

Published in final edited form as:

*Environ Res*. 2017 January ; 152: 51–58. doi:10.1016/j.envres.2016.09.021.

# Prenatal Phthalate Biomarker Concentrations and Performance on the Bayley Scales of Infant Development-II in a Population of Young Urban Children

**Brett T. Doherty**[a], **Stephanie M. Engel**[a], **Jessie P. Buckley**[a], **Manori J. Silva**[b], **Antonia M. Calafat**[b], and **Mary S. Wolff**[c]

[a]Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA 135 Dauer Drive, 2101 McGavran-Greenberg Hall, CB #7435, Chapel Hill, NC, USA, 27599

[b]National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, Georgia, USA 1600 Clifton Road Atlanta, GA, USA, 30329

[c]Department of Preventive Medicine, Icahn School of Medicine at Mount Sinai, New York, New York, USA 1 Gustave Lane, Levy Plaza, New York, NY, USA, 10029

## Abstract

**Introduction—**Evidence suggests prenatal phthalate exposures may have neurodevelopmental consequences. Our objective was to investigate prenatal exposure to phthalates and cognitive development in a cohort of young urban children.

**Materials and Methods—**We recruited pregnant women in New York City from 1998 to 2002 and measured concentrations of nine phthalate metabolites in urine collected in late pregnancy. We administered a neurodevelopmental screening instrument, the Bayley Scales of Infant Development II (BSID-II), to children who returned for follow-up at approximately 24 months

**Corresponding Author**, Brett T. Doherty, Department of Epidemiology, Gillings School of Global Public Health. 135 Dauer Drive, 2101 McGavran-Greenberg Hall, CB #7435, UNC Chapel Hill, Chapel Hill, NC 27599 USA. Telephone: (508) 259-5194. Fax: (919) 966-2089. bdoherty@live.unc.edu.



Competing Financial Interests Declaration

The authors declare no competing financial interests.

Institutional Review Board Approval

The Mount Sinai Children's Environmental Health Study was approved by the Institutional Review Board of the Mount Sinai School of Medicine. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research. This present analysis was approved by the Institutional Review Board of the University of North Carolina at Chapel Hill (IRB # 14-0746).

Disclaimers

All of the authors have read and approved the paper and it has not been published previously nor is it being considered by any other peer-reviewed journal. 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.

Color Printing

Color printing is NOT required.

(n=276). We estimated associations between phthalate metabolite concentrations in maternal urine and BSID-II indices (Mental Development Index (MDI), Psychomotor Development Index (PDI)).

**Results—**We observed no associations between phthalate metabolite concentrations and performance on the MDI or PDI in boys and girls combined. We did, however, observe evidence of effect measure modification by sex. We observed several negative associations between metabolite concentrations and both MDI and PDI scores among girls, suggesting poorer performance across multiple metabolites, with estimates equal to a 2-3 point decrease in score per ln-unit increase in creatinine-standardized metabolite concentration. Conversely, we observed multiple weakly positive associations among boys, equal to a 1-2 point increase in score per ln-unit increase in metabolite concentration. The strongest associations were for the metabolites mono-n-butyl phthalate, monoisobutyl phthalate, monobenzyl phthalate, and mono(3-carboxylpropyl) phthalate (MCPP).

**Conclusions—**Girls of mothers with higher urinary concentrations of MCPP and metabolites of dibutyl phthalates had lower MDI scores on the BSID-II. These same biomarker concentrations were often associated with improved scores among boys. We observed similar results for MnBP, MCPP, and MBzP on the PDI. Given the prevalence of phthalate exposures in reproductive aged women, the implications of potential neurotoxicity warrant further investigation.

### Keywords

Phthalates; Bayley Scales of Infant Development; Neurodevelopment; Endocrine Disruptor; Prenatal

## 1. Introduction

Phthalates are high production volume chemicals with many industrial applications and are used to produce a diverse array of commercial and consumer products, including packaging and storage materials, medical supplies, medication coatings, building materials, cosmetics, and personal care products. Phthalates are weakly bound to these products and can easily contaminate other materials and the environment. Human exposure to phthalates occurs via dermal absorption, ingestion of certain medications and contaminated foods, inhalation, and intravenously through medical tubing and devices (NRC 2008; CHAP 2014). Inside the body, phthalates are rapidly metabolized and are primarily excreted in urine (Koch et al. 2006; M. J. Silva et al. 2003; Hogberg et al. 2008). While phthalates do not accumulate significantly in the body, their ubiquity and consistent use results in frequent exposure. Biomonitoring surveys of the United States population consistently detect phthalate metabolites in greater than 90% of those surveyed (CDC 2015). Vulnerable populations, such as women of reproductive age, infants and young children, and racial and ethnic minorities, may be more exposed to phthalates than persons of other demographic strata (CDC 2015; Manori J. Silva et al. 2003).

Certain phthalates are biologically active compounds that exhibit endocrine disrupting properties, including anti-androgenicity (CHAP 2014; NTP 2000) and associations with thyroid hormone concentrations (Boas et al. 2010; Kuo et al. 2015; Johns et al. 2015), both of which can impair the development and function of physiologic systems (NRC 2008;

CHAP 2014; Miodovnik et al. 2014). The hormonal environment *in utero* regulates many neurodevelopmental processes (Gore et al. 2014) and alterations in maternal endocrine function (de Escobar et al. 2004; Haddow et al. 1999) or fetal sex hormone concentrations during pregnancy (Gore et al. 2014) can affect neurologic development in offspring. Consequently, it is hypothesized that maternal exposure to phthalates during the prenatal period could affect neurologic development in offspring.

To date, several epidemiologic studies have investigated the relationship between prenatal exposure to phthalates and child cognitive (Factor-Litvak et al. 2014; Whyatt et al. 2012; Polanska et al. 2014; Tellez-Rojo et al. 2013; Kim et al. 2011) and behavioral (Whyatt et al. 2012; Engel et al. 2010; Swan et al. 2010; Lien et al. 2015; Gascon et al. 2015; Kobrosly et al. 2014) development. These studies suggest potential neurodevelopmental consequences of prenatal phthalate exposures, though there has been limited overlap in the neurodevelopmental outcomes assessed and child ages included across studies. Among the studies addressing childhood cognitive development among children two to three years of age, only two considered differential responses by child sex, and among these, the results are somewhat conflicting. These data gaps and inconsistencies hinder a synthesis of the evidence.

We investigated prenatal exposure to phthalates and cognitive and psychomotor development in children at approximately 24 months of age in the Mount Sinai Children's Environmental Health cohort.

