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Publication Date

2024-08-01

DOI

10.1016/j.ijheh.2024.114425

Peer reviewed

HHS Public Access

Author manuscript

Int J Hyg Environ Health. Author manuscript; available in PMC 2024 October 17.

Published in final edited form as:

Int J Hyg Environ Health. 2024 August ; 261: 114425. doi:10.1016/j.ijheh.2024.114425.

Exposure to endocrine disrupting chemicals including phthalates, phenols, and parabens in infancy: Associations with neurodevelopmental outcomes in the MARBLES study

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Abstract

Background: Endocrine disrupting chemicals (EDCs) are widely used compounds with the potential to affect child neurodevelopmental outcomes including autism spectrum disorders (ASD). We aimed to examine the urinary concentrations of biomarkers of EDCs, including phthalates, phenols, and parabens, and investigate whether exposure during early infancy was associated with increased risk of later ASD or other non-typical development (Non-TD) or adverse cognitive development.

Methods: This analysis included infants from the Markers of Autism Risks in Babies—Learning Early Signs (MARBLES) study, a high-risk ASD cohort (n = 148; corresponding to 188 urine samples). Thirty-two EDC biomarkers were quantified in urine among infants 3 and/or 6 months of age. Trends in EDC biomarker concentrations were calculated using least square geometric

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CRediT authorship contribution statement

Jennie Sotelo-Orozco: Writing – original draft, Visualization, Formal analysis. **Antonia M. Calafat:** Writing – review & editing, Validation, Methodology, Investigation. **Julianne Cook Botelho:** Investigation, Formal analysis. **Rebecca J. Schmidt:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Irva Hertz-Picciotto:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Deborah H. Bennett:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2024.114425.

means. At 36 months of age, children were clinically classified as having ASD (n = 36), nontypical development (Non-TD; n = 18), or typical development (TD; n = 81) through a clinical evaluation. Trinomial logistic regression analysis was used to test the associations between biomarkers with ASD, or Non-TD, as compared to children with TD. In single analyte analysis, generalized estimating equations were used to investigate the association between each EDC biomarkers and longitudinal changes in cognitive development using the Mullen Scales of Early Learning (MSEL) over the four assessment time points (6, 12, 24, and 36 months of age). Additionally, quantile g-computation was used to test for a mixture effect.

Results: EDC biomarker concentrations generally decreased over the study period, except for mono-2-ethyl-5-carboxypentyl terephthalate. Overall, EDC biomarkers at 3 and/or 6 months of age were not associated with an increased risk of ASD or Non-TD, and a few showed significant inverse associations. However, when assessing longitudinal changes in MSEL scores over the four assessment time points, elevated monoethyl phthalate (MEP) was significantly associated with reduced scores in the composite score (β = −0.16, 95% CI: 0.31, −0.02) and subscales of fine motor skills (β = −0.09, 95%CI: 0.17, 0.00), and visual reception (β = −0.11, 95% CI: 0.23, 0.01). Additionally, the sum of metabolites of di (2-ethylhexyl) terephthalate (ΣDEHTP) was associated with poorer visual reception (β = −0.09, 95% CI: 0.16, −0.02), and decreased composite scores (β = −0.11, 95% CI: 0.21, −0.01). Mixtures analyses using quantile g-computation analysis did not show a significant association between mixtures of EDC biomarkers and MSEL subscales or composite scores.

Conclusion: These findings highlight the potential importance of infant exposures on cognitive development. Future research can help further investigate whether early infant exposures are associated with longer-term deficits and place special attention on EDCs with increasing temporal trends and whether they may adversely affect neurodevelopment.

Keywords

Endocrine disrupting chemicals; Autism spectrum disorder; Infants; Biomarkers; Phthalate; Cognitive

1. Introduction

Autism Spectrum Disorder (ASD) is a complex neurological disorder characterized by deficits in social communication and repetitive behaviors. Over the last two decades, the frequency of autism has climbed and is currently estimated to affect 1 in 36 children in the United States (Maenner et al., 2023). Furthermore, boys are disproportionately affected by ASD, with an estimated 4:1 male-to-female ratio (Maenner et al., 2023; Wingate et al., 2014). Although the causes of ASD remains unknown, ASD is multifactorial and likely caused by gene-environment interactions (Hallmayer et al., 2011); a number of such interactions have already been identified (Volk et al., 2022). Environmental exposures during critical periods of development may be associated with adverse neurodevelopmental outcomes. For example, air pollution and pesticide exposure have previously been associated with increased ASD risk (Engel et al., 2011; Shelton et al., 2014; Volk et al., 2011, 2013). Additionally, environmental exposure to endocrine-disrupting chemicals (EDCs) might also

be related to neurodevelopment and ASD (Barkoski et al., 2019; Moosa et al., 2018; Shin et al., 2018).

EDCs comprise several classes of chemicals that are ubiquitously found in modern environments (Crinnion, 2010; Engel et al., 2010; Mustieles et al., 2015; Radke et al., 2020). Phthalates, for example, are extensively used in personal-care products and cosmetics as well as plasticizers in many consumer products including indoor residential flooring and plastics, vinyl tiles, and shower curtains (Carlstedt et al., 2013; da Silva Oliveira et al., 2017; Latini, 2005; Swan, 2008). Phenols are also broadly used in household and personal care products; some are used in the production of polycarbonate plastics and epoxy resins, such as bisphenol A (BPA) (Dekant and Völkel, 2008; Vandenberg et al., 2007). Similarly, parabens are low-cost, broad-spectrum antimicrobials and antiseptic preservatives commonly used in cosmetics, personal-care products, and pharmaceuticals to suppress microbial growth and extend product life (Darbre and Harvey, 2008; Wei et al., 2021). Although increased awareness of the potential negative health effects of some EDCs has decreased the use and availability of some chemicals, others are on the rise (Buckley et al., 2020; Meeker et al., 2009; Zota et al., 2014). Di (2-ethylhexyl) phthalate (DEHP), for example, is increasingly being replaced by di (2-ethylhexyl) terephthalate (DEHTP), a structural isomer of DEHP, and the non-phthalate alternative 1,2-cyclohexane dicarboxylic acid-diisononyl ester (DINCH) (Silva et al., 2015). However, some evidence in animal models and in vitro studies suggests the non-phthalate alternative DINCH, for instance, may also interfere with the endocrine system (Campioli et al., 2015, 2017; Schaffert et al., 2021). Given their pervasive presence in our environments, EDCs and replacement chemicals require further investigation.

