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Prenatal and early childhood exposure to phthalates and childhood behavior at age 7 years

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ABSTRACT

Background: Emerging evidence suggests that phthalate exposure may be associated with behavior problems in children and that these associations may be sex specific.

Methods: In a follow up study of 411 inner-city minority mothers and their children, mono-n-butyl phthalate (MnBP), monobenzyl phthalate (MBzP), monoisobutyl phthalate (MiBP), monethyl phthalate (MEP) and four di-2-ethylhexyl phthalate metabolites (DEHP) were quantified in maternal urine samples collected during the third trimester and in child urine samples at ages 3 and 5 years. The Conners' Parent Rating Scale-Revised: Long Form (CPRS) and Child Behavior Checklist (CBCL) were administered to the mothers to assess children's behavior problems at 7 years of age. The analysis included children with available measures of CBCL, CPRS and phthalates measured in maternal urine. We performed both Quasi-Poisson regression and a mixture analysis using Weighted Quantile Sum(WQS) regression to assess the risk for CPRS scores and for internalizing and externalizing behaviors (from the CBCL) following intra-uterine exposure to the phthalate metabolites for boys and girls separately.

Results: Among boys, increases in in anxious-shy behaviors were associated with prenatal exposure to MBzP (Mean Ratio [MR] = 1.20, 95%CI 1.05–1.36) and MiBP (Mean Ratio (MR) = 1.22, 95%CI 1.02–1.47). Among girls, increases in perfectionism were associated with MBzP (MR = 1.15, 95%CI 1.01–1.30). In both boys and girls, increases in psychosomatic problems were associated with MiBP (MR = 1.28, 95%CI 1.02–1.60), and MnBP (MR = 1.28, 95%CI 1.02–1.59), respectively. Among girls, decreased hyperactivity was associated with two DEHP metabolites, mono(2-ethyl-5-oxohexyl) phthalate (MR = 0.83, 95%CI 0.71–0.98) and mono(2-ethyl-5-hydroxyhexyl) phthalate (MR = 0.85, 95%CI 0.72–0.99). Using weighted Quantile Sum logistic regression, no associations were found between the Weighted Quantile Sum (WQS) of phthalate metabolites and CPRS scores or externalizing and internalizing behaviors. Nonetheless, when the analysis was performed separately for DEHP and non-DEHP metabolites significant associations were found between the WQS of DEHP metabolites and social problems in boys (OR = 2.15, 95%CI 1.13–4.06, p-value = 0.02) anxious-shy problems in girls (OR = 2.19, 95%CI 1.15–4.16, p = 0.02), and emotional lability problems in all children (OR = 0.61, 95%CI 0.38–0.97, p = 0.04). MEHP and MEOHP were the most highly weighted DEHP metabolites in WQS mixture. The analysis performed with CBCL scale corroborated these associations.

Conclusion: Concentration of non-DEHP metabolites was associated with anxious-shy behaviors among boys. DEHP phthalate metabolites were associated with decreased hyperactivity and impulsivity among girls on CPRS

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scores. These findings lend further support to the adverse associations between prenatal phthalate exposure and childhood outcomes, and clearly suggest that such associations are sex and mixture specific.

1. Introduction

A growing body of evidence suggests that phthalates, a family of high-volume synthetic chemicals (Sathyanarayana, 2008), have adverse effects on human health. Due to their ubiquitous nature, exposure to phthalates is almost inevitable (Centers for Disease Control and Prevention, 2019). Phthalate exposure during pregnancy in humans is well-documented (Woodruff et al., 2011); phthalate metabolites are readily detected in the urine of pregnant women (Adibi et al., 2003; Woodruff et al., 2011), and have been also detected in amniotic fluid (Jensen et al., 2015, 2012). Some phthalate metabolites pass through the placenta and enter fetal circulation (Adibi et al., 2010; Mose et al., 2007). The fetus is particularly vulnerable to environmental toxicants as it undergoes rapid growth and brain development (Rice and Barone 2000). Although most brain development occurs prior to birth, maturation processes in the brain, such as myelination, synapse formation, synapse pruning, and refining connectivity continue throughout childhood (de Graaf-Peters and Hadders-Algra 2006; Lesser and Pope 2007). Several studies find associations between early life exposure to phthalates and neurobehavioral deficits in children (Braun et al., 2014; Ejaredar et al., 2015; Factor-Litvak et al., 2014; Gascon et al., 2015; Miodovnik et al., 2011; Whyatt et al., 2012). Thus, biological plausibility and empirical evidence suggest that prenatal and early postnatal exposure to phthalates may have adverse consequences for child development.

Phthalates are endocrine disruptor chemicals (EDC), and likely operate through multiple mechanisms including perturbations of testosterone homeostasis (Bellinger 2013; Braun et al., 2014). Experimental data on rodents indicate that phthalates can disrupt sex-specific development. During fetal rat development, phthalate exposure inhibits testosterone production in males by affecting the size and number of fetal Leydig cells resulting in genital abnormalities (Howdeshell et al., 2008; Wang et al., 2017). Female rats exposed prenatally to phthalates also have reproductive malformations, albeit at lower rates than male rats (Hannas et al., 2013; Mylchreest et al., 1998). Several cohort studies, and a meta-analysis suggest that in utero exposure to phthalates decreases anogenital distance and impairs penile development among boys (Chiu et al., 2018; Wineland et al., 2018; Swan et al., 2005; Suzuki et al., 2012; Sathyanarayana et al., 2015). These sex-specific reproductive alterations observed after prenatal exposure to phthalates provide evidence that prenatal phthalate exposure may also disrupt behavior in a sex dependent manner (Swan et al., 2010). Since prenatal testosterone masculinizes the human reproductive system and the brain (Swaab, 2007; Wilson and Davies, 2007), prenatal exposure to phthalates may be associated with sex-specific behavioral alterations.

To date seventeen published studies, in thirteen birth cohorts examined associations between prenatal phthalate exposure and child behavior (Jankowska et al., 2019a, 2019b; Braun et al., 2014; Engel et al., 2010; Gascon et al., 2015; Kim et al., 2018; Kobrosly et al., 2014; Lien et al., 2015; Messerlian et al., 2017; Minatoya et al., 2018; Miodovnik et al., 2011; Percy et al., 2016; Philippat et al., 2017; Singer et al., 2017; Stroustrup et al., 2018; Swan et al., 2010; Whyatt et al., 2012). Although there are inconsistencies in findings, taken together the evidence suggests the presence of sex-specific associations between specific prenatal concentrations of phthalate metabolites and specific behavior outcomes, with associations varying by child age at assessment.

To better understand the relationship between phthalates and child behavior, we expand our previous results (Whyatt et al., 2012) to evaluate associations between prenatal and postnatal (child ages 3 and 5 years) exposure to phthalates and subsequent behavior problems reported by the mother at child age 7 years. We *a priori* posit that the associations will vary by sex of the child.