## 2. Methods

### 2.1 The Mount Sinai Children's Environmental Health Center birth cohort

Between March 1998 and March 2002 the Mount Sinai Children's Environmental Health Center enrolled primiparous women seeking prenatal care from the Mount Sinai Diagnostic and Treatment Center and two private practices located on the Upper East Side of Manhattan. Women were eligible to participate if they were pregnant with a singleton pregnancy, had had their first prenatal care visit prior to 26 weeks of gestation, did not have a serious chronic condition (i.e., diabetes, hypertension, thyroid disease), did not acquire a serious pregnancy complication, and did not report consuming more than two alcoholic beverages per day or use illicit drugs. Of 1,450 women who satisfied initial eligibility criteria and approached for enrollment, 479 enrolled and completed a baseline questionnaire that collected information pertaining to pregnancy characteristics, demographics, lifestyle factors, and environmental and occupational exposures. Mothers gave birth at Mount Sinai School of Medicine (MSSM) and birth outcome information were available from the MSSM perinatal database. After birth, a total of 404 mother-child pairs were eligible to continue participation (Berkowitz et al. 2002; Berkowitz et al. 2003).

We invited mothers and children to return for follow-up evaluations at the Mount Sinai Hospital at approximately 24 months (n=276). At this time, we collected additional information on the child and home environment, and administered the Bayley Scales of Infant Development-II (BSID-II).

The Mount Sinai Children's Environmental Health Study was approved by the Institutional Review Board of the Mount Sinai School of Medicine. This analysis was approved by the Institutional Review Board of the University of North Carolina at Chapel Hill. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research.

### 2.2 Measurement of phthalate metabolite concentrations

We collected a spot urine sample from mothers during a prenatal care visit that occurred between 25 and 40 weeks gestation (Median: 31, IQR: 29-34). We stored these urine samples at -20°C until analysis, and shipped them overnight on dry ice to the CDC in Atlanta, GA for measurement of concentrations of nine phthalate metabolites: mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-n-butyl phthalate (MnBP), mono-isobutyl phthalate (MiBP), monoethyl phthalate (MEP), monobenzyl phthalate (MBzP), and mono(3-carboxypropyl) phthalate (MCPP). Metabolite concentrations were expressed in micrograms per liter. We summed four metabolites (MEHP, MEHHP, MECPP, MEOHP) of di(2-ethylhexyl) phthalate (DEHP) to produce a molar sum (∑DEHP) expressed in micromoles per liter. Analytical procedures used to measure phthalate metabolite concentrations in the Mount Sinai Children's Environmental Health Study have been described before (Silva et al. 2008; Kato et al. 2005). We measured urinary dilution using creatinine concentration, and we excluded participants (n=2) who had extremely dilute urine (creatinine <10 mg/dL).

We imputed metabolite concentrations below the limit of detection (LOD) with values equal to the LOD divided by the square root of two (Lubin et al. 2004). We natural-log (ln) transformed the creatinine-standardized metabolite concentrations in statistical models to improve the normality of their distributions.

### 2.3 Measurement of neurodevelopment

One of four trained examiners administered the Bayley Scales for Infant Development-II (BSID-II) in either English or Spanish to children who returned at the 24-month examination (n=276). The BSID-II is a validated and widely-used neurodevelopmental screening instrument designed to measure cognitive and psychomotor development in children ages 1 to 42 months. The BSID-II produces two indices: the Mental Development Index (MDI) that assesses memory, habituation, problem solving, early number concepts, generalization, classification, vocalizations, language, and social skills, and the Psychomotor Development Index (PDI) that assesses gross- and fine-motor skills. The MDI and PDI normative scores are standardized to have a population mean of 100 and standard deviation of 15; valid MDI/PDI scores range between 50 and 150 (Bayley 1993). Participating children with a valid score on either the MDI or PDI were included in our analysis (n=258).

### 2.4 Statistical analyses

We calculated descriptive statistics to quantitatively describe the baseline population (n=404) and the population included in the present analysis (n=258). We used t-tests and chi-square tests of association to assess differences in characteristics between these two

populations. We calculated descriptive statistics to characterize distributions of the phthalate metabolite concentrations, and similarly calculated descriptive statistics of the children's MDI and PDI scores and compared these between certain informative strata using t-tests.

To investigate our primary hypothesis, we used general linear models to estimate the change in MDI or PDI score per unit increase in ln-transformed creatinine standardized metabolite concentrations. Dependent variables in all models were either MDI or PDI scores modeled as continuous variables. We adjusted for the following confounding variables selected on the basis of a directed acyclic graph (Greenland et al. 1999; Rothman et al. 2008): maternal race (White/Black/Hispanic/other), pre-pregnancy Body Mass Index (BMI, $kg/m^2$), maternal age in years, maternal education (less than high school/high school/some college/college graduate), supportive qualities of child's home environment (Home Observation for Measurement of the Environment (HOME) score) (Caldwell BM 1979), child's age at testing in months, maternal marital status (single/married/living with partner), and duration of breastfeeding in months. We used covariate-adjusted standardization plus covariate adjustment to account for urine dilution and adjust for potential confounding by creatinine, a method proposed by O'Brien et al. (O'Brien et al. 2015). Specifically, we used maternal age, BMI, education, race, and sex of the child to predict creatinine and compute the creatinine ratio used to standardize the phthalate metabolite concentrations. We also included creatinine as an adjustment variable in all analyses. This procedure is expected to account for variation in creatinine attributable to the adjustment factors, and also block potential confounding by associations between creatinine and the outcome (here, neurologic development).

We estimated these associations in the full cohort and among boys and girls separately by fully stratifying by sex. To conduct a formal test of heterogeneity of associations by sex, we specified a model that included product terms between sex and all covariates (which is functionally equivalent to stratification by sex) and evaluated the sex-metabolite product term. We considered there to be potentially meaningful effect measure modification if the product term p-value was below 0.10.

We conducted additional sensitivity analyses. First, we repeated our analyses using the standard creatinine-adjusted approach described by Barr et al. (Barr et al. 2005). Second, because race was a strong predictor of phthalate exposure and BSID-II scores in our population, we repeated analyses restricted to Black and Hispanic participants (n=199). And third, to assess the validity of our linearity assumptions and investigate alternative dose-response shapes, we estimated associations between MDI or PDI scores and tertile of creatinine-standardized metabolite concentrations. We did not additionally adjust for urinary creatinine in categorical analyses.

We used SAS 9.4 (Cary, NC) for statistical analyses.

# 3. Results

## 3.1 Study population

Mothers who participated in the Mount Sinai Children's Environmental Health Study were diverse with respect to age, race, and educational attainment (Table 1). As compared to White mothers (n=56), non-White (Black, Hispanic, or other; n=202) mothers were younger (median age 21 years non-White vs. 33 years White), less likely to have graduated from college (7% non-White vs. 97% White), and less likely to be married at enrollment (11% married non-White vs. 91% married White). We observed no important differences in the distributions of important characteristics between the baseline cohort (n=404) and the subset included in our analyses (n=258).

## 3.2 Phthalate metabolite concentrations

In Table 2 we provide descriptive statistics of the phthalate metabolite concentrations we measured in third trimester maternal urine. With the exception of MEHP, all metabolites were detected in >95% of the mothers. We observed that phthalate metabolite concentrations among non-White mothers in our population were 1.4 to 3.1 times concentrations among White mothers (Supplemental Table 1). Correlations among phthalate metabolite concentrations were moderate to strong: Spearman correlation coefficients for metabolites of different parent compounds ranged from 0.33 to 0.80, while Spearman correlations for the four metabolites of DEHP ranged from 0.81 to 0.98 (Supplemental Table 2). Creatinine standardization had little impact on the distributions of the metabolite concentrations (not shown).