Several epidemiological studies have examined the relationship between early exposure to EDCs and adverse child neurodevelopmental outcomes, especially during the gestational period. In the Markers of Autism Risks in Babies – Learning Early Signs (MARBLES), a high-risk ASD pregnancy cohort, several publications have already investigated maternal exposure during pregnancy to several EDCs in association with child neurodevelopment. Gestational monoethyl phthalate (MEP) urinary concentrations were associated with an increased risk of non-typical development (Non-TD) in children (Shin et al., 2018). Additionally, using a mixture analysis, a pregnancy phenol and paraben mixture was significantly associated with an increased risk of Non-TD, and borderline-significant risk for ASD (Barkoski et al., 2019). These findings are consistent with other epidemiology studies (Braun et al., 2014; Kim et al., 2021a; Mustieles et al., 2015; Oulhote et al., 2020). For example, maternal urinary concentrations of mono (2-ethyl-5--hydroxyhexyl) phthalate (MEHHP) and mono (2-ethyl-5-oxohexyl) phthalate (MEOHP) during the second trimester of pregnancy were found to be associated with autistic traits as measured on the social communication questionnaire (SCQ) at the age of 4; boys showed a stronger association with MEHHP and MEOHP than girls (Kim et al., 2021a). Additionally, a review article found negative impacts on neurobehavioral functioning (including aggressive behavior, attention deficit, hyperactivity disorder, depression, and anxiety impairments) were associated with in-utero BPA exposure (Mustieles et al., 2015). These studies highlight the vulnerability of the gestational period and possible sex differences. However, limited studies have investigated the effects of EDC exposure during early childhood and adverse

neurodevelopment; some of which have been conducted in children ranging from 2 to 18 years of age (depending on the specific study), in relation to attention-deficit hyperactivity disorder (Chopra et al., 2014; Park et al., 2014, 2015; Watkins et al., 2021), or other adverse development (Bennett et al., 2022; Daniel et al., 2020; Li et al., 2020).

Therefore, the present study aimed to quantify urinary concentrations of biomarkers of select EDCs, including phthalates, phenols, and parabens during early infancy (measured at 3–6 months). As there are so few measurements in the extant literature of these chemicals in early childhood, we first characterized correlations between biomarkers and assessed trends over the study period. The MARBLES study is an enriched-risk ASD cohort that follows pregnant women who have an older child with ASD and therefore younger siblings have a high probability of developing ASD, or other developmental concerns without ASD, although many will develop typically. As such, we sought to investigate whether urinary biomarkers in infants were associated with later development of ASD or Non-TD and further explored their associations with cognitive composite scores or subscales for motor and language skills and visual perception. We hypothesized that elevated concentrations of EDC biomarkers in a child's urine would correlate with an increased risk of ASD or Non-TD as compared to typically developing (TD) children and poorer cognitive scores.

2. Methods

2.1. Study participants

Study participants in the present analysis are from the MARBLES Study (Hertz-Picciotto et al., 2018)—a high-risk ASD cohort that began recruiting families in 2006. The MARBLES study follows pregnant women who are at an increased risk for delivering another infant(s) with ASD, primarily because they previously delivered a child who developed ASD (Ozonoff et al., 2011). Details of the MARBLES study have previously been published (Hertz-Picciotto et al., 2018). The MARBLES study has been approved by the State of California Department of Developmental Services and the institutional review board at the University of California Davis, and informed consent was obtained before enrollment from all participants. The analysis of de-identified specimens at the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute human subjects' research.

For the present study, we investigated concentrations of EDC biomarkers in infants' urine collected between the ages of 3–6 months in association with neurodevelopmental diagnostic classification at 36 months. Beginning in late 2008, we attempted to collect urine using a pediatric U-bag at 3- and 6-month study visits, with some samples available at both time points. In total, samples from 148 participants (collected at 3 months (n = 79), 6 months (n = 29), or both (n = 40); corresponding to 188 urine samples) were available for this analysis. For exposure assessment, we computed the average biomarker concentration for participants with samples collected at both 3 and 6 months or used whichever timepoint was available at 3 or 6 months for participants who only provided one sample.

In the MARBLES study, children are evaluated for ASD by a licensed clinical psychologist who has achieved reliability on the assessment instruments: the gold-standard Autism Diagnostic Observation Schedules (ADOS) at 36 months of age (Lord et al., 2012), and

the Mullen Scale of Early Learning (MSEL) (Mullen, 1995) which assesses cognitive skills on four subscales including visual reception, fine motor, receptive language, and expressive language, as well as a composite score at 6, 12, 24, and 36 months of age. Both ADOS and MSEL scores were used to determine final neurodevelopmental outcomes as previously described (Ozonoff et al., 2014; Schmidt et al., 2021). Children with ADOS scores greater than the ASD cutoff and who meet DSM-5 criteria for ASD are classified as ASD. Children without ASD but who have elevated ADOS scores (within 3 points of the ASD cutoff), and/or low MSEL subscales (i. e., two or more subdomain scores greater than 1.5 standard deviations below average or at least one subdomain score that is greater than 2 standard deviations below average) are classified as non-typically developing (Non-TD). The remaining participants who did not meet the criteria for ASD or Non-TD are classified as typically developing (TD).