2. Method

2.1. Study design

Our study population derives from the Columbia Center for Children's Environmental Health (CCCEH) longitudinal birth cohort of 727 pregnant women who delivered between 1998 and 2006. Enrollment and exclusion criteria have been described previously (Perera et al., 2003). The CCCEH cohort was restricted to nonsmoking women 18–35 years of age who self-identified as either African American or Dominican and who had resided in northern Manhattan or the South

Bronx in New York City for at least 1 year at the time of recruitment. Women were excluded if they used illicit drugs, had diabetes, hypertension, or known HIV, or had their first prenatal visit after the 20th week of pregnancy. The institutional review boards at the Columbia University Medical Center and the Centers for Disease Control and Prevention (CDC) approved the study. Informed consent was obtained from all participating mothers, and written informed assent was obtained from all children starting at age 7 years.

Women were included in this analysis if phthalate metabolite concentrations had been measured in spot urine sample collected during pregnancy and if the women completed either the Conners' Parent Rating Scale (CPRS) (Conners, 1997) or the Child Behavior Checklist (CBCL) (Achenbach and Rescorla, 2001) when the child was 7 years of age. The eligibility criteria yielded a total sample of 322 mother–child pairs which were included in the primary analysis. After applying the same eligibility criteria for the analysis of postnatal exposure to phthalates, 234 mother–child pairs at age 3 and 293 at age 5 were included in the secondary analysis. The subjects included in this report were similar to the ineligible CCCEH subjects with respect to basic demographic characteristics (maternal race/ethnicity, maternal age, prenatal marital status, education level, household income, proportion on Medicaid), maternal demoralization assessed at the prenatal interview (Dohrenwend et al., 1978), child sex, gestational age, and birth weight. Moreover, the participating children were also similar to the ineligible children with respect to the mean Child Behavior Checklist scores and the mean Bayley Scales of Infant Development II assessed at the age 3–year visit (Whyatt et al., 2012).

2.2. Urine sample collection and phthalate metabolite measurements

Spot urine samples were collected during the third trimester of pregnancy (mean ± SD: 34.0 ± 3.0 weeks; median 33.9 weeks). We also collected child spot urine samples at the age 3 (n = 240) and age 5 years (n = 275) visits. The urinary phthalate metabolite concentrations were measured at the CDC as previously described (Kato et al., 2005). Each analytical run included calibration standards, reagent blanks, and quality control samples. To correct for urinary dilution, we used specific gravity (Hauser et al., 2004), measured with a handheld refractometer (Atago PAL 10-S; Atago U.S.A. Inc., Bellevue, WA).

In this study we focus on the following phthalate metabolites: mono-n-butyl phthalate (MnBP), monobenzyl phthalate (MBzP), mono-*iso*-butyl phthalate (MiBP), the main metabolites of di-n-butyl phthalate (DnBP), butylbenzyl phthalate (BBzP) and di-isobutyl phthalate (DiBP), respectively; monoethyl phthalate (MEP), the metabolite of diethyl

phthalate (DEP) and several other high molecular weight phthalates, and the sum of four metabolites of DEHP [ΣDEHP; mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), and mono-2-ethyl-5-carboxpentyl phthalate (MECPP)].

For concentrations of phthalate metabolites below the limit of detection (LOD), we assigned a value of LOD/2 (Hornung and Reed, 1990) as we have done previously (Factor-Litvak et al., 2014). We also calculated the sum of molar concentrations for both DEHP and non-DEHP metabolites by converting concentrations (in ng/mL) into their respective molar concentrations (Meeker and Ferguson, 2014). To assess reliability, we calculated intraclass correlation coefficients (ICCs) for the phthalate metabolites in serial spot urine samples collected biweekly from 48 women in the CCCEH cohort over 6–8 weeks late in pregnancy (n = 135 samples, 2 to 4 repeats per woman). Adjusting for specific gravity, the ICCs were 0.77 for MBzP, 0.65 for MnBP, and 0.60 for MiBP and ranged from 0.27 to 0.42 for the DEHP metabolites (Whyatt et al., 2012).

2.3. Measures of child behavior outcomes

Trained research assistants administered two behavior surveys, the CPRS (Conners, 1997) and the CBCL (Achenbach and Rescorla, 2001), to the mothers in English or Spanish, depending on the mother's language of choice. The CPRS is a comprehensive standardized checklist for obtaining parental reports of behaviour problems among children aged 3–17 years. The instrument is used extensively to assess general psychopathology. It contains 80 five-point Likert items (0 = not true at all; 4 = very much true) assessing 7 factors: oppositional, cognitive problems/inattention, hyperactivity, anxious/shy, perfectionism, social problems, and psychosomatic and provides 14 subscale scores; higher scores indicate more problems. The CPRS has good internal consistency, test–retest reliability, and construct validity (Conners et al., 1998). Externalizing and attention problem behaviors were characterized by the following subscales: oppositional, cognitive problems, hyperactivity, attention deficit hyperactivity disorder (ADHD) index, global restless/impulsive and global index total, DSM-IV inattentive, DSM-IV hyperactive/impulsive scale, and DSM-IV index total. Internalizing behavior problems were characterized by: anxious/shy, perfectionism, social problems, psychosomatic problems, and emotional lability.

The CBCL (Achenbach and Rescorla, 2001) was administered as a complementary instrument. The CBCL contains 118 Likert-point items (0 = Not True; 1 = Somewhat or Sometimes true; 2 = Very True or Often True) and contains 9 subscales: Anxious/depressed, withdrawn/depressed, somatic problems, thought problems, attention problems, rule-breaking behavior, aggressive behavior and other problems. The CBCL provides scores for internalizing (Sum of Anxious/depressed, Withdrawn/depressed, Somatic problems scores) and externalizing behaviors (Sum of rule-breaking behavior, aggressive behavior) and total problems (sum of all subscales). As is the case with the CPRS, higher scores on the CBCL suggest more problems. Several CBCL subscales correlate with CPRS subscales (Achenbach and Ruffle, 2000; Conners et al., 1998). The CBCL subscale raw scores were analyzed as counts. The CBCL was completed by 322 mothers of whom 303 (95%) also completed the CPRS.

2.4. Covariates

We obtained information on potential confounders using questionnaires administered to the mother during pregnancy and at postnatal intervals and by review of maternal and infant medical records. Variables of interest included maternal race/ethnicity, maternal education, marital status, household income, parity, child sex, gestational age, birth weight, breastfeeding, prenatal exposure to tobacco smoke in the home, prenatal alcohol consumption, and prenatal psychosocial factors including maternal self-report of hardship during pregnancy, and satisfaction with overall living conditions. Maternal demoralization at the child's age 7 years visit was measured by the 27-item Psychiatric Epidemiology Research Instrument-Demoralization Scale (Dohrenwend et al., 1978). For seven women who were missing measures of maternal demoralization at child age 7 years, we substituted maternal demoralization measured at child age 5 years, because the two demoralization scores were strongly correlated (Spearman correlation coefficient $r = 0.68$, $p < 0.0001$). The quality of proximal care-taking environment was measured by the Home Observation for Measurement of the Environment (HOME) scale at mean child age 39.2 ± 7.1 months (n = 312) (Caldwell and Bradley, 1979; Caldwell et al., 1984). Twenty mother–child pairs were missing HOME scale scores. These values were imputed using a linear regression model maternal race/ethnicity, education and IQ, and household income as predictors (model $R^2 = 0.13$; n = 310). Maternal intelligence was assessed in the postnatal period using the Test of Non-Verbal Intelligence, third edition (Brown et al., 1990), a language-free measure of general intelligence, which is relatively stable and free of cultural bias.

