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Running title: Prenatal Phthalates and Child Development

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Abbreviations:

BBzP - butylbenzyl phthalate

BPA - bisphenol A

BSID II - The Bayley Scales of Infant Development II

CBCL - Child Behavior Checklist

CCCEH - Columbia Center for Children's Environmental Health

CDC - Centers for Disease Control and Prevention

CI - confidence interval

DEHP - di-2-ethylhexyl phthalate

DiBP - di-isobutyl phthalate

DnBP - di-n-butyl phthalate

HMWP - high molecular weight phthalates

HOME - Home Observation for Measurement of the Environment

ICC - intraclass correlation coefficient

LMWP - low molecular weight phthalates

LOD - limit of detection

MBzP - mono-benzyl phthalate

MDI - Mental Development Index

MEHP – mono-2-ethylhexyl phthalate

MiBP - mono-isobutyl phthalate

MnBP - mono-*n*-butyl phthalate

ng/ml - nanogram per milliliter

PAH - polycyclic aromatic hydrocarbon

PDI - Psychomotor Development Index

Abstract

Background: Research suggests prenatal phthalate exposures affect child executive function and behavior.

Objectives: To evaluate associations between phthalate metabolite concentrations in maternal prenatal urine and mental, motor and behavioral development in children at age 3 years.

Methods: Mono-*n*-butyl phthalate (MnBP), mono-benzyl phthalate (MBzP), mono-isobutyl phthalate (MiBP) and 4 di-2-ethylhexyl phthalate metabolites were measured in a spot urine sample collected from 319 women during the 3rd trimester. At child age 3 years, the Mental Development Index (MDI) and Psychomotor Development Index (PDI) were measured using the Bayley Scales of Infant Development II, and behavior problems were assessed by maternal report on the Child Behavior Checklist.

Results: Child PDI scores decreased with increasing $\log_e$MnBP (estimated adjusted coefficient [β]= -2.81 [95% confidence interval (CI) -4.63, -1.0]) and $\log_e$MiBP (β= -2.28 [95% CI -3.90, -0.67]); odds of motor delay increased significantly (estimated adjusted odds ratios (OR)=1.64 [95%CI 1.10, 2.44] and 1.82 [95% CI 1.24, 2.66 per $\log_e$MnBP and $\log_e$MiBP). In girls, MDI scores decreased with increasing $\log_e$MnBP (β= -2.67 `[95% CI -4.70, -0.65]); the child sex difference in odds of mental delay was significant (p=0.037). The OR for clinically withdrawn behavior were 2.23 (95% CI 1.27, 3.92) and 1.57 (95% CI 1.07, 2.31) per $\log_e$ unit increase in MnBP and MBzP, respectively; for clinically internalizing behaviors, the OR was 1.43 (95% 1.01, 1.90) per $\log_e$ unit increase in MBzP. Significant child sex differences were seen in associations between MnBP and MBzP and behaviors in internalizing domains ($p<0.05$).

Conclusion: Certain prenatal phthalate exposures may decrease child mental and motor development and increase internalizing behaviors.

Introduction

Phthalates are a class of high production volume chemicals widely used in consumer products (Sathyanarayana 2008). Biomonitoring studies have established unequivocally that exposures in the United States are ubiquitous (CDC 2011). Concentrations of certain phthalates in maternal urine during pregnancy have been associated with adverse child cognitive and/or behavioral development (Engel et al. 2010; Miodovnik et al. 2010; Swan 2010). Cross-sectional studies report associations between phthalate metabolites in children's urine and behavioral problems and reduced IQ postnatally (Cho et al. 2010; Kim et al. 2009). Experimental research is limited but has shown adverse effects of prenatal exposure of rats to di-2-ethylhexyl phthalate (DEHP) and di-n-butyl phthalate (DnBP) on pup learning, memory and behavior (Arcadi et al. 1998; Li et al. 2009; Tanaka 2002, 2005).

Phthalates have short biologic half-lives, with most metabolites eliminated within 24 hours (Wittassek and Angerer 2008). Once absorbed, phthalates are rapidly metabolized into monoesters; some monoesters can undergo further transformations into more hydrophilic oxidative metabolites; metabolites are eliminated mainly in urine (Heudorf et al. 2007). Epidemiologic studies measure phthalate metabolites in urine as internal dosimeters of exposure because urinary enzymatic activity is negligible and most of the metabolites present arise from elimination of endogenous phthalates, rather than from external contamination with phthalates during collection and processing. The aim of the current study is to evaluate the association between child mental, psychomotor and behavioral development following prenatal exposures to 4 phthalates: DnBP, di-isobutyl phthalate (DiBP), butylbenzyl phthalate (BBzP), and DEHP.