In total, 121 of the 148 participants included in our analysis received a final neurodevelopmental outcome and completed the MARBLES study (ASD = 26, Non-TD = 17, and TD = 78). However, 27 participants did not receive a final diagnosis at the time of analysis, due to loss of follow-up, moving out of state, or having missed site visits at 36 months due to COVID-19 limitations. If the ADOS-2 could not be administered using standard procedures due to COVID-19 pandemic restrictions, when possible, an alternative measure was used to inform clinical best estimate (CBE) diagnostic classification, including non-standard administration of the ADOS-2 with use of personal protective equipment (PPE; e.g., masks; n = 1), the Brief Observation of Symptoms of Autism (BOSA; n = 12) (Lord et al., 2020), or the TELE-ASD-PEDS (TAP; n = 1) (Wagner et al., 2021). As such, neurodevelopmental outcomes using alternative assessments were obtained for an additional 14 of the 27 children missing neurodevelopmental diagnosis (ASD = 10, Non-TD = 1, TD = 3). The alternative diagnostic process is detailed in the supplemental materials. Therefore, 1) data from all 148 participants, corresponding to 188 urine samples from 3 and/or 6 months, were used to determine the detection frequency of EDCs, 2) similarly data from all 148 participants were utilized in temporal trends analysis of biomarkers, 3) only data from those with final neurodevelopmental outcomes (n = 135; ASD = 36, Non-TD = 18, and TD = 81) were used to assess ASD/Non-TD risk in association with biomarkers, and 4) data from those with available MSEL scores at each assessment time point (6 months, 12 months, 24 months, and/or 36 months) were used to investigate the associations with cognitive skills (n = 106–124, Supplementary Table 1).

2.2. Quantification of urinary concentrations of EDC biomarkers

Urinary concentrations of EDC biomarkers were measured at the Centers for Disease Control and Prevention (CDC) using analytical methods described before (Silva et al., 2007; Ye et al., 2005). First, urine (100 μL) was incubated to hydrolyze the conjugates of the target biomarkers, followed by pre-concentration by online solid phase extraction, separation by high-performance liquid chromatography, and detection by isotope dilution-tandem mass spectrometry of target biomarkers. Limits of detection (LODs) were in the low ng/mL (parts per billion) range. Analytical measurements at the CDC laboratory, which is certified to comply with the requirements set forth in the Clinical Laboratory Improvement Act of 1988 (CLIA ‘88), were conducted following strict CLIA guidelines to assure the

accuracy and reliability of results. For example, each analytical run also included high- and low-concentration quality control materials (QCs) and reagent blanks; concentrations of the QCs were evaluated using standard statistical probability rules (Caudill et al., 2008). The CDC laboratory has used this analytical approach for the analyses of tens of thousands of biological specimens, including those collected as part of the ongoing National Health and Nutrition Examination Survey (NHANES). Noteworthy, the CDC laboratory analyzed 21 blinded duplicates randomly selected from the study samples for additional quality assurance. Replicate analysis for individual pairs showed good agreement: the average relative percent difference was 6.7% (Supplementary Table 2).

In total, we measured triclocarban, eight phenols (BPA, bisphenol S (BPS), bisphenol F (BPF), benzophenone-3, dihydroxyavobenzone, triclosan, 2,4-dichlorophenol, 2,5-dichlorophenol), methyl-paraben, ethyl-paraben, propyl-paraben, butyl-paraben, 3-hydroxy n-butyl paraben (HBP), mono-carboxyisooctyl ester (MCOCH), mono-hydroxy-isononyl ester (MHiNCH), two metabolites of the non-phthalate plasticizer 1,2-cyclohexane dicarboxylic acid-diisononyl ester (DINCH): mono-carboxyisooctyl ester (MCOCH), mono-hydroxy-isononyl ester (MHiNCH), and 16 phthalate metabolites: mono-2-ethyl-5-carboxypentyl phthalate (MECPP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), mono-2-ethylhexyl phthalate (MEHP), mono-3-carboxypropyl phthalate (MCPP), mono-n-butyl phthalate (MBP), mono-hydroxybutyl phthalate (MHBP), mono-isobutyl phthalate (MiBP), mono-hydroxy-isobutyl phthalate (MHiBP), monobenzyl phthalate (MBzP), monoethyl phthalate (MEP), monooxononyl phthalate (MONP), mono-carboxyisooctyl phthalate (MCOP), mono-carboxyisononyl phthalate (MCNP), mono-2-ethyl-5-carboxypentyl terephthalate (MECPTP), mono-2-ethyl-5-hydrohexyl terephthalate (MEHHTP). Because some phthalate metabolites originate from the same parent compound, the molar sum of the following metabolites was used in subsequent analysis to assess exposure to the parent compound: Σ DEHP (Di (2-ethylhexyl) phthalate) = MEHHP + MEOHP + MECPP, Σ DBP (Di-n-butyl phthalate) = MBP + MHBP, Σ DiBP (Di-isobutyl phthalate) = MiBP + MHiBP, Σ DEHTP (Di (2-ethylhexyl) terephthalate) = MECPTP + MEHHTP.

2.3. Statistical analysis

Correlations between EDC biomarkers were calculated using Spearman correlation. For biomarkers with a detection frequency ≥75% (Hornung and Reed, 1990), we explored temporal trends by computing the least square geometric mean (LSGM) of EDC biomarker concentrations (natural-log transformed to account for the right skewness of biomarker data) to compare the urinary concentrations across different years of birth (2008–2019) similar to (Zota et al., 2014). From the regression models, LSGM of biomarker concentrations by year was calculated as exp (LSGM), with 95% confidence intervals (CI) as exp (LSGM ± 1.96 X SE), where SE is the standard error. The Mann-Kendall test was used to test for monotonic trends in biomarker concentrations in the study period. P-values <0.05 were considered statistically significant.