## 3.3 Performance on the BSID-II

Children's ages at time of testing ranged from 22 to 42 months (Median: 25; IQR: 23-27). Children's MDI scores ranged from 50 to 126 (Median: 88; IQR: 78-98) and PDI scores ranged from 50 to 129 (Median: 95; IQR: 87-103). We observed that distributions of MDI scores varied considerably across strata of race and maternal education, whereas distributions of PDI scores were homogenous across these characteristics (Table 3).

## 3.4 Associations between metabolite concentrations and BSID-II scores

In the full cohort, we observed no strong associations between individual phthalate metabolites or metabolite sums (i.e., ∑DEHP) and performance on the MDI or PDI. We did, however, observe strong evidence of effect measure modification by sex: associations were generally null or negative among girls (suggesting neurodevelopmental harm) and null or positive among boys (suggesting neurodevelopmental benefit). Beta estimates were often larger and less precise for girls than for boys. Phthalate-MDI associations differed most strongly for MCPP, MBzP, and metabolites of dibutyl phthalates, while phthalate-PDI associations differed most strongly for MnBP, MBzP, and MCPP (all interaction $p < 0.10$).

Concentrations of MnBP, MiBP, and MCPP in maternal urine during pregnancy were negatively associated with MDI scores among girls, such that a unit increase in metabolite concentrations was associated with a 2 to 2.5 point decrease in MDI score (Table 4a). Conversely, similar increases in maternal urinary concentrations of MnBP, MCPP, and

MBzP were associated with a 1.5 to 2 point increase in MDI score among boys. These estimates were slightly more precise for boys than for girls, with standard errors (SEs) ranging from 0.71 to 1.01 for boys and 0.82 to 1.19 for girls - a result of there being more boys than girls in our study population.

Concentrations of MnBP, MCPP, and MBzP in maternal urine during pregnancy were associated with poorer performance on the PDI among girls, with an estimated 2 to 3 point decrease in PDI score per unit increase in metabolite concentration (Table 4b). Conversely, a unit increase in MnBP concentration was associated with a 1.92 (95% CI: 0.31, 3.54) point increase in PDI score among boys. These beta estimates were, again, more precise among boys than girls, with SEs ranging from 0.70 to 0.98 for boys and 0.86 to 1.24 for girls.

In sensitivity analyses, models using the traditional creatinine adjustment approach (without standardization) described by Barr et al. yielded no material differences in associations' magnitudes and confidence intervals (data not shown) (Barr et al. 2005). We also considered models that additionally adjusted for maternal IQ and found little differences in estimates of association, although precision was decreased due to a substantial reduction in sample size as a result of 15% of the population missing maternal IQ. Upon restriction to Black and Hispanic participants (n=199) we observed that the direction and magnitude of most of our associations remained unchanged, though estimates became somewhat less precise in this smaller sample size (Supplemental Table 3). The results of our categorical analyses supported our assumption of linearity in some instances, but not all. Some associations demonstrated threshold-type (e.g., MCPP and MDI/PDI among girls) and non-monotonic (e.g., MiBP and MDI among girls) exposure-response shapes (Supplemental Table 4). Because of the small size of each tertile, however, these estimates were very imprecise (SEs from 2.55 to 3.02). In light of this imprecision and to facilitate comparison with other published reports, we opted to focus on the linear estimates described above.

## 4. Discussion

### 4.1 Primary results

Girls of mothers with higher urinary concentrations of MCPP and metabolites of dibutyl phthalates had lower MDI and PDI scores on the BSID-II at age 24 months. Also, higher MBzP concentrations were associated with lower PDI scores among girls. These same biomarker concentrations were often associated with improved performance among boys, of a smaller magnitude but often greater precision due to the larger number of boys in our study population.

Our study of primiparous urban women adds to the growing number of observational studies of prenatal phthalate exposure and cognitive development in young children (Whyatt et al. 2012; Polanska et al. 2014; Tellez-Rojo et al. 2013; Gascon et al. 2015). With few exceptions, investigators who reported results for girls and boys separately generally observed weakly negative associations among girls and null or weakly positive associations among boys. Figure 1 provides comparisons of our results with two such studies, including Tellez-Rojo et al. who investigated prenatal phthalate exposure in relation to performance on the BSID-II among 135 children ages 2 to 3 years living in Mexico City between 1997 and

2003, and Whyatt et al. who investigated this association among 319 children at approximately 3 years of age living in New York City between 1996 and 2006 (Figure 1) (Whyatt et al. 2012; Tellez-Rojo et al. 2013). The implications of these differences in associations among boys and girls are unclear, but are echoed in sexually dimorphic associations across a range of outcomes in relation to phthalate exposure, including anogenital distance (Swan et al. 2015), behavior (Engel et al. 2010; Swan et al. 2010; Kobrosly et al. 2014; Swan et al. 2015), and growth (Valvi et al. 2015; Kim et al. 2016; Buckley et al. 2016; Maresca et al. 2015). Additional experimental evidence would improve our understanding of how phthalates might influence sexually dimorphic neurologic development in human populations, although plausible biological mechanisms have been noted (Miodovnik et al. 2011).

### 4.2 Biological Mechanism

Although a specific mechanism linking maternal phthalate exposure to neurologic development is not yet established, extensive evidence suggests that phthalates can interfere with systems and processes essential to the development of the fetal brain and nervous system (CHAP 2014; Miodovnik et al. 2014; Lyche et al. 2009). One hypothesis is that phthalates and/or their metabolites directly interact with sex-hormones, either in the mother or the developing fetus. The plausibility of this mechanistic relationship is supported by consistent observations of antiandrogenic effects exposure to some phthalates in boys and the frequent observations of sex-specific outcomes in observational studies, including our own (NRC 2008; CHAP 2014; Miodovnik et al. 2014). Although direct mechanistic support for the relevance of this pathway for the developing brain and nervous system is lacking, the importance of sex hormones in brain development is well-described (Gore et al. 2014; Nguon et al. 2005), although the impact of prenatal anti-androgenic chemical exposures on the brain development of female offspring is an understudied area. However, the consequences of an altered hormonal environment on fetal brain development, though unclear, might be expected to differ between boys and girls. Another hypothesis contends that phthalate exposure affects thyroid function in the mother or fetus —an important determinant of neurodevelopment in offspring (de Escobar et al. 2004; Haddow et al. 1999). In support of this hypothesis are observational studies that have reported associations between phthalate exposure and thyroid function in pregnant women (Johns et al. 2015; Sathyanarayana et al. 2014; Huang et al. 2007) and children (Boas et al. 2010), and there is strong evidence from animal models that phthalates and their metabolites can disrupt the production and function of certain thyroid hormones (CHAP 2014; Miodovnik et al. 2014; Lyche et al. 2009). In addition to any endocrine-mediated effects, some phthalates and their metabolites are reported to interact with peroxisome proliferator-activated receptors, a class of nuclear receptors involved in many physiologic processes central to neurodevelopment, including cellular reproduction and differentiation (Miodovnik et al. 2014; Lyche et al. 2009). Additionally prenatal phthalate exposures may be associated with gestational duration and fetal growth (Marie et al. 2015), which in turn are strongly related to neurologic development. It is therefore biologically plausible that maternal exposure to phthalates during pregnancy could affect neurologic development in offspring.