Methods

We selected 319 pregnant inner-city women, who delivered between 1999-2006, from the longitudinal birth cohort of 727 mothers and newborns being conducted by the Columbia Center for Children's Environmental Health (CCCEH). Enrollment and exclusion criteria have been described previously (Perera et al. 2003). The CCCEH cohort was restricted to non-smoking women 18-35 years old 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 one year prior to pregnancy. 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 study was approved by the Columbia University Medical Center and Centers for Disease Control and Prevention (CDC) IRB. Study procedures were explained at enrollment and each woman signed a IRB-approved consent form. We selected women (n=319) for participation in the current study if phthalate metabolite concentrations had been measured in spot urine samples collected during pregnancy, if the child had completed the Bayley Scales of Infant Development II (BSID-II, n=297) or the mother had completed the Child Behavior Checklist (CBCL, n=286) at the child age 3 year visit, and data were available on model covariates. The 319 subjects did not differ significantly from the remaining subjects in the CCCEH cohort in terms of basic demographics (race/ethnicity, maternal prenatal marital status and education level, household income, proportion on Medicaid or other public assistance) or on child sex, gestational age and birth weight (all p-values >0.05).

Questionnaire and medical record data. A trained bi-lingual interviewer administered a 45-minute questionnaire to each woman in her home during the 3rd trimester of pregnancy,

collecting information on demographics, race/ethnicity, home characteristics and residential history, history of active and passive smoking, occupational history, marital status, education and income level, prenatal alcohol and drug use, and maternal psychosocial conditions. We also abstracted information from the mothers' and infants' medical records following delivery, including gestational age, infant sex, birth weight, length and head circumference, complications of pregnancy, medication use and delivery method.

Urine sample collection and phthalate measurements. We collected a spot urine sample from the women during the 3rd trimester of pregnancy (average 33.1±3.0 weeks gestation; median 33 weeks). Samples were stored at Columbia University at -80 ^{0}C, shipped to CDC on dry ice, and stored at -70 ^{0}C until analysis. The urinary phthalate metabolite concentrations were measured at CDC as described (Kato et al. 2005). Each analytical run included calibration standards, reagent blanks, and quality control samples. We used specific gravity to correct for urinary dilution as recommended for phthalates (Hauser et al. 2004). Specific gravity was measured using a handheld refractometer (Atago PAL 10-S, Bellevue, WA). As a measure of 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, 2-4 repeats per woman). Adjusting for specific gravity, ICCs were: 0.77 for MzBP, 0.65 for MnBP, 0.60 for MiBP, and ranged from 0.27-0.42 for the DEHP metabolites.

Measures of child mental, psychomotor and behavioral development. The BSID-II (Bayley 1993) provides a developmental quotient (raw score/child chronological age) from which a

continuous Mental Developmental Index (MDI) and a Psychomotor Development Index (PDI) are generated. The raw scores are converted to a normalized scale with a mean of 100 and standard deviation of 15. Scores can be analyzed continuously (with higher scores indicating better development) or children can be classified as normal or at risk of delay (scores ≤ 85). The child age at the administration of the BSID-II averaged 36.4±1.7 (range 27-42) months. Cohort children were tested under controlled conditions by trained bilingual research assistants; inter-rater reliability has been described (Rauh et al. 2006). Behavioral problems were measured through maternal report of the 99-item CBCL for ages 1.5 to 5 years, which provides an early indicator of potential behavioral problems in young children (Achenbach 2000). The 99 items are summed into 7 syndrome scales, with 4 scales (emotionally reactive, anxious/depressed, somatic complaints, withdrawn) subsequently summed into internalizing behaviors and 2 (attention and aggressive behaviors) summed into externalizing behaviors. The CBCL scales can be analyzed as continuous scores, or the children can be classified in the normal, borderline or clinical range based on predetermined cut points (Achenbach 2000). The child age at the administration of the CBCL averaged 36.6±2.8 (range 33-48) months.

Model covariates. Prenatal psychosocial factors included maternal self-report of hardship during pregnancy (lack of food, clothing, housing, gas or electricity, or medicines) and satisfaction with overall living conditions. Maternal demoralization was measured by the 27-item Psychiatric Epidemiology Research Instrument-Demoralization Scale (Dohrenwend et al. 1978). Maternal intelligence was assessed postnatally by the Test of Non-Verbal Intelligence-Third Edition (Brown 1990), a 15-minute, language-free measure of general intelligence, which is relatively stable and free of cultural bias. The quality of proximal care-taking environment was measured

by the Caldwell and Bradley's Home Observation for Measurement of the Environment (HOME scale) (Caldwell 1979) at child age 38.4±6.2 months. Prenatal alcohol consumption and exposure to environmental tobacco smoke were measured by maternal self-report. Eight polycyclic aromatic hydrocarbons (PAH) were quantified in 48-hour maternal 3rd trimester personal air samples and summed (Perera et al. 2003). Bisphenol A (BPA) was measured in the maternal prenatal spot urine samples at CDC as described (Ye et al. 2005).

