isotope dilution calibration. Two quality

control materials (low and high concentrations) and one blank sample were analyzed concurrently with each set of 28 unknown samples. In addition, quality assurance materials were included in the sample analyses, which included NIST SRM 3672 and 3673 (one of each per 100 samples) and blinded quality controls (6 each of 2 materials per 100 samples). LEADER also participates in a bi-annual proficiency-testing program, known as the German External Quality Assessment Scheme (G-EQUAS), to ensure the accuracy and reproducibility of the analytical methods used in this study. Specifications of the method are provided in Table S1. The intra-batch relative standard deviation (RSD) for each analyte included in this analysis ranged from 2 – 5 and the inter-batch RSDs ranged from 5 – 6.

## Statistical Analysis

Mothers provided a variable number of samples per trimester. Across both $2^{nd}$ and $3^{rd}$ trimesters mothers contributed between 1 and 10 samples. The samples were a combination of spot, 24-hour, and pooled samples. Chemicals used in further analyses had at least 50% of samples with concentrations above the study-wide LOD among samples with complete covariate data (N=693 samples) (Table S1). These chemicals included BPA, ETPB, MEPB, and PRPB. Thirty-three samples were missing values for at least one of these biomarker concentrations due to an interfering substance and these samples were removed from further analyses (leaving N=660). Each sample had a unique specific gravity (SG) measurement; therefore machine-read phenol and paraben metabolite concentrations were standardized for SG using the following formula described in Hauser et al. (2004): $C_{SG} = C \times [(SG_{median} - 1)/(SG - 1)]$ (Hauser et al. 2004). $C_{SG}$ is the specific gravity corrected biomarker concentration, C is the measured concentration of the specified biomarker concentration, and $SG_{median}$ (0.012) is the median specific gravity across all of the MARBLES study samples.

A weighted average of each urinary biomarker concentration was calculated using the following formula: $C_{avg} = [(n_p)(C_p) + (C_i)]/(n_p + n_i)$ where $n_p$ is the number of samples that were pooled, $n_i$ is the number of individual samples (spots or 24-hour), $C_p$ is the SG-corrected phenol or paraben biomarker concentration from a pooled sample, and $C_i$ is the SG-corrected concentration from an individual sample.

Since machine-read values were provided, negative values arise legitimately near the LOD, which is by definition +/− 33% of the ‘blank’ level. This resulted in some negative average concentrations. So that all values were positive and nonzero, the urinary biomarker concentrations with a minimum average concentration value of zero or below were monotonically transformed by adding the minimum and 0.01. To account for right skewedness of biomarker data, log transformed values of the final average concentrations were used.

Single chemical trinomial logistic regression models, using log transformed and quantiled concentrations, were used to calculate the odds of ASD and non-TD, each relative to TD. Ordinal quantiles of log-transformed concentrations were used to compare with mixture models that used quantiles. Models were adjusted for covariates that were selected *a priori*, and included pre-pregnancy body mass index (BMI), peri-conceptional prenatal vitamin use (yes/no), homeowner status (yes/no), birth year, and child’s sex. Year of birth was centered at the study population’s average birth year. Trinomial logistic regression was used as

opposed to binomial logistic regression to simultaneously estimate two regression coefficients, one for each chemical in relation to ASD and the second for each chemical in relation to non-TD. This approach allowed for the effect of covariates to differ by each of the three outcomes, and maintained the entire sample size. See appendix for further details.

Weighted quantile sum (WQS) regression was used to test for a mixture effect of the measured environmental phenols and parabens on child neurodevelopmental outcomes while accommodating a complex correlation pattern among the analyte components (Figure S1). An average weight was estimated for each chemical that maximized the difference between each group. The weights were estimated from 100 bootstraps. The mixture index was calculated for each subject using $WQS = \Sigma W_I Q_I$, where WQS is the mixture term representing the environmental phenol and paraben mixture in this study. The $W_i$ is the mean weight per compound averaged across all 100 bootstrap subsets, and $Q_I$ is the quartiled environmental phenol or paraben measurement per subject/compound. Since quartiles were used and the weights sum to 1, an individual's WQS index can range from 0 to 3. The estimated WQS index was then analyzed in a standard generalizable linear model to obtain odds ratios using the following formula: $g(\mu) = \alpha + \beta_1 WQS + \delta Z$. Where $g$ indicates a logit link function, $\mu$ is the log odds of ASD or non-TD compared to TD, $\alpha$ is the intercept, $\beta_1 WQS$ is the phenol/paraben mixture index and associated beta estimate, and $\delta Z$ is some vector of covariates. When the WQS index is significant, it identifies major and minor contributors to the outcome among analytes in a mixture of correlated environmental chemicals (Carrico et al. 2015). Weights above a target threshold of 1/c for c analytes (e.g., for c=4, 1/4= 25%) were used to identify important analytes when the corresponding beta coefficient was significant. In this study we have three outcomes therefore a trinomial WQS regression was applied to ASD and non-TD compared to TD using. This is an expansion of WQS that follows similar principles to trinomial logistic regression (Appendix).

Two weighted indices of phenol and paraben mixtures were estimated across 100 bootstraps simultaneously for each logit. It is preferable to split the data into a training set to estimate the weights per component and a validation set for testing the significance of the regression coefficient associated with the index, given that the weight estimation is stable across a variety of splits. Here, we attempted to split the data such that 40% were in the training set and 60% were in the validation set, and ran the analysis using 4 random splits. ETPB and PRPB consistently had the highest weights for ASD; however, there was no consistency in highest weight components for non-TD. Due to this instability of weights associated with non-TD, likely attributed to a small sample size, we did not split the data into a training and validation set.

The main WQS analysis examined beta estimates that were constrained in the positive direction to detect increased risk of non-TD and ASD compared to TD. As a sensitivity analysis, WQS regression was also used with negatively constrained betas. Constraining in both the positive and negative direction allows for the detection of phenol components that are lower or higher in TD compared to non-TD and ASD when the mixture effect is significant.

Sex-specific differences were examined in both single analyte and WQS regression analyses. Similar to the previous analyses, the positively constrained model was the main analysis and the negatively constrained model was analyzed as a sensitivity analysis. From the sex-stratified WQS analysis, weight contributions of the chemical mixture's effect were calculated, such that boy and girl contributions together add to 100%. Further, sex-specific single chemical weight contributions (relative contributions) were calculated to compare the weights of analytes in the mixtures affecting boys and girls differently, where relative contributions in boys added to 100% and likewise for girls. These relative contributions were calculated by dividing the sex-specific analyte weight by the sum of the sex-specific analyte weights of the total mixture.

Trimester-specific differences were examined by re-calculating weighted averages using either only 2$^{nd}$ trimester samples or only 3$^{rd}$ trimester samples. Both single analyte and WQS regression analyses were conducted separately by trimester.

An alpha of 0.05 was the criterion for statistical significance. P-values between 0.05 and 0.12 were considered borderline significant. All statistical analyses were conducted with SAS statistical analysis software version 9.4 (SAS, Cary, NC, USA) and an independent programmer validated the results in this study.

