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Associations of maternal and paternal preconception and maternal pregnancy urinary phthalate biomarker and bisphenol A concentrations with offspring autistic behaviors: The PEACE study

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ABSTRACT

Background: Environmental chemical exposures *in utero* may play a role in autism development. While preconception risk factors for autism are increasingly being investigated, little is known about the influence of chemical exposures during the preconception period, particularly for paternal exposures.

Methods: In 195 children from the Preconception Environmental exposures And Childhood health Effects (PEACE) cohort born to parents recruited from a fertility clinic in Boston, Massachusetts between 2004 and 2017, we quantified concentrations of 11 phthalate metabolites and bisphenol A (BPA) in urine samples collected from mothers and fathers before conception and mothers throughout pregnancy. When children were 6–15 years old, parents completed the Social Responsiveness Scale (SRS) questionnaire assessing autistic behaviors. We used linear mixed effect models to estimate covariate-adjusted associations of phthalate biomarker and BPA concentrations, separately for maternal preconception ($n = 179$), paternal preconception ($n = 121$), and maternal pregnancy ($n = 177$), with SRS *T*-scores, based on age and gender, in offspring. We used quantile g-computation models for mixture analyses and evaluated modification by selected dietary factors.

Results: The mean SRS *T*-score was 47.7 (±7.4), lower than the normative mean of 50. In adjusted models for individual biomarkers or mixtures, few associations were observed and estimates were generally negative (e.g., lower SRS *T*-scores) and imprecise. We observed associations of higher mono-isobutyl phthalate (MiBP) concentrations measured in maternal preconception and paternal preconception periods with lower SRS *T*-scores ($\beta_{maternal\_precon} = -1.6$, 95% CI -2.7; −0.4; $\beta_{paternal\_precon} = -2.9$, 95% CI -4.6; −1.2) for each $\log_e$ increase. In a subset of participants with maternal preconception nutrition information, we generally observed stronger inverse associations with higher folate and iron intake, particularly for folate intake and MiBP concentrations.

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Conclusions: Urinary phthalate biomarker and BPA concentrations during preconception (maternal and paternal) and pregnancy (maternal) were not associated with adverse autistic behaviors in these children. Larger studies are needed to elucidate the observed associations, while considering interactions between maternal nutrition and chemical exposures.

1. Introduction

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder that is characterized by challenges with social skills, communication deficits, and restricted, repetitive, and stereotyped patterns of behaviors and interests (Faras et al., 2010). As ASD includes a spectrum of disorders, there is wide variation in the manifestation of symptoms and phenotypes. In recent years, the prevalence of autism has increased worldwide (Zeidan et al., 2022; Neggers, 2014) and approximately 1 in 36 United States (U.S.) children has ASD according to 2018 data (Maenner et al., 2023). While ASD has a genetic basis, genetics alone does not completely explain the risk of developing ASD and there is increasing evidence that early life risk factors are involved (Lyall et al., 2017). More recently, it has been suggested that *in utero* environmental chemical exposures may play a role in the development of ASD (Braun, 2017; Schug et al., 2015).

The developing fetus is especially susceptible to endocrine disrupting chemicals (EDCs), as they can impact critical processes of fetal brain development and lead to increased risk of neurodevelopmental disorders in the offspring (Braun, 2017; Braun et al., 2013; Ejaredar et al., 2015; Radke et al., 2020), by altering hormonal function and epigenetic mechanisms (La Merrill et al., 2020; Zhang et al., 2024). Phthalates and bisphenol A (BPA) are chemicals with recognized endocrine disrupting properties and have been shown to pass the placental barrier, potentially exposing the fetus (Ejaredar et al., 2015; Schettler, 2006; Vandenberg et al., 2007; Mose et al., 2007; Bräuner et al., 2022). Phthalates are widely used in various consumer and personal care products such as shampoo, hair spray and soap, but also in building materials, furniture, and medical devices (Schettler, 2006; Sathyanarayana, 2008; Chou and Wright, 2006). Phthalates have short biological half-lives (hours) but the presence of several phthalate metabolites in many everyday products results in chronic and widespread human exposure, including exposure to pregnant women and persons of childbearing age (Sathyanarayana, 2008; Chou and Wright, 2006; Woodruff et al., 2011; Wang et al., 2019). BPA is one of the highest volume chemicals worldwide and used to produce epoxy resins and plastic products such as toys, food can linings, drinking containers, and medical equipment among others (Vandenberg et al., 2007). Both BPA and phthalates have been increasingly investigated in relation to autistic traits due to their ubiquitous exposure and potential neurotoxicity (Braun, 2017; Braun et al., 2013; Ejaredar et al., 2015; Radke et al., 2020; Jeddi et al., 2016). One proposed mechanism of BPA action is through its hormone-like properties, particularly its ability to bind to estrogen receptors, thereby interfering with various neurological functions (Schug et al., 2015; Costa and Cairrao, 2024; Schwartzer et al., 2013; Alonso-Magdalena et al., 2006). Similarly, some phthalates can interfere with estrogen and thyroid activity in pregnant women that are critical for fetal brain development (Schug et al., 2015; Jeddi et al., 2016; de et al.; Ghisari et al., 2009; Testa et al., 2012). Indeed, available epidemiological evidence suggests that gestational exposure to BPA or phthalates may influence childhood behavioral problems and ASD in later life (Kim et al., 2021; Alampi et al., 2021; Patti et al., 2021; Day et al., 2021; Ponsonby et al., 2020; Oulhote et al., 2020; Braun et al., 2014; Miodovnik et al., 2011; Larsson et al., 2009); but studies have also reported a lack of association (Patti et al., 2021; Mustieles et al., 2023; Haggerty et al., 2021; Hyland et al., 2019; Shin et al.).

Although phthalates and BPA are among the most extensively studied endocrine disrupting chemicals, their effects during the preconception period on offspring health remain relatively unexplored (Braun et al., 2017). The Childhood Autism Risks from Genetics and Environment study explored risk factors for autism starting from preconception (Hertz-Picciotto et al., 2006), and other studies have examined maternal and fetal epigenetic changes, which may increase the risk of autism (Zhu et al., 2022; Schroeder et al., 2016; Bakulski et al., 2021). However, evidence addressing the influence of paternal exposures is lacking. Emerging studies support that paternal environmental exposures can induce genetic mutations and epigenic alternations in sperm, which can subsequently affect health of the offspring (Duty et al., 2003; Feinberg et al., 2015; Meeker et al., 2009), including early autism risk (Feinberg et al., 2015). The exact biological mechanisms underlying these paternal exposures are unclear, but may include epigenetic modifications transmitted through sperm DNA, histones, and RNA, all of which are critical to programming early embryonic development (Jenkins and Carrell, 2012) and in turn impact brain development.

In the present study, we investigated maternal and paternal preconception urinary concentrations of phthalate biomarkers and BPA as well as maternal pregnancy urinary concentrations in relation to autistic behaviors in offspring measured with the Social Responsiveness Scale-2 (SRS) (Constantino and Gruber, 2005).

2. Material and methods

2.1. Study design and data source

This analysis included data from 195 children (and their 164 mothers and 99 fathers) participating in the Preconception Environmental exposures And Childhood health Effects (PEACE) study. The PEACE study is an ongoing prospective study cohort established in 2018 to investigate the impact of maternal and paternal preconception and maternal pregnancy exposures to endocrine disrupting chemicals on the risk of childhood neurodevelopmental disorders, obesity, and cardiometabolic outcomes. Beginning in 2018, we recruited children born to a parent(s) who participated in the Environment and Reproductive Health (EARTH) study between late 2004 to 2017 (Messerlian et al., 2018). In the EARTH study, couples were recruited while attending the fertility clinic at the Massachusetts General Hospital in Boston. Recruitment occurred before or at the beginning of fertility evaluation and treatment. Multiple urine samples were collected from both mothers and fathers before conception and later from mothers throughout the pregnancy. These pregnancies included both natural and assisted conceptions. The liveborn children were enrolled into the PEACE study from age 6 years on, at which time information on autistic behaviors was obtained using the SRS questionnaire. Additional information on birth characteristics and relevant covariate data such as preconception diet (e.g. on daily folic acid/folate and iron intake), socio-economic status, medical history, and physical activity were extracted from medical records and other standardized baseline and follow-up questionnaires.

2.2. Study participants

Among 438 mothers from the EARTH study who met the inclusion criteria, 174 unique mothers with multiple children (twins or siblings) agreed to participate in the PEACE study (Fig. 1). (Leader et al., 2024) We excluded children with no available urine sample from either preconception or pregnancy ($n = 9$) and further excluded triplets ($n = 1$ set), providing for a total of 195 children and their 164 participating mothers and 99 participating fathers. Of the 195 children ultimately included in this analysis, 179 had maternal preconception urinary

phthalate metabolite or BPA measures, 121 had paternal preconception measures and 177 had maternal pregnancy measures available. A total of 105 children had phthalate metabolite and BPA measures available from all three exposure windows. Additionally, 161 children had measures available for both maternal preconception and pregnancy, and 113 children had measures available for both maternal preconception and paternal preconception (Data not shown).

2.3. Sample collection and chemical analyses

Multiple urine samples were available from each mother: up to two during preconception (for each cycle or at EARTH study entry) and up to three urine samples during pregnancy (one from each trimester). A cycle was defined as the start of either a new intrauterine insemination or in vitro fertilization cycle. Participating fathers provided one preconception urine sample when attending their partner's fertility treatment or at EARTH study entry for medically unassisted pregnancies. For both mothers and fathers, preconception samples were only used for the cycle associated with a pregnancy of a child included in the PEACE follow-up. All samples were collected in polypropylene cups. Research staff measured specific gravity (SG) of each urine sample using a handheld refractometer (National Instrument Company Inc) and afterwards divided into aliquots in polypropylene cryovials. The urine samples were stored at −80 °C for up to three years and shipped on dry ice overnight to the Centers for Disease Control and Prevention (CDC, Atlanta, Georgia) for the measurement of phthalate metabolites and BPA. At controlled subfreezing temperatures, concentrations phthalate metabolites and BPA in urine are stable (Calafat et al., 2009).