Statistical analysis. To examine the relationship between prenatal exposure to the four phthalates (assessed from the urinary metabolite concentrations) and BSID-II and/or CBCL outcomes, linear models were used for the continuous outcomes and logistic models for categorical outcomes. The few phthalate metabolites concentrations below the limit of detection (LOD) were assigned a value of half LOD. Metabolite concentrations were right-skewed and were transformed using the natural logarithm. From a pool of covariates known or suspected of being associated with the phthalate concentrations or BSID-II or CBCL outcomes (Eskenazi et al. 2007; Rauh et al. 2006; Wasserman et al. 2003; Whyatt et al. 2009), we selected those remaining significant or marginally significant ($p < 0.10$) in the regression model for at least one of the outcome variables in the same set. Model covariates for the BSID II outcomes were: child sex (boy versus girl), race/ethnicity (Dominican versus African American), the quality of proximal care-taking environment (continuous HOME scale), gestational age (in weeks), maternal marital status (never versus ever married), maternal prenatal alcohol use (yes/no), and urine specific gravity. Maternal IQ was not controlled as it was not significant once the HOME scale had been added to the model and did not appreciably change the magnitude of the exposure BSID-II outcome relationships. Covariates in the linear models for CBCL outcomes were: child

age in months at the time of test administration, child sex, race/ethnicity, maternal IQ (categorized as described below), maternal satisfaction with overall living conditions (yes/no), maternal perceived hardship (yes/no), maternal demoralization (continuous scale), maternal prenatal PAH exposure (categorized as described below), maternal prenatal urinary $\log_e$ BPA concentrations and specific gravity. Multinominal logistic regression was used to analyze the association between the urinary phthalate metabolite concentrations and whether the child fell in the normal, borderline or clinical range on the CBCL scales. The analyses were conducted only on the four scales that had at least 15 subjects (≥5% of the sample) in each cell. The control variables were: child's age in months at test administration, child sex, mother's satisfaction, maternal demoralization and urine specific gravity. Urine specific gravity was standardized ((individual subject SG-mean SG)/standard deviation) before inclusion in all models. Additional variables assessed as confounders but not controlled were: maternal education, prenatal environmental tobacco smoke, year and season of urine collection, and umbilical cord lead and chlorpyrifos. To evaluate whether language of test administrations (Spanish versus English) acted as an effect modifier, we conducted stratified analyses and tested the interaction terms between the phthalate metabolites and language of administration; results were comparable to those presented here and none of the interaction terms were significant. Missing values for the following 5 covariates were inputted: (1) 3 missing values for maternal demoralization were inputted by a linear regression model with maternal education, maternal satisfaction and maternal hardship as predictors (model R^2=0.15, n=285); (2) 12 missing observations from the HOME scale were inputted by linear regression with race/ethnicity, maternal education and IQ and household income as predictors (model R^2=0.18, n=294). Maternal hardship during pregnancy (yes/no) had 5 missing observations which were inputted based on a logistic

regression with race/ethnicity, maternal demoralization, maternal satisfaction, and prenatal PAH as predictors (model R^2=0.18, n=285). For maternal IQ and PAH exposure, both with >5% missing values, we categorized the observed data and added an additional category for missing values. We excluded subjects with missing values for gestational age (n=4) and BPA concentrations (n=9), as these could not be imputed. We also conducted analyses: (1) prior to imputation of the missing values; and (2) after removing subjects with very dilute urine (specific gravity <1.007, n=15) or concentrated urine (specific gravity >1.03, n=7); and results were comparable to those presented here. Results from the linear models and the logistic model of BSID-II outcomes are presented for the total cohort and also after stratifying by child sex. Due to small sample sizes in at risk categories, logistic regression models were adjusted to remove covariates not significantly related to the outcome. Sex difference in the effect of the exposure variable was detected by the Wald test. Sample size was too small to stratify by child sex in the multinominal logistic regression analyses for CBCL outcomes. Results were considered significant at $p<0.05$. Analyses were conducted using SAS 9.2.

Results

Table 1 shows subject demographics and distributions of model covariates and outcome variables. Girls scored significantly higher than boys ($p<0.01$, group t-test) on both the mental (MDI scores 93.1±10.9 versus 88.8±11.5) and psychomotor (PDI scores 101.4± 12.4 versus 96.3±14.3) tests, whereas boys had significantly higher scores than girls ($p=0.03$) on attention problems (2.9±2.1 versus 2.4±1.8). There were no other significant differences between boys and girls on the remaining scales (data not shown). Table 2 shows the distribution of the urinary phthalate metabolite concentrations. Metabolites were detected in 84%-100% of the urine

samples. As expected, the four DEHP metabolite concentrations, adjusted for specific gravity, were highly correlated (Spearman's correlation r-values ranged from 0.68-0.97) and were converted into their molecular weights and summed (∑DEHP). The correlations between the DEHP and non-DEHP metabolite concentrations were weaker (r-values ranged from 0.14-0.29). MnBP, MiBP and MBzP concentrations were also correlated (r-values ranged from 0.42-0.63), suggesting potential common exposure sources. There was no significant difference in the phthalate metabolites concentration in maternal urine during pregnancy by child sex (data not shown).