## Results

TD children tended to be born in earlier years, have a higher socioeconomic status, and had a higher proportion of white non-Hispanic participants compared to ASD and non-TD children. Mothers of TD children were more likely to have taken prenatal vitamins during the peri-conceptional period, and had a pre-pregnancy BMI lower than mothers of children with ASD and non-TD (Table 1). These findings are consistent with previous research on the protective association between prenatal vitamin use (Guo et al. 2019; Schmidt et al. 2019; Suren et al. 2013), and studies of higher pre-pregnancy BMI and ASD risk (Lei et al. 2019; Windham et al. 2019).

Four environmental phenols or parabens had 58% or more samples above LOD, specifically BPA, ETPB, MEPB and PRPB. Table 2 shows the distributions of chemical concentrations stratified by outcome and provides the percentage of concentrations above the LOD. Mothers of children with non-TD and ASD had higher concentrations of ETPB and PRPB during the 2$^{nd}$/3$^{rd}$ trimester compared to mothers of TD children. Median pregnancy average urinary ETPB, MEPB, and PRPB concentrations from pregnant women in the MARBLES Study were generally less than median urinary concentrations from a nationally representative sample of pregnant women and women of childbearing age from NHANES 2007–2014 (CDC, 2007 – 2014) (Table 11). Lower phenol analyte concentrations may reflect differences in personal care product use between MARBLES Study participants and the NHANES population. In contrast, MARBLES Study urinary BPA concentrations, which primarily reflect dietary exposures from food and drink packaging, were similar to NHANES (Table 11).

Single analyte analyses, using log-transformed averages, indicate that higher levels of BPA are significantly associated with a decreased risk of ASD compared to TD (OR=0.59, 95% CI: 0.36, 0.96). There are also borderline associations between increased levels of ETPB and risk of non-TD and ASD (OR= 1.19, 95% CI: 0.97, 1.45 and OR=1.23, 95% CI: 0.99, 1.53) respectively; Table 3). When log-transformed average concentrations were quartiled there were no significant associations; however, MEPB was associated with a borderline increased risk with non-TD (OR=1.35, 95% CI: 0.99, 1.86) and ETPB was borderline positively associated with ASD (OR=1.32, 95% CI: 0.94, 1.87). Sex-stratified single chemical analyses also revealed no significant associations (Table 4).

The mixture analysis using trinomial WQS regression with positively constrained betas and adjusted for covariates showed that the odds of a non-TD diagnosis were 1.58 times the odds of a TD diagnosis, for every 1 unit increase (on a scale from 0 – 3 indicating the weighted sum of quartiles) in the phenol and paraben mixture (p=0.032). MEPB contributed the majority of the weight in the mixture, which is consistent with results from single analyte analyses. There was a borderline significant association between the phenol mixture and increased risk of ASD compared to TD (OR=1.46, 95% CI: 1.45, 1.49) (Table 5). The sensitivity analysis of constraining betas in the negative direction resulted in no significant associations (Table S2).

In a WQS analysis stratified by sex with positively constrained betas, there was a significant association between the phenol mixture and increased risk of non-TD compared to TD (Table 6; OR=3.18, 95% CI: 3.09, 3.28). The summed chemical weight contributions for girls dominated the total weight of the index (66% for girls and 35% for boys). For both boys and girls, MEPB contributed the largest relative weight and together explain almost 50% of the overall mixture effect. There was a borderline significant association in the stratified analysis comparing ASD to TD among boys (OR=2.14, 95% CI: 2.09, 2.19). Conversely, the summed analyte weight contribution for boys was larger than the weight contribution for girls (59% for boys and 41% for girls). The relative contribution of ETPB for boys was 69%. ETPB and PRPB had the largest relative weight contributions in the mixture for girls. When betas were constrained in the negative direction, there were no significant associations (Table S3).

The single chemical analyses of 2$^{nd}$ trimester SG-corrected averaged concentrations had a borderline significant association between higher ETPB concentrations and ASD risk (OR = 1.31, 95% CI: 1.00, 1.71; Table 7). There was a borderline significant association for higher BPA concentrations and reduced risk of non-TD (OR = 0.67, 95% CI: 0.43, 1.06). When chemicals were quartiled, higher BPA concentrations continued to show a borderline reduced risk with non-TD (OR = 0.69, 95% CI: (0.47, 1.02) and ASD (OR = 0.72, 95% CI: 0.48, 1.09). The positively constrained WQS analyses revealed a borderline significant mixture effect on increased risk of ASD (OR=1.60, 95% CI: 1.57, 1.62; Table 8) that was weighted primarily by ETPB, which contributed to 72% of the mixture effect.

The single chemical analyses for 3$^{rd}$ trimester samples revealed borderline significant associations between higher ETPB concentrations and risk of non-TD (OR= 1.22, 95% CI: 0.97, 1.52; Table 9) and ASD (OR=1.23, 95% CI: 0.97, 1.58). In addition, there was a

borderline significant association between increased PRPB concentrations and non-TD (OR= 1.18, 95% CI: 0.96, 1.44). When chemicals were quartiled, there were borderline associations between higher MEPB and PRPB concentrations and risk of Non-TD. Examining 3rd trimester samples only, the phenol and paraben mixture was significantly associated with non-TD (OR= 1.57, 95% CI: 1.55, 1.60; Table 10), which was weighted primarily by MEPB and ETPB (combined weight was 69%).

## Discussion

To our knowledge this is the first study to investigate pregnancy environmental phenol and paraben exposures in relation to clinical ASD diagnosis. Mixtures of environmental phenols and parabens were significantly associated with an increased risk for non-TD and borderline associated with ASD risk (Table 5). The MARBLES Study has also investigated pregnancy phthalates, another type of EDC, and found pregnancy phthalate exposures increased risk for non-TD development in this population (Shin et al. 2018). In single analyte analyses, higher average ETPB concentrations were borderline associated with non-TD risk, and a suggestive association was observed for quintiles of ETPB and ASD (Table 3). For the mixtures analysis ETPB contributed a higher weight for ASD risk but this association was not statistically significant. Exposure to environmental phenols and parabens is widespread and due to the common exposure sources and similar modes of action, a mixtures approach may be more appropriate for studying the effects of these EDC exposures on neurodevelopment.

A mixtures approach is a rational choice for evaluating risks in relation to exposures and it is realistic of exposure assessment since phenols and parabens are often not found in isolation and have complex correlations. WQS regression identifies components of the mixture that are more important than others. Results for ETPB and MEPB were consistent for single chemical and WQS analysis irrespective of child's neurodevelopmental outcome. In addition, WQS regression was able to detect sex differences between mixture effects. Sex differences in stratified single chemical analyses may have been missed due to small sample sizes per sex and outcome. Conversely, the WQS regression is a preferable method to maintain power because it utilizes the full sample size to produce weights per chemical and sex with both outcomes simultaneously.

We were unable to split the data into two independent data sets – one for training and one for validation. Ideally, one subset of the data should be used to estimate the weights of the WQS regression index through bootstrap sampling (training), and the second dataset should be used to test the significance of resulting index (validation). Since weights were estimated in the same data used to test for significance, our findings may be prone to type 1 error. Additionally, WQS regression measures a mixture effect from an empirical index but does not measure interaction effects among individual components. Other techniques, such as Bayesian kernel machine regression (Bobb et al. 2015), are more suited for examining interactions.