Concentrations of 11 phthalate metabolites and BPA were quantified for each urine sample using solid-phase extraction coupled with high-performance liquid chromatography-isotope dilution tandem mass spectrometry. The analytical approaches have previously been described in detail (Silva et al., 2007; Ye et al., 2005). The CDC laboratory is certified to comply with the requirements set forth in the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88). Analytical measurements are conducted following strict quality control/quality

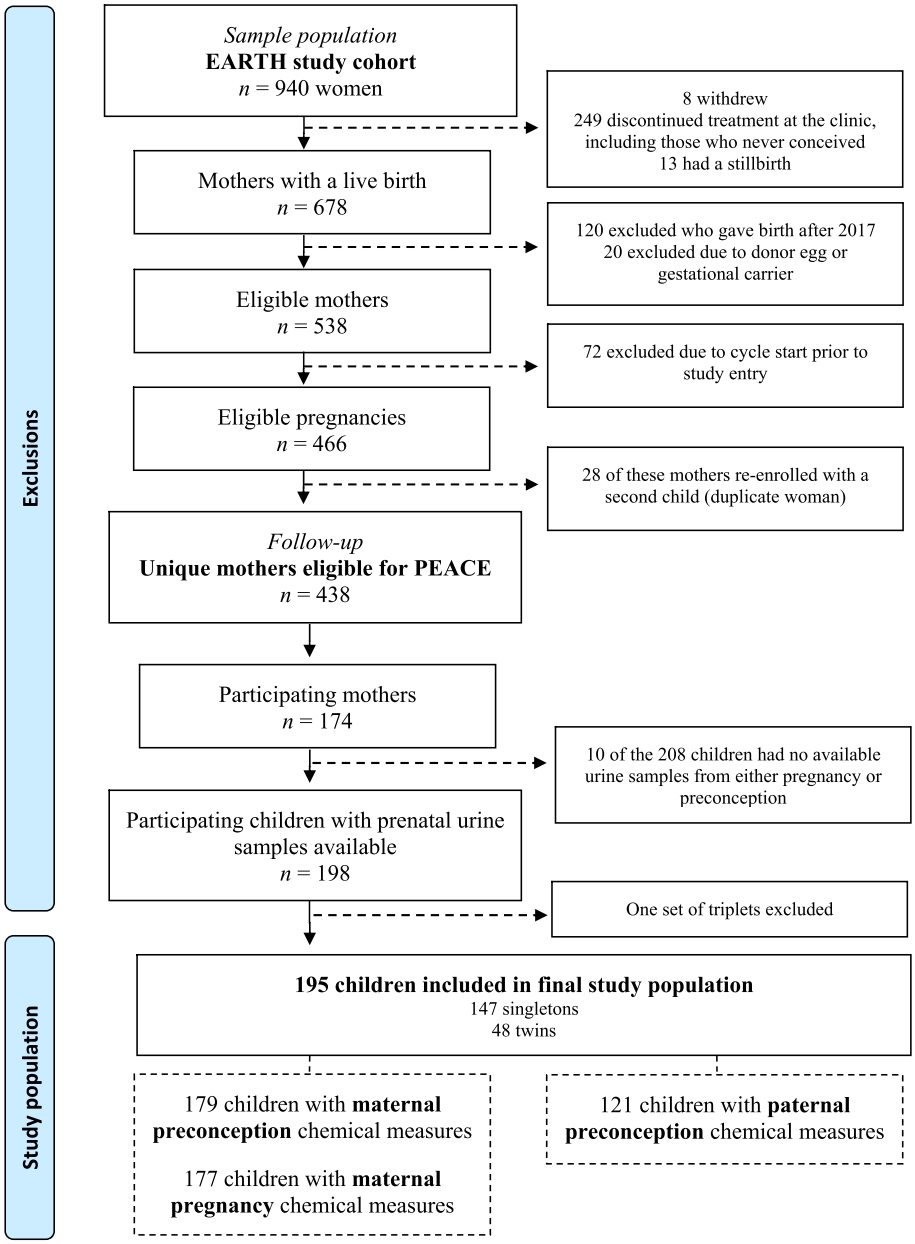

Fig. 1. Flowchart of the PEACE study participation (n = 195 children).

assurance (QC/QA) CLIA guidelines and include participation in proficiency testing programs to continuously demonstrate the accuracy and precision of the analytical methods. Furthermore, along with the study samples, each analytical run includes a set of calibrators, reagent blanks, and high- and low-concentration QC materials. Concentrations of the QCs are evaluated using standard statistical probability rules (Caudill et al., 2008). The CDC methods and QA/QC approach are public and have been used since the early 2000s for analyses of tens of thousands of biological specimens, including those collected as part of CDC's ongoing National Health and Nutrition Examination Survey (NHANES). NHANES data have provided the most comprehensive assessment of Americans' exposure to phthalates and BPA for decades (see www.cdc.gov/exposurereport).

The 11 phthalate monoester metabolites measured included the following seven: monoethyl phthalate (MEP), mono-n-butyl phthalate (MnBP), mono-isobutyl phthalate (MiBP), monobenzyl phthalate (MBzP), mono(3-carboxypropyl) phthalate (MCPP), monocarboxyoctyl phthalate (MCOP), monocarboxyisononyl phthalate (MCNP), as well as four metabolites of di(2-ethylhexyl) phthalate (DEHP): mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono(2-ethyl-5-carboxypentyl) phthalate (MECPP). The limit of detection (LOD) ranged from 0.4 to 1.2 μg/L depending on the specific chemical biomarker, as presented in Table 2. Concentrations below the LOD for BPA or any of the phthalate metabolites were assigned a value equal to the LOD divided by the square root of two prior to adjustment by SG (Hornung and Reed, 1990). The molar sum of the four DEHP metabolites was calculated after dividing the concentration of each metabolite by its respective molecular weight: ΣDEHP = [(MEHP * (1/278.34)) + (MEHHP * (1/294.34)) + (MEOHP * (1/292.33)) + (MECPP * (1/308.33))]. Subsequently, we quantified ΣDEHP in μg/L of MECPP by multiplying the molar sum by the molecular weight of MECPP (308.33) (Leader et al., 2024).

Analyses at the CDC were performed at 16 different times from 2007 to 2017. While the analytical approach and QA/QC protocols remained the same, the sensitivity of methods, e.g., LODs, for the various biomarkers measured changed slightly over the years. In this study, we used the highest LOD for each biomarker (Table 2). Additionally, in the earlier years, methods for measuring MCOP and MCNP were not yet developed, for which reason, there are fewer samples in our study with measurements for these metabolites.

2.4. Social Responsiveness Scale-2 (SRS)

The SRS questionnaire is a valid and reliable tool for assessing autistic behaviors in children by measuring children's social awareness, cognition, communication, motivation, and autistic mannerism (Constantino and Gruber, 2005; Bölte et al., 2008). The parent-answered questionnaire comprises 65 questions scored on a 4-point rating scale (i.e., Likert-style). We used age- and gender-specific *T*-scores (normative population mean: 50, standard deviation: 10 points) derived from US normative data. Higher scores indicate a greater degree of autistic symptoms and behaviors, with *T*-scores ≥60 considered indicative of clinically significant deficiencies in reciprocal social behavior, and *T*-scores ≥76 considered consistent with a clinical diagnosis of ASD (Braun et al., 2014).

The SRS was predominately filled out by the mothers ($n = 189/195$, 97%). Due to the COVID-19 pandemic, the follow-up visits of participating children and their parents to assess SRS were conducted in person prior to March 2020, followed by virtual participation due to pandemic restrictions on in-person visits. This meant that the SRS questionnaires were completed using paper copies up to and including March 2020 ($n = 68/195$, 35%) and after using online platforms ($n = 127/195$, 65%).

2.5. Covariates

Covariates were identified *a priori* through a Directed Acyclic Graph based on existing literature and biological relevance (Fig. S1). Covariates were identified as confounders if associated with the exposure, a risk factor of the outcome, and not part of the causal pathway. We also included variables not associated with exposure, but predictors of SRS scores (i.e., precision variables). We identified the following covariates as confounding factors in the association, while considering available data: Parental age, parental educational status, parental body mass index (BMI) pre-pregnancy, maternal smoking history, child sex, child age, child race, birth year, parity, and mode of conception.

2.6. Statistical approach

We provided descriptive statistics to assess whether relevant study characteristics differed by SRS scores (<60 *T* vs. ≥60 *T*). We further provided median BPA and phthalate biomarker concentrations in each of the three exposure windows (maternal preconception, paternal preconception, and maternal pregnancy). To reduce the influence of outliers, all urinary biomarker concentrations were $\log_e$-transformed prior to statistical analyses. If more than one urine sample was collected within an exposure window (maternal preconception or maternal pregnancy), we used the average of the $\log_e$-transformed SG-adjusted concentrations to reflect that specific exposure window. We used Spearman's correlation coefficients to assess correlations between individual biomarkers within and across exposure windows. These were visualized as correlation plots using R. In main regression analyses, we investigated the crude and adjusted associations of one unit increase in prenatal phthalate biomarker and BPA urinary concentrations with total SRS *T*-scores in offspring, separately for each exposure window. These were performed using linear mixed effect models while considering cluster effects to account for correlations between twins in our study population. Adjusted analyses accounted for parental age (continuous), parental educational status (high school and college; post-graduate), parental body mass index (BMI) pre-pregnancy (<25; 25–30; ≥30 kg/m^2), maternal smoking history (never; current/ever), child sex (boy; girl), child age (continuous), child race (white; other races such as black, Hispanic etc.), birth year (2006-9; 2010-3; 2014-7), parity (nulliparous; multiparous), and mode of conception (natural; assisted conception). The two missing values for maternal BMI were replaced with the most frequent value.