The Conners' Adult ADHD Rating Scale (CAARS), a quantitative measure of current ADHD symptomatology in adults (Conners et al., 1999), was used to control for maternal ADHD symptoms. The CAARS is a reliable and valid measure of current adult ADHD symptomatology (Erhardt et al., 1999). Among the 8 subscales of the CAARS, we chose Inattention/Memory subscale as the control variable since it was more correlated with the CPRS items than the other CAARS subscales.

2.5. Statistical analysis

Summary statistics were calculated to describe the sample characteristics. The Chi-square and the Wilcoxon rank sum tests were used to compare included and excluded observations. To evaluate whether exposure patterns in the sample represented exposure in the United States, we compared the prenatal phthalate metabolite concentrations measured in the CCCEH study population to those measured in girls of reproductive age and pregnant women who participated in the National Health and Nutrition Examination Survey (NHANES) (CDC/NCHS NCHS (Centers for Disease Control and Prevention/ National Center for Health Statistics) 2019) in 1999–2000, 2001–2002, 2003–2004, and 2005–2006. We used publicly accessible data from NHANES 1999–2006 to calculate the geometric mean concentrations and 95% confidence intervals (CI). To account for the sampling scheme, we used the recommended techniques to estimate the variances (National Center for Health Statistics, 2011).

Scores on the CPRS and CBCL were considered as count data, as mothers indicated whether or not their child exhibited the problem.

Data analysis was performed in two consecutives stages for both prenatal and postnatal models: First, we used Quasi-Poisson regression models for count data with a logarithmic link function to connect outcome mean with a predictor of exposure and covariates, in order to assess the associations between each individual metabolite and behavioral outcomes. We also examined the associations between behavioral outcomes and the sum of molar concentrations of both DEHP and non-DEHP metabolites separately. This model results in estimates of the Mean Ratio (MR) of outcome score per unit increase in the predictor with 95% Confidence interval was derived from the coefficient of exposure variable. These analyses were performed for single phthalate metabolite concentrations, without adjustment for concentrations of other phthalate metabolites.

Second, recognizing that the concentrations of the different metabolites were positively correlated, we performed mixture analysis using logistic Weighted Quantile sum (WQS) regression models. These models considered three mixtures: all phthalate metabolites, DEHP metabolites and non-DEHP metabolites. Results of the WQS regression also indicates the relative importance of individual metabolites related to specific outcomes, and thus identifies what is called the 'bad actors'. In this analysis outcomes were not normally distributed, therefore we

modeled dichotomous variables of the studied outcomes, comparing values above to values below the median of the scale.

Since previous studies reported a different pattern of brain development between boys and girls, both the Quasi-Poisson regression and the WQS regression models were stratified by sex as in our a priori hypotheses. Because the phthalate metabolite's distributions were right skewed, we used a logarithmic transformation to reduce the influence of extreme values and improve model fitting.

Covariates in all the prenatal and postnatal models were identical. We chose potential confounders based on the previous literature along with a directed acyclic graph (DAG), to identify the minimal set. We considered in the DAG all variables associated with phthalate exposure and with the CPRS or the CBCL (Chen et al., 2007; Gaysina et al., 2013; Perez-Lobato et al., 2016; Roy et al., 2011). The final regression models contained the following covariates: maternal race/ethnicity, maternal demoralization at child age 7 years, child age at time of CPRS or CBCL assessment, prenatal specific gravity, and CAARS inattention/memory (for CPRS outcomes only). All analyses were performed using MASS and gWQS packages in R.

3. Results

The prenatal and postnatal concentrations of the log transformed phthalate metabolites, adjusted for specific gravity, are presented in Table 1, Fig. 1 and Appendixes 1a-2b. The metabolites of both DEHP and non-DEHP phthalates were highly correlated with Spearman correlation coefficient (r) ranging from 0.36 to 0.98 (Fig. 2, Appendix 3). The DEHP and nonDEHP metabolites of phthalates measured at age 3 and age 5 were also highly correlated (Appendixes 4 and 5) The sum of molar concentration of DEHP metabolites was correlated with the sum of molar concentration of non-DEHP metabolites with r = 0.46(data not shown).

Of the 322 pregnancies included in the analysis, 169 (52.5%) resulted in female offspring. Approximately two thirds of mothers were married, 40% had high school education, and 30% consumed alcohol. All the women were not smoking by definition to be included in CCCEH cohort. Maternal and children's characteristics were similar among included and excluded observations except that children not eligible for this analysis were older (Appendix 6).

Comparisons of the phthalate metabolite geometric means from the current study with those from NHANES for years 1999–2006 are summarized in Table 1. The geometric mean concentrations of MEHP, MBzP, and MnBP were significantly higher in CCCEH pregnant women compared to those in the NHANES pregnant women or women of reproductive age (18–40 years). For MEHHP, the geometric mean concentration was similar between the CCCEH and the NHANES women.

Appendix 7 presents a comparison of CPRS and CBCL scores and of phthalate metabolites concentrations between boys and girls. Boys had significantly higher scores on scales of emotional lability, oppositional and cognitive problems. Also, hyperactivity, impulsivity, inattention and total ADHD scores were also higher among boys in both CPRS and CBCL scores.

3.1. CPRS scores at age 7 years

Among girls, MEHP, MEOHP and MEHHP were associated with lower scores of hyperactivity and impulsivity (MR = 0.86, 95%CI 0.76–0.97; MR = 0.87, 95%CI 0.76–1.01, p = 0.06 and MR = 0.90, 95%CI 0.78–1.03, p = 0.12 respectively). No associations were found between prenatal phthalate metabolites concentrations and inattention scores or the total ADHD score (Fig. 3).

3.1.1. Internalizing behaviors at age 7 years

The mean ratios for internalizing behaviors for one unit increase in the log-transformed concentration of phthalate metabolites are presented in Fig. 4. We found increased concentrations of MBzP and MiBP to be associated with anxious- shy behavior in boys (MR = 1.20, 95%CI 1.05–1.36) and (MR = 1.22, 95%CI 1.02–1.47), respectively). Higher concentrations of MBzP (MR = 1.15, 95%CI 1.01–1.30) were associated with higher scores of perfectionism among girls. Higher concentrations of MiBP (MR = 1.28, 95%CI 1.02–1.60) and MnBP (MR = 1.28, 95%CI 1.02–1.59) were associated with higher scores on psychosomatic problems for boys and girls, respectively. Higher concentrations of MnBP (MR = 1.34, 95%CI 0.99–1.82) and MEHP (MR = 1.35, 95%CI 1.07–1.7) were associated with social problems among boys but not girls.

3.1.2. Externalizing and attention related behaviors at age 7 years

Fig. 5 describes the associations between phthalate metabolite concentrations and externalizing behaviors. No associations were found between any prenatal phthalate metabolite and scores of attention related and externalizing behaviors among boys. Higher concentrations of MEOHP (MR = 0.83, 95%CI 0.71–0.98), MEHHP (MR = 0.85, 95%CI 0.72–0.99), MECPP (MR = 0.84, 95%CI 0.70–1.01, p = 0.09) and MEHP (MR = 0.84, 95%CI 0.73–0.95) were associated with lower scores of hyperactivity among girls. Higher concentrations of MEHP

Table 1
Urinary phthalate metabolite concentrations (ng/mL) in CCCEH women during the third trimester of pregnancy (n = 322), and comparison with females from NHANES.