There have been several studies on BPA exposures and child neurodevelopment, but more limited work on the environmental phenols and parabens included in this study. In the present study results from single analyte analyses showed log-transformed pregnancy

average BPA was associated with reduced ASD risk but this finding did not hold for quartile analyses or when stratified by child's sex, indicating the BPA exposure association with ASD likely was a random fluctuation. Our lack of a finding with BPA is consistent with two other studies on pregnancy BPA exposures which both found some EDCs, not including BPA, were associated with increased ASD symptoms (Braun et al. 2014b; Miodovnik et al. 2011). In the present study, children were clinically assessed for ASD at 3 years on gold standard ASD assessments (Lord et al. 2008) by trained clinicians with established reliability, adding further credence to the null findings (previous research has investigated ASD symptoms using parental report at ages 4 – 9 years).

In the mixtures analyses we observed a significantly higher risk for non-TD compared to TD with girls contributing greater weight to the overall risk (Table 5). Child's sex may modify the effects of EDC exposures on neurodevelopment, which is relevant for ASD research given the higher rate of diagnosis in males compared to females. Rodent studies provide evidence for sex differences in the way EDCs can alter neurodevelopment across the brain (reviewed by (Rebuli and Patisaul 2016)). Additionally, sex-specific effects on behavioral outcomes have been reported in mice with low-level pregnancy exposure to EDC mixtures (Sobolewski et al. 2014). Epidemiology studies have also reported sex differences when studying pregnancy EDC exposures on child behavioral outcomes, including autism-like behaviors that were evaluated on autism screening tools such as the Social Responsiveness Scale (SRS) and Child Behavior Checklist (CBCL) (Braun et al. 2009; Harley et al. 2013; Perera et al. 2012; Philippat et al. 2017). In a prospective cohort study, pregnancy *trans*-nonachlor, an EDC, was positively associated with higher SRS scores in girls but not boys (Braun et al. 2014b). Another study found pregnancy BPA exposures were associated with higher scores on the CBCL at 5 years among boys but not girls (Perera et al. 2012). Sex differences occur in ASD prevalence where males are about four times more likely to be diagnosed with ASD compared to females but the reasons for this disparity are not fully understood (Baio et al. 2018). One proposed mechanism is through sex hormones, such as prenatal androgens, that can act on the developing brain resulting in sex differences (Baron-Cohen et al. 2005). Evidence in rodents suggests that gestational phenol exposure to BPA can increase embryonic brain expression of estrogen, aryl hydrocarbon, retinoic acid, and retinoid X receptors and other regulatory factors important during development in a sex-specific manner (Richter et al. 2007).

This study had several limitations. Given the small sample size we cannot rule out null results for environmental phenol and paraben exposures on ASD risk. The MARBLES study is an enriched risk cohort and it is unclear how exposures may affect this high-risk sample differently from the general population because the ASD cases may have a higher genetic risk compared to cases from low-risk populations. Pregnancy urinary paraben concentrations from pregnant women in the MARBLES Study were lower than those reported for women from a nationally representative U.S. sample for overlapping years (Centers for Disease Control and Prevention 2017), and pregnant women recruited from a fertility clinic in Boston, MA (Braun et al. 2014a). However, other environmental phenols in this population were similar to women from a nationally representative U.S. sample (Centers for Disease Control and Prevention 2017). This may reflect a difference in personal care products used

by pregnant women in the MARBLES Study compared to participants sampled from these other populations.

Timing of EDC exposures may be important for studying neurodevelopmental effects (Frye et al. 2012). Pregnancy BPA exposures especially during early pregnancy (≤ 16 weeks) have been shown to affect child behaviors at age 2 years in girls but not boys (Braun et al. 2009). In the present study the majority of women enrolled during their 2nd trimester, therefore we did not have an adequate sample size to assess 1st trimester exposures. However, MARBLES participants' 2nd trimester samples would be comparable to the timing effects reported for BPA and triclosan. There are likely multiple critical windows of increased susceptibility for ASD risk over the prenatal and early postnatal periods. Epidemiology studies provide evidence for critical windows of exposures such as air pollution exposures during the 3rd trimester and early postnatal period or maternal fever in the 2nd trimester have been shown to increase risk for ASD (Lyall et al. 2014). In the present study, analyses examining only 2nd or only 3rd trimester samples resulted in a borderline significant mixture effect on ASD for the 2nd trimester and a significant mixture effect on non-TD for the 3rd trimester. This suggests that timing of exposures to phenols and parabens should be considered when studying the effects on child neurodevelopment.

This study had several strengths. First, the prospective design allowed for multiple urinary biomarkers, which is an improvement over previous studies of prenatal EDC exposures and ASD. Expert clinicians and experienced study staff administered assessments including gold-standard tools for ASD diagnosis, and the study collected abundant data on various types of risk factors, which were controlled for in the analyses.

In the present study we found pregnancy environmental phenol and paraben exposures may increase risk for non-TD development in this high-risk population. Additionally, we report a suggestive association for environmental phenols with ASD risk, for which future research with a large sample is needed. Parabens are commonly used as preservatives in cosmetics and food (US Centers for Disease Control and Prevention 2017b), and often more than one paraben is found in a single product which makes the mixtures approach appropriate for this analysis.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## References:


Aker AM, Watkins DJ, Johns LE, Ferguson KK, Soldin OP, Anzalota Del Toro LV, et al. 2016 Phenols and parabens in relation to reproductive and thyroid hormones in pregnant women. Environmental research 151:30–37. [PubMed: 27448730]

Aker AM, Johns L, McElrath TF, Cantonwine DE, Mukherjee B, Meeker JD. 2018 Associations between maternal phenol and paraben urinary biomarkers and maternal hormones during pregnancy: A repeated measures study. Environment international 113:341–349. [PubMed: 29366524]

Andersen SL, Laurberg P, Wu CS, Olsen J. 2014 Attention deficit hyperactivity disorder and autism spectrum disorder in children born to mothers with thyroid dysfunction: A danish nationwide cohort study. BJOG : an international journal of obstetrics and gynaecology 121:1365–1374. [PubMed: 24605987]

Baio J, Wiggins L, Christensen DL, Maenner MJ, Daniels J, Warren Z, et al. 2018 Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, united states, 2014. MMWR Surveillance Summaries 67:1.

Barkoski J, Bennett D, Tancredi D, Barr DB, Elms W, Hertz-Picciotto I. 2018 Variability of urinary pesticide metabolite concentrations during pregnancy in the marbles study. Environmental research 165:400–409. [PubMed: 29860212]

Baron-Cohen S, Knickmeyer RC, Belmonte MK. 2005 Sex differences in the brain: Implications for explaining autism. Science 310:819–823. [PubMed: 16272115]

Becerra TA, Wilhelm M, Olsen J, Cockburn M, Ritz B. 2013 Ambient air pollution and autism in los angeles county, california. Environ Health Perspect 121:380–386. [PubMed: 23249813]

Berger K, Gunier RB, Chevrier J, Calafat AM, Ye X, Eskenazi B, et al. 2018 Associations of maternal exposure to triclosan, parabens, and other phenols with prenatal maternal and neonatal thyroid hormone levels. Environmental research 165:379–386. [PubMed: 29803919]

Bernal J, Nunez J. 1995 Thyroid hormones and brain development. European journal of endocrinology 133:390–398. [PubMed: 7581959]

Bernal J. 2005 Thyroid hormones and brain development. Vitamins and hormones 71:95–122. [PubMed: 16112266]

Bobb JF, Valeri L, Claus Henn B, Christiani DC, Wright RO, Mazumdar M, et al. 2015 Bayesian kernel machine regression for estimating the health effects of multi-pollutant mixtures. Biostatistics (Oxford, England) 16:493–508.