We performed mixture analyses using quantile g-computation (qgcomp) models from the R package qgcomp to investigate the joint effects of multiple chemical exposures on SRS scores while accounting for correlations between exposures (performed for total mixture and for only phthalate biomarkers). The qgcomp model transforms all exposure biomarker concentrations into quantiles, set at quartiles in our analyses, and estimates the effect of simultaneously increasing all biomarkers by one quantile (Keil et al., 2020). It utilizes an optimally weighted mixture approach to identify notable chemical contributors to the overall mixture association. An advantage of the qgcomp model is that it does not require the directional homogeneity assumption. Of note, we were not able to account for twin correlations in these mixture models.

In supplementary analysis, we explored differences in associations according to child sex and separately for singletons. We also explored associations according to daily maternal preconception folate and iron intake (total from both diet and supplements) in a reduced sample (Kim et al., 2021; Oulhote et al., 2020; Haggerty et al., 2021; Goodrich et al., 2018; Schmidt et al., 2017). Folate and iron intakes were assessed at study entry using a validated food frequency questionnaire (Yuan et al., 2017, 2018) and calculated by summing the contributions of relevant food and supplement items, while taking into account the brand, type, and dose of dietary supplements used, as previously described (Kadir et al., 2022; Jiménez-Cardozo et al., 2023). Stratified nutrition models were only performed for the maternal preconception window, as we did

not collect data on iron and folate during pregnancy, which could have changed from preconception to pregnancy; and the paternal nutrition data were subject to a high number of missing values in an already modest sample of participating fathers. For maternal preconception iron intake (median: 31 mg/day; 25-75th percentile: 18–41 mg/day), we used cut-offs based on recommendations (27 mg/day) (National Institutes of Health, 2023). For maternal preconception folate intake (median: 1780 μg/day; 25-75th percentile: 1162–2317 μg/day), we used median cut-offs to achieve more balanced groups, as less than 3% fell below the recommended intake of 600 μg/day (National Institutes of Health, 2022). To assess potential effect modification (i.e., MnBP, MiBP and MBzP), we used Johnson-Neyman plots from the R package 'interactions' to further explore nutritional modification of associations between individual phthalate biomarkers and SRS *T*-scores. Johnson-Neyman plots were used to visualize interactions between a continuous exposure and modifier by graphing the modifier value (x-axis) against the beta for the exposure and outcome association (y-axis). This allowed us to estimate the magnitude of any modifying effect across the range of modifier values in our cohort and determine what level(s) of modifier would attenuate associations between phthalate biomarker concentrations and SRS scores to null (Bauer et al., 2005). Lastly, as an explorative analysis, we assessed associations using binary clinical cut-offs of SRS *T*-scores (<60 vs. ≥60) in a simplified covariate-adjusted model due to practical constraints related to the limited number of children in the at-risk SRS group.

All statistical analyses were performed in STATA version 18.0 or in R when specified.

2.7. Ethical considerations

The PEACE cohort study used for these analyses was approved by the Harvard T.H. Chan School of Public Health institutional review board. The EARTH study in which mothers originally participated in was approved by the Human Subject Committees of the Harvard T.H. Chan School of Public Health, Massachusetts General Hospital and the CDC. We obtained verbal (for children <7 years of age) or written (children >7 years of age) assent from all participating children as well as written informed consent from their guardians.

3. Results

A total of 195 children (including 24 twin sets) and their 164 mothers and 99 fathers were included in this analysis (Table 1a–b). A larger proportion of mothers had a post-graduate education (70%) compared with fathers (46%). Most mothers had a normal BMI at baseline (69%) in contrast to fathers (28%), while smoking status at baseline was comparable between mothers and fathers. The majority of children were first born (84%), vaginally delivered (54%), born to term (76.4%), and after medically assisted conception (75%). More children were boys (55%) and predominately non-Hispanic white (86%). The mean age at follow-up was 9.6 years (±1.9). The mean SRS *T*-score was 47.7 (±7.4), and 13 (7%) had an SRS *T*-score ≥60.

Table 1a
Parental demographic characteristics in the PEACE study.

Total	Maternal	Paternal
	n (%) or mean ± SD	*n* (%) or mean ± SD
	164 (100)	99 (100)
Parental age at conception (years)	34.4 ± 3.6	35.3 ± 4.3
Parental educational status		
High school and college	49 (29.9)	54 (54.6)
Post-graduate	115 (70.1)	45 (45.5)
Parental baseline (pre-pregnancy) BMI		
<25 kg/m2	114 (68.9)	28 (28.3)
25 to <30 kg/m2	36 (22.0)	52 (52.5)
≥30 kg/m2	13 (7.9)	19 (19.2)
Missing	*2 (1.2)*	-
Parental baseline smoking status		
Never smoker	123 (75.0)	65 (65.7)
Current/Ever smoker	41 (25.0)	34 (34.3)

Table 1b
Child demographics and pregnancy characteristics in the PEACE study (n = 195).

	Total children	Total SRS scores (T)		
		Children <60 T	Children ≥60 T	
	n (%) or mean ± SD	*n* (%) or mean ± SD	*n* (%) or mean ± SD	P-value
Total	195 (100)	182 (93.3)	13 (6.7)	
Mean SRS T-scores	47.7 ± 7.4	46.3 ± 5.1	67.8 ± 5.6	
Before March 2020, n = 68	46.9 ± 6.0	46.2 ± 4.9	63.3 ± 3.5	
After March 2020, n = 127	48.2 ± 8.1	46.4 ± 5.2	69.2 ± 5.6	
Child demographics				
Age at follow-up	9.6 ± 1.9	9.6 ± 1.8	9.3 ± 2.3	0.735
Sex assigned at birth				
Boy	108 (55.4)	99 (54.4)	9 (69.2)	0.299
Girl	87 (44.6)	83 (45.6)	4 (30.8)	
Race				
White	167 (85.6)	156 (85.7)	11 (84.6)	0.913
Other	28 (14.4)	26 (14.3)	2 (15.4)	
Parity				
Nulliparous (first born)	163 (83.6)	152 (83.5)	11 (84.6)	0.918
Multiparous	32 (16.4)	30 (16.5)	2 (15.4)	
Twins (24 sets)				
Birth year				
2006–2009	63 (32.3)	61 (33.5)	2 (15.4)	0.321
2010–2013	95 (48.7)	88 (48.4)	7 (53.9)	
2014–2017	37 (19.0)	33 (18.1)	4 (30.8)	
Pregnancy characteristics				
Gestational age at birth				
Preterm (<37 weeks)	30 (15.4)	28 (15.4)	2 (15.4)	0.998
Normal term (≥37 weeks)	149 (76.4)	139 (76.4)	10 (76.9)	
Missing	16 (8.2)	15 (8.2)	1 (7.7)	
Mode of conception				
Natural conception	48 (24.6)	46 (25.3)	2 (15.4)	0.424
Assisted conception	147 (75.4)	136 (74.7)	11 (84.6)	
Mode of delivery				
Vaginally born	105 (53.9)	98 (53.9)	7 (53.9)	0.853
Cesarian section born	81 (41.5)	76 (41.8)	5 (38.5)	
Unknown/Missing	9 (4.6)	8 (4.4)	1 (7.7)	

The highest median phthalate biomarker concentrations were observed for MEP, ΣDEHP, and MCOP across exposure windows (Table 2). All biomarkers were detected in a minimum of 75% of samples. Moderate to strong correlations were seen between individual phthalate metabolites within the same exposure window (up to 0.8) (Fig. S2). The highest correlations were generally between MCOP, MCNP, and MCPP. Correlations across exposure windows were generally weak, but correlations were consistently higher when comparing the same metabolites across windows (up to 0.65).

Generally, *maternal preconception* phthalate biomarker and BPA concentrations were not associated with SRS *T*-scores, although estimated mean changes for each $\log_e$ increase in concentrations were almost always negative and imprecise. In adjusted models, we observed an association between higher MiBP and MCOP concentrations with lower SRS *T*-scores ($\beta_{MiBP} = -1.58$, 95% CI -2.72; −0.43, $n = 179$ and $\beta_{MCOP} = -1.11$, 95% CI -2.09; −0.13, $n = 165$). In adjusted *paternal preconception* models, only MiBP was significantly associated with lower

Table 2
Limit of detection (LOD) and urinary specific-gravity standardized concentrations (μg/L) of phthalate metabolites and bisphenol A presented as medians (25-75th percentiles) according to samples from maternal pregnancy and parental preconception.