Trinomial logistic regression models were used to simultaneously estimate adjusted relative risk ratio (RRR) and 95% CIs, one for each biomarker with ASD and Non-TD, as previously

described (Barkoski et al., 2019). Because participants provided a variable number of urine samples at 3 and/or 6 months, we computed the average biomarker concentration for samples collected at both 3 and 6 months or used whichever timepoint was available at 3 or 6 months for participants who only provided one sample for regression analysis. Subsequently, biomarkers detected in ≥75% (Hornung and Reed, 1990) of participants were modeled as continuous natural-log transformed variables in our models. For these biomarkers, concentrations below the LOD were assigned a value of the LOD divided by the square root of 2. Additionally, we modeled biomarkers detected in 50–74% of participants as three-level categorical variables, with the lowest category defined as participants with concentrations < LOD and the remaining two categories created by dichotomizing participants at the median of concentrations ≥ LOD. Lastly, we modeled concentrations of biomarkers detected in 25–49% of participants as binary variables dichotomized as < LOD or ≥ LOD. Biomarker concentrations with detection frequencies below 25% were excluded from further statistical analysis: ethyl-paraben, butyl-paraben, MCOCH, HBP, dihydroxyavobenzone, triclocarban, MEHP, and BPF. All models were adjusted for covariates selected a priori based on a directed acyclic graph (DAG) (Supplementary Fig. 1). Covariates adjusted in the final models included the child's year of birth (continuous, to account for the temporal trend of EDC biomarkers), the child's sex (female, male), maternal age at the child's birth (continuous), maternal pre-pregnancy body mass index (BMI; (<25, 25–30, >30 kg/m^2) and parental homeowner status (yes/no), as a proxy for socioeconomic status and family wealth. The child's birth year was centered by subtracting the mean birth year. Given the exploratory nature of the study and low sample size, we did not adjust for multiple comparisons, and p-values <0.05 were considered statistically significant. As a sensitivity analysis, trinomial logistic regression models were also run excluding participants with alternative diagnostic assessments (n = 14; see Supplementary Materials). Furthermore, given a subset of participants from the present analysis (n = 53) had maternal prenatal EDC data available as previously described (Barkoski et al., 2019; Shin et al., 2018), as a sensitivity analysis, we also ran trinomial logistic regression models further adjusting our models for maternal EDC urinary concentrations when available. We also examined maternal prenatal vitamin use during the first month of pregnancy as a possible effect modifier in a stratified analysis and by adding interaction product terms in separate trinomial logistic regression models, based on previous studies reporting effect modification of associations between maternal prenatal vitamin use and cognitive development in children (Schmidt et al., 2017; Shin et al., 2018).

For biomarkers with a detection frequency ≥75% (Hornung and Reed, 1990), generalized estimating equations (GEE) (Liang and Zeger, 1986) were used to investigate the association between urinary EDC biomarkers and longitudinal changes in MSEL (composite and T-scores for each subscale) at each assessment time point (6, 12, 24, and 36 months). GEE models were performed using the "Proc Genmod" function in SAS using a linear link and autoregressive correlation structure, adjusting for the same covariate set as before with age at assessment included in the model in the main effects and with an interaction term with EDC concentrations, with the interaction term considered statistically significant when $p < 0.10$. As a secondary analysis, multiple linear regression models were also used to investigate the association between urinary EDC biomarkers and MSEL scores (composite and T-scores for

each subscale) at each assessment time point individually. The adjusted estimated regression coefficients (β) and 95% confidence intervals (CI) are presented. Finally, for these same biomarkers, quantile g-computation (which allows for examining the effects of an exposure mixture by estimating the effects of increasing all exposures by one quantile) (Keil et al., 2020) was used to assess the exposure to mixtures of EDC biomarkers and MSEL subscales and composite scores, adjusted for the same covariates as the other models. Quantile g-computation was implemented using the R package *qgcomp* with gaussian distribution, and 500 boot iterations to calculate CIs. All statistical analyses were conducted in SAS software version 9.4 (Institute Inc. Carny, NC, USA) and R version 4.2.2. (R Foundation for Statistical Computing, Vienna, Austria) with RStudio.

3. Results

Table 1 shows the study population demographics for MARBLES participants included in our study. The population included 36 ASD, 18 Non-TD, and 81 TD participants. Approximately 63% of study participants were male and 37% were female. Thirty-seven percent of children were non-Hispanic white, 30% were Hispanic, and 32% were non-Hispanic, non-white. Approximately 55% of mothers reported taking prenatal vitamins in the first month of pregnancy. Furthermore, 56% of mothers were 35 years or older at the time of the child's birth, about 53% of mothers had a bachelor's degree or higher, and approximately 62% of parents were homeowners.

The detection frequencies and concentrations of the thirty-two EDCs measured in this study are presented in Table 2. MECPP was detected in 100% of samples, additionally, eight phthalates (MECPTP, MiBP, MCOP, MEP, MHiBP, MBP, MBzP, and MEOHP) and benzophenone-3 were detected in over 90% of samples. The highest median concentrations were observed for benzophenone-3 (10.8 ng/mL), methyl-paraben (10.4 ng/mL), and MEP (8.9 ng/mL). As expected, MEOHP, MEHHP, and MECPP (phthalate metabolites originating from the same parent compound, DEHP) were highly correlated (Spearman's rho (r) = 0.94–0.98) (Supplementary Fig. 2). As were MHBP and MBP (r = 0.92), and MHiBP and MiBP (r = 0.92), which originate from DBP and DiBP, respectively. When examining temporal trends (Supplementary Fig. 3), most biomarkers decreased over the study period including significant negative trends for methyl-paraben ($\tau = -0.58$, $p = 0.01$) and propyl-paraben ($\tau = -0.65$, $p < 0.01$). However, MECPTP significantly increased over the study period ($\tau = 0.81$, $p < 0.01$).