Braun JM, Yolton K, Dietrich KN, Hornung R, Ye X, Calafat AM, et al. 2009 Prenatal bisphenol a exposure and early childhood behavior. Environmental health perspectives 117:1945–1952. [PubMed: 20049216]

Braun JM. 2012 Endocrine disrupting compounds, gonadal hormones, and autism. Developmental medicine and child neurology 54:1068. [PubMed: 22788982]

Braun JM, Just AC, Williams PL, Smith KW, Calafat AM, Hauser R. 2014a Personal care product use and urinary phthalate metabolite and paraben concentrations during pregnancy among women from a fertility clinic. Journal of exposure science & environmental epidemiology 24:459–466. [PubMed: 24149971]

Braun JM, Kalkbrenner AE, Just AC, Yolton K, Calafat AM, Sjodin A, et al. 2014b Gestational exposure to endocrine-disrupting chemicals and reciprocal social, repetitive, and stereotypic behaviors in 4- and 5-year-old children: The home study. Environmental health perspectives 122:513–520. [PubMed: 24622245]

Braun JM. 2017 Early-life exposure to edcs: Role in childhood obesity and neurodevelopment. Nature reviews Endocrinology 13:161–173.

Braun JM, Muckle G, Arbuckle T, Bouchard MF, Fraser WD, Ouellet E, et al. 2017 Associations of prenatal urinary bisphenol a concentrations with child behaviors and cognitive abilities. Environmental health perspectives 125:067008. [PubMed: 28657891]

Carrico C, Gennings C, Wheeler DC, Factor-Litvak P. 2015 Characterization of weighted quantile sum regression for highly correlated data in a risk analysis setting. Journal of agricultural, biological, and environmental statistics 20:100–120.

Centers for Disease Control and Prevention. 2017 Fourth national report on human exposure to environmental chemicals.

Colborn T. 2004 Neurodevelopment and endocrine disruption. Environmental health perspectives 112:944–949. [PubMed: 15198913]

Croen LA, Grether JK, Yoshida CK, Odouli R, Hendrick V. 2011 Antidepressant use during pregnancy and childhood autism spectrum disorders. Arch Gen Psychiatry 68:1104–1112. [PubMed: 21727247]

Eskenazi B, Marks AR, Bradman A, Harley K, Barr DB, Johnson C, et al. 2007 Organophosphate pesticide exposure and neurodevelopment in young mexican-american children. Environ Health Perspect 115:792–798. [PubMed: 17520070]

Food and Drug Administration (FDA). 2016 Safety and effectiveness of consumer antiseptics; topical antimicrobial drug products for over-the-counter human use.

Frye CA, Bo E, Calamandrei G, Calza L, Dessi-Fulgheri F, Fernandez M, et al. 2012 Endocrine disrupters: A review of some sources, effects, and mechanisms of actions on behaviour and neuroendocrine systems. Journal of neuroendocrinology 24:144–159. [PubMed: 21951193]

Guo BQ, Li HB, Zhai DS, Ding SB. 2019 Maternal multivitamin supplementation is associated with a reduced risk of autism spectrum disorder in children: A systematic review and meta-analysis. Nutrition research (New York, NY) 65:4–16.

Hallmayer J, Cleveland S, Torres A, Phillips J, Cohen B, Torigoe T, et al. 2011 Genetic heritability and shared environmental factors among twin pairs with autism. Archives of general psychiatry 68:1095–1102. [PubMed: 21727249]

Harley KG, Gunier RB, Kogut K, Johnson C, Bradman A, Calafat AM, et al. 2013 Prenatal and early childhood bisphenol a concentrations and behavior in school-aged children. Environmental research 126:43–50. [PubMed: 23870093]

Harrington RA, Lee L-C, Crum RM, Zimmerman AW, Hertz-Picciotto I. 2014 Prenatal ssri use and offspring with autism spectrum disorder or developmental delay. Pediatrics 133:E1241–E1248. [PubMed: 24733881]

Hauser R, Meeker JD, Park S, Silva MJ, Calafat AM. 2004 Temporal variability of urinary phthalate metabolite levels in men of reproductive age. Environmental health perspectives 112:1734–1740. [PubMed: 15579421]

Hertz-Picciotto I, Schmidt RJ, Walker CK, Bennett DH, Oliver M, Shedd-Wise KM, et al. 2018 A prospective study of environmental exposures and early biomarkers in autism spectrum disorder: Design, protocols, and preliminary data from the marbles study. Environmental health perspectives 126:117004. [PubMed: 30465702]

Jackson-Browne MS, Papandonatos GD, Chen A, Calafat AM, Yolton K, Lanphear BP, et al. 2018 Identifying vulnerable periods of neurotoxicity to triclosan exposure in children. Environmental health perspectives 126:057001. [PubMed: 29727133]

Khan A, Harney JW, Zavacki AM, Sajdel-Sulkowska EM. 2014 Disrupted brain thyroid hormone homeostasis and altered thyroid hormone-dependent brain gene expression in autism spectrum disorders. Journal of physiology and pharmacology : an official journal of the Polish Physiological Society 65:257–272.

Krakowiak P, Walker CK, Bremer AA, Baker AS, Ozonoff S, Hansen RL, et al. 2012 Maternal metabolic conditions and risk for autism and other neurodevelopmental disorders. Pediatrics 129:E1121–E1128. [PubMed: 22492772]

Landrigan PJ, Lambertini L, Birnbaum LS. 2012 A research strategy to discover the environmental causes of autism and neurodevelopmental disabilities. Environ Health Perspect 120:A258–A260. [PubMed: 22543002]

Larsson M, Weiss B, Janson S, Sundell J, Bornehag C-G. 2009 Associations between indoor environmental factors and parental-reported autistic spectrum disorders in children 6–8 years of age. Neurotoxicology 30:822–831. [PubMed: 19822263]

Lei XY, Li YJ, Ou JJ, Li YM. 2019 Association between parental body mass index and autism spectrum disorder: A systematic review and meta-analysis. European child & adolescent psychiatry 28:933–947. [PubMed: 30470952]

Lord C, Rutter M, Dilavore PC, Risi S. 2008 Ados: Autism diagnostic observation schedule:Hogrefe Boston.

Lyall K, Schmidt RJ, Hertz-Picciotto I. 2014 Maternal lifestyle and environmental risk factors for autism spectrum disorders. International journal of epidemiology.

Miodovnik A, Engel SM, Zhu C, Ye X, Soorya LV, Silva MJ, et al. 2011 Endocrine disruptors and childhood social impairment. Neurotoxicology 32:261–267. [PubMed: 21182865]

Mortensen ME, Calafat AM, Ye X, Wong LY, Wright DJ, Pirkle JL, et al. 2014 Urinary concentrations of environmental phenols in pregnant women in a pilot study of the national children's study. Environmental research 129:32–38. [PubMed: 24529000]

Mullen EM. 1995 Mullen scales of early learning. Circle Pines, Minnesota: American Guidance Serviecs, Inc.