		CCCEH Study 1999–2006			NHANES Females 1999–2006	
					Aged 18–40 years[b]	Pregnant[c]
Metabolite	**LOD[a]**	**Percent > LOD[a]**	**Range**	**Geometric mean (95% CI)**	**Geometric mean(95% CI)**	
ΣDEHP			21.3–18,652	294.7 (260.0, 334.0)	NA	NA
MEHP	1.20	83.6	< LOD to 613	5.2 (4.5, 6.0)	3.5 (3.2, 3.9)**	3.9 (3.1, 4.9)*
MEHHP	0.70	100	1.1–1,750	22.1 (19.3, 25.4)	22.9 (20.3, 25.9)	19.6 (14.9, 25.8)
MEOHP	0.70	100	0.7–1,320	18.4 (16.1, 21.0)	15.7 (13.9, 17.6)	15.9 (12.4, 20.5)
MECPP	0.60	100	3.0–1,840	39.1 (34.7, 44.1)	NA	NA
Non-DEHP						
MBzP	0.22	99.7	< LOD to 550	13.3 (11.5, 15.4)	9.8 (8.9, 10.8)**	9.2 (7.2, 11.6)*
MiBP	0.30	99.7	< LOD to 374	9.1 (8.1, 10.2)	4.3 (4.0, 4.7)	3.2 (2.6, 4.0)
MnBP	0.60	100	1.2–1,110	37.4 (33.3, 42.0)	23.1 (21.4, 25.0)	18.5 (14.9, 22.9)**
MEP	0.53	100	7.8–6,046	160.4 (140.4, 183.2)	152.9 (135.7, 172.3)	123.7 (35.6, 150.0)
MCPP	0.20	94.5	< LOD–32.7	2.0 (1.8, 2.2)	2.2 (2.0, 2.5)	1.8 (1.4, 2.3)

*p < 0.05; **p < 0.00001 indicating differences between CCCEH and NHANES females.
[a] LOD is the limit of detection in ng/mL.
[b] Females 18–40 years of age; n = 1686 for MEHP, MnBP, MBzP, and MEP; n = 1270 for MEHHP, MiBP, MEOHP, and MCPP.
[c] Pregnant females; n = 416 for MEHP, MnBP, MBzP, and MEP; n = 311 for MEHHP, MiBP, MEOHP, and MCPP.

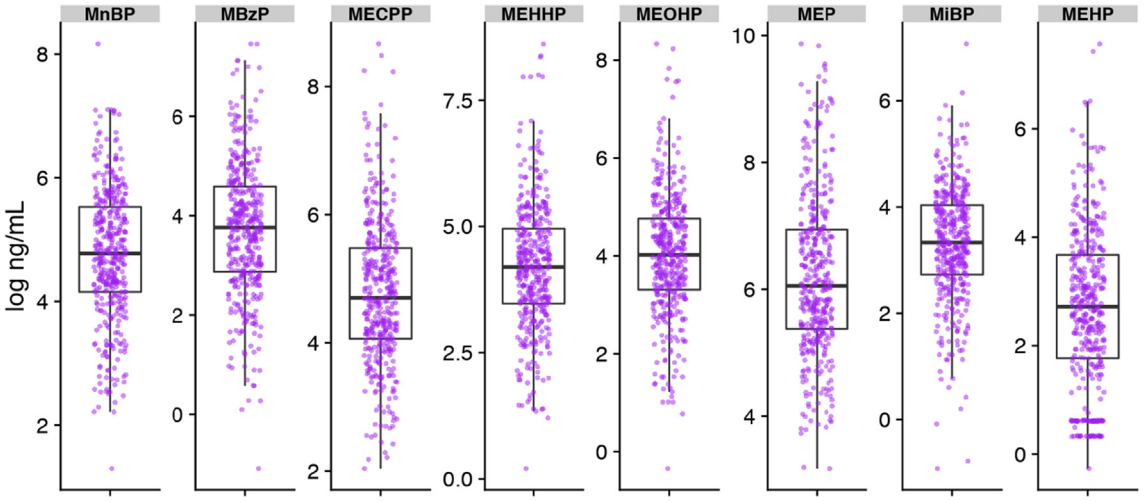

Fig. 1. Concentrations of the log transformed prenatal phthalate metabolites, adjusted for specific gravity.

was also associated with lower scores on the ADHD index in CPRS scale (MR = 0.9, 95%CI 0.8–1.03).

3.1.3. Mixtures analysis using Weighted Quantile sum (WQS) regression models

The results from the logistic WQS regression models for prenatal phthalate metabolites and the dichotomized CPRS scores are presented in Fig. 6 and Appendixes 8–12 respectively. No associations were found between the WQS of phthalate metabolites and binary outcomes for CPRS scores (data not shown). However, when the analysis was performed separately for DEHP and non-DEHP metabolites (Appendixes 8,9), significant associations were found between the weighted quantile sum of DEHP metabolites and social problems in boys (OR = 2.15, 95%CI 1.13–4.06, p-value = 0.02) (Fig. 6A), anxious shy problems (OR = 2.19, 95% CI 1.15–4.16, p = 0.02) in girls (Fig. 6B), and lower odds of emotional lability problems in all children (OR = 0.61, 95%CI 0.38–0.97, p = 0.04) (Fig. 6C). We did not find any significant associations between the weighted quantile sum of non-DEHP metabolites and behavioral outcomes in boys or girls. The most highly weighted DEHP metabolites in all three models were MEHP and MEOHP (Appendixes 10–12).

3.1.4. Sub-analysis for postnatal exposure on 3 and 5 years of age

The concentrations of MEP measured among 3-year-old boys were associated with higher scores on externalizing and inattentive behavior indices (cognitive problems, hyperactivity, global index, impulsivity