Table 3 presents the results from adjusted trinomial linear regression models investigating EDC biomarker concentrations at 3 and/or 6 months of age in association with ASD and Non-TD risk, as compared to TD controls. Overall, most associations between EDC biomarkers during early infancy with ASD and Non-TD outcomes were null. However, we found a significant inverse association between ASD risk with ΣDEHTP concentrations (RRR = 0.50; 95% CI [0.30, 0.83]), methyl-paraben (RRR = 0.79; 95% CI [0.63, 0.99], the 2nd tertile (vs. the 1st tertile) of MCNP (RRR = 0.23; 95% CI [0.07, 0.76]), and the 3rd tertile of BPA (RRR = 0.29; 95% CI [0.09, 0.90]). Similarly, a significant inverse association between Non-TD risk and the 3rd tertile of BPA was also found (RRR = 0.26; 95% CI [0.07, 0.92]). Sensitivity analysis excluding children (n = 14) with alternative neurodevelopmental

diagnosis did not change our results and similarly showed no increased risk for ASD or Non-TD risk (Supplementary Table 3); neither did further adjusting our models for maternal prenatal EDC urinary concentrations, when available (n = 53) (Supplementary Table 4). Lastly, results from models stratified by maternal prenatal vitamin use during the first month of pregnancy also did not show a significantly increased risk for ASD or Non-TD (Supplementary Table 5).

Moving beyond neurodevelopmental diagnosis, when we examined the association between EDC biomarkers at 3 and/or 6 months of age and MSEL across the four assessment time points utilizing GEE models, we found two phthalate biomarkers were significantly associated with poorer scores on the MSEL (Fig. 1). Notably, increased MEP was associated with poorer composite score (β = −0.16, 95%CI [−0.31, −0.02]) and two subscales including fine motor (β = −0.09, 95%CI [−0.17, 0.002]), and visual reception (β = −0.11, 95% CI [−0.23, 0.01]). Additionally, ∑DEHTP was associated with poorer composite score (β = −0.11, 95%CI [−0.21, −0.01]), and reduced scores in visual reception (β = −0.09, 95% CI [−0.16, −0.02]). However, propyl-paraben and methyl-paraben were both positively correlated with fine motor, the latter of which was also positively correlated with composite score (β = 0.06, 95% CI [0.00, 0.12]). See Supplementary Table 6 for all β and 95% CIs. When analyses were further stratified by each assessment timepoint (Supplementary Table 7), similarly, increased MEP concentrations were associated with poorer fine motor at 24 months (β = −1.99; 95% CI [−3.75, −0.23]), and composite scores at 36 months (β = −5.20; 95% CI [−9.27, −1.13]), and MBzP, MCOP, and ∑DEHP were also significantly associated with poorer receptive language at 12 months (Supplementary Fig. 4). Lastly, using quantile g-computation to investigate a mixtures effect, we did not find a significant estimated effect on any MSEL subscales or composite scores in association with a mixture of EDC biomarkers; although notably a consistent trend for decreased receptive language was observed in association with a mixture of EDC biomarkers (Supplementary Table 8).

4. Discussion

To better understand the potential effect of early postnatal EDC exposure on child neurodevelopment, we measured select urinary EDC biomarkers (including phthalates, phenols, and parabens) at 3 and/or 6 months of age in this high-ASD-risk cohort. When we investigated the temporal trajectories of biomarkers during our study period from 2008 to 2019, we found significant decreasing trends in methyl-paraben and propyl-paraben. Consistent with our study, Kim et al. also previously found significant decreasing time trends for methyl-paraben and propyl-paraben in urine samples from moms of the MARBLES study, while they were pregnant (Kim et al., 2021b). Additionally, Shin et al. also examined temporal trends of phthalate metabolites among mothers in the MARBLES study, during pregnancy, and found that concentrations for MBzP, MEP, ∑DBP, and ∑DEHP decreased but reported ∑DiBP and MCOP increased over their study period from 2007 to 2017. Similarly, we found infant postnatal phthalate metabolite concentrations of MBzP, MEP, DBP metabolite (MBP), and DEHP metabolites (MECPP, MEHHP, and MEOHP), as well as MCOP, tended to decrease over time, although none reached statistical significance. However, we also found that MECPTP, a major metabolite of DEHTP, significantly increased over the study period and was detected in about 99% of our infant samples.

Similar to our results, a previous study also found that MECPTP increased over the study period (2014–2015) and was detectable in 100 percent of urine samples in Portuguese children (n = 107) ages 4–17 years (Lessmann et al., 2017). DEHTP is increasingly used as a replacement plasticizer for other phthalates such as DEHP, as it is a structural isomer of DEHP (Silva et al., 2015). Although DEHTP exposure has not been reported to induce major adverse effects in older rodent models at certain doses (Barber and Topping, 1995; Wirnitzer et al., 2011), additional research is necessary to further investigate possible adverse effects in humans due to DEHTP's ubiquitous presence in our environments and potentially increasing exposures, especially in infants. Nonetheless, regulations with perhaps contribution from behavioral changes induced by increased consumer awareness about the potential adverse health effects of some EDCs, together, may have successfully contributed to decreasing body burden of some phthalates in the last decades (Zota et al., 2014).