Mustieles V, Perez-Lobato R, Olea N, Fernandez MF. 2015 Bisphenol a: Human exposure and neurobehavior. Neurotoxicology 49:174–184. [PubMed: 26121921]

National Research Council 2000 Scientific frontiers in developmental toxicology and risk assessment. 0309070864. Washington, DC:National Academies Press.

Ozonoff S, Young GS, Carter A, Messinger D, Yirmiya N, Zwaigenbaum L, et al. 2011 Recurrence risk for autism spectrum disorders: A baby siblings research consortium study. Pediatrics 128:e488–495. [PubMed: 21844053]

Ozonoff S, Young GS, Belding A, Hill M, Hill A, Hutman T, et al. 2014 The broader autism phenotype in infancy: When does it emerge? Journal of the American Academy of Child and Adolescent Psychiatry 53:398–407.e392. [PubMed: 24655649]

Perera F, Vishnevetsky J, Herbstman JB, Calafat AM, Xiong W, Rauh V, et al. 2012 Prenatal bisphenol a exposure and child behavior in an inner-city cohort. Environmental health perspectives 120:1190–1194. [PubMed: 22543054]

Philippat C, Nakiwala D, Calafat AM, Botton J, De Agostini M, Heude B, et al. 2017 Prenatal exposure to nonpersistent endocrine disruptors and behavior in boys at 3 and 5 years. Environmental health perspectives 125:097014. [PubMed: 28937960]

Rebuli ME, Patisaul HB. 2016 Assessment of sex specific endocrine disrupting effects in the prenatal and pre-pubertal rodent brain. The Journal of steroid biochemistry and molecular biology 160:148–159. [PubMed: 26307491]

Richter CA, Birnbaum LS, Farabollini F, Newbold RR, Rubin BS, Talsness CE, et al. 2007 In vivo effects of bisphenol a in laboratory rodent studies. Reproductive toxicology (Elmsford, NY) 24:199–224.

Roberts EM, English PB, Grether JK, Windham GC, Somberg L, Wolff C. 2007 Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the california central valley. Environmental health perspectives 115:1482–1489. [PubMed: 17938740]

Roberts EM, English PB, Grether JK, Windharn GC, Somberg L, Wolff C. 2007 Maternal residence near agricultural pesticide applications and autism spectrum disorders among children in the california central valley. Environ Health Perspect 115:1482–1489. [PubMed: 17938740]

Rochester JR, Bolden AL. 2015 Bisphenol s and f: A systematic review and comparison of the hormonal activity of bisphenol a substitutes. Environmental health perspectives 123:643–650. [PubMed: 25775505]

Sandin S, Lichtenstein P, Kuja-Halkola R, Larsson H, Hultman CM, Reichenberg A. 2014 The familial risk of autism. Jama 311:1770–1777. [PubMed: 24794370]

Sandin S, Lichtenstein P, Kuja-Halkola R, Hultman C, Larsson H, Reichenberg A. 2017 The heritability of autism spectrum disorder. Jama 318:1182–1184. [PubMed: 28973605]

Schmidt RJ, Tancredi DJ, Ozonoff S, Hansen RL, Hartiala J, Allayee H, et al. 2012 Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the charge (childhood autism risks from genetics and environment) case-control study. Am J Clin Nutr 96:80–89. [PubMed: 22648721]

Schmidt RJ, Iosif AM, Guerrero Angel E, Ozonoff S. 2019 Association of maternal prenatal vitamin use with risk for autism spectrum disorder recurrence in young siblings. JAMA psychiatry.

Shelton JA, Geraghty EM, Tancredi DJ, Delwiche LD, Schmidt RJ, Ritz B, et al. 2014 Neurodevelopmental disorders and prenatal residential proximity to agricultural pesticides: The charge study. Environ Health Perspect 122:1103–1109. [PubMed: 24954055]

Shin HM, Schmidt RJ, Tancredi D, Barkoski J, Ozonoff S, Bennett DH, et al. 2018 Prenatal exposure to phthalates and autism spectrum disorder in the marbles study. Environmental health : a global access science source 17:85. [PubMed: 30518373]

Sobolewski M, Conrad K, Allen JL, Weston H, Martin K, Lawrence BP, et al. 2014 Sex-specific enhanced behavioral toxicity induced by maternal exposure to a mixture of low dose endocrine-disrupting chemicals. Neurotoxicology 45:121–130. [PubMed: 25454719]

Suren P, Roth C, Bresnahan M, Haugen M, Hornig M, Hirtz D, et al. 2013 Association between maternal use of folic acid supplements and risk of autism spectrum disorders in children. Jama 309:570–577. [PubMed: 23403681]

US Centers for Disease Control and Prevention. 2017a Bisphenol a (bpa) factsheet. Available: https://www.cdc.gov/biomonitoring/BisphenolA_FactSheet.html [accessed August 30 2018].

US Centers for Disease Control and Prevention. 2017b Parabens factsheet. Available: https://www.cdc.gov/biomonitoring/Parabens_FactSheet.html [accessed August 30 2018].

Volk HE, Lurmann F, Penfold B, Hertz-Picciotto I, McConnell R. 2013 Traffic-related air pollution, particulate matter, and autism. Jama Psychiatry 70:71–77. [PubMed: 23404082]

Volk HE, Kerin T, Lurmann F, Hertz-Picciotto I, McConnell R, Campbell DB. 2014 Autism spectrum disorder interaction of air pollution with the met receptor tyrosine kinase gene. Epidemiology 25:44–47. [PubMed: 24240654]

Windham GC, Zhang L, Gunier R, Croen LA, Grether JK. 2006 Autism spectrum disorders in relation to distribution of hazardous air pollutants in the san francisco bay area. Environ Health Perspect 114:1438–1444. [PubMed: 16966102]

Windham GC, Anderson M, Lyall K, Daniels JL, Kral TVE, Croen LA, et al. 2019 Maternal pre-pregnancy body mass index and gestational weight gain in relation to autism spectrum disorder and other developmental disorders in offspring. Autism research : official journal of the International Society for Autism Research 12:316–327. [PubMed: 30575327]

Woodruff TJ, Zota AR, Schwartz JM. 2011 Environmental chemicals in pregnant women in the united states: Nhanes 2003–2004. Environmental health perspectives 119:878–885. [PubMed: 21233055]

- First study to assess both individual and mixtures of pregnancy environmental phenol and paraben urinary metabolites in relation to children's risk for developing autism spectrum disorder (ASD).
- Mixtures of environmental phenols and parabens were significantly associated with an increased risk for nontypical development and borderline associated with ASD risk.
- Environmental phenols are often not found in isolation and they have complex correlations so the mixtures approach in this study is a rational choice for evaluating risks in relation to exposures.