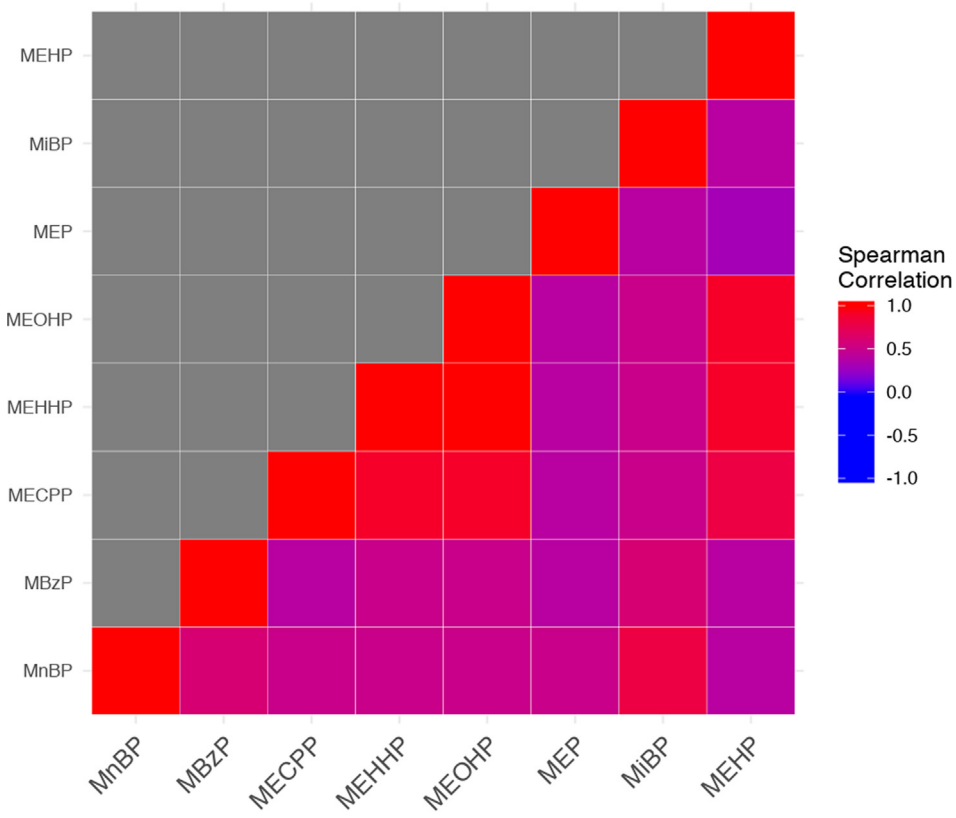

Fig. 2. Spearman correlation coefficients for associations between prenatal phthalate metabolites, adjusted for specific gravity.

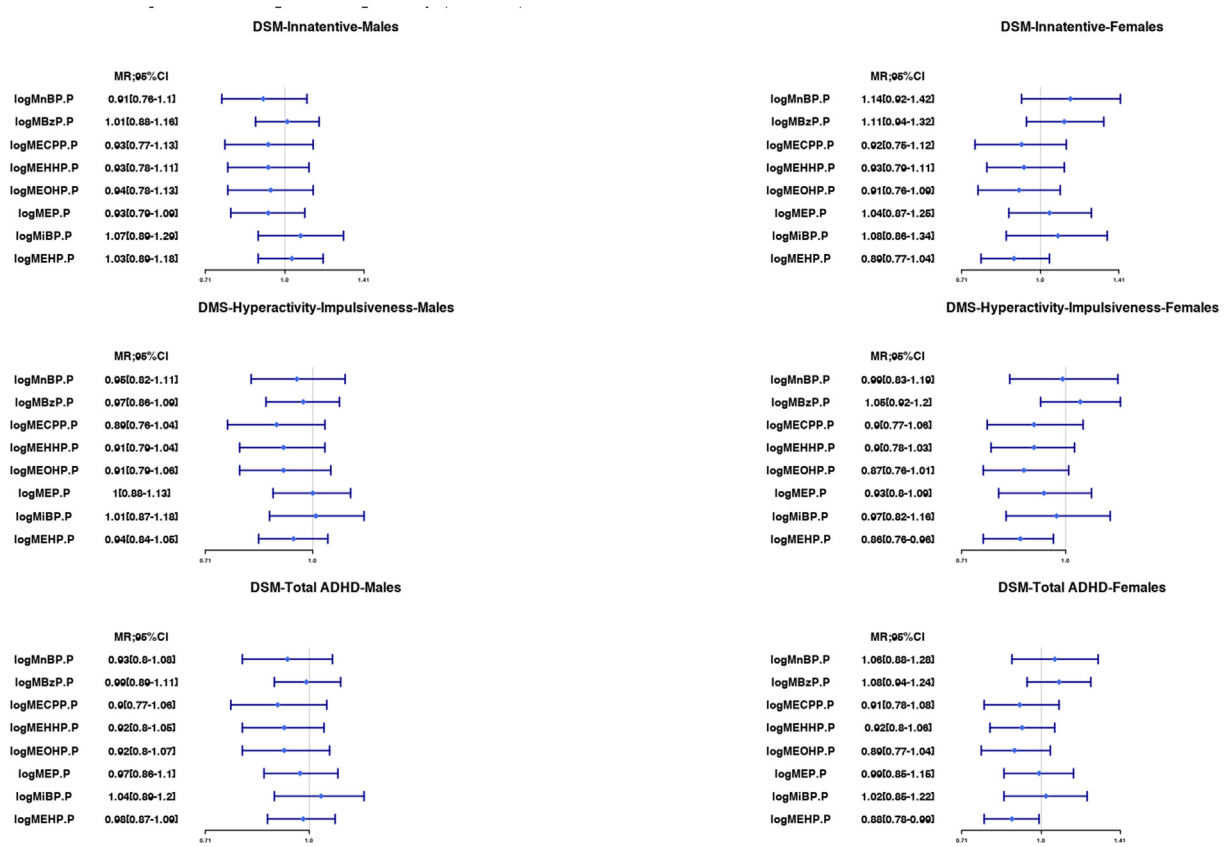

Fig. 3. Mean Ratio's for CPRS scores for one unit increase in the log-transformed concentration of phthalate metabolite in maternal urine, adjusted for specific gravity (n = 322).

and ADHD index) (Appendixes 13 and 14). Higher concentrations of MBzP among 3 year old girls were associated with higher oppositional, cognitive, impulsivity and ADHD index problems from externalizing behaviors scale and social problems scores from internalizing behaviors scale in CPRS (Appendix 15).

Higher concentrations of MiBP in five-year-old boys, was associated with lower Conners' Global Index score (Appendixes 16 and 17). Among girls, MECPP, MEHHP, MEOHP, MEHP and MiBP concentrations were associated with increases in social problems and emotional lability (Appendix 18).

The results from the logistic WQS regression models for the dichotomized CPRS scores at age 3 and age 5 are presented in Appendixes 19–22. At age 3, the WQS of DEHP metabolites were associated with cognitive problems among females. Nonetheless, nonDEHP metabolites were not associated with any of the CPRS behavioral outcomes among boys or girls. At age 5, we found that WQS of DEHP phthalates exposures were significantly associated with higher odds of higher than median scores on emotional lability scale. Non-DEHP phthalates were not associated with any of the CPRS behavioral outcomes among boys or girls (Appendixes 21 and 22).

3.2. CBCL scores at age 7 years

The association between maternal and postnatal phthalate exposure and CBCL score at age 7 are presented as the secondary outcomes of our study. Prenatal exposure to MiBP were associated with higher scores on internalizing problems, externalizing problems and total problems score only among boys (Appendixes 23 and 24). Prenatal exposure to MnBP and MBzP were associated with somatic and withdrawn problems among girls respectively. Exposure to higher levels of MEP at age 3 was associated with higher scores of externalizing and total problems in boys (Appendix 26). At age 5, exposure to higher levels of MECPP, MEHJP MEOHP and MEHP were associated with higher externalizing, internalizing, and total CBCL scores among girls but not boys (Appendixes 27 and 28).

4. Discussion

In this follow-up study of inner-city minority children, we found significant associations between prenatal urinary phthalate metabolite concentrations and maternal reported behavior problems in children at seven years of age. In general, exposure to non-DEHP phthalates was associated with anxious-shy behaviors among boys, and the concentrations of DEHP metabolites were associated with decreased hyperactivity among girls.

The hyperactivity and impulsivity scores from CPRS were decreased among girls prenatally exposed to DEHP.