While previous studies have also examined several phthalate metabolites in early postnatal life, to our knowledge, this is the first study to measure triclocarban, phenols, and parabens at 3 and/or 6 months of age, as well as investigate a broader range of phthalate and phthalate alternative biomarkers than previously investigated concerning neurodevelopment. Therefore, this study adds to the limited literature focusing on EDC exposure during early infancy and provides updated exposure data as our study period was from 2008 to 2019. Sathyanarayana et al. previously reported urinary concentrations of select phthalate metabolites among infants aged 2–28 months born between 2000 and 2005, and found higher concentrations for MBP, MBzP, MEHHP, MEOHP, MEP, and MHBP than the concentrations we observed, while MECPP had a similar range to ours, and MiBP was lower (Sathyanarayana et al., 2008). Interestingly, they found certain phthalates concentrations of MEP and MiBP, for example, were significantly associated with the reported use of baby lotion, infant powder, and baby shampoo (as reported by mothers' questionnaires about infant product use), particularly among children younger than 8 months. In a pilot study conducted between January to March 2000, Brock et al. reported four phthalate metabolites in 12–18 month infants (n = 19) (Brock et al., 2002); the reported concentrations were also higher than what we found in our 3 to 6-month infants. As infants develop, they begin to move around, crawl, and have increased hand-to-mouth behaviors with the potential for increased exposure to phthalate sources in the environment (Carlstedt et al., 2013). In contrast, younger infants and newborns with relatively limited mobility are exposed predominantly to dermal contact with baby care products (Fisher et al., 2019; Sathyanarayana et al., 2008), or oral ingestion (Cao, 2010; Fromme et al., 2011; Henderson et al., 2020). While differences in study populations and ages of study participants certainly contribute to discrepancies in phthalate concentrations we observed compared to previous studies, decreasing temporal trends (previously discussed) may also be a contributing factor. Supplementary Table 9 presents a comparison of the phthalate metabolite concentrations in the present study compared to some concentrations previously reported among infants.

When we analyzed EDC biomarker concentrations at 3 and/or 6 months in association with risk of ASD or Non-TD, we found that the results of our analysis did not support our hypothesis that early postnatal EDC concentrations were associated with adverse neurodevelopmental diagnosis. Most of our findings were null, and a few were opposite the direction we expected, such as for ∑DEHTP and ASD risk, and BPA and ASD/Non-TD

risk. Given the MARBLES study is an enriched-risk ASD cohort, it is possible that some of the inverse associations we observed may be related to the genetic component of ASD which may have a stronger effect in our study population than the associations between environmental exposures and child neurodevelopmental outcomes. Another study using a different high risk-ASD cohort of mothers who had a child with ASD also reported null or inverse associations between maternal urinary phthalate concentrations and ASD-related behaviors using the Social Responsiveness Scales (SRS) at 3–8 years of age (Patti et al., 2021); their gestational phthalate metabolite concentrations were very similar to those previously reported in MARBLES moms (Shin et al., 2020). Furthermore, due to the small sample size and large confidence intervals in the present study, we cannot rule out null/inverse results for EDC exposures on adverse neurodevelopmental outcome risk and further research in the general population is necessary.

Although we did not find EDCs in early infancy were associated with increased risk of ASD or Non-TD, interestingly, when we further investigated the association between EDC biomarkers and longitudinal cognitive developmental scores, we found that several phthalate biomarkers, but not phenols or parabens, were associated with reduced scores in MSEL subscales and composite scores. Most notably, MEP concentrations were associated with poorer fine motor, visual reception, and composite scores on the MSEL. Additionally, ∑DEHTP, which previously had an inverse association with ASD risk, was associated with poorer visual reception and composite scores. Thus, an overall pattern that was most consistent across several phthalates was reduced scores in visual reception and composite scores. Very few studies have examined postnatal phthalate exposure in association with cognitive scores. One study by Jankowska et al. found postnatal MEP and MBP levels at 2 years of age were inversely associated with cognition at 7 years old; prenatal MEP levels were also associated with an increased risk of peer relationship problems at early school age (Jankowska et al., 2019). Postnatal urinary DEHP levels (and its metabolites) have also previously been associated with poorer IQ scores (Cho et al., 2010; Huang et al., 2015) and decreased cognition (Radke et al., 2020). In the context of the present study, EDC exposure and in particular phthalates throughout early life—both prenatal (Engel et al., 2010; Jankowska et al., 2019; Miodovnik et al., 2011) and infancy — may have negative impacts on both cognitive development and behavioral outcomes, i.e. both periods of exposure should be considered risky for the child.

Though others have also considered a broad range of EDCs in association with neurodevelopmental outcomes, previous studies have largely focused on the prenatal period (Braun et al., 2014). As such, this study is unique in that it adds to the extant literature by focusing on early infancy and investigating some of the youngest age groups previously reported on—therefore providing more up-to-date biomarker concentrations, and highlighting that even during the early infancy period, exposure to these EDCs is already occurring. Additionally, a strength of our investigation is that ASD diagnosis was confirmed by trained psychologists using the gold standard diagnostic instruments. However, there were several limitations in our study, including a limited sample size for ASD and Non-TD outcomes which limited the statistical power and resulted in large confidence intervals. Nevertheless, the findings of several statistically significant associations indicate that we actually had adequate power for some of our hypotheses. Another limitation of

this investigation is that our study participants are from a high-risk ASD population, i.e. they have a family history, e.g., an older sibling with ASD. As such, study participants (even TD controls) were at an elevated risk for ASD because of their family history of this condition. Therefore, our findings, especially related to the risk of ASD, may not be generalizable to the greater population with this condition, the majority of whom do not have a family history. Additionally, the parents may have been more motivated to avoid products with chemicals, given that they already have a child with ASD and thus may be aware of potential risk factors. Nevertheless, because phthalates are pervasive and have such a plethora of sources (shampoos, lotions, cosmetics, packaged food, home building materials, medications), it can take exceptional vigilance to measurably reduce one's body burden of this class of chemicals.

Overall, we found many of these EDC biomarkers were already detectable in the urine of infants at 3 and/or 6 months of age. While temporal trends suggest exposure to most of these EDCs is decreasing in the population, exposure to replacement phthalates, such as DEHTP, seems to be increasing and is of concern given the associations we found with ∑DEHTP and poorer visual reception and composite scores on the MSEL. The current study revealed a mixed set of consistent and discordant findings compared to previous studies that examined associations between EDCs and diagnosis of ASD or another developmental diagnosis, however, a clear pattern emerged in the analyses of cognitive function in association with select phthalates, but not other EDCs. Notably, higher MEP concentrations were associated with a pattern of poorer skills for the composite, fine motor, and visual reception. In light of consistency regarding MEP from the more abundant literature in relation to gestational exposures, these findings highlight the potential importance of early infant exposures in cognitive development. Further research can shed light on the particular sources of infant body burdens of phthalates in the current time period, investigate whether those early postnatal exposures are associated with longer-term deficits, identify cofactors that may modify the impacts, and place special attention on the increasing replacement chemicals, such as DEHTP, and whether they adversely affect neurodevelopment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors would like to thank all the MARBLES Study participants and staff for helping make this research possible.