No significant associations were found between maternal concentrations of MBzP or ∑DEHP and 3-year-old child's mental (MDI) or psychomotor (PDI) scores or mental or motor delay (all p-values > 0.05, Table 3 and Table 4)). As seen from Table 3, we found a significant inverse association between $\log_e$MnBP (β= -2.81 [95% CI -4.63, -1.0]) and $\log_e$MiBP (β= -2.28 [95% CI -3.90, -0.67]) concentrations and child PDI. Among girls, $\log_e$MnBP concentrations were associated with decreases in child MDI (β= -2.67 [95% CI -4.70, -0.65]). The odds of psychomotor delay (scores ≤ 85) increased with concentrations of $\log_e$MnBP (OR=1.64 [95% CI 1.10, 2.44]) and $\log_e$MiBP (OR=1.82 [95% CI 1.24, 2.66]) (Table 4). There were no significant child sex differences in associations between the phthalates and motor delay. There was a significant child sex difference between $\log_e$MnBP and the risk of mental delay (p=0.037, Table 4).

No significant associations were found between ∑DEHP metabolite concentrations and any CBCL outcome (all p-values > 0.05, Tables 5 and 6). None of the phthalate metabolite

concentrations were associated with child sleep problems or with scales in the externalizing domains (all p-values > 0.05, data not shown). As seen from Table 5, among the total cohort, log_eMnBP concentrations were significantly associated with increases in somatic complaints (B= 0.54 [95% CI 0.19, 0.90]), withdrawn behavior (B=0.40 [95% CI 0.05, 0.74]) and internalizing behaviors (B=1.45 [95% CI 0.40, 2.50]). Log_eMiBP concentrations were significantly associated with increases in emotionally reactive behavior (B=0.32 [95% CI 0.01, 0.62]). Log_eMBzP concentrations were associated with significant increases in withdrawn behavior (B=0.31 [95% CI 0.07, 0.55]) and internalizing behaviors (B=0.83 [95% CI 0.11, 1.56]). Associations between phthalate concentrations and internalizing behaviors varied somewhat by child sex. As seen from Table 5, among boys only, log_eMnBP concentrations were significantly associated with emotionally reactive behavior, somatic complaints, withdrawn behavior and internalizing behaviors. Among girls only, log_eMBzP concentrations were significantly associated with anxious/depressed behavior, somatic complaints, withdrawn behavior and internalizing behaviors. The child sex difference was significant for MnBP on emotionally reactive behavior (p=0.03) and for MBzP on anxious depressed behavior (p=0.035), somatic complaints (p=0.01) and internalizing behaviors (p=0.04) but not for the other scales (p-values ranged from 0.12-0.99, Table 5).

Table 6 shows estimated odds ratios for exposure and scores on the borderline and clinical ranges on the CBCL. We observed significantly increased odds ratios for the association between MnBP and MBzP concentrations and scores in the clinical range for withdrawn behavior (OR=2.23 [95% CI 1.27, 3.92] and OR=1.57 [95% CI 1.07, 2.31], respectively for each log unit increase). Finally, we found increased odds ratios for scoring in the borderline range for

internalizing behaviors related to MiBP concentrations (OR=1.98 [95% CI 1.24, 3.23] per log unit increase) and MBzP concentrations (OR=1.38 [95% CI 1.01, 1.90] per log unit increase), and for scoring in the clinical range on internalizing behaviors related to MBzP concentrations (OR=1.43 [95% 1.01, 1.90] per log unit increase).

Discussion

This is one of the few epidemiologic studies to estimate effects of prenatal phthalate exposures on child cognitive and behavioral development, and, to our knowledge, is the only study to look at these associations during the preschool years. MnBP and MiBP metabolite concentrations during pregnancy were significantly associated with decreases in psychomotor development and with increased odds of psychomotor delay. In girls, but not boys, maternal prenatal MnBP was also associated with a significant decrease in child age 3 mental development. Further, MnBP, MiBP, and MBzP were significantly associated with increases in a number of behavioral problems in the internalizing domains and increased odds that the child would score in the clinical range. Geometric mean concentrations of the phthalate metabolites measured here were 1.3-2.7 times higher than in a representative sample of pregnant U.S. women sampled in 2003-04 (Woodruff 2011).

In addition to our study, Engel and colleagues evaluated associations between prenatal phthalate metabolite concentrations and outcomes on the Behavioral Rating Inventory of Executive Function (BRIEF) and the Behavior Assessment System for Children-Parent Rating Scales (BASC-PRS) at child ages 4-9 years (n=171) (Engel et al. 2010). The metabolites were divided into high molecular weight phthalates (HMWP, predominantly DEHP metabolites) and low