No associations were found for CPRS scores in a mixture analysis performed using WQS regression models that contained the entire group of phthalate metabolites. When the models were evaluated separately for DEHP and non-DEHP metabolites, an association was found between DEHP metabolites and social problems among boys, between DEHP metabolites and anxious-shy behavior among girls. Further, concentrations of DEHP urine metabolites measured at age 3 were associated with higher cognitive problems among girls and at 5 years of age were associated with higher scores on emotional lability problems among all the children.

These results demonstrate that the inclusion of compounds that hold different biological activity in the same WQS models may result in a bias towards the null hypothesis and reinforce the thought that thinking about biological activity is imperative when considering mixtures of environmental chemicals.

Our findings indicate that while prenatal exposure to non-DEHP phthalates were associated with internalizing behaviors among boys,

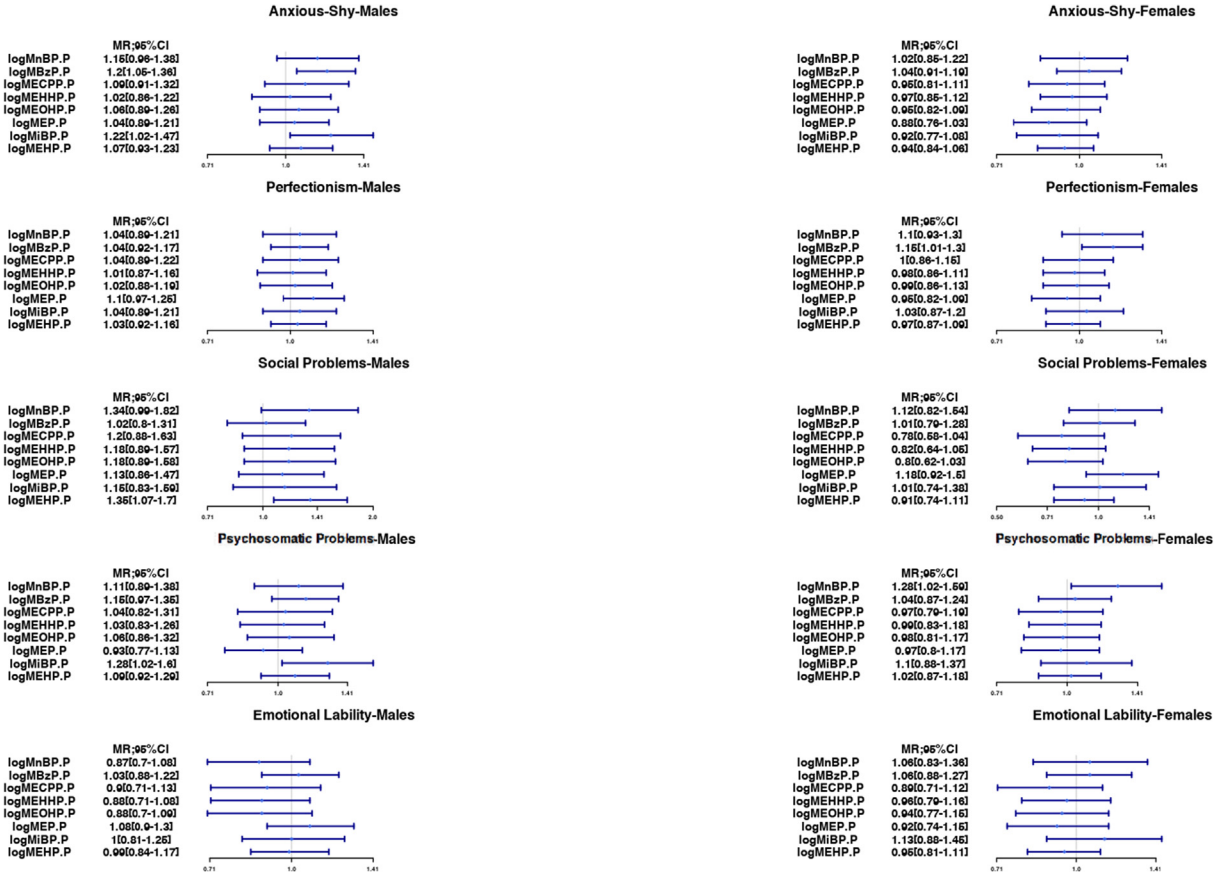

Fig. 4. Mean Ratio's for internalizing behaviors in CPRS for one unit increase in the log-transformed concentration of phthalate metabolite in maternal urine, adjusted for specific gravity (n = 322).

postnatal exposure to nonDEHP phthalates at age 5 were associated with a range of internalizing behaviors among girls. These results are corroborated by the associations found between DEHP and nonDEHP metabolites and internalizing and externalizing behaviors on the CBCL questionnaire among boys and girls.

Of the seventeen published studies of prenatal phthalate exposure and child behavior (Jankowska et al., 2019a, 2019b; Braun et al., 2014; Engel et al., 2010; Gascon et al., 2015; Kim et al., 2018; Kobrosly et al., 2014; Lien et al., 2015; Messerlian et al., 2017; Minatoya et al., 2018; Miodovnik et al., 2011; Percy et al., 2016; Philippat et al., 2017; Singer et al., 2017; Stroustrup et al., 2018; Swan et al., 2010; Whyatt et al., 2012), most described positive associations between prenatal phthalate metabolite concentrations and externalizing behaviors, predominantly among boys. We detected decreased hyperactivity among girls and increased internalizing behaviors among boys whose mothers were exposed to DEHP during pregnancy.

Our results are similar to the earlier findings regarding the behavior of the children from same cohort at age 3 (Whyatt et al., 2012), where we found that higher exposure to non-DEHP phthalates were associated with higher scores on somatic complaints, withdrawn behavior, emotionally reactive, and total internalizing behaviors scores on CBCL. In a recent study in Polish mother and Child Cohort, prenatal exposure to MEP and exposure to MEP and mono-methyl phthalate at age 7 were associated with higher odds of peer relationship problems (Jankowska et al., 2019a, 2019b). Some associations between prenatal phthalate exposure and internalizing behaviors were reported. Engel et al. (2010) found that higher low-molecular-weight phthalate metabolite concentrations were associated with higher depression scores in children 4–9 years; Lien et al. (2015) found positive, albeit non-significant, associations between MnBP concentrations and somatic complaints, anxious/depressed, and internalizing problems in children 8 years; and Kobrosly et al. (2014) found positive associations between DEHP metabolites and somatic complaints 6–10 years boys. Studying only boys, Philippat et al. (2017) found that prenatal MnBP and MBzP concentrations were positively associated with internalizing behavior at 3 years of age. Investigating gender typical play behavior among children 3–6 years, Swan et al. (2010) observed that DEHP, DBP, and DiBP metabolites were associated with reduced male-typical play behavior in boys, but none of the phthalate metabolites were associated with play behavior in girls. Among 8-year old children, Percy et al. (2016) also observed that MiBP concentrations were associated with less male gender-related play behaviors in boys and MEP concentrations were associated with more gender appropriate play in girls. Thus, there is growing evidence that prenatal phthalate exposure might be associated with internalizing behaviors (Bailey et al., 2007; Chaplin and Aldao, 2013). Our findings showed increased anxious-shy scores among 7 year old boys prenatally exposed to non-DEHP phthalates, and therefore corroborate previous studies.