Chemicals		Max LOD[b] μg/L	Maternal preconception			Paternal preconception			Maternal pregnancy		
			n	%≥LOD	Median(25–75th p.)	*n*	%≥LOD	Median(25–75th p.)	*n*	%≥LOD	Median(25–75th p.)
monoethyl phthalate	MEP	1.2	179	100%	59 (22–132)	121	100%	31 (14–125)	177	100%	34 (16–94)
mono-n-butyl phthalate	MnBP	0.6	179	100%	13 (6.7–23)	121	99.2%	10.8 (5.6–16)	177	100%	12 (7.1–19)
mono-isobutyl phthalate	MiBP	0.8	179	97.8%	7.0 (3.9–15)	121	98.3%	6.2 (3.4–9.8)	177	98.9%	6.6 (3.9–10.6)
monobenzyl phthalate	MBzP	0.8	179	90.5%	4.1 (1.8–7.1)	121	82.6%	3.2 (1.4–6.5)	177	95.5%	3.1 (2.1–4.6)
mono(3-carboxypropyl) phthalate	MCPP	0.4	179	99.4%	3.3 (1.7–8.3)	121	99.2%	3.1 (1.6–6.0)	177	98.9%	3.4 (1.9–6.5)
monocarboxyoctyl phthalate	MCOP	0.7	165	100%	22 (9.5–67)	110	100%	25 (6.8–82)	158	100%	25 (12–52)
monocarboxyisononyl phthalate	MCNP	0.6	165	100%	4.1 (2.3–7.7)	110	98.2%	3.4 (2.0–7.1)	158	100%	3.6 (2.3–5.7)
ΣDEHP[a]					45 (25–88)			48 (28–86)	177		43 (30–85)
mono(2-ethylhexyl) phthalate	MEHP	1.2	179	79.3%	2.2 (1.2–4.0)	121	75.2%	2.5 (1.2–4.7)	177	79.7%	2.4 (1.4–4.6)
mono(2-ethyl-5-hydroxyhexyl) phthalate	MEHHP	1.0	179	100%	12 (5.9–26)	121	97.5%	13 (7.1–24)	177	100%	12 (7.4–24)
mono(2-ethyl-5-oxohexyl) phthalate	MEOHP	1.0	179	99.4%	8.1 (4.1–17)	121	95.9%	8.7 (4.3–15)	177	100%	8.7 (5.4–18)
mono(2-ethyl-5-carboxypentyl) phthalate	MECPP	0.6	179	100%	22 (12–41)	121	100%	20 (11–42)	177	100%	18 (12–38)
Bisphenol A	BPA	0.4	179	97.8%	1.3 (0.87–2.2)	121	100%	1.4 (0.87–2.5)	177	96.0%	1.2 (0.78–1.8)

[a] The molar sum of ΣDEHP was calculated by dividing each metabolite concentration by its molecular weight and then summing: ΣDEHP = [(MEHP * (1/278.34)) + (MEHHP * (1/294.34)) + (MEOHP * (1/292.33)) + (MECPP * (1/308.33))].
[b] We have reported the max LOD used during the period of chemical analyses in the samples.

SRS *T*-scores (β = −2.88, 95% CI -4.56; −1.21, *n* = 121). Other adjusted associations of paternal preconception phthalate biomarker and BPA concentrations with SRS *T*-scores suggested inverse relationships, but were not significant. In adjusted *maternal pregnancy* models, a similar pattern of results with generally imprecise and inverse associations was observed, except for MBzP (β = 0.59, 95% CI -0.85; 2.02, *n* = 177) (Table 3). The crude and adjusted models were comparable.

When analyzing the mixture of all included biomarkers and the mixture of only phthalate biomarkers, both were inversely associated with SRS *T*-scores for the preconception windows, but these were attenuated and not significant in adjusted models (Table 3). In the full sample, thereby excluding MCOP and MCNP, the mixture results for phthalate biomarkers were essentially the same, although effect estimates appeared smaller for the preconception models and slightly larger for maternal pregnancy models using the larger set of phthalate biomarkers. The individual contributions of different phthalate biomarkers to the total mixture effect differed across exposure windows (Figure S3). For maternal preconception, the largest negative contributions were from MiBP and MCOP, while for paternal preconception, MCOP and MnBP showed the most important negative contributions, with a large positive contribution from MCPP. For maternal pregnancy, MnBP and MCPP contributed with the largest negative contributions, with a positive contribution from MBzP. Notably, ΣDEHP had a positive contribution across all exposure windows.

In supplementary analyses, we observed some sex-specific differences in associations, but these were small, not statistically significant, and not consistent across biomarkers or exposure windows (Table S1). In general, maternal preconception associations appeared more negative for boys compared to girls, except for MEP and MCNP, where the patterns were opposite. For paternal preconception, the interaction *p*-value for BPA was near-significant ($P = 0.10$). For maternal pregnancy, the sex differences were less consistent and not statistically significant ($P > 0.25$). When restricting our analysis to singletons, results were not appreciably different, with only marginal differences in associations that did not show a consistent pattern, and none were statistically significant (Table S2). When investigating binary cut-offs of SRS *T*-scores, the findings generally aligned with those from our main analyses examining continuous SRS scores (Table S3). The differences in statistical significance of some associations could be due to the small sample of children in the clinical at-risk group.

In a subset of participants (*n* = 147) examining modification by maternal preconception nutrition (folate and iron), we generally observed weaker and fewer negative associations of phthalate biomarker and BPA concentrations with SRS *T*-scores with lower daily folate intake (<1780 μg/day), except for MCNP, compared with higher folate intake (≥1780 μg/day) (Fig. 2). The pattern of results for maternal iron intake was similar (using 27 mg/day as cut-offs), except for MCPP and MCNP. Consistently, associations of MnBP, MiBP, and MBzP appeared more negative in the high intake groups for both folate and iron intake compared to low intake groups. But overall, none of the interaction *P*-values were statistically significant, although few at a more liberal level of 0.2 (MBzP for folate intake: $P = 0.14$; and MCNP and ΣDEHP for iron intake: $P = 0.12$ and 0.11, respectively) (Table S4). The Johnson-Neyman plots showed positive associations between phthalate biomarkers (MnBP, MiBP, and MBzP) and SRS scores among participants with lower folate and iron intake (Figure S4). The interactions of MiBP with folate and iron were statistically significant ($P < 0.05$) when folate levels were above 1943 μg/day and when iron levels were 36–50 mg/day.

4. Discussion

In children from the prospective PEACE study, we did not find consistent evidence that preconception (maternal or paternal) or pregnancy (maternal) urinary phthalate biomarker or BPA concentrations, either individually or as a mixture, were related to offspring autistic

Table 3
Associations between total SRS scores (continuous) with one loge increase in phthalate metabolite and bisphenol A concentrations measured in urine samples from preconception (maternal and paternal) and pregnancy (maternal) in addition to mixtures assesed using quantile g-computation models.

Chemicals		Maternal preconception			Paternal preconception			Maternal pregnancy		
		Difference in SRS scores, β (95% CI)			Difference in SRS scores, β (95% CI)			Difference in SRS scores, β (95% CI)		
		n	Crude	Adjusted[a]	*n*	Crude	Adjusted[b]	*n*	Crude	Adjusted[a]
monoethyl phthalate	MEP	179	−0.57 (−1.37; 0.23)	−0.21 (−1.13; 0.72)	121	−0.54 (−1.55; 0.47)	−0.12 (−1.24; 1.00)	177	−0.30 (−1.20; 0.60)	−0.14 (−1.13; 0.85)
mono-n-butyl phthalate	MnBP	179	−0.82 (−2.03; 0.40)	−0.46 (−1.78; 0.87)	121	**−1.70 (-3.23; -0.18)**	−1.65 (−3.36; 0.07)	177	−0.68 (−2.09; 0.72)	−0.91 (−2.51; 0.70)
mono-isobutyl phthalate	MiBP	179	**−1.14 (-2.22; -0.06)**	**−1.58 (-2.73; -0.43)**	121	**−2.30 (-3.79; -0.81)**	**−2.88 (-4.56; -1.21)**	177	0.10 (−1.24; 1.44)	−0.05 (−1.60; 1.51)
monobenzyl phthalate	MBzP	179	−0.32 (−1.35; 0.71)	−0.11 (−1.21; 0.98)	121	−0.62 (−1.83; 0.58)	−1.01 (−2.29; 0.27)	177	0.52 (−0.79; 1.83)	0.59 (−0.85; 2.02)
mono(3-carboxypropyl) phthalate	MCPP	179	−0.71 (−1.68; 0.26)	−0.23 (−1.31; 0.86)	121	−0.66 (−1.65; 0.34)	−0.37 (−1.56; 0.81)	177	−0.73 (−1.76; 0.31)	−0.73 (−1.99; 0.53)
monocarboxyoctyl phthalate	MCOP	165	**−0.95 (-1.74; -0.17)**	**−1.11 (-2.09; -0.13)**	110	−0.87 (−1.77; 0.04)	−0.98 (−2.18; 0.23)	158	−0.42 (−1.42; 0.57)	−0.89 (−2.19; 0.42)
monocarboxyisononyl phthalate	MCNP	165	−0.44 (−1.53; 0.66)	0.07 (−1.13; 1.28)	110	−1.15 (−2.54; 0.24)	−0.31 (−1.89; 1.27)	158	−0.14 (−1.48; 1.20)	−0.17 (−1.69; 1.36)
ΣDEHP	179	−0.49 (−1.61; 0.62)	−0.13 (−1.60; 1.34)	121	−0.59 (−1.66; 0.48)	−0.54 (−1.90; 0.82)	177	−0.43 (−1.63; 0.77)	−0.48 (−2.06; 1.10)	
Mixtures of phthalate biomarkers	165	**−1.83 (-3.56; -0.11)**	−1.02 (−2.82; 0.78)	110	**−3.50 (-5.75; -1.25)**	−2.35 (−4.94; 0.24)	158	−0.57 (−2.38; 1.24)	−0.56 (−2.52; 1.41)	
Mixtures without MCOP and MCNP[c]		179	**−1.60 (-3.11; -0.09)**	−0.76 (−2.41; 0.89)	121	**−2.47 (-4.52; -0.42)**	−2.01 (−4.27; 0.25)	177	−0.69 (−2.32; 0.95)	−0.83 (−2.62; 0.96)
Bisphenol A	BPA	179	−0.33 (−1.83; 1.18)	0.10 (−1.55; 1.75)	121	−1.06 (−2.35; 0.22)	−0.82 (−2.21; 0.56)	177	−0.32 (−1.87; 1.23)	−0.37 (−2.18; 1.44)
Total mixtures of all chemicals	165	**−2.03 (-3.85; -0.22)**	−1.25 (−3.15; 0.64)	110	**−3.82 (-6.16; -1.47)**	−2.57 (−5.33; 0.18)	158	−0.64 (−2.51; 1.23)	−0.66 (−2.68; 1.37)	

[a] Adjusted for maternal age, maternal education, maternal BMI, maternal smoking, child sex, child age, child race, birth year, parity and mode of conception.
[b] Adjusted for paternal age, paternal education, paternal BMI, maternal smoking, child sex, child age, child race, birth year, parity and mode of conception.
[c] Mixtures of phthalates without MCOP and MCNP were assessed to maintain the full sample size. Mixtures with the inclusion of MCOP and MCOP resulted in a slightly reduced sample size.