### 4.3 Strengths and Limitations

Our study possesses several strengths. First, the Mount Sinai Children's Environmental Health Center enrolled a racially and socioeconomically diverse urban cohort and, as such, represents a wide range of potential phthalate exposure profiles. Second, our study was restricted to primiparous women and collected detailed information on important sociodemographic qualities, such as the child's home environment, which improved control of confounding by these important predictors of neurodevelopment. Third, the longitudinal nature of our study permits temporal ordering of the exposure-outcome relationship. Lastly, the urine samples were collected during the sensitive prenatal period, an etiologically significant period for brain development.

Our study also possesses certain limitations. Phthalates have relatively short biological half-lives, and the single spot urine sample we collected during the third trimester may not be representative of participants' exposure to phthalates over the entire course of pregnancy. Other investigators have reported weak to moderate correlations between phthalate metabolite concentrations measured in urine at different times in pregnancy, which vary depending on the specific phthalate in question; higher molecular weight phthalates such as DEHP tend to demonstrate greater variability than lower molecular weight phthalates, such as DBPs (Adibi et al. 2008; Braun et al. 2012). We suspect any bias resulting from measurement error of phthalate metabolite concentrations to be non-differential with respect to important qualities (i.e., child's neurologic development, phthalate exposure, confounding variables). Second, over a third of our baseline population did not return for 24-month follow-up which introduces the possibility of selection bias. However, we identified no notable differences between women who did and did not return for follow-up with respect to the characteristics we measured at baseline. Similarly, the participation rate at baseline for our study was low (33%), which potentially limits the generalizability of our results or possibly introduced bias; we did not collect information on non-participants and cannot speculate as to the nature of such bias. Third, although the BSID-II is a validated and widely-used neurologic instrument with appreciable test-retest and inter-rater reliability (Tieman et al. 2005; O'Grady and Dusing 2015), its greatest predictive validity is at the extremes of the performance distribution. Additionally, the MDI and PDI are summary measures of complex neurodevelopmental qualities and they cannot be used to make inferences about specific physiologic pathways or mechanisms.

Lastly, the associations we observed could have been influenced by uncontrolled or residual confounding. Although we controlled for multiple factors including maternal race, HOME Score, maternal education, and other factors, there may be important unmeasured factors that we did not include. One plausible source of residual confounding is by other correlated neurotoxicants, or correlated phthalate metabolites, because we estimated associations for each metabolite individually. Despite the potential for such uncontrolled confounding, the effect estimates produced by the single-metabolite models displayed considerable variability, even for correlated metabolites on the same outcome (e.g., MDI in girls), which suggests that our results were not overly influenced by such confounding. Additionally, in other analyses within the Mount Sinai cohort, confounding by co-exposure to other phthalates was found to be minimally influential (Buckley et al. 2015). Another potential source of residual

confounding is income and/or material wealth. Specifically, material wealth is associated with patterns of personal consumption, which are related to phthalate exposures (Schettler 2006), and also ability to provide a supportive environment for a child's neurologic development. We attempted to control for such confounding by including proxy variables, such as educational attainment, marital status, race, and HOME score in our models, though other more directly related measures (e.g., income) could have potentially improved our analyses. We suspected our population had marked socioeconomic differences by race, and repeated our analyses among Black and Hispanic participants only to assess whether our results were consistent among this presumably more socioeconomically homogenous population. In this analysis, we observed similar associations to results in the full cohort, suggesting residual confounding by race (and presumably material wealth) was modest.

## 5. Conclusions

In a diverse population of urban mother-child pairs, we found evidence that maternal exposure to phthalates in late pregnancy was associated with her child's mental and psychomotor development, and observed decrements in girls but not boys. Specifically, greater concentrations of MCPP (a non-specific metabolite of several phthalates), MBzP, and MiBP were associated with poorer performance on the MDI among girls and improved performance among boys. We observed similar trends for MnBP, MCPP, and MBzP on the PDI. The results of our observational study and others like it suggest that prenatal exposure to certain phthalates may influence neurodevelopment in young children, and consequently that efforts to reduce phthalate exposure among pregnant women are prudent.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgements

We are grateful for the invaluable contributions of Gertrud Berkowitz for the development of the Mount Sinai New York Children's Environmental Health cohort, and for the staff, students, participants, and families who made this study possible. The authors thank Ella Samandar, James Preau, and John Reidy for technical assistance in measuring the concentrations of phthalate metabolites.

Funding Sources

This research was supported by National Institute for Environmental Health Sciences and Environmental Protection Agency Children's Center [grant numbers ES09584 and R827039], The New York Community Trust, Agency for Toxic Substances and Disease Registry, Centers for Disease Control and Prevention (CDC), and Association for Prevention Teaching and Research. BTD was supported by a training grant from the National Institute of Environmental Health Sciences [grant number T32 ES007018].

## References

NRC.. Phthalates and Cumulative Risk Assessment - The Tasks Ahead. National Research Council; Washington, DC, USA: 2008.

CHAP.. Report to the US consumer Product Safety Commission by the CHRONIC HAZARD ADVISORY PANEL ON PHTHALATES AND PHTHALATE ALTERNATIVES. Directorate for Health Sciences; Bethesda, MD, USA: 2014.

Koch HM, Preuss R, Angerer J. Di(2-ethylhexyl)phthalate (DEHP): human metabolism and internal exposure-- an update and latest results. Int J Androl. 2006; 29(1):155–165. discussion 181-155. [PubMed: 16466535]

Silva MJ, Barr DB, Reidy JA, Kato K, Malek NA, Hodge CC, et al. Glucuronidation patterns of common urinary and serum monoester phthalate metabolites. Arch Toxicol. 2003; 77(10):561–567. [PubMed: 14574443]

Hogberg J, Hanberg A, Berglund M, Skerfving S, Remberger M, Calafat AM, et al. Phthalate diesters and their metabolites in human breast milk, blood or serum, and urine as biomarkers of exposure in vulnerable populations. Environ Health Perspect. 2008; 116(3):334–339. [PubMed: 18335100]

CDC.. Fourth National Report on Human Exposure to Environmental Chemicals. Centers for Disease Control; Atlanta, GA: 2015.

Silva MJ, Barr DB, Reidy JA, Malek NA, Hodge CC, Caudill SP, et al. Urinary Levels of Seven Phthalate Metabolites in the U.S. Population from the National Health and Nutrition Examination Survey (NHANES) 1999-2000. Environmental Health Perspectives. 2003; 112(3):331–338.