The variability in study results may, in part, result from differences in the distribution patterns of phthalate metabolites and their characterization, the underlying characteristics of the populations studied, the behavior instruments used, and the child age at assessment. The timing of the urine collection used to quantify phthalate metabolites is critical since phthalates have a short half-life (Braun et al., 2013; Hoppin et al., 2002) and use of phthalate − containing products might vary before and throughout pregnancy (Cantonwine et al., 2014; Smith et al., 2012). Urine was collected preconceptionally in one study (Messerlian et al., 2017). Among the remaining studies, except for a few studies (Gascon et al., 2015; Percy et al., 2016), which collected urine samples in two time points, all studies based prenatal phthalate exposure on a single spot urine collected during pregnancy; two at the end of the second trimester (Kobrosly et al., 2014; Swan et al., 2010) and

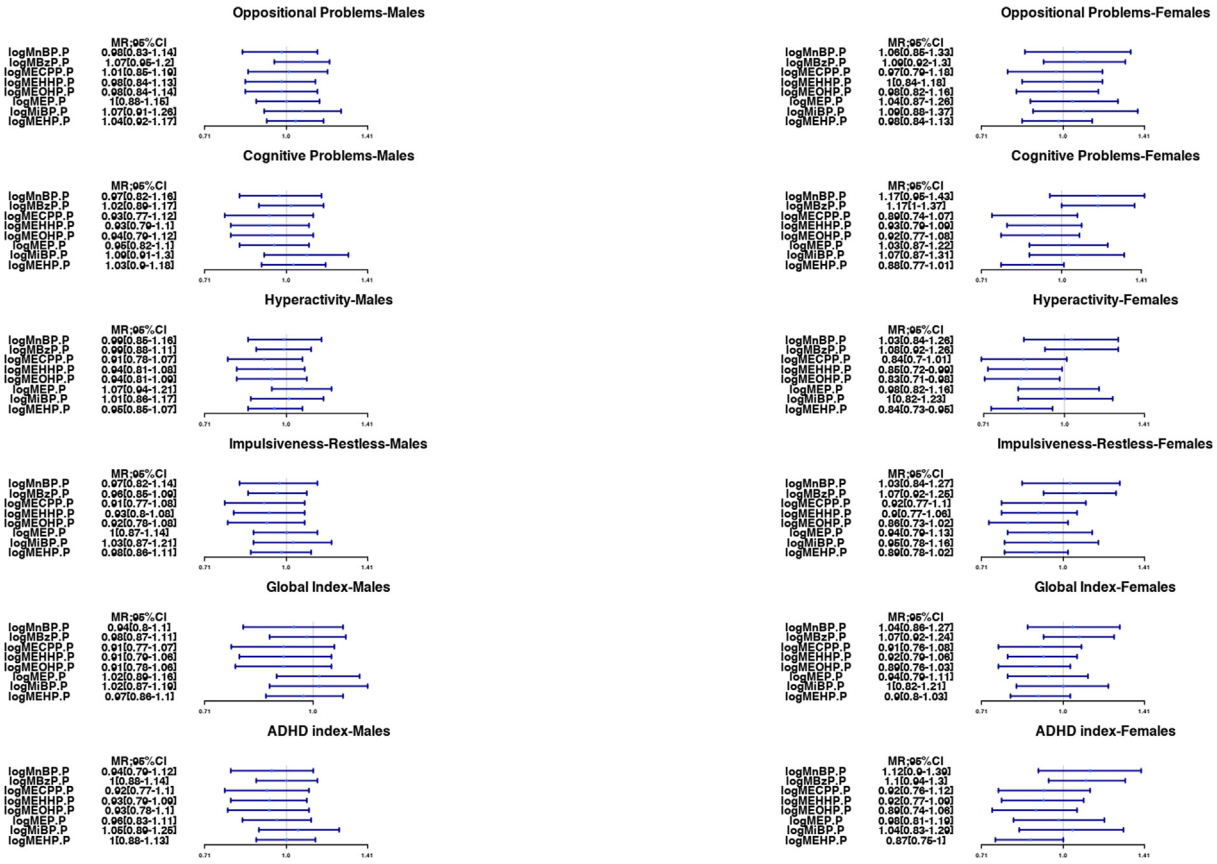

Fig. 5. Mean Ratio's for attention related and externalizing behaviors in CPRS for one unit increase in the log-transformed concentration of phthalate metabolites in maternal urine, adjusted for specific gravity (n = 322).

others, including CCCEH, during the third trimester (Engel et al., 2010; Lien et al., 2015; Whyatt et al., 2012). The concentrations of phthalate metabolites also varied across the studies; the concentrations of phthalate metabolites of the CCCEH pregnant women were generally higher than those in the other studies. The underlying socio-demographic characteristics of the populations may have influenced the results since phthalate-containing product use varies between different socioeconomic classes (Belova et al., 2013; Casas et al., 2011; Tyrrell et al., 2013; Valvi et al., 2015). Further, both child behavior and maternal perceptions of behaviors (Bradley and Corwyn, 2002; Brooks-Gunn and Duncan, 1997; Ellen and Turner, 1997) vary by culture, ethnicity, region and socioeconomic status.

The wide range of the children's ages in the studies, from preschoolers to preadolescents, may have also contributed to inconsistent findings across studies. Behavior problems vary with child age; externalizing behavior generally declines during early childhood among both sexes (Miner and Clarke-Stewart, 2008), while internalizing behavior begins to increase among girls (Shanahan et al., 2014). We note that different scales measure slightly different constructs of behavior, and the use of these different scales may, in part, explain differences in results across studies.

Our findings are biologically plausible, based on the current understanding of neurodevelopment. The brain of the fetus and/or infant is particularly vulnerable to environmental exposures due to its rapid growth and development during the prenatal and early childhood periods. While the brain growth spurt occurs during the third trimester (Lodygensky et al., 2010), brain development, particularly the cerebral cortex, continues during the early years of life (de Graaf-Peters and Hadders-Algra, 2006). The developing brain is dependent on sex steroids, particularly testosterone, and thyroid hormones for maturation (McEwen, 1992). These hormones organize the neural architecture, inducing sexual dimorphism of brain structure and function. As such, a range of exposures leading to alterations in the prenatal hormonal milieu may disrupt brain development. Experimental data show that early exposure to phthalates can disrupt hippocampal function and structure (Holahan and Smith, 2015), a brain region thought to be associated with internalizing behaviors, in particular anxiety and depression (Gray and McNaughton, 2003). Our results suggest that child behavior depends on the interplay between the specific phthalate exposures, including its magnitude (estimated from the phthalate metabolite concentration) and timing, as well as the sex of child. However, a better understanding of biological mechanisms and a more precise mapping of exposure effects on specific brain regions await further study.

Our results are consistent with experimental studies (Boberg et al., 2011; Carbone et al., 2013; Dai et al., 2015). Carbone et al. (2013) showed that perinatal exposure to DEHP increased anxiety-like behavior in male rats and testosterone reversed its anxiogenic effects, while DEHP had no effect in female rats. Dai et al. (2015) showed that perinatal low levels of DEHP increased anxiety-like behavior in pubertal male and adult female offspring. Boberg et al. (2011) showed that diisononyl phthalate (DiNP) perinatally exposed female rats performed better on spatial learning task than female controls and performed comparably to control boys; suggesting that DiNP and possibly other phthalates have the potential to masculinize behavior in exposed girls. Thus, animal studies provide evidence that prenatal exposure to some phthalates may feminize the male brain and masculinize the female brain.