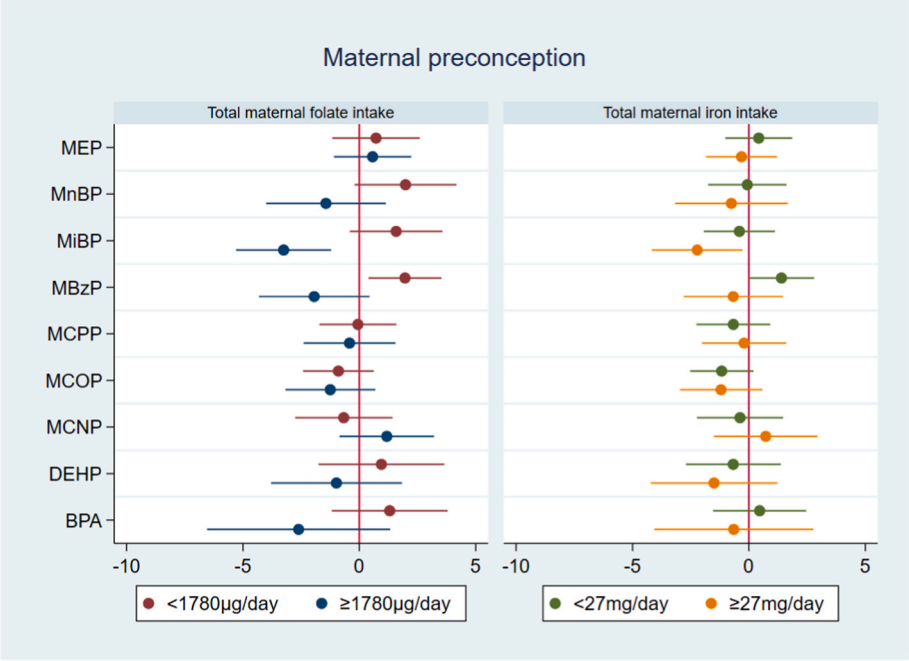

Fig. 2. Maternal preconception association models between urinary concentrations of phthalate biomarkers and bisphenol A with autistic behaviors of the offspring according to low and high intake groups of daily maternal preconception folate and iron intake, separately.

behaviors. Although the individual associations suggested imprecise inverse associations, the overall pattern of results essentially supported a null association, with the possible exception of MiBP. In our evaluation of effect modification by micronutrient intake, associations were consistently more inverse with higher levels of folate intake and, to some extent, iron intake, suggesting a potential protective effect of these micronutrients.

Our findings showing no harmful associations of phthalates and BPA throughout preconception and pregnancy on autistic behaviors in offspring are to some extent consistent with previous publications examining gestational phthalate and BPA exposure biomarkers in relation to autistic behaviors (Mustieles et al., 2023; Haggerty et al., 2021; Hyland et al., 2019; Shin et al.; Yu et al., 2024; Tsai et al., 2023). While some initial, early studies supported this association (Miodovnik et al., 2011; Larsson et al., 2009), the majority of later studies have not replicated these findings. Some studies have observed individual effects of specific biomarkers (Patti et al., 2021; Oulhote et al., 2020; Shin et al.) or associations that depended on timing of exposure biomarker assessments (Day et al., 2021), age of follow-up (Kim et al., 2021), sex of the child (Kim et al., 2021; Oulhote et al., 2020; Braun et al., 2014; Haggerty et al., 2021), or folic acid intake (Oulhote et al., 2020). The generally inverse associations observed in our study, although imprecise, contradicted our hypothesis. But interestingly, in a 2021 study by Patti et al. (2021), the authors compared associations in two separate cohorts, the Health Outcomes and Measures of the Environment (HOME) Study and the Early Autism Risk Longitudinal Investigation (EARLI) study, and observed opposite directions of findings: generally inverse associations in the EARLI study and generally positive associations in the HOME study. Similar to our study, pregnant women from EARLI had lower median phthalate biomarker concentrations and children had lower median SRS scores compared to mother-child pairs in HOME. The limited proportion of children in the at-risk SRS category in our study (7%) and in EARLI (12%) in contrast to HOME (17%), as well as lower concentrations of some phthalate metabolites as compared to HOME (e.g., median MEP: 152 ng/mL and MnBP: 26 ng/mL in HOME) may have attenuated the detection of any associations even if a causal relationship is true. Noteworthy, the two initial studies to demonstrate an association of phthalate biomarkers and autism differed from our study. The first study utilized PVC flooring as proxy of airborne phthalates (Larsson et al., 2009), while the other involved a cohort of pregnant women with significantly higher urinary concentrations of MEP (median: 372 µg/L) and MnBP (median: 33 µg/L) (Miodovnik et al., 2011). Other studies have also found inverse or null associations and explained their lack of detecting associations as due to a small sample size (Haggerty et al., 2021), or use of high-risk population with a genetic component of autism, possibly masking the associations (Patti et al., 2021; Shin et al.; Yu et al., 2024). These reasons may also apply to our study, as we were similarly hampered by a modest sample size. While we did not have an autism-enriched risk cohort by design, our study participants comprised couples with known fertility issues who possibly have certain diet and lifestyle that may make them more sensitive to exposures to BPA/phthalates or other factors relevant to this study. Alternatively, they may also have a lower use of BPA/phthalate-containing personal care products, resulting in lower exposure. Either way, BPA and phthalate concentrations in our study did not differ significantly from levels reported in most other studies.

Most previous studies did not consider mixtures, despite the fact that environmental chemicals rarely occur in isolation and the interactions of multiple chemicals, even at low doses, can induce synergistic effects (Kortenkamp, 2007, 2014). However, we did not find any robust evidence of an association in our mixture analyses. Notably, the relatively insubstantial contribution of MiBP to the total mixture for paternal preconception indicates that the statistically significant association of MiBP with SRS scores was confounded by other correlated phthalate biomarkers. The discrepancy suggests that the single pollutant model may not fully account for correlations between chemicals that are handled more effectively in mixture models and further highlight the importance of considering chemical mixtures rather than relying solely on single pollutant models. Unfortunately, we were not able to account for correlations between twin pairs in our mixture models, making the comparison of results for single pollutant and mixture models more challenging.

Indeed, we do not believe that the observed protective effect of phthalates and BPA, individually nor as a mixture, on autistic behaviors in our study represents a true association, and we speculate that the generally inverse associations we observed (i.e., higher chemical

biomarker concentrations with lower SRS scores) may be due to other factors or unknown or unmeasured confounding. To support this, our supplementary analyses revealed some effect modifications by maternal preconception folate and iron intake. On average, among mothers with higher folate or iron levels, there were stronger inverse associations of SRS scores for most phthalate biomarker concentrations (i.e., better SRS scores as compared to mothers with lower folate or iron). It is plausible that a preconception diet with sufficient intake of iron and folate plays an important protective role in early neurodevelopmental processes (Schmidt et al., 2014). Folate, in particular, is essential for DNA methylation (Crider et al., 2012) and is required for proper nucleotide biosynthesis, both of which are critical during sensitive periods of development and rapid cell growth such as during pregnancy (Miller, 2008; Irvine et al., 2022). Iron is also considered vital for many neurodevelopmental processes, albeit less is known about the specific mechanisms (Cortés-Albornoz et al., 2021). Notably, experimental evidence points to possible diet-induced alterations in methylation and gene expression (Cortés-Albornoz et al., 2021; Geraghty et al., 2015; Zhang, 2015; Green and Marsit, 2015). Therefore, maternal nutrients during pregnancy may have interactive effects on brain development, potentially mitigating any adverse effects of EDC exposures (Singh and Li, 2012). These findings are consistent with a similar study by Oulhote et al. who showed that adequate prenatal folic acid supplementation levels (≥400 μg/day) consistently and significantly attenuated the positive associations between first trimester phthalate biomarkers and SRS (Oulhote et al., 2020). The potentially modifying effects by folate are also supported by two other studies investigating air pollution and pesticides in relation to autism (Goodrich et al., 2018; Schmidt et al., 2017). Altogether, there may be opportunities for reducing any risk of autism from environmental exposures by ensuring sufficient levels of micronutrient supplementation and these results suggest that the nutritional "background" of study populations should be considered when interpreting results across studies.

The concentrations of phthalate biomarkers and BPA in our study were comparable to those reported in studies with similar sample collection periods (Shin et al.; Yu et al., 2024; Stevens et al., 2022; Arbuckle et al., 2018) and largely similar to those reported for pregnant women surveyed in NHANES cycles overlapping with ours (2005–2016). (Centers for Disease Control and Prevention (CDC)) However, the urine concentrations of selected phthalate biomarkers, specifically MEP, MnBP, and MBzP, were lower compared to pregnant women from NHANES cycle 2003–2004 (Woodruff et al., 2011), HOME (Yu et al., 2024), and Mount Sinai Children's Environmental Health Study (MSCEH) (Miodovnik et al., 2011) cohorts with earlier sample collections (from 1998 to 2006). This may reflect declining concentrations of some phthalate biomarkers in the U.S. populations, and could explain why studies using data from HOME and MSCEH were able to detect positive associations. To address this, we adjusted for birth year in all analyses.