**Table 1:**

Characteristics of mothers and children included in the analysis, by 3-year clinical outcome, from the MARBLES Study with phenol and paraben metabolite concentrations from pregnancy urine samples (n=207).

| | TD (n=106) n (%) | ASD (n=47) n (%) | non-TD (n=54) n (%) | ASD vs TD p-value[a] | non-TD vs TD p-value[a] |
|---|---|---|---|---|---|
| Child Sex | | | | 0.06≠ | 0.12≠ |
| Male | 55 (51.89) | 32 (68.09) | 35 (64.81) | | |
| Female | 51 (48.11) | 15 (31.91) | 19 (35.19) | | |
| Child Race | | | | 0.34 | 0.02* |
| White | 78 (73.58) | 31 (65.96) | 30 (55.56) | | |
| Other | 28 (26.42) | 16 (34.04) | 24 (44.44) | | |
| Home Ownership | | | | 0.24 | 0.63 |
| Yes | 67 (63.21) | 25 (46.81) | 32 (59.26) | | |
| No | 39 (36.79) | 22 (53.19) | 22 (40.74) | | |
| Prenatal Vitamin Use[c] | | | | <0.01* | 0.40 |
| Yes | 90 (84.91) | 30 (63.83) | 43 (79.63) | | |
| No | 16 (15.09) | 17 (36.17) | 11 (20.37) | | |
| Maternal Metabolic Conditions | | | | 0.42 | 0.45 |
| No metabolic condition | 83 (78.3) | 34 (72.34) | 45 (83.33) | | |
| Any metabolic condition | 23 (21.7) | 13 (27.66) | 9 (16.67) | | |
| Sample Type (N=660) | 346 (49.93) | 157 (22.66) | 190 (27.42) | 0.05≠ | 0.84 |
| 24 hour | 47 (13.58) | 33 (21.02) | 26 (13.68) | | |
| Pooled | 114 (32.95 | 39 (24.84) | 58 (30.53) | | |
| Spot | 185 (53.47) | 85 (54.14) | 106 (55.79) | | |

| | Mean | SD | Mean | SD | Mean | SD | p-value[b] | p-value[b] |
|---|---|---|---|---|---|---|---|---|
| Maternal Age | 34.02 | 5.48 | 35.36 | 5.30 | 34.27 | 4.34 | 0.16 | 0.77 |
| Maternal BMI | 25.72 | 5.74 | 28.09 | 7.97 | 27.51 | 8.00 | 0.07≠ | 0.15 |
| Birth year | 2010.1 | 2.14 | 2010.8 | 1.91 | 2010.8 | 2.15 | 0.03* | 0.04* |

Note: TD = typical development, non-TD = nontypical development, ASD = autism spectrum disorder

[a] Pearson's chi-squared

[b] Two sample t-test

[c] during the peri-conceptional period

≠ P-value between 0.05 – 0.12 is borderline statistically significant.

* P-value <0.05 is statistically significant.

LOD = Limit of Detection

**Table 2:**

Maternal pregnancy urinary phenol biomarker concentrations among the 660 samples from 207 mother-child pairs in the MARBLES Study included in this analysis.

| | Urinary[a] Biomarkers (ng/mL) | N Samples | 25th Percentile | Median | 75th Percentile | 95th Percentile |
|---|---|---|---|---|---|---|
| **ASD N=47** | BPA | 178 | <LOD | 1.03 | 1.83 | 3.73 |
| | BPF | 178 | <LOD | <LOD | <LOD | 8.36 |
| | BPS | 174 | <LOD | <LOD | <LOD | 3.25 |
| | TCC | 178 | <LOD | <LOD | <LOD | 1.99 |
| | TCS | 178 | <LOD | <LOD | 34.06 | 192.47 |
| | MEPB | 178 | 13.84 | 47.14 | 132.80 | 627.87 |
| | ETPB | 178 | <LOD | 0.65 | 2.74 | 52.68 |
| | PRPB | 178 | 2.28 | 8.07 | 27.19 | 139.46 |
| | BUPB | 178 | <LOD | <LOD | <LOD | 10.39 |
| **non-TD N=54** | BPA | 149 | <LOD | 0.91 | 1.42 | 5.45 |
| | BPF | 149 | <LOD | <LOD | <LOD | 13.11 |
| | BPS | 149 | <LOD | <LOD | <LOD | 2.01 |
| | TCC | 149 | <LOD | <LOD | <LOD | 6.24 |
| | TCS | 149 | <LOD | <LOD | 43.11 | 258.56 |
| | MEPB | 149 | 8.52 | 30.78 | 99.35 | 362.46 |
| | ETPB | 149 | <LOD | 1.11 | 4.44 | 61.08 |
| | PRPB | 149 | 1.23 | 6.31 | 20.38 | 87.84 |
| | BUPB | 149 | <LOD | <LOD | <LOD | 13.18 |
| **TD N=106** | BPA | 333 | <LOD | 0.98 | 1.95 | 4.84 |
| | BPF | 333 | <LOD | <LOD | <LOD | 13.07 |
| | BPS | 333 | <LOD | <LOD | <LOD | 2.23 |
| | TCC | 333 | <LOD | <LOD | <LOD | 1.49 |
| | TCS | 333 | <LOD | 15.14 | 55.86 | 310.78 |
| | MEPB | 333 | 14.97 | 35.30 | 107.68 | 449.87 |
| | ETPB | 333 | <LOD | 0.64 | 2.62 | 27.13 |
| | PRPB | 333 | 2.50 | 7.63 | 24.26 | 110.56 |
| | BUPB | 331 | <LOD | <LOD | <LOD | 7.70 |

TD = typical development, non-TD = nontypical development, ASD = autism spectrum disorder

[a] average pregnancy concentration

**Table 3:**

Results from analyses of averaged single urinary biomarker concentrations, log-transformed and quantiled (into quartiles), in generalized logit models (ordered by outcome category with TD as the reference level) adjusted for covariates[a] (N=207).

| Urinary biomarker[a] | Outcome | Estimate | SE | P-value | OR[b] (95% CI) |
|---|---|---|---|---|---|
| Natural log transformed concentrations | | | | | |
| BPA | ASD | −0.53 | 0.25 | 0.03* | 0.59 (0.36, 0.96) |
| ETPB | ASD | 0.21 | 0.11 | 0.06≠ | 1.23 (0.99, 1.53) |
| MEPB | ASD | −0.06 | 0.11 | 0.61 | 0.94 (0.76, 1.17) |
| PRPB | ASD | 0.11 | 0.12 | 0.34 | 1.12 (0.89, 1.41) |
| BPA | non-TD | −0.11 | 0.24 | 0.65 | 0.90 (0.57, 1.42) |
| ETPB | non-TD | 0.17 | 0.10 | 0.09≠ | 1.19 (0.97, 1.45) |
| MEPB | non-TD | 0.11 | 0.12 | 0.35 | 1.11 (0.89, 1.40) |
| PRPB | non-TD | 0.15 | 0.11 | 0.18 | 1.16 (0.93, 1.45) |
| Quartiled concentration | | | | | |
| BPA | ASD | −0.09 | 0.17 | 0.59 | 0.91 (0.66, 1.27) |
| ETPB | ASD | 0.28 | 0.18 | 0.11≠ | 1.32 (0.94, 1.87) |
| MEPB | ASD | 0.08 | 0.17 | 0.65 | 1.08 (0.78, 1.51) |
| PRPB | ASD | 0.16 | 0.17 | 0.34 | 1.18 (0.84, 1.65) |
| BPA | non-TD | 0.05 | 0.16 | 0.73 | 1.06 (0.78, 1.44) |
| ETPB | non-TD | 0.17 | 0.16 | 0.30 | 1.18 (0.86, 1.62) |
| MEPB | non-TD | 0.30 | 0.16 | 0.06≠ | 1.35 (0.99, 1.86) |
| PRPB | non-TD | 0.21 | 0.16 | 0.19 | 1.24 (0.90, 1.70) |

[a]Adjusted for pre-pregnancy body mass index, periconceptional prenatal vitamin use (yes/no), homeowner status (yes/no), birth year, and child's sex.