Funding sources

Funding for this work was provided in part by the National Institute of Health (NIH) [grant numbers P0ES11269, R01ES020392, R01ES028089, P30ES023513, R01ES025574, R24ES028533, and U24ES928533]; U.S. EPA STAR [grant numbers RD-829388 & RD-833292]; and the UC Davis MIND Institute's Intellectual and Developmental Disabilities Research Center [grant number P50HD103526]. Additionally, this work was supported by a grant from the Simons Foundation (SFARI #863967, RJS).

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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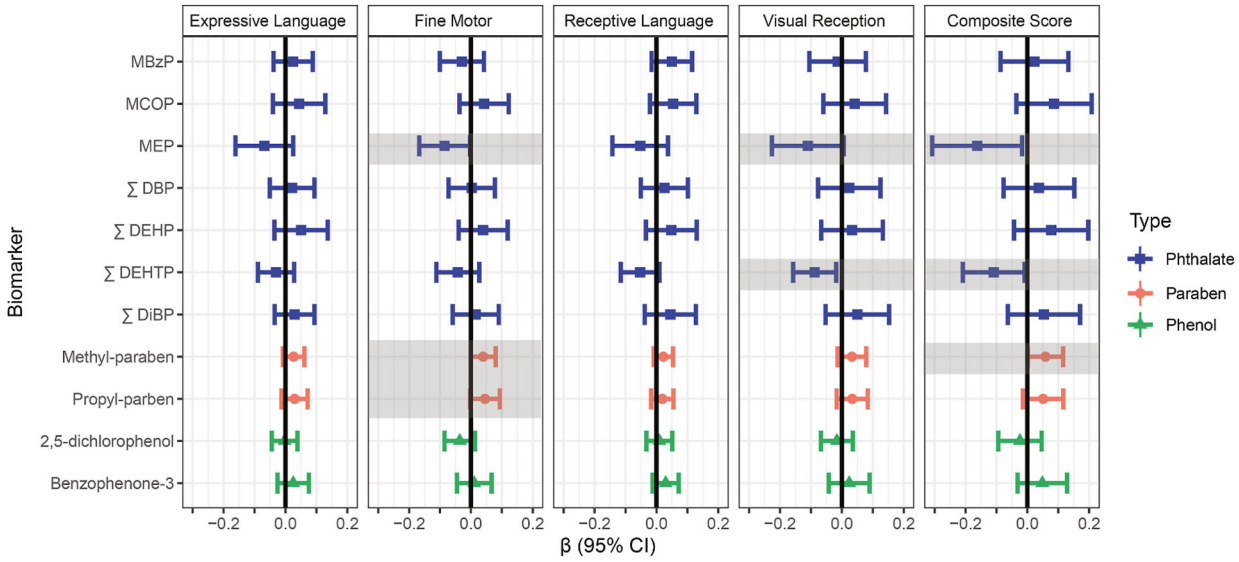

Fig. 1.

Longitudinal changes (β) and 95% confidence intervals (CI) in MSEL Composite Scores and T-scores of subscales of children over the four assessment time points in association with select phthalate (MBzP, MCOP, MEP, ΣDBP, ΣDEHP, ΣDEHTP, ΣDiBP), paraben (methyl-paraben and propyl-paraben), and phenol (2,5-dichlorophenol and benzophenone-3 biomarker concentrations in generalized estimating equations. Models were adjusted for the child's birth year, the child's sex, maternal age, maternal pre-pregnancy BMI, and parental homeownership. Shaded areas represent associations with a p-value <0.10 for interaction between age of assessment and EDCs concentrations.

Table 1

Characteristics of study participants (n = 148).

	Frequency (%)
Neurodevelopmental outcome [a]	
ASD	36 (26.67)
Non-TD	18 (13.33)
TD	81 (60.00)
Child's sex	
Female	55 (37.16)
Male	93 (62.84)
Child's race/ethnicity	
Non-Hispanic, white	55 (37.16)
Hispanic, any race	45 (30.41)
Non-Hispanic, non-white	48 (32.43)
Child's birth year	
2008–2011	25 (16.89)
2012–2015	71 (47.97)
2016–2019	52 (35.14)
Maternal vitamin intake in the first month of pregnancy [a]	
No	61 (44.9)
Yes	75 (55.15)
Maternal pre-pregnancy BMI (kg/m^2) [a]	
<25	60 (44.44)
25–30	45 (33.33)
≥30	30 (22.22)
Maternal education	
Some college or less	69 (46.62)
Bachelor's degree	45 (30.41)
Graduate or professional degree	34 (22.97)
Homeownership status [a]	
No	55 (38.19)
Yes	89 (61.81)
Mother's age at the time of child's birth	
<35	65 (43.92)
≥35	83 (56.08)

[a] Missing (n): neurodevelopmental diagnosis (13); maternal prenatal vitamin intake (12); Pre-pregnancy BMI (13); Homeownership status (<5).

Table 2

Distribution of EDC biomarker concentrations (ng/mL) for study participants collected at 3 and/or 6 months for 148 study participants, corresponding to 188 urine samples available.