NTP.. Monograph on the Potential Human Reproductive and Developmental Effects of DBP. US Department of Health and Human Services; 2000.

Boas M, Frederiksen H, Feldt-Rasmussen U, Skakkebaek NE, Hegedus L, Hilsted L, et al. Childhood exposure to phthalates: associations with thyroid function, insulin-like growth factor I, and growth. Environ Health Perspect. 2010; 118(10):1458–1464. [PubMed: 20621847]

Kuo FC, Su SW, Wu CF, Huang MC, Shiea J, Chen BH, et al. Relationship of urinary phthalate metabolites with serum thyroid hormones in pregnant women and their newborns: a prospective birth cohort in Taiwan. PLoS One. 2015; 10(6):e0123884. [PubMed: 26042594]

Johns LE, Ferguson KK, Soldin OP, Cantonwine DE, Rivera-Gonzalez LO, Del Toro LV, et al. Urinary phthalate metabolites in relation to maternal serum thyroid and sex hormone levels during pregnancy: a longitudinal analysis. Reprod Biol Endocrinol. 2015; 13:4. [PubMed: 25596636]

Miodovnik A, Edwards A, Bellinger DC, Hauser R. Developmental neurotoxicity of ortho-phthalate diesters: review of human and experimental evidence. Neurotoxicology. 2014; 41:112–122. [PubMed: 24486776]

Gore AC, Martien KM, Gagnidze K, Pfaff D. Implications of prenatal steroid perturbations for neurodevelopment, behavior, and autism. Endocr Rev. 2014; 35(6):961–991. [PubMed: 25211453]

de Escobar GM, Obregon MJ, del Rey FE. Maternal thyroid hormones early in pregnancy and fetal brain development. Best Pract Res Clin Endocrinol Metab. 2004; 18(2):225–248. [PubMed: 15157838]

Haddow JE, Palomaki GE, Allan WC, Williams JR, Knight GJ, Gagnon J, et al. Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med. 1999; 341(8):549–555. [PubMed: 10451459]

Factor-Litvak P, Insel B, Calafat AM, Liu X, Perera F, Rauh VA, et al. Persistent Associations between Maternal Prenatal Exposure to Phthalates on Child IQ at Age 7 Years. PLoS One. 2014; 9(12):e114003. [PubMed: 25493564]

Whyatt RM, Liu X, Rauh VA, Calafat AM, Just AC, Hoepner L, et al. Maternal prenatal urinary phthalate metabolite concentrations and child mental, psychomotor, and behavioral development at 3 years of age. Environ Health Perspect. 2012; 120(2):290–295. [PubMed: 21893441]

Polanska K, Ligocka D, Sobala W, Hanke W. Phthalate exposure and child development: the Polish Mother and Child Cohort Study. Early Hum Dev. 2014; 90(9):477–485. [PubMed: 25038557]

Tellez-Rojo MM, Cantoral A, Cantonwine DE, Schnaas L, Peterson K, Hu H, et al. Prenatal urinary phthalate metabolites levels and neurodevelopment in children at two and three years of age. Sci Total Environ. 2013; 461-462:386–390. [PubMed: 23747553]

Kim Y, Ha EH, Kim EJ, Park H, Ha M, Kim JH, et al. Prenatal exposure to phthalates and infant development at 6 months: prospective Mothers and Children's Environmental Health (MOCEH) study. Environ Health Perspect. 2011; 119(10):1495–1500. [PubMed: 21737372]

Engel SM, Miodovnik A, Canfield RL, Zhu C, Silva MJ, Calafat AM, et al. Prenatal phthalate exposure is associated with childhood behavior and executive functioning. Environ Health Perspect. 2010; 118(4):565–571. [PubMed: 20106747]

Swan SH, Liu F, Hines M, Kruse RL, Wang C, Redmon JB, et al. Prenatal phthalate exposure and reduced masculine play in boys. Int J Androl. 2010; 33(2):259–269. [PubMed: 19919614]

Lien YJ, Ku HY, Su PH, Chen SJ, Chen HY, Liao PC, et al. Prenatal exposure to phthalate esters and behavioral syndromes in children at 8 years of age: Taiwan Maternal and Infant Cohort Study. Environ Health Perspect. 2015; 123(1):95–100. [PubMed: 25280125]

Gascon M, Valvi D, Forns J, Casas M, Martinez D, Julvez J, et al. Prenatal exposure to phthalates and neuropsychological development during childhood. Int J Hyg Environ Health. 2015; 218(6):550–558. [PubMed: 26095249]

Kobrosly RW, Evans S, Miodovnik A, Barrett ES, Thurston SW, Calafat AM, et al. Prenatal phthalate exposures and neurobehavioral development scores in boys and girls at 6-10 years of age. Environ Health Perspect. 2014; 122(5):521–528. [PubMed: 24577876]

Berkowitz GS, Obel J, Deych E, Lapinski R, Godbold J, Liu Z, et al. Exposure to Indoor Pesticides during Pregnancy in a Multiethnic, Urban Cohort. Environmental Health Perspectives. 2002; 111(1):79–84.

Berkowitz GS, Wetmur JG, Birman-Deych E, Obel J, Lapinski RH, Godbold JH, et al. In Utero Pesticide Exposure, Maternal Paraoxonase Activity, and Head Circumference. Environmental Health Perspectives. 2003; 112(3):388–391.

Silva MJ, Preau JL Jr. Needham LL, Calafat AM. Cross validation and ruggedness testing of analytical methods used for the quantification of urinary phthalate metabolites. J Chromatogr B Analyt Technol Biomed Life Sci. 2008; 873(2):180–186.

Kato K, Silva MJ, Needham LL, Calafat AM. Determination of 16 phthalate metabolites in urine using automated sample preparation and on-line preconcentration/high-performance liquid chromatography/tandem mass spectrometry. Anal Chem. 2005; 77(9):2985–2991. [PubMed: 15859620]

Lubin JH, Colt JS, Camann D, Davis S, Cerhan JR, Severson RK, et al. Epidemiologic Evaluation of Measurement Data in the Presence of Detection Limits. Environmental Health Perspectives. 2004; 112(17):1691–1696. [PubMed: 15579415]

Bayley, N. Bayley Scales of Infant Development. 2 ed.. Psychological Corporation; San Antonio, TX: 1993.

Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. Epidemiology. 1999; 10(1):37–48. [PubMed: 9888278]

Rothman, KJ.; Greenland, S.; Lash, TL. Modern Epidemiology. 3rd Edition. 3 ed. Lippincott Williams & Wilkins; 2008.

Caldwell, BMBR. Home Observation for Measurement of the Environment. University of Arkansas Press; Little Rock, AK: 1979.