[b]The OR represents the odds of being diagnosed with ASD or non-TD compared to TD for a one natural log unit change or one quartile change in chemical concentration.

≠P-value between 0.05 – 0.12 is borderline statistically significant.

*P-value <0.05 is statistically significant.

**Table 4:**

Results from analyses of averaged single urinary biomarker concentrations quantiled (into quartiles), in generalized logit models (ordered by outcome category with TD as the reference level) adjusted for covariates, stratified by gender.

| | | Females (n=85) | | | | Males (n=122) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Urinary biomarker** | **Outcome** | **Estimate** | **SE** | **P-value** | **OR[a] (95% CI)** | **Estimate** | **SE** | **P-value** | **OR[a] (95% CI)** | **Interaction[b] p-value** |
| BPA | ASD | 0.04 | 0.30 | 0.90 | 1.04 (0.58, 1.86) | −0.10 | 0.22 | 0.66 | 0.91 (0.59, 1.40) | 0.96 |
| ETPB | ASD | 0.05 | 0.35 | 0.88 | 1.05 (0.53, 2.08) | 0.32 | 0.22 | 0.14 | 1.37 (0.90, 2.10) | 0.41 |
| MEPB | ASD | −0.19 | 0.35 | 0.58 | 0.83 (0.42, 1.63) | 0.09 | 0.21 | 0.67 | 1.09 (0.73, 1.65) | 0.55 |
| PRPB | ASD | 0.10 | 0.32 | 0.76 | 1.10 (0.59, 2.07) | 0.09 | 0.22 | 0.67 | 1.10 (0.72, 1.68) | 0.85 |
| BPA | non-TD | 0.08 | 0.24 | 0.73 | 1.09 (0.68, 1.73) | 0.11 | 0.22 | 0.63 | 1.11 (0.72, 1.72) | 0.96 |
| ETPB | non-TD | 0.40 | 0.28 | 0.15 | 1.50 (0.87, 2.57) | 0.10 | 0.21 | 0.62 | 1.11 (0.74, 1.67) | 0.35 |
| MEPB | non-TD | 0.42 | 0.27 | 0.12[≠] | 1.53 (0.90, 2.60) | 0.25 | 0.20 | 0.21 | 1.29 (0.87, 1.92) | 0.51 |
| PRPB | non-TD | 0.32 | 0.26 | 0.21 | 1.38 (0.83, 2.29) | 0.16 | 0.21 | 0.45 | 1.17 (0.77, 1.78) | 0.58 |

[a]The OR represents the odds of being diagnosed with ASD or non-TD compared to TD for a one quartile change in chemical concentration.

[b]Interaction between child's sex and urinary phenol biomarker concentration in a generalized logit model (ordered by outcome category with TD as the reference level) adjusted for pre-pregnancy body mass index, prenatal vitamin use (yes/no), homeowner status (yes/no), birth year.

[≠]P-value between 0.05 – 0.12 is borderline statistically significant.

**Table 5:**

Results from the trinomial WQS regression, with positively constrained betas, for the two logit models with urinary biomarker concentrations quantiled (into quartiles) in generalized logit models (ordered by outcome category with TD as the reference level) adjusted for covariates[a] (N=207).

| | ASD vs TD | non-TD vs TD |
|---|---|---|
| **OR[b] (95% CI)** | **1.47 (1.45, 1.49)** | **1.58 (1.56, 1.60)** |
| **P value** | **0.098[ǂ]** | **0.032[*]** |
| **Urinary biomarker[a]** | **Weights (%)** | **Weights (%)** |
| BPA | 10.8 | 17.5 |
| ETPB | 55.4 | 16.8 |
| MEPB | 3.8 | 52.1 |
| PRPB | 30.0 | 13.5 |

[a]Adjusted for pre-pregnancy body mass index, prenatal vitamin use (yes/no), homeowner status (yes/no), birth year, and child's sex.

[b]The OR represents the odds of having ASD or non-TD compared to TD for a one unit increase (on a scale from 0 to 3) in the WQS index score.

[ǂ]P-value between 0.05 – 0.12 is borderline statistically significant.

[*]P-value <0.05 is statistically significant.

**Table 6:**

Results from the stratified trinomial WQS regression, with positively constrained betas, for the two logit models with urinary biomarker concentrations quantiled (into quartiles) in a generalized logit model adjusted for covariates[a] (N=207).

| | ASD vs TD | | | | non-TD vs TD | | | |
|---|---|---|---|---|---|---|---|---|
| **OR[b] (95% CI)** | **2.14 (2.09, 2.19)** | | | | **3.18 (3.09, 3.28)** | | | |
| **P value** | **0.054[≠]** | | | | **0.015[*]** | | | |
| **Urinary biomarker** | **Boys Weights (%)** | **Relative Contribution** | **Girls Weights (%)** | **Relative Contribution** | **Boys Weights (%)** | **Relative Contribution** | **Girls Weights (%)** | **Relative Contribution** |
| BPA | 3.0 | 5.1 | 7.4 | 18.2 | 8.0 | 23.2 | 11.7 | 17.8 |
| ETPB | 41.2 | 69.5 | 14.9 | 36.4 | 4.6 | 13.3 | 17.3 | 26.4 |
| MEPB | 3.7 | 6.2 | 2.0 | 4.8 | 16.5 | 47.8 | 27.6 | 42.2 |
| PRPB | 11.4 | 19.2 | 16.6 | 40.6 | 5.4 | 15.6 | 8.9 | 13.6 |
| Sum | 59.2 | | 40.8 | | 34.5 | | 65.5 | |

[a] Adjusted for pre-pregnancy body mass index, prenatal vitamin use (yes/no), homeowner status (yes/no), birth year, and child's sex.

[b] The OR represents the odds of having ASD or non-TD compared to TD for a one unit increase (on a scale from 0 to 3) in the WQS index score.

[≠] P-value between 0.05 – 0.12 is borderline statistically significant.

[*] P-value <0.05 is statistically significant.

**Table 7:**

Results from analyses of averaged single urinary biomarker concentrations, log-transformed and quantiled (into quartiles), in generalized logit models (ordered by outcome category with TD as the reference level) adjusted for covariates[a], using only 2nd trimester samples (N=141).

| Urinary biomarker[a] | Outcome | Estimate | SE | P-value | OR[b] (95% CI) |
|---|---|---|---|---|---|
| Natural log transformed concentrations | | | | | |
| BPA | ASD | −0.29 | 0.25 | 0.25 | 0.75 (0.45, 1.23) |
| ETPB | ASD | 0.27 | 0.14 | 0.05≠ | 1.31 (1.00, 1.71) |
| MEPB | ASD | −0.13 | 0.15 | 0.39 | 0.88 (0.66, 1.18) |
| PRPB | ASD | 0.09 | 0.15 | 0.55 | 1.09 (0.82, 1.46) |
| BPA | non-TD | −0.39 | 0.23 | 0.09≠ | 0.67 (0.43, 1.06) |
| ETPB | non-TD | −0.03 | 0.13 | 0.79 | 0.97 (0.75, 1.24) |
| MEPB | non-TD | −0.07 | 0.14 | 0.64 | 0.94 (0.71, 1.24) |
| PRPB | non-TD | −0.10 | 0.13 | 0.46 | 0.91 (0.70, 1.17) |
| Quartiled concentrations | | | | | |
| BPA | ASD | −0.32 | 0.21 | 0.12≠ | 0.72 (0.48, 1.09) |
| ETPB | ASD | 0.32 | 0.22 | 0.13 | 1.38 (0.91, 2.11) |
| MEPB | ASD | −0.04 | 0.21 | 0.86 | 0.96 (0.64, 1.45) |
| PRPB | ASD | 0.11 | 0.21 | 0.59 | 1.12 (0.75, 1.68) |
| BPA | non-TD | −0.37 | 0.20 | 0.06≠ | 0.69 (0.47, 1.02) |
| ETPB | non-TD | −0.25 | 0.19 | 0.20 | 0.78 (0.53, 1.14) |
| MEPB | non-TD | −0.07 | 0.19 | 0.72 | 0.93 (0.64, 1.36) |
| PRPB | non-TD | −0.13 | 0.19 | 0.49 | 0.88 (0.60, 1.27) |