molecular weight phthalates (LMWP, including MnBP, MiBP). No significant associations were found between urinary concentrations of the HMWP metabolites and most of the outcomes, except for adaptability. However, urinary concentrations of LMWP were significantly associated with poorer scores on aggression, attention, conduct problems, depression and externalizing problems. Most of these associations were specific to boys. LMWP concentrations were also positively associated with poorer scores on the global executive composite index and emotional control scale, as well as social cognition, social communication and social awareness (Miodovnik et al. 2010). A second longitudinal birth cohort study by Swan et al. (Swan 2010) evaluated associations between maternal prenatal phthalate metabolite concentrations and behavior at child ages 3.6-6 years (n=145) on the Pre-School Activities Inventory, a validated instrument to assess sexually dimorphic play behavior. MnBP, MiBP, and the DEHP metabolite concentrations were associated with less masculine play behavior among boys. Two cross-sectional studies reported that DEHP and/or DnBP metabolites in child urine were positively associated with behavioral problems and inversely associated with IQ among Korean children (Cho et al. 2010; Kim et al. 2009). Comparisons of these results to our findings are challenging, as study designs, metabolite concentrations and outcome measures differed, and the children were evaluated at different ages. The latter could be particularly important as parents' awareness of the internal states of their children may vary between school-age versus younger children. However, significant correlations between anxious/depressed, somatic complaints, withdrawn and internalizing behaviors by parental report on the CBCL, and depression and attention problems by parental report on the BASC-PRS, have been seen among both preschoolers and older children (Doyle et al., 1997; Reynolds and Kamphaur, 1992), providing evidence of some comparability between our findings and those of Engel and colleagues (2010) discussed above.

Experimental studies in laboratory rodents are similarly limited but have found significant associations between prenatal DEHP exposure and significant increases in time to perform the beam walking test (Arcadi et al. 1998), decrease in surface righting, and acceleration in the swimming direction tests (Tanaka 2002, 2005); and between prenatal DnBP exposure and depressed surface righting, shortened forepaw grip time, as well as inhibition in special learning and memory at low doses, but enhanced spatial learning and memory at high doses. Sex by treatment effects were seen for a number of domains and males appeared more sensitive (Li et al. 2009).

In terms of potential mechanism, associations between exposures to DEHP, DnBP and BzBP and modulation of thyroid function or reductions in circulating thyroid hormone levels have been seen in experimental studies (Breous et al. 2005; Hinton et al. 1986; Howarth et al. 2001; O'Connor et al. 2002; Pereira et al. 2007; Poon et al. 1997; Price et al. 1998; Sugiyama et al. 2005) as well as several epidemiologic studies (Boas 2010, Huang et al. 2007; Meeker et al. 2007; Meeker et al., 2011) and could be one mechanism given the critical role that thyroid hormone plays in fetal and early postnatal brain development (Attree 1992; Hendrich et al. 1984; Porterfield and Hendrich 1993). Furthermore, modulation of testosterone production by phthalates in the male fetus could be another potential mechanism whereby the compounds could disrupt sexually dimorphic behaviors. Experimental data have established that DnBP, DiBP, BBzP, and DEHP are all equally potent at inhibiting testosterone production in male rats during fetal development (Howdeshell 2008), and that this disruption results in abnormalities in genital development (Swan, 2008). However, testosterone also plays a critical role in male brain development. Testosterone synthesized by the testis diffuses into the brain where it is converted

to estradiol by aromatase in specific male brain regions (Roselli et al. 2009). The estradiol is thought to organize the brain along a masculine phenotype with resultant male sexual behaviors (Wu et al. 2009). Reduction of testosterone production by phthalates during fetal development has been hypothesized as a mechanism for the feminization in play behavior observed among boys (Swan et al. 2010). Preliminary data also suggest that phthalates can modulate aromatase activity itself and this could be yet a third potential mechanism affecting brain development. Estradiol is synthesized de novo in both male and female brains from cholesterol, a reaction catalyzed by aromatase, and is essential for brain development in both sexes (Roselli et al. 2009; McCarthy 2009; Bakker J. 2010). Only one prior experimental study has shown a link between phthalate exposure and modulations of aromatase activity in the developing brain (Andrade et al. 2006). Phthalates have also been shown to modulate aromatase activity or expression in other tissues (Lovekamp-Swan and Davis 2003; Adibi et al. 2010). Finally, experimental studies suggest that prenatal DEHP exposure alters transfer of essential fatty acids across the placenta and decreases lipid content in fetal brains. This could be another mechanism whereby phthalate exposure might alter brain development (Xu et al. 2007, 2008).

In addition to the uncertainty over potential mechanisms whereby prenatal phthalates exposure might be affecting child mental, motor or behavior development, additional limitations in our study results should be noted. In particular, while prior results, as well as our own study findings, suggest that phthalate-outcome relationships are likely to be sexually dimorphic, mechanistic understanding of the child sex-by-phthalate interactions is in its infancy. It is certainly possible that mothers are more likely to be concerned about, and thus report, internalizing behaviors in boys compared to girls. However, reporting basis is unlikely to have influenced our results as

MnBP was associated with internalizing behaviors in boys but not girls, while MBzP was associated with internalizing behaviors in girls but not boys and a number of the child sex differences were statistically significant. More importantly, assessment of mental, motor and behavioral development during the preschool years is challenging (Burchinal et al. 2000; Sternberg 2001). Our findings may also be compromised by the reliability of the biomarkers used to characterize DEHP exposure, as the ICCs in repeat urine samples were lower for the DEHP metabolites than for metabolites of the other 3 phthalates examined. Moreover, prior epidemiologic studies are extremely limited, and none have estimated effects of prenatal phthalate exposure on these outcomes during the preschool years, making the comparison of our findings to the prior results difficult.