O'Brien KM, Upson K, Cook NR, Weinberg CR. Environmental Chemicals in Urine and Blood: Improving Methods for Creatinine and Lipid Adjustment. Environ Health Perspect. 2015

Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect. 2005; 113(2):192–200. [PubMed: 15687057]

Swan SH, Sathyanarayana S, Barrett ES, Janssen S, Liu F, Nguyen RH, et al. First trimester phthalate exposure and anogenital distance in newborns. Hum Reprod. 2015; 30(4):963–972. [PubMed: 25697839]

Valvi D, Casas M, Romaguera D, Monfort N, Ventura R, Martinez D, et al. Prenatal Phthalate Exposure and Childhood Growth and Blood Pressure: Evidence from the Spanish INMA-Sabadell Birth Cohort Study. Environ Health Perspect. 2015; 123(10):1022–1029. [PubMed: 25850106]

Kim JH, Park H, Lee J, Cho G, Choi S, Choi G, et al. Association of diethylhexyl phthalate with obesity-related markers and body mass change from birth to 3 months of age. J Epidemiol Community Health. 2016

Buckley JP, Engel SM, Braun JM, Whyatt RM, Daniels JL, Mendez MA, et al. Prenatal phthalate exposures and body mass index among 4 to 7 year old children: A pooled analysis. Epidemiology. 2016

Maresca MM, Hoepner LA, Hassoun A, Oberfield SE, Mooney SJ, Calafat AM, et al. Prenatal Exposure to Phthalates and Childhood Body Size in an Urban Cohort. Environ Health Perspect. 2015

Miodovnik A, Engel SM, Zhu C, Ye X, Soorya LV, Silva MJ, et al. Endocrine disruptors and childhood social impairment. Neurotoxicology. 2011; 32(2):261–267. [PubMed: 21182865]

Lyche JL, Gutleb AC, Bergman A, Eriksen GS, Murk AJ, Ropstad E, et al. Reproductive and developmental toxicity of phthalates. J Toxicol Environ Health B Crit Rev. 2009; 12(4):225–249. [PubMed: 20183522]

Nguon K, Ladd B, Baxter MG, Sajdel-Sulkowska EM. Sexual dimorphism in cerebellar structure, function, and response to environmental perturbations. Prog Brain Res. 2005; 148:341–351. [PubMed: 15661202]

Sathyanarayana S, Barrett E, Butts S, Wang C, Swan SH. Phthalate exposure and reproductive hormone concentrations in pregnancy. Reproduction. 2014; 147(4):401–409. [PubMed: 24196015]

Huang PC, Kuo PL, Guo YL, Liao PC, Lee CC. Associations between urinary phthalate monoesters and thyroid hormones in pregnant women. Hum Reprod. 2007; 22(10):2715–2722. [PubMed: 17704099]

Marie C, Vendittelli F, Sauvant-Rochat MP. Obstetrical outcomes and biomarkers to assess exposure to phthalates: A review. Environ Int. 2015; 83:116–136. [PubMed: 26118330]

Adibi JJ, Whyatt RM, Williams PL, Calafat AM, Camann D, Herrick R, et al. Characterization of phthalate exposure among pregnant women assessed by repeat air and urine samples. Environ Health Perspect. 2008; 116(4):467–473. [PubMed: 18414628]

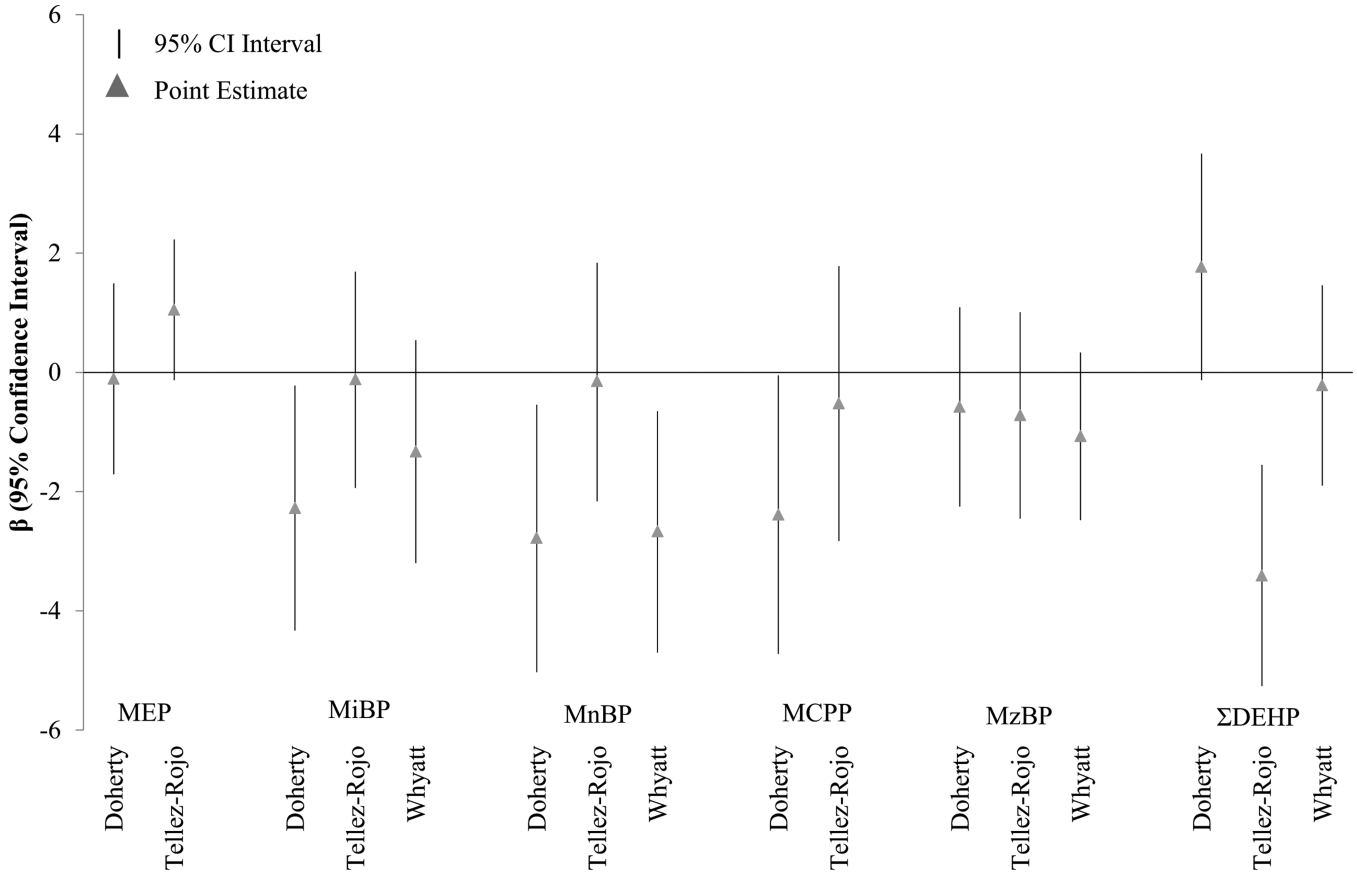
Braun JM, Smith KW, Williams PL, Calafat AM, Berry K, Ehrlich S, et al. Variability of urinary phthalate metabolite and bisphenol A concentrations before and during pregnancy. Environ Health Perspect. 2012; 120(5):739–745. [PubMed: 22262702]