We are unaware of other studies examining relations of preconception chemical biomarkers with autistic behaviors in offspring. Three retrospective case-control studies using occupation as a proxy of preconception chemical exposures have supported a positive association with autism risk, but are of lower confidence given the study design, sample size, and use of proxy variables (Coleman, 1976; Felicetti, 1981; McCanlies et al., 2012; Rossignol et al., 2014). Further, efforts to understand the potential influences of paternal preconception exposures on autism are lacking. We were hampered by a limited sample of participating fathers to make firm conclusions on the paternal preconception models, but other studies have suggested that potential effects of paternal environmental exposures on offspring health may be mediated by epigenetic modifications passed on to the offspring through sperm DNA (Braun et al., 2017; Feinberg et al., 2015). Of interest, two studies based on the same EARTH preconception samples have associated specific urinary preconception phthalate biomarkers with other aspects of child behavior and neurodevelopment at younger ages (Leader et al., 2024; Messerlian et al., 2017). Additional studies on chemical concentrations using preconception and paternal data can help expand our knowledge on the potentially very early environmental risk factors of autism.

We acknowledge that we explored three exposure windows separately, and disentangling the effect of paternal preconception exposure from maternal preconception or pregnancy exposure is difficult. Understanding the couple's exposure patterns and exposure sources are needed in order to identify any joint effects across windows or within couples to account for correlations because of shared environmental characteristics, particularly in light of how different exposure sources and routes may impact differently on the concentrations and subsequent associations (Braun et al., 2017).

4.1. Strengths and limitations

Our study is based on a unique cohort that provided an opportunity for studying both preconception and pregnancy urinary biomarkers of exposure with extensive data on relevant covariates. This allowed for investigating multiple windows of susceptibility for the effects of phthalates and BPA on autistic behaviors, including the understudied potential influence of paternal exposures. Adequately measuring biomarkers of exposure to non-persistent chemicals such as phthalates and BPA can be challenging due to their short biological half-life and relatively large within-person variability of concentrations (Radke et al., 2020; Verner et al., 2020). However, urine is the optimal matrix for this purpose and the multiple maternal samples per exposure window is a particular strength of our study, likely limiting exposure misclassification. The use of qgcomp models to estimate mixtures allows us to quantify mixture effects while accounting for co-pollutant correlations. Further, the SRS questionnaire is a validated tool for assessing a continuous measure of autistic behaviors and symptoms. The measure is characterized by a high sensitivity, although concerns have been raised regarding the specificity (Aldridge et al., 2012).

The modest sample size and limited number of children in the at-risk SRS group may have reduced our statistical power, particularly for the stratified analyses. In addition, our study participants included a rather selected group of women that were recruited through a fertility clinic and agreed to participate in studies both when pregnant (in EARTH) and again many years later (in PEACE). This selection is supported by the generally well-educated study participants and thus, generalizability of our findings may be restricted to people in the higher social classes. Further, our findings may not be generalized to children of parents without infertility concerns. We attempted to control for the most relevant covariates such as ART and education status, but we cannot rule out potential residual confounding as we were sometimes limited to rather broad categories of variables, e.g., maternal smoking (ever vs. never), and moreover, we were not able to adjust for parental autistic behaviors, which would have been an imperfect proxy for children's genetic risk of ASD-related behaviors. Since many SRS questionnaires were filled out in the aftermath of the COVID-19 pandemic, a plausible scenario is that this overall change in people's living situation (being more at home and alone) may have affected the way the parents answered the questions or how closely they noticed and perceived the behaviors of their children. Importantly, we did not observe any significant shift in SRS scores when comparing before and after March 2020, and a comparison of online vs. paper-based testing found no significant differences in median values (Čandrlić et al., 2014). Another limitation is the inclusion of twin sets that may introduce complexity in the interpretation of findings given the shared genetic and environmental characteristics between twins. We acknowledge the risk of spurious findings in our study, but in additional explorative analyses (on maternal psychopathology, parental physical activity, maternal BMI, etc.), we did not find any confounding or modifying effects that can explain our inverse trends and few statistically significant associations (Results not shown). We also explored models with different levels of

covariate adjustment and considered tertiles of exposure biomarkers; however, these methodological adjustments did not significantly change the results (Results not shown).

5. Conclusions

In our study, urinary concentrations of phthalate biomarkers and BPA measured during preconception (maternal and paternal) and pregnancy (maternal) were not associated with adverse autistic behaviors in the offspring measured by SRS scores. Some evidence suggested that maternal micronutrient intake modified the associations and possibly play a protective role in the development of autistic behaviors. Further, larger studies in other cohorts are needed to fully elucidate the associations, while considering interactions with maternal nutrition during pregnancy. Studies should also prioritize exploring the potential importance of the preconception period in the development of autistic behaviors.

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CRediT authorship contribution statement

Cecilie Skaarup Uldbjerg: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Jordana Leader:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Data curation, Conceptualization. **Lidia Minguez-Alarcon:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Data curation, Conceptualization. **Olivia Chagnon:** Resources, Investigation, Data curation. **Ramace Dadd:** Resources, Investigation, Data curation. **Jennifer Ford:** Resources, Investigation, Data curation. **Elvira Fleury:** Writing – review & editing, Validation, Software, Methodology. **Paige Williams:** Writing – review & editing, Validation, Methodology. **Anders Juul:** Writing – review & editing, Supervision. **David C. Bellinger:** Writing – review & editing, Validation, Methodology. **Antonia M. Calafat:** Writing – review & editing, Resources, Methodology, Investigation. **Russ Hauser:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Joseph M. Braun:** Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention (CDC). Use of trade name is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the U.S. Department of Health and Human Services. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.120253.

Data availability

The data that has been used is confidential.

References

Alampi, J.D., Lanphear, B.P., Braun, J.M., et al., 2021. Association between gestational exposure to toxicants and autistic behaviors using bayesian quantile regression. Am. J. Epidemiol. 190 (9). https://doi.org/10.1093/AJE/KWAB065.

Aldridge, F.J., Gibbs, V.M., Schmidhofer, K., Williams, M., 2012. Investigating the clinical usefulness of the social responsiveness scale (SRS) in a tertiary level , autism spectrum disorder specific assessment clinic. J. Autism Dev. Disord. 42, 294–300. https://doi.org/10.1007/s10803-011-1242-9.

Alonso-Magdalena, P., Morimoto, S., Ripoll, C., Fuentes, E., Nadal, A., 2006. The estrogenic effect of bisphenol A disrupts pancreatic beta-cell function in vivo and induces insulin resistance. Environ. Health Perspect. 114 (1), 106–112. https://doi.org/10.1289/ehp.8451.

Arbuckle, T.E., Agarwal, A., MacPherson, S.H., et al., 2018. Prenatal exposure to phthalates and phenols and infant endocrine-sensitive outcomes: the MIREC study. Environ. Int. 120 (September), 572–583. https://doi.org/10.1016/j.envint.2018.08.034.

Bakulski, K.M., Dou, J.F., Feinberg, J.I., et al., 2021. Autism-associated DNA methylation at birth from multiple tissues is enriched for autism genes in the early autism risk longitudinal investigation. Front. Mol. Neurosci. 14, 775390. https://doi.org/10.3389/fnmol.2021.775390.

Bauer, D.J., Curran, P.J., Thurstone, L.L., 2005. Probing interactions in fixed and multilevel regression: inferential and graphical techniques. Multivariate Behav. Res. 40 (3), 373–400. https://doi.org/10.1207/s15327906mbr4003_5.

Bölte, S., Poustka, F., Constantino, J.N., 2008. Assessing autistic traits: cross-cultural validation of the social responsiveness scale (SRS). Autism Res. 1 (6), 354–363. https://doi.org/10.1002/AUR.49.

Braun, J.M., 2017. Early-life exposure to EDCs: role in childhood obesity and neurodevelopment. Nat. Rev. Endocrinol. 13 (3), 161–173. https://doi.org/10.1038/NRENDO.2016.186.

Braun, J.M., Sathyanarayana, S., Hauser, R., 2013. Phthalate exposure and children's health. Curr. Opin. Pediatr. 25 (2), 247–254. https://doi.org/10.1097/MOP.0B013E32835E1EB6.

Braun, J.M., Kalkbrenner, A.E., Just, A.C., et al., 2014. Gestational exposure to endocrine-disrupting chemicals and reciprocal social, repetitive, and stereotypic behaviors in 4- and 5-year-old children: the HOME study. Environ. Health Perspect. 122 (5), 513. https://doi.org/10.1289/EHP.1307261.

Braun, J.M., Messerlian, C., Hauser, R., 2017. Fathers matter: why it's time to consider the impact of paternal environmental exposures on children's health. Curr Epidemiol reports 4 (1), 46–55. https://doi.org/10.1007/S40471-017-0098-8.

Bräuner, E.V., Uldbjerg, C.S., Lim, Y.-H., et al., 2022. Presence of parabens, phenols and phthalates in paired maternal serum, urine and amniotic fluid. Environ. Int. 158, 106987. https://doi.org/10.1016/J.ENVINT.2021.106987.

Calafat, A.M., Weuve, J., Ye, X., et al., 2009. Exposure to bisphenol A and other phenols in neonatal intensive care unit premature infants. Environ. Health Perspect. 117 (4), 639–644. https://doi.org/10.1289/ehp.0800265.

Čandrlić, S., Katić, M.A., Dlab, M.H., 2014. Online vs. Paper-Based Testing: A Comparison of Test Results. 2014 37th Int Conv Inf Commun Technol Electron Microelectron MIPRO 2014 - Proc, pp. 657–662. https://doi.org/10.1109/MIPRO.2014.6859649. Published online.