Conclusion

Results presented here suggest that prenatal exposure to DnBP, DiBP and BBzP may adversely affect child mental, motor and behavioral development during the preschool years. These findings raise a public health concern, but given the limitations discussed above, should be interpreted with caution and additional research is warranted. This is especially true in light of recent epidemiologic findings among elementary school age children showing significant negative correlations between internalizing behaviors (anxious/depressed and withdrawn symptoms) on the CBCL and intellectual function, language, visual construction skills, attention, processing speed, executive function, aspects of learning and memory, psychomotor coordination and basic academic skills (Lundy et al. 2010). We will continue to follow children in the current cohort to assess associations between prenatal as well as postnatal phthalate exposures and child mental, motor and behavioral development during the elementary school years.

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Table 1. Subject demographics, distribution of model covariates and outcome variables n=319

Maternal age (years) (n=319)[a]	25.5±4.9	Maternal hardship (%)[c] (n=313)[c]	43.1%
Ethnicity (%) (n=319)		Maternal satisfaction (% satisfied) (n=319)[d]	27.0%
African American	33.5%	Prenatal alcohol (%) (n=308)[e]	27.9%
Dominican or other Hispanic	66.5%	Child sex (% female) (n=319)	52.7%
Maternal Education (%)[b] (n=319)		HOME scale [a] (n=301)	39.3±6.3
< High School degree	37.0%	**Outcome variables**	
High School diploma or GED	36.7%	**BSID II**	
>High School	26.3%	MDI (n=297)[a]	91.3±11.3
Marital Status (%) (n=319)		PDI (n=296) [a]	99.1±13.6
Never married	67.4%	**CBCL** (n=286)	
Married [b]	26.9%	Emotionally reactive [a]	1.9±2.1
Separated, widowed, divorced	5.6%	Anxious/depressed [a]	3.2±2.5
Household Income (%) (n=295)		Somatic complaints [a]	2.5±2.4
< $10,000	41.7%	Withdrawn behavior [a]	2.0±2.2
$10,000-$30,000	43.0%	Sleep Problems [a]	2.8±2.4
>$30,000	15.3%	Attention Problems [a]	2.7±1.9
Maternal demoralization (n=316)[a]	1.1±0.7	Aggressive problems [a]	10.1±6.9
Maternal IQ (n=291) [a]	84.4±13.2	Internalizing behavior [a]	9.5±6.9
Prenatal PAH in air (ng/m^3) (n=303) [a]	3.4±9.0	Externalizing behavior [a]	12.8±8.3
Prenatal BPA in urine (ng/ml) (n=308) [a]	3.2±4.4		

[a]Mean±standard deviation
[b] Includes living with same partner for > 7 years
[c] Lack of food, clothing, housing, gas or electricity, or medicines
[d] Maternal self-report of satisfaction with overall living conditions
[e] Reported drinking any alcohol during pregnancy

Table 2. Distribution of phthalate metabolites in maternal spot urine during the 3rd trimester of pregnancy (ng/ml), n=319

Metabolite	%>LOD	Geometric mean (95% CI)	Range
DEHP			
Mono-2-ethylhexyl phthalate (MEHP)	84%	5.1 (4.3, 6.0)	<LOD-613
Mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	100%	23.0 (20.1, 26.3)	1.1-1750
Mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP)	100%	19.2 16.8, 22.0)	0.7-1320
Mono-2-ethyl-5-carboxypentyl phthalate (MECPP)	100%	40.2 (35.6, 45.4)	3.0-1840
Non-DEHP			
Monobenzyl phthalate (MBzP)	99.7%	19.0 (16.4, 22.0)	<L0D-1110
Mono-isobutyl phthalate (MiBP)	99.4%	9.3 (8.3, 10.5)	<LOD-374
Mono-*n*-butyl phthalate (MnBP)	100%	38.0 (33.9, 42.6)	0.2-785

LOD: limit of detection (in ng/ml); 0.9-1.5 for MEHP, 0.3 for MBzP and MiBP

Table 3. Estimated coefficient of the predictor (maternal urinary phthalate metabolite concentrations) in the linear models for child MDI and PDI on the Bayley Scales on Infant Development II