[a]Adjusted for pre-pregnancy body mass index, prenatal vitamin use (yes/no), homeowner status (yes/no), birth year, and child's sex.

[b]The OR represents the odds of being diagnosed with ASD or non-TD compared to TD for a one natural log unit change or one quartile change in chemical concentration.

≠P-value between 0.05 – 0.12 is borderline statistically significant.

**Table 8:**

Results from the trinomial WQS regression, with positively constrained betas, for the two logit models with urinary biomarker concentrations quantiled (into quartiles) in generalized logit models (ordered by outcome category with TD as the reference level adjusted for covariates[a], using only 2nd trimester samples (N=141).

| | ASD vs TD | non-TD vs TD |
|---|---|---|
| **OR[b] (95% CI)** | **1.60 (1.57, 1.62)** | **0.89 (0.87, 0.90)** |
| **P value** | **0.065[≠]** | **0.608** |
| **Urinary biomarker** | **Weights (%)** | **Weights (%)** |
| BPA | 5.6 | 12.1 |
| ETPB | 72.2 | 13.5 |
| MEPB | 0.8 | 54.6 |
| PRPB | 21.4 | 19.8 |

[a]Adjusted for pre-pregnancy body mass index, prenatal vitamin use (yes/no), homeowner status (yes/no), birth year, and child's sex.

[b]The OR represents the odds of having ASD or non-TD compared to TD for a one unit increase (on a scale from 0 to 3) in the WQS index score.

[≠]P-value between 0.05 – 0.12 is borderline statistically significant.

**Table 9:**

Results from analyses of averaged single urinary biomarker concentrations, log-transformed and quantiled (into quartiles), in generalized logit models (ordered by outcome category with TD as the reference level) adjusted for covariates[a], using only 3rd trimester samples (N=198).

| Urinary biomarker | Outcome | Estimate | SE | P-value | OR[b] (95% CI) |
|---|---|---|---|---|---|
| Natural log transformed concentrations | | | | | |
| BPA | ASD | −0.41 | 0.29 | 0.16 | 0.66 (0.37, 1.18) |
| ETPB | ASD | 0.21 | 0.12 | 0.09[≠] | 1.23 (0.97, 1.58) |
| MEPB | ASD | 0.07 | 0.12 | 0.54 | 1.07 (0.86, 1.35) |
| PRPB | ASD | 0.12 | 0.11 | 0.25 | 1.13 (0.92, 1.40) |
| BPA | non-TD | −0.08 | 0.26 | 0.76 | 0.92 (0.55, 1.54) |
| ETPB | non-TD | 0.20 | 0.11 | 0.08[≠] | 1.22 (0.97, 1.52) |
| MEPB | non-TD | 0.13 | 0.11 | 0.24 | 1.14 (0.92, 1.42) |
| PRPB | non-TD | 0.16 | 0.10 | 0.11[≠] | 1.18 (0.96, 1.44) |
| Quartiled concentrations | | | | | |
| BPA | ASD | −0.05 | 0.18 | 0.78 | 0.95 (0.67, 1.34) |
| ETPB | ASD | 0.31 | 0.18 | 0.09[≠] | 1.36 (0.95, 1.95) |
| MEPB | ASD | 0.12 | 0.18 | 0.51 | 1.12 (0.80, 1.59) |
| PRPB | ASD | 0.18 | 0.18 | 0.30 | 1.20 (0.85, 1.71) |
| BPA | non-TD | 0.06 | 0.16 | 0.72 | 1.06 (0.77, 1.46) |
| ETPB | non-TD | 0.28 | 0.17 | 0.10[≠] | 1.32 (0.95, 1.84) |
| MEPB | non-TD | 0.26 | 0.16 | 0.12[≠] | 1.29 (0.94, 1.78) |
| PRPB | non-TD | . 0.23 | 0.17 | 0.17 | 1.26 (0.91, 1.74) |

[a]Adjusted for pre-pregnancy body mass index, prenatal vitamin use (yes/no), homeowner status (yes/no), birth year, and child's sex.

[b]The OR represents the odds of being diagnosed with ASD or non-TD compared to TD for a one natural log unit change or one quartile change in chemical concentration.

[≠]P-value between 0.05 – 0.12 is borderline statistically significant.

**Table 10:**

Results from the trinomial WQS regression, with positively constrained betas, for the two logit models with urinary biomarker concentrations quantiled (into quartiles) in generalized logit models (ordered by outcome category with TD as the reference level) adjusted for covariates[a], using only 3rd trimester samples (N=198).

| | ASD vs TD | non-TD vs TD |
|---|---|---|
| **OR[b] (95% CI)** | **1.46 (1.44, 1.49)** | **1.57 (1.55, 1.60)** |
| **P value** | **0.108[≠]** | **0.048[*]** |
| **Urinary biomarker** | **Weights (%)** | **Weights (%)** |
| BPA | 10.8 | 16.9 |
| ETPB | 55.8 | 34.4 |
| MEPB | 6.9 | 34.8 |
| PRPB | 26.5 | 13.9 |

[a]Adjusted for pre-pregnancy body mass index, prenatal vitamin use (yes/no), homeowner status (yes/no), birth year, and child's sex.

[b]The OR represents the odds of having ASD or non-TD compared to TD for a one unit increase (on a scale from 0 to 3) in the WQS index score.

[≠]P-value between 0.05 – 0.12 is borderline statistically significant.

[*]P-value <0.05 is statistically significant.

**Table 11:**

Urinary environmental phenol analyte concentrations from the MARBLES Study and National Health and Nutrition Examination Survey (NHANES) populations 2007 −2014.

| | **MARBLES Study (N=207)**[a] | **NHANES 2007– 2014 pregnant women (N=85)**[b] | **NHANES 2007– 2014 women of child-bearing age (N=2,848)**[b,c] |
|---|---|---|---|
| | Median | Median | Median |
| BPA (ng/ml) | 1.16 | 1.65 | 1.74 |
| ETPB (ng/ml) | 0.85 | 2.12 | 2.37 |
| MEPB (ng/ml) | 50.97 | 128.75 | 135.90 |
| PRPB (ng/ml) | 11.10 | 23.33 | 25.26 |

[a] Specific-gravity corrected pregnancy average urinary concentrations

[b] Creatinine corrected urinary concentrations

[c] Child-bearing age ranges from 12–51 years