Caudill, S.P., Schleicher, R.L., Pirkle, J.L., 2008. Multi-rule quality control for the age-related eye disease study. Stat. Med. 27 (20), 4094–4106. https://doi.org/10.1002/sim.3222.

Centers for Disease Control and Prevention (CDC). National Health and Nutrition Examination Survey Data. National Center for Health Statistics (NCHS). https://www.cdc.gov/nchs/nhanes/index.htm.

Chou, K., Wright, R.O., 2006. Phthalates in food and medical devices. J. Med. Toxicol. 2 (3), 126. https://doi.org/10.1007/BF03161027.

Coleman, M., 1976. The Autistic Syndromes. North-Holland Publishing.

Constantino, J., Gruber, C., 2005. Social Responsiveness Scale. Manual.

Cortés-Albornoz, M.C., García-Guáqueta, D.P., Velez-van-Meerbeke, A., Talero-Gutiérrez, C., 2021. Maternal nutrition and neurodevelopment: a scoping review. Nutrients 13 (10). https://doi.org/10.3390/nu13103530.

Costa, H.E., Cairrao, E., 2024. Effect of bisphenol A on the neurological system: a review update. Arch. Toxicol. 98 (1), 1–73. https://doi.org/10.1007/s00204-023-03614-0.

Crider, K.S., Yang, T.P., Berry, R.J., Bailey, L.B., 2012. Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. Adv. Nutr. 3 (1), 21–38. https://doi.org/10.3945/an.111.000992.

Day, D.B., Collett, B.R., Barrett, E.S., et al., 2021. Phthalate mixtures in pregnancy, autistic traits, and adverse childhood behavioral outcomes. Environ. Int. 147. https://doi.org/10.1016/J.ENVINT.2020.106330.

deCock M, Maas YG, van de Bor M, Margot van de Bor C. Does perinatal exposure to endocrine disruptors induce autism spectrum and attention deficit hyperactivity disorders? Review. doi:10.1111/j.1651-2227.2012.02693.x.
Duty, S.M., Singh, N.P., Silva, M.J., et al., 2003. The relationship between environmental exposures to phthalates and DNA damage in human sperm using the neutral comet assay. Environ. Health Perspect. 111 (9), 1164. https://doi.org/10.1289/EHP.5756.
Ejaredar, M., Nyanza, E.C., Ten Eycke, K., Dewey, D., 2015. Phthalate exposure and childrens neurodevelopment: a systematic review. Environ. Res. 142, 51–60. https://doi.org/10.1016/J.ENVRES.2015.06.014.
Faras, H., Al Ateeqi, N., Tidmarsh, L., 2010. Autism spectrum disorders. Ann. Saudi Med. 30 (4), 295. https://doi.org/10.4103/0256-4947.65261.
Feinberg, J.I., Bakulski, K.M., Jaffe, A.E., et al., 2015. Paternal sperm DNA methylation associated with early signs of autism risk in an autism-enriched cohort. Int. J. Epidemiol. 44 (4), 1199–1210. https://doi.org/10.1093/IJE/DYV028.
Felicetti, T., 1981. Parents of autistic children: some notes on a chemical connection. Milieu Ther. 1 (1), 13–16.
Geraghty, A.A., Lindsay, K.L., Alberdi, G., McAuliffe, F.M., Gibney, E.R., 2015. Nutrition during pregnancy impacts offspring's epigenetic status-evidence from human and animal studies. Nutr. Metab. Insights 8 (Suppl. 1), 41–47. https://doi.org/10.4137/NMI.S29527.
Ghisari, M., Bonefeld Jorgensen, E.C., 2009. Effects of plasticizers and their mixtures on estrogen receptor and thyroid hormone functions. Toxicol. Lett. 189 (1), 67–77. https://doi.org/10.1016/j.toxlet.2009.05.004.
Goodrich, A.J., Volk, H.E., Tancredi, D.J., et al., 2018. Joint effects of prenatal air pollutant exposure and maternal folic acid supplementation on risk of autism spectrum disorder. Autism Res. 11 (1), 69–80. https://doi.org/10.1002/aur.1885.
Green, B.B., Marsit, C.J., 2015. Select prenatal environmental exposures and subsequent alterations of gene-specific and repetitive element DNA methylation in fetal tissues. Curr Environ Heal reports 2 (2), 126–136. https://doi.org/10.1007/s40572-015-0045-0.
Haggerty, D.K., Strakovsky, R.S., Talge, N.M., et al., 2021. Prenatal phthalate exposures and autism spectrum disorder symptoms in low-risk children. Neurotoxicol. Teratol. 83. https://doi.org/10.1016/J.NTT.2021.106947.
Hertz-Picciotto, I., Croen, L.A., Hansen, R., Jones, C.R., van de Water, J., Pessah, I.N., 2006. The CHARGE study: an epidemiologic investigation of genetic and environmental factors contributing to autism. Environ. Health Perspect. 114 (7), 1119–1125. https://doi.org/10.1289/ehp.8483.
Hornung, R.W., Reed, L.D., 1990. Estimation of average concentration in the presence of nondetectable values. Appl. Occup. Environ. Hyg 5 (1), 46–51. https://doi.org/10.1080/1047322X.1990.10389587.
Hyland, C., Mora, A.M., Kogut, K., et al., 2019. Prenatal exposure to phthalates and neurodevelopment in the CHAMACOS cohort. Environ. Health Perspect. 127 (10). https://doi.org/10.1289/EHP5165.
Irvine, N., England-Mason, G., Field, C.J., Dewey, D., Aghajafari, F., 2022. Prenatal folate and choline levels and brain and cognitive development in children: a critical narrative review. Nutrients 14 (2). https://doi.org/10.3390/nu14020364.
Jeddi, M.Z., Janani, L., Memari, A.H., Akhondzadeh, S., Yunesian, M., 2016. The role of phthalate esters in autism development: a systematic review. Environ. Res. 151, 493–504. https://doi.org/10.1016/J.ENVRES.2016.08.021.
Jenkins, T.G., Carrell, D.T., 2012. The sperm epigenome and potential implications for the developing embryo. Reproduction 143 (6), 727–734. https://doi.org/10.1530/REP-11-0450.
Jiménez-Cardozo, N., Mitsunami, M., Mínguez-Alarcón, L., et al., 2023. Iron intake in relation to ovarian reserve among women seeking infertility treatment. Hum. Reprod. 38 (8), 1613–1620. https://doi.org/10.1093/humrep/dead118.
Kadir, M., Hood, R.B., Mínguez-Alarcón, L., et al., 2022. Folate intake and ovarian reserve among women attending a fertility center. Fertil. Steril. 117 (1), 171–180. https://doi.org/10.1016/j.fertnstert.2021.09.037.
Keil, A.P., Buckley, J.P., O'Brien, K.M., Ferguson, K.K., Zhao, S., White, A.J., 2020. A quantile-based g-computation approach to addressing the effects of exposure mixtures. Environ. Health Perspect. 128 (4). https://doi.org/10.1289/EHP5838.
Kim, J.I., Lee, J., Lee, K.S., et al., 2021. Association of phthalate exposure with autistic traits in children. Environ. Int. 157. https://doi.org/10.1016/J.ENVINT.2021.106775.
Kortenkamp, A., 2007. Ten years of mixing cocktails: a review of combination effects of endocrine-disrupting chemicals. Environ. Health Perspect. 115 (Suppl. 1), 98–105. https://doi.org/10.1289/ehp.9357.
Kortenkamp, A., 2014. Low dose mixture effects of endocrine disrupters and their implications for regulatory thresholds in chemical risk assessment. Curr. Opin. Pharmacol. 19, 105–111. https://doi.org/10.1016/j.coph.2014.08.006.
La Merrill, M.A., Vandenberg, L.N., Smith, M.T., et al., 2020. Consensus on the key characteristics of endocrine-disrupting chemicals as a basis for hazard identification. Nat. Rev. Endocrinol. 16 (1), 45–57. https://doi.org/10.1038/s41574-019-0273-8.
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 (5), 822–831. https://doi.org/10.1016/J.NEURO.2009.01.011.
Leader, J., Minguez-Alarcon, L., Williams, P.L., et al., 2024. Paternal and maternal preconception and maternal pregnancy urinary phthalate metabolite and BPA concentrations in relation to child behavior. Environ. Int. 183.
Lyall, K., Croen, L., Daniels, J., et al., 2017. The changing epidemiology of autism spectrum disorders. Annu Rev Public Heal 38, 81–102. https://doi.org/10.1146/annurev-publhealth-031816-044318.
Maenner, M.J., Warren, Z., Williams, A.R., et al., 2023. Prevalence and characteristics of autism spectrum disorder among children aged 8 Years - autism and developmental disabilities monitoring network, 11 sites, United States, 2020. MMWR Surveill Summ 72 (SS-2), 1–14. https://doi.org/10.15585/mmwr.ss7202a1.
McCanlies, E.C., Fekedulegn, D., Mnatsakanova, A., et al., 2012. Parental occupational exposures and autism spectrum disorder. J. Autism Dev. Disord. 42 (11), 2323–2334. https://doi.org/10.1007/s10803-012-1468-1.
Meeker, J.D., Sathyanarayana, S., Swan, S.H., 2009. Phthalates and other additives in plastics: human exposure and associated health outcomes. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364 (1526), 2097–2113. https://doi.org/10.1098/RSTB.2008.0268.
Messerlian, C., Bellinger, D.C., Minguez-Alarcon, L., et al., 2017. Paternal and maternal preconception urinary phthalate metabolite concentrations and child behavior. Env Res 158, 720–728. https://doi.org/10.1016/j.envres.2017.07.032.
Messerlian, C., Williams, P.L., Ford, J.B., et al., 2018. The environment and reproductive health (EARTH) study: a prospective preconception cohort. Hum Reprod Open 2018 (2), 1–11. https://doi.org/10.1093/HROPEN/HOY001.
Miller, A.L., 2008. The methylation, neurotransmitter, and antioxidant connections between folate and depression. Altern Med Rev 13 (3), 216–226.
Miodovnik, A., Engel, S.M., Zhu, C., et al., 2011. Endocrine disruptors and childhood social impairment. Neurotoxicology 32 (2), 261–267. https://doi.org/10.1016/J.NEURO.2010.12.009.
Mose, T., Mortensen, G.K., Hedegaard, M., Knudsen, L.E., 2007. Phthalate monoesters in perfusate from a dual placenta perfusion system, the placenta tissue and umbilical cord blood. Reprod. Toxicol. 23 (1), 83–91. https://doi.org/10.1016/j.reprotox.2006.08.006.
Mustieles, V., Rolland, M., Pin, I., et al., 2023. Early-life exposure to a mixture of phenols and phthalates in relation to child social behavior: applying an evidence-based prioritization to a cohort with improved exposure assessment. Environ. Health Perspect. 131 (8). https://doi.org/10.1289/EHP11798.
National Institutes of Health, 2022. Folate: fact sheet for health professionals. National Institutes of Health (NIH) Office of Dietary Supplements (ODS). https://ods.od.nih.gov/factsheets/Folate-HealthProfessional/. (Accessed 23 April 2024).
National Institutes of Health, 2023. Iron: fact sheet for health professionals. National Institutes of Health (NIH) Office of Dietary Supplements (ODS). https://ods.od.nih.gov/factsheets/Iron-HealthProfessional/. (Accessed 23 April 2024).
Neggers, Y.H., 2014. Increasing prevalence, changes in diagnostic criteria, and nutritional risk factors for autism spectrum disorders. ISRN Nutr. Published online 1–14. https://doi.org/10.1155/2014/514026.
Oulhote, Y., Lanphear, B., Braun, J.M., et al., 2020. Gestational exposures to phthalates and folic acid, and autistic traits in Canadian children. Environ. Health Perspect. 128 (2). https://doi.org/10.1289/EHP5621.
Patti, M.A., Newschaffer, C., Eliot, M., et al., 2021. Gestational exposure to phthalates and social responsiveness scores in children using quantile regression: the EARLI and HOME studies. Int J Environ Res Public Health 18 (3), 1–17. https://doi.org/10.3390/IJERPH18031254.
Ponsonby, A.L., Symeonides, C., Saffery, R., et al., 2020. Prenatal phthalate exposure, oxidative stress-related genetic vulnerability and early life neurodevelopment: a birth cohort study. Neurotoxicology 80, 20–28. https://doi.org/10.1016/J.NEURO.2020.05.006.
Radke, E.G., Braun, J.M., Nachman, R.M., Cooper, G.S., 2020. Phthalate exposure and neurodevelopment: a systematic review and meta-analysis of human epidemiological evidence. Environ. Int. 137. https://doi.org/10.1016/J.ENVINT.2019.105408.
Rossignol, D.A., Genuis, S.J., Frye, R.E., 2014. Environmental toxicants and autism spectrum disorders: a systematic review. Transl. Psychiatry 4 (2). https://doi.org/10.1038/tp.2014.4.
Sathyanarayana, S., 2008. Phthalates and children's health. Curr. Probl. Pediatr. Adolesc. Health Care 38 (2), 34–49. https://doi.org/10.1016/J.CPPEDS.2007.11.001.
Schettler, T., 2006. Human exposure to phthalates via consumer products. Int. J. Androl. 29 (1), 134–139. https://doi.org/10.1111/J.1365-2605.2005.00567.X.
Schmidt, R.J., Tancredi, D.J., Krakowiak, P., Hansen, R.L., Ozonoff, S., 2014. Maternal intake of supplemental iron and risk of autism spectrum disorder. Am. J. Epidemiol. 180 (9), 890–900. https://doi.org/10.1093/aje/kwu208.
Schmidt, R.J., Kogan, V., Shelton, J.F., et al., 2017. Combined prenatal pesticide exposure and folic acid intake in relation to autism spectrum disorder. Env Heal Perspect 125 (9), 097007.
Schroeder, D.I., Schmidt, R.J., Crary-Dooley, F.K., et al., 2016. Placental methylome analysis from a prospective autism study. Mol. Autism. 7, 51. https://doi.org/10.1186/s13229-016-0114-8.
Schug, T.T., Blawas, A.M., Gray, K., Heindel, J.J., Lawler, C.P., 2015. Elucidating the links between endocrine disruptors and neurodevelopment. Endocrinology 156 (6), 1941–1951. https://doi.org/10.1210/EN.2014-1734.
Schwartzer, J.J., Koenig, C.M., Berman, R.F., 2013. Using mouse models of autism spectrum disorders to study the neurotoxicology of gene-environment interactions. Neurotoxicol. Teratol. 36, 17–35. https://doi.org/10.1016/j.ntt.2012.08.007.
Shin H-M, Schmidt RJ, Tancredi D, et al. Prenatal Exposure to Phthalates and Autism Spectrum Disorder in the MARBLES Study. doi:10.1186/s12940-018-0428-4.
Silva, M.J., Samandar, E., Preau, Jr.JL., Reidy, J.A., Needham, L.L., Calafat, A.M., 2007. Quantification of 22 phthalate metabolites in human urine. J. Chromatogr. B 860 (1), 106–112.
Singh, S., Li, S.S.-L., 2012. Epigenetic effects of environmental chemicals bisphenol A and phthalates. Int. J. Mol. Sci. 13 (8), 10143–10153. https://doi.org/10.3390/ijms130810143.
Stevens, D.R., Bommarito, P.A., Keil, A.P., et al., 2022. Urinary phthalate metabolite mixtures in pregnancy and fetal growth: findings from the infant development and the environment study. Environ. Int. 163 (December 2021), 107235. https://doi.org/10.1016/j.envint.2022.107235.