Tieman BL, Palisano RJ, Sutlive AC. Assessment of motor development and function in preschool children. Mental retardation and developmental disabilities research reviews. 2005; 11(3):189–196. [PubMed: 16161086]

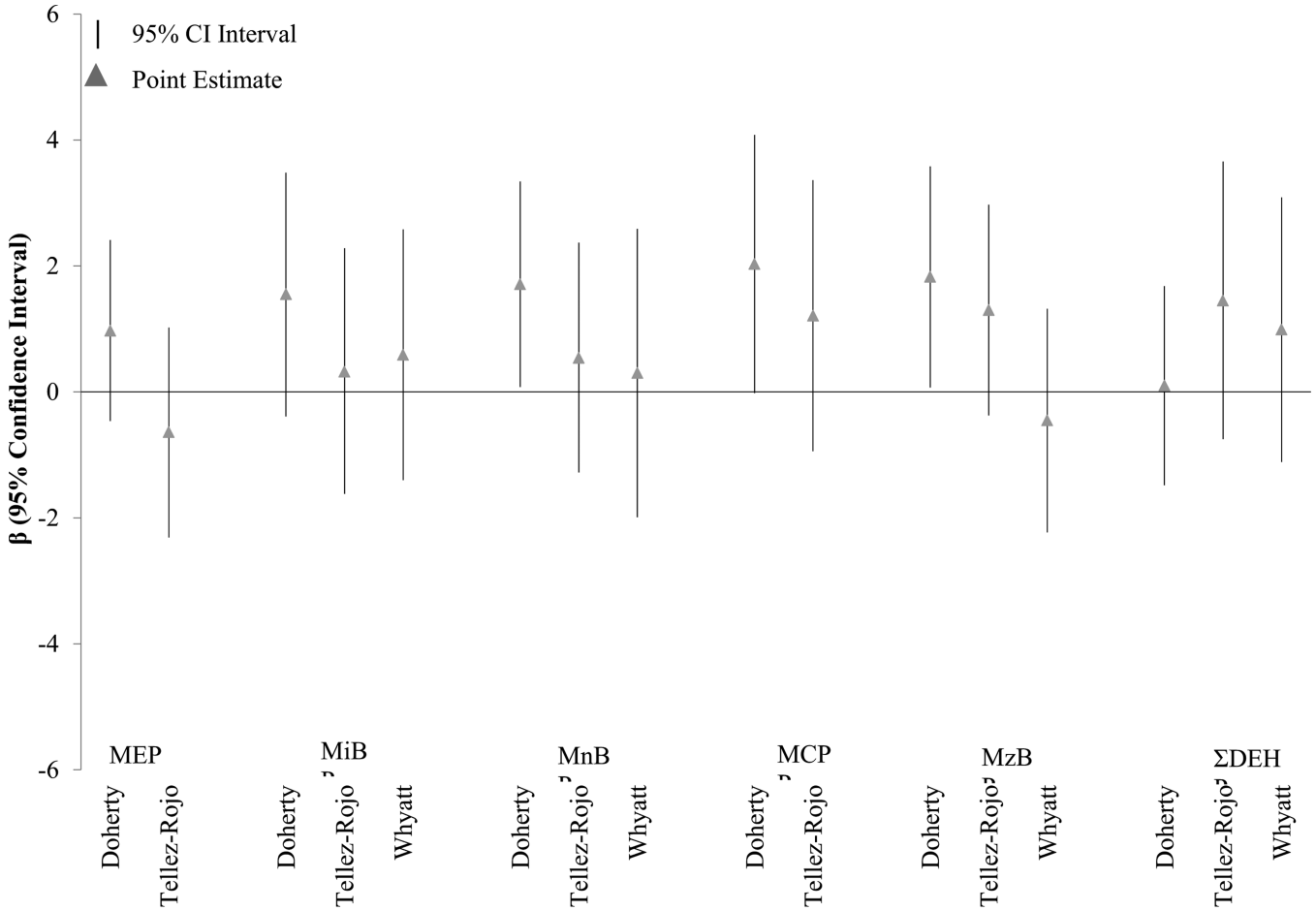
O'Grady MG, Dusing SC. Reliability and validity of play-based assessments of motor and cognitive skills for infants and young children: a systematic review. Phys Ther. 2015; 95(1):25–38. [PubMed: 25169918]

Buckley JP, Engel SM, Mendez MA, Richardson DB, Daniels JL, Calafat AM, et al. Prenatal Phthalate Exposures and Childhood Fat Mass in a New York City Cohort. Environ Health Perspect. 2015

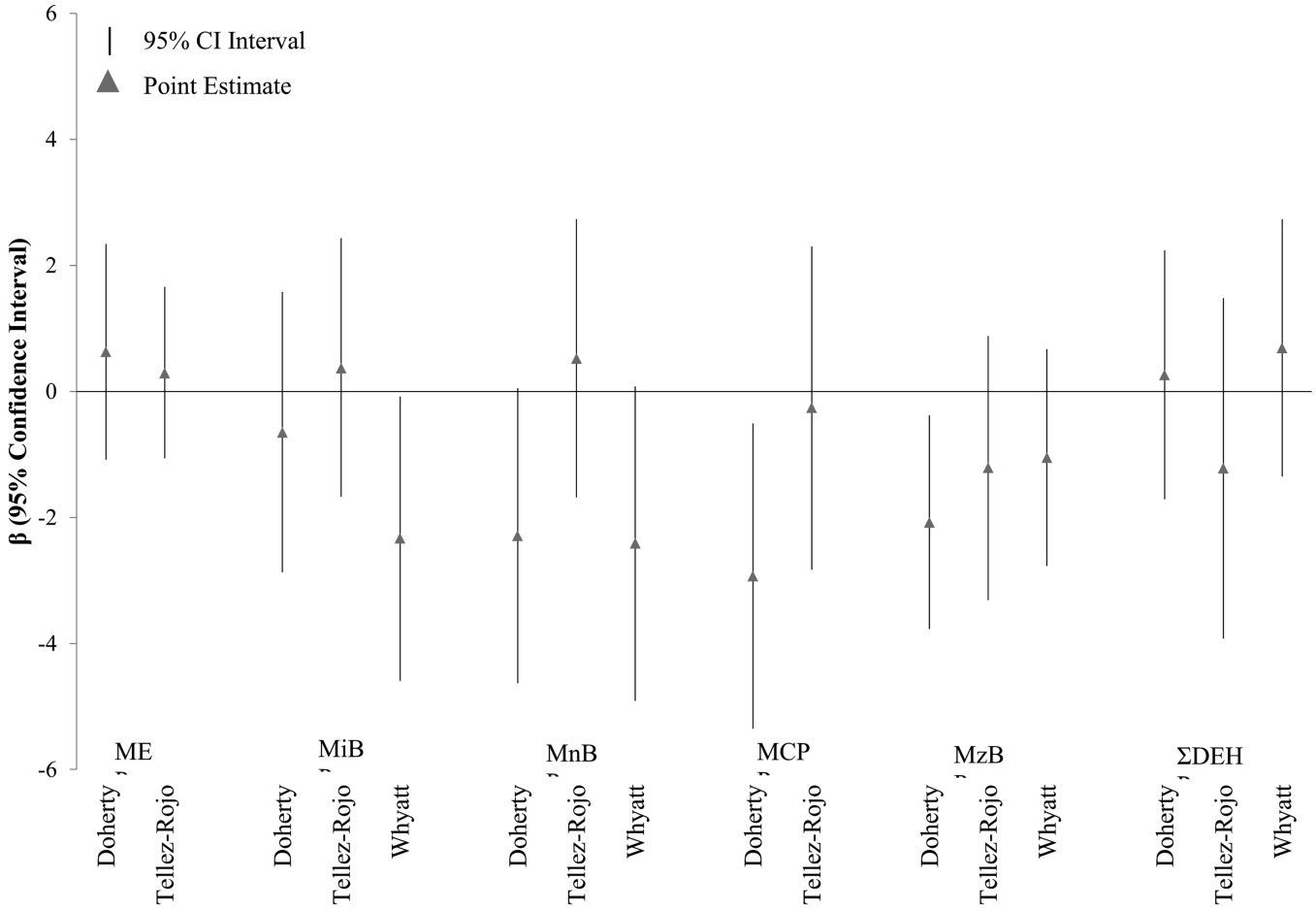
Schettler T. Human exposure to phthalates via consumer products. Int J Androl. 2006; 29(1):134–139. discussion 181-135. [PubMed: 16466533]