	Psychomotor Development Index (PDI)			
	Total (n=296)	**Girls (n=156)**	**Boys (n=140)**	**Child sex difference**
Metabolite	β (95% CI)	β (95% CI)	β (95% CI)	p-value
$\log_e$ MnBP	-2.81 (-4.63, -1.0)**	-2.41 (-4.91, 0.08)	-3.08 (-5,82,-0.33)*	0.72
$\log_e$ MiBP	-2.28 (-3.90, -0.67)**	-2.33 (-4.59, - 0.08)*	-2.21 (-4.61, 0.19)	0.94
$\log_e$ MBzP	-0.92 (-2.23, 0.40)	-1.05 (-2.77, 0.67)	-0.57 (-2.74, 1.60)	0.73
$\log_e$ ∑DEHP	1.31 (-0.26, 2.89)	0.69 (-1.35, 2.73)	2.33 (-0.21, 4.87)	0.32
	Mental Development Index			
	Total (n=297)	**Girls (n=157)**	**Boys (n=140)**	**Child sex difference**
Metabolite	β (95% CI)	β (95% CI)	β (95% CI)	p-value
$\log_e$ MnBP	-1.12 (-2.62, 0.39)	-2.67 (-4.70, -0.65)**	0.30 (-1.99, 2.59)	0.054
$\log_e$ MiBP	-0.28 (-1.62, 1.05)	-1.33 (-3.20, 0.54)	0.59 (-1.40, 2.58)	0.16
$\log_e$ MBzP	-0.73 (-1.80, 0.34)	-1.07 (-2.48, 0.33)	-0.45 (-2.23, 1.32)	0.59
$\log_e$ ∑DEHP	0.35 (-0.94, 1.64)	-0.22 (-1.90, 1.46)	0.99 (-1.11, 3.09)	0.37

Sex difference was detected by Wald test. Models controlled for specific gravity, race/ethnicity, maternal marital status, maternal prenatal alcohol consumption, gestational age, the quality of proximal care-taking environment (HOME scale) and child sex (total analyses). *$p<0.05$, **$p\leq0.01$

Table 4. Odds ratio (OR) that the child would score a risk of mental or psychomotor delay for each log_e change in phthalate metabolite concentrations in maternal urine

	PDI (52/296 had score ≤ 85)[a]	**Girls (19/156)[b]**	**Boys (33/140)[c]**	**Sex difference**
Metabolite	OR (95% CI)	OR (95% CI)	OR (95% CI)	p-value
Log_e MnBP	1.64 (1.10, 2.44)*	1.57 (0.84, 2.94)	1.58 (0.95, 2.61)	0.99
Log_e MiBP	1.82 (1.24, 2.66)**	1.98 (1.02, 3.83)*	1.80 (1.13, 2.87)*	0.82
log_e MBzP	1.08 (0.81, 1.44)	1.25 (0.80, 1.95)	0.96 (0.66, 1.39)	0.38
log_e ∑DEHP	0.96 (0.68, 1.36)	1.13 (0.68, 1.87)	0.88 (0.56, 1.40)	0.48
	MDI (83/297 had score ≤ 85)[d]	**Girls (34/157)[e]**	**Boys (49/140)[f]**	
	OR (95% CI)	OR (95% CI)	OR (95% CI)	
Log_e MnBP	0.93 (0.66, 1.31)	1.44 (0.84, 2.47)	0.68 (0.43, 1.07)	0.037
Log_e MiBP	0.89 (0.66, 1.20)	0.98 (0.62, 1.56)	0.87 (0.60, 1.28)	0.71
log_e MBzP	0.89 (0.69, 1.15)	0.94 (0.66, 1.35)	0.89 (0.64, 1.25)	0.83
log_e ∑DEHP	0.79 (0.58, 1.08)	0.95 (0.61, 1.47)	0.71 (0.45, 1.10)	0.35

All logistic regression models controlled for specific gravity. Sex difference was detected by Wald test.
[a]The models for PDI≤85 also controlled for maternal marital status, gestational age, the quality of proximal care-taking environment (HOME scale) and child sex. [b]The models for PDI≤85 with girls also controlled for HOME scale. [c]The models for PDI≤85 with boys also controlled for HOME scale and maternal marital status. [d]The models for MDI≤85 also controlled for race/ethnicity, maternal marital status, maternal prenatal alcohol consumption, gestational age, HOME scale and child sex. [e]The models for MDI≤85 with girls also controlled for HOME scale and maternal prenatal alcohol consumption. [f]The models for MDI≤85 with boys also controlled for race/ethnicity and gestational age.
*$p < 0.05$, **$p < 0.01$.

Table 5. Estimated coefficient of the predictor (maternal urine phthalate concentrations) in the linear models for internalizing behaviors on the CBCL at child age 3 years