Class	Biomarker[a]	LOD (ng/mL)	Detection frequency	Percentiles of concentrations (ng/mL)		
				25th	50th	75th
Phthalate	MBP	0.4	94.7	1.9	4.2	9.3
	MBzP	0.3	93.6	0.6	1.6	3.9
	MCNP	0.2	67.0	<LOD	0.3	0.6
	MCOP	0.3	97.9	0.7	1.4	3.0
	MCPP	0.4	61.2	<LOD	0.6	1.1
	MECPP	0.4	100	2.7	5.6	10.1
	MECPTP	0.2	99.5	2.0	4.7	11.3
	MEHHP	0.4	87.8	0.6	1.4	3.0
	MEHHTP	0.4	50.0	<LOD	0.4	1.1
	MEOHP	0.2	91.5	0.6	1.2	2.6
	MEP	1.2	96.8	5.1	8.9	16.7
	MHBP	0.4	74.5	<LOD	0.9	2.0
	MHiBP	0.4	96.8	1.4	2.8	5.6
	MiBP	0.8	98.9	3.3	5.8	11.7
	MONP	0.4	39.9	<LOD	<LOD	0.7
Phthalate Alternative	MHiNCH	0.4	29.3	<LOD	<LOD	0.5
Paraben	Methyl-paraben	1.0	85.6	2.1	10.4	84.6
	Propyl-paraben	0.1	87.8	0.2	0.8	3.2
Phenol	2,4-dichlorophenol	0.1	69.1	<LOD	0.2	0.3
	2,5-dichlorophenol	0.1	76.6	0.1	0.2	0.6
	Benzophenone-3	0.4	98.4	3.6	10.8	30.2
	BPA	0.2	59.6	<LOD	0.2	0.5
	BPS	0.1	51.6	<LOD	0.1	0.3
	Triclosan	1.7	41.0	<LOD	<LOD	3.9

[a]Compounds detected in less than 25% of the population (detection frequency %): Ethyl-paraben (23.9%), Butyl-paraben (21.3%), MCOCH (21.3%), HBP (19.7%), Dihydroxyavobenzone (14.4%), Triclocarban (13.8%), MEHP (13.3%), and BPF (7.4%).

Int J Hyg Environ Health. Author manuscript; available in PMC 2024 October 17.

Table 3

Adjusted relative risk ratios (RRR) and 95% confidence intervals (CI) from trinomial logistic regression analysis of EDC biomarker concentrations and neurodevelopmental outcome (ASD = 36, Non-TD = 18, TD = 81), with TD as the reference level. P < 0.05 are shown in bold.

Biomarker	ASD (n = 36)			Non-TD (n = 18)		
	RRR[a]	(95% CI)	p-value	RRR[a]	(95% CI)	p-values
Continuous (natural log-transformed)						
Phthalate						
MBzP	0.81	(0.56, 1.17)	0.25	1	(0.64, 1.55)	1.00
MCOP	0.73	(0.44, 1.19)	0.21	0.83	(0.46, 1.50)	0.54
MEP	1.14	(0.74, 1.76)	0.56	1.08	(0.63, 1.85)	0.78
Σ DBP	0.68	(0.45, 1.03)	0.07	0.9	(0.54, 1.49)	0.67
Σ DEHP	0.87	(0.55, 1.38)	0.55	0.86	(0.49, 1.52)	0.60
Σ DEHTP	**0.5**	**(0.30, 0.83)**	**0.01**	0.77	(0.45, 1.32)	0.35
Σ DiBP	0.66	(0.42, 1.03)	0.07	0.67	(0.38, 1.20)	0.18
Paraben						
Methyl-paraben	**0.79**	**(0.63, 0.99)**	**0.04**	1.04	(0.81, 1.33)	0.76
Propyl-paraben	0.78	(0.60, 1.01)	0.06	0.92	(0.69, 1.22)	0.55
Phenol						
Benzophenone-3	0.75	(0.56, 1.00)	0.05	1.1	(0.80, 1.52)	0.56
2,5-dichlorophenol	0.86	(0.61, 1.21)	0.39	1.09	(0.76, 1.56)	0.63
***Tertile (compared to* 1st)**						
Phthalate						
MCNP—2nd	**0.23**	**(0.07, 0.76)**	**0.02**	1.54	(0.25, 9.40)	0.64
MCNP—3rd	0.51	(0.18, 1.47)	0.21	3.03	(0.57, 16.07)	0.19
MCPP—2nd	0.48	(0.17, 1.37)	0.17	0.25	(0.05, 1.19)	0.08
MCPP—3rd	0.42	(0.14, 1.22)	0.11	0.59	(0.17, 2.05)	0.40
Phenol						
BPA—2nd	1.08	(0.37, 3.15)	0.88	0.28	(0.05, 1.51)	0.14
BPA—3rd	**0.29**	**(0.09, 0.90)**	**0.03**	**0.26**	**(0.07, 0.92)**	**0.04**
BPS—2nd	0.49	(0.15, 1.64)	0.25	0.66	(0.16, 2.83)	0.58
BPS—3rd	0.38	(0.12, 1.18)	0.09	0.43	(0.11, 1.76)	0.24

Biomarker	ASD (n = 36)			Non-TD (n = 18)		
	RRR[a]	(95% CI)	p-value	RRR[a]	(95% CI)	p-values
2,4-dichlorophenol—2nd	1.34	(0.37, 4.87)	0.66	1.77	(0.23, 13.49)	0.58
2,4-dichlorophenol—3rd	0.98	(0.36, 2.69)	0.97	2.85	(0.68, 11.97)	0.15
Detect vs. Non-Detect						
Phthalate						
MONP	0.42	(0.16, 1.07)	0.07	0.87	(0.29, 2.63)	0.80
Phthalate alternative						
MHiNCH	0.67	(0.26, 1.78)	0.43	1.83	(0.62, 5.42)	0.28
Phenol						
Triclosan	1.27	(0.49, 3.31)	0.63	2.78	(0.81, 9.58)	0.10

[a]Adjusted for the child's birth year, the child's sex, maternal age, maternal pre-pregnancy BMI, and parental homeownership.