There are several potential mechanisms underlying the observed associations between prenatal phthalate exposure and child behavior; many of which are derived from animal literature. Phthalates may alter normal testosterone homeostasis, necessary for normal brain

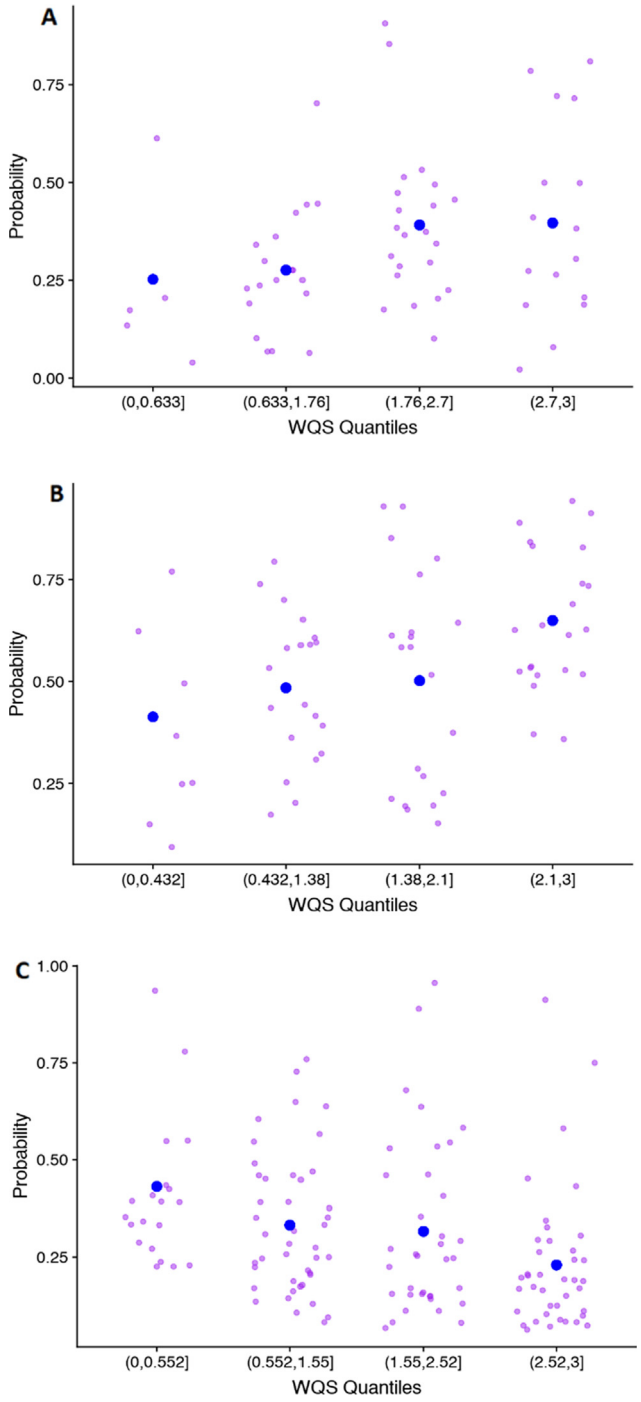

Fig. 6. (A) Weighted Quantile Sum of DEHP metabolites and the probability for social problems among boys (B) Weighted Quantile Sum of DEHP metabolites and the probability for anxious-shy behavior problems among girls (C) Weighted Quantile Sum of DEHP metabolites and the probability for emotional lability problems among all children.

development (Roselli et al., 2009; Wilson and Davies, 2007; Wu et al., 2009). They may also suppress aromatase enzyme activity and interfere with estrogen synthesis, critical for sexual differentiation of the brain. Animal studies show that DEHP exposure during gestation induced changes in aromatase activity that varied with sex of offspring (Andrade et al., 2006). Phthalates may inhibit fatty acid metabolism, also essential for brain development (Wainwright, 2002). Rosenberger et al. observed that brain lipid content of PPARβ-null mice was altered in female mice only, providing support for sex differential associations. Other mechanisms include phthalate-mediated modulation of thyroid functioning (Boas et al., 2010; Breous et al., 2005; Hinton et al., 1986; Howarth et al., 2001; Meeker et al., 2007; 2010; Moriyama et al., 2002; O'Connor et al., 2002; Sugiyama et al., 2005) and disruption of brain dopaminergic activity, linked to inattention and hyperactivity (Matsuda et al., 2012; Tanida et al., 2009).

Our study has several strengths. First, it is a prospective evaluation of a large birth cohort with assessment of phthalate exposure during late pregnancy as well as during two time points in early childhood, thereby allowing us to evaluate patterns and timing of associations between phthalate exposure and child behavior. Second, the sample size was sufficient to examine boys and girls separately. Third, two complementary behavior instruments, the Conners' Parent Rating Scale and the Child Behavior Checklist, were used. As with most studies, limitations exist. We were unable to identify a critical prenatal developmental window because urine samples were collected once in the third trimester. We lacked clinical diagnoses of behavior problems. While restriction of the sample to inner-city African American and Hispanic residents likely reduced generalizability, it also minimized residual confounding by socioeconomic status and race. Finally, the multiple comparisons in this study might have increased the chance of Type I error.

Given the observational nature of this study, we cannot infer a causal relationship between prenatal and early childhood exposure to certain phthalates and specific behavior outcomes. We have observed consistent associations between prenatal phthalate exposure and behavior outcomes at two time-points during childhood–at three years of age (Whyatt et al., 2012) and at seven years of age. Increases in internalizing behaviors were associated with prenatal concentrations of BzBP metabolites among girls and associated with prenatal concentrations of DBP, BzBP, and DiBP metabolites among boys.

5. Conclusion

In conclusion, our results augment the current literature, suggesting that exposure to some phthalates in late pregnancy and in early childhood may affect child behavior. Furthermore, our findings suggest the exposure in prenatal period and early childhood affect child behavior in a sex-specific manner. Further studies are needed to explore the critical windows of phthalate exposure for boys and girls and the mechanism of their effect on child behavioral development.

6. 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 names 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

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CRediT authorship contribution statement

Sharon Daniel: Conceptualization, Methodology, Formal analysis, Writing - original draft, Visualization. **Arin A. Balalian:** Conceptualization, Methodology, Formal analysis, Writing - original draft. **Beverly J. Insel:** Conceptualization, Formal analysis. **Xinhua Liu:** Methodology, Writing - review & editing, Supervision. **Robin M. Whyatt:** Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition. **Antonia M. Calafat:** Resources,

Writing - review & editing. **Virginia A. Rauh:** Conceptualization, Writing - review & editing. **Frederica P. Perera:** Writing - review & editing. **Lori A Hoepner:** Software, Data curation, Writing - review & editing. **Julie Herbstman:** Conceptualization, Writing - review & editing. **Pam Factor-Litvak:** Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition.

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.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105894.

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