Testa, C., Nuti, F., Hayek, J., et al., 2012. Di-(2-ethylhexyl) phthalate and autism spectrum disorders. ASN Neuro 4 (4), 223–229. https://doi.org/10.1042/AN20120015.

Tsai, T.L., Hsieh, C.J., Wu, M.T., Chen, M.L., Kuo, P.H., Wang, S.L., 2023. Co-exposure to toxic metals and phthalates in pregnant women and their children's mental health problems aged four years — taiwan Maternal and Infant Cohort Study (TMICS). Environ. Int. 173 (February), 107804. https://doi.org/10.1016/j.envint.2023.107804.

Vandenberg, L.N., Hauser, R., Marcus, M., Olea, N., Welshons, W.V., 2007. Human exposure to bisphenol A (BPA). Reprod. Toxicol. 24 (2), 139–177. https://doi.org/10.1016/J.REPROTOX.2007.07.010.

Verner, M.A., Salame, H., Housand, C., et al., 2020. How many urine samples are needed to accurately assess exposure to non-persistent chemicals? The biomarker reliability assessment tool (brat) for scientists, research sponsors, and risk managers. Int J Environ Res Public Health 17 (23), 1–10. https://doi.org/10.3390/ijerph17239102.

Wang, Y., Zhu, H., Kannan, K., 2019. A review of biomonitoring of phthalate exposures. Toxics 7 (2). https://doi.org/10.3390/TOXICS7020021.

Woodruff, T.J., Zota, A.R., Schwartz, J.M., 2011. Environmental chemicals in pregnant women in the United States: NHANES 2003-2004. Environ. Health Perspect. 119 (6), 878–885. https://doi.org/10.1289/EHP.1002727.

Ye, X., Kuklenyik, Z., Needham, L.L., Calafat, A.M., 2005. Automated on-line column-switching HPLC-MS/MS method with peak focusing for the determination of nine environmental phenols in urine. Anal. Chem. 77 (16), 5407–5413. https://doi.org/10.1021/ac050390d.

Yu, E.X., Braun, J.M., Lyall, K., et al., 2024. A mixture of urinary phthalate metabolite concentrations during pregnancy and offspring social responsiveness scale scores. Epidemiology 35 (1), 84–93. https://doi.org/10.1097/EDE.0000000000001682.

Yuan, C., Spiegelman, D., Rimm, E.B., et al., 2017. Validity of a dietary questionnaire assessed by comparison with multiple weighed dietary records or 24-hour recalls. Am. J. Epidemiol. 185 (7), 570–584. https://doi.org/10.1093/aje/kww104.

Yuan, C., Spiegelman, D., Rimm, E.B., et al., 2018. Relative validity of nutrient intakes assessed by questionnaire, 24-hour recalls, and diet records as compared with urinary recovery and plasma concentration biomarkers: findings for women. Am. J. Epidemiol. 187 (5), 1051–1063. https://doi.org/10.1093/aje/kwx328.

Zeidan, J., Fombonne, E., Scorah, J., et al., 2022. Global prevalence of autism: a systematic review update. Autism Res. 15 (5), 778–790. https://doi.org/10.1002/AUR.2696.

Zhang, N., 2015. Epigenetic modulation of DNA methylation by nutrition and its mechanisms in animals. Anim Nutr (Zhongguo xu mu shou yi xue hui). 1 (3), 144–151. https://doi.org/10.1016/j.aninu.2015.09.002.

Zhang, Y., Wang, B., Sun, W., et al., 2024. Paternal exposures to endocrine-disrupting chemicals induce intergenerational epigenetic influences on offspring: a review. Environ. Int. 187, 108689. https://doi.org/10.1016/j.envint.2024.108689.

Zhu, Y., Gomez, J.A., Laufer, B.I., et al., 2022. Placental methylome reveals a 22q13.33 brain regulatory gene locus associated with autism. Genome Biol. 23 (1), 46. https://doi.org/10.1186/s13059-022-02613-1.