Metabolite	Total cohort (n=277)	Girls (n=148)	Boys (n=129)	Sex difference
	β (95% CI)	β (95% CI)	β (95% CI)	p-value
Emotionally reactive				
$\log_e$ MnBP	0.25 (-0.09, 0.58)	-0.02 (-0.50, 0.45)	0.71 (0.22, 1.19)**	0.03
$\log_e$ MiBP	0.32 (0.01, 0.62)*	0.34 (-0.11, 0.78)	0.42 (-0.005, 0.85)	0.79
$\log_e$ MBzP	0.21 (-0.02, 0.44)	0.26 (-0.05, 0.57)	0.34 (-0.008, 0.69)	0.72
$\log_e$ ∑DEHP	-0.0002 (-0.27, 0.27)	0.07 (-0.30, 0.44)	-0.007 (-0.5, 0.4)	0.79
Anxious/Depressed				
$\log_e$ MnBP	0.26 (-0.11, 0.65)	0.41 (-0.11, 0.94)	0.17 (-0.40, 0.75)	0.54
$\log_e$ MiBP	0.12 (-0.23, 0.47)	0.16 (-0.34, 0.66)	0.12 (-0.38, 0.61)	0.91
$\log_e$ MBzP	0.22 (-0.04, 0.48)	0.51 (0.17, 0.85)**	-0.05 (-0.46, 0.35)	0.035
$\log_e$ ∑DEHP	0.18 (-0.13, 0.49)	0.18 (-0.23, 0.59)	0.19 (-0.32, 0.70)	0.98
Somatic complaints				
$\log_e$ MnBP	0.54 (0.19, 0.90)**	0.43 (-0.06, 0.91)	0.77 (0.21, 1.33)**	0.35
$\log_e$ MiBP	0.25 (-0.08, 0.58)	0.24 (-0.22, 0.70)	0.31 (-0.18, 0.81)	0.83
$\log_e$ MBzP	0.09 (-0.15, 0.34)	0.42 (0.10, 0.73)**	-0.23 (-0.63, 0.17)	0.01
$\log_e$ ∑DEHP	-0.06 (-0.35, 0.23)	-0.08 (-0.45, 0.30)	-0.12 (-0.62, 0.39)	0.90
Withdrawn behavior				
$\log_e$ MnBP	0.40 (0.05, 0.74)*	0.47 (-0.03, 0.98)	0.56 (0.09, 1.03)*	0.79
$\log_e$ MiBP	0.28 (-0.04, 0.60)	0.47 (-0.007, 0.94)	0.36 (-0.05, 0.77)	0.74
$\log_e$ MBzP	0.31 (0.07, 0.55)**	0.61 (0.29, 0.93)***	0.24 (-0.09, 0.58)	0.12
$\log_e$ ∑DEHP	-0.04(-0.32, 0.25)	-0.04 (-0.44, 0.36)	0.15 (-0.28, 0.57)	0.52
Internalizing behavior				
$\log_e$ MnBP	1.45 (0.40, 2.50)**	1.29 (-0.15, 2.72)	2.21 (0.66, 3.76)**	0.39
$\log_e$ MiBP	0.97 (-0.002, 1.94)	1.20 (-0.15, 2.55)	1.21 (-0.16, 2.56)	0.99
$\log_e$ MBzP	0.83 (0.11, 1.56)*	1.79 (0.88, 2.69)**	0.29 (-0.83, 1.42)	0.04
$\log_e$ ∑DEHP	0.08 (-0.78, 0.95)	0.13 (-0.99, 1.26)	0.21 (-1.21, 1.63)	0.93

Sex difference was detected by Wald test.

Models controlled for ethnicity, maternal IQ, maternal demoralization, maternal hardship and maternal satisfaction during pregnancy, maternal prenatal exposure to PAH and bisphenol A, child sex, child age in months at the time of the CBCL administration and specific gravity. *$p<0.05$, **$p\leq0.01$, ***$p<0.001$.

Table 6. Odds ratio (OR) the child would score in the borderline or clinical compared to normal range on internalizing behaviors for each log_e unit increase in maternal phthalate metabolite concentrations (n=286)

Metabolite	Borderline[a] OR (95% CI)	Clinical[a] OR (95% CI)
Somatic complaints	**N=35**	**N=18**
log_e MnBP	1.32 (0.84, 2.08)	1.37 (0.73, 2.56)
log_e MiBP	1.29 (0.84, 1.99)	0.76 (0.42, 1.36)
log_e MBzP	0.83 (0.59, 1.15)	1.20 (0.78, 1.86)
log_e ∑DEHP	0.88 (0.58, 1.33)	0.96 (0.57, 1.61)
Withdrawn behavior	**N=15**	**N=23**
log_e MnBP	0.60 (0.31, 1.16)	2.23 (1.27, 3.92)**
log_e MiBP	0.81 (0.44, 1.51)	1.62 (0.97, 2.73)
log_e MBzP	0.79 (0.48, 1.28)	1.57 (1.07, 2.31)*
log_e ∑DEHP	0.67 (0.35, 1.30)	0.98 (0.62, 1.55)
Internalizing behavior	**N=31**	**N=37**
log_e MnBP	1.31 (0.82, 2.10)	1.44 (0.92, 2.25)
log_e MiBP	1.98 (1.24, 3.23)**	1.41 (0.91,. 2.18)
log_e MBzP	1.38 (1.01, 1.90)*	1.43 (1.01, 1.90)*
log_e ∑DEHP	1.26 (0.85, 1.86)	1.19 (0.82, 1.72)

Models controlled for maternal demoralization and maternal satisfaction during pregnancy, child sex, child age in months at the time of the CBCL administration and specific gravity. *$p<0.05$, **$p\leq0.01$