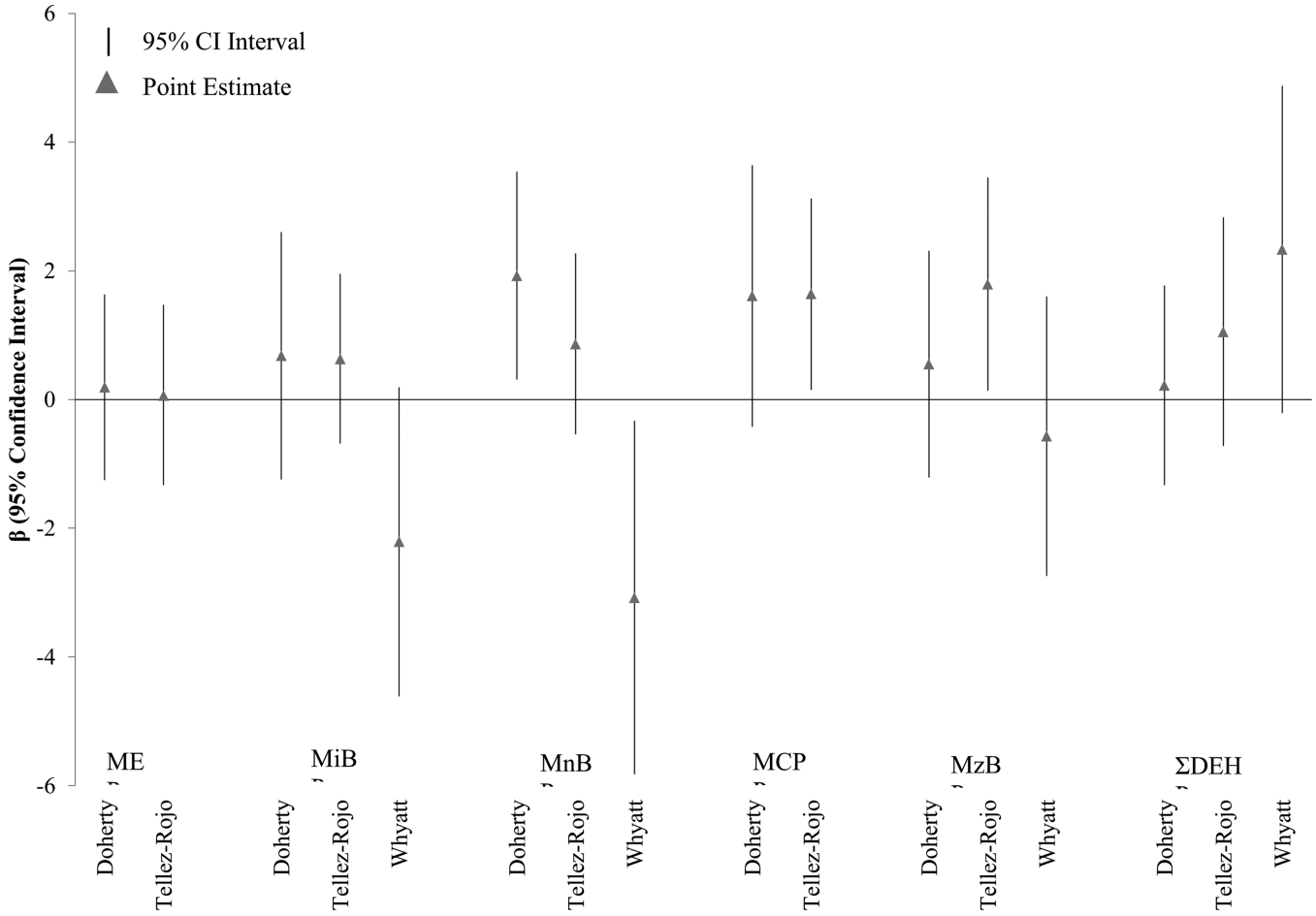

**a.** Females and MDI




**b.** Males and MDI




**c.** Females and PDI




**d.** Males and PDI

**Figure 1.**

*a.* Comparisons of point estimates and 95% confidence intervals for associations between phthalate metabolite concentrations in maternal urine during pregnancy and offspring's performance on the MDI among girls in three prospective birth cohort studies.

*b.* Comparisons of point estimates and 95% confidence intervals for associations between phthalate metabolite concentrations in maternal urine during pregnancy and offspring's performance on the MDI among boys in three prospective birth cohort studies.

*c.* Comparisons of point estimates and 95% confidence intervals for associations between phthalate metabolite concentrations in maternal urine during pregnancy and offspring's performance on the PDI among girls in three prospective birth cohort studies.

*d.* Comparisons of point estimates and 95% confidence intervals for associations between phthalate metabolite concentrations in maternal urine during pregnancy and offspring's performance on the PDI among boys in three prospective birth cohort studies.

## Table 1

Demographic characteristics of participants in the Mount Sinai Children's Environmental Health Study, 1998-2002.

| | Original cohort (*n*=404) | | Analysis population (*n*=258) | | |
|---|---|---|---|---|---|
| **Characteristic** | **Median (IQR)** | ***n* (%)** | **Median (IQR)** | ***n* (%)** | **p-Value[a]** |
| Maternal age at enrollment (years) | 22 (19-29) | | 22 (19-30) | | 0.2 |
| Body mass index (kg/m$^2$) | 23 (20-25) | | 23 (20-25) | | 0.9 |
| Maternal race/ethnicity | | | | | |
| White | | 86 (21) | | 56 (22) | 0.9 |
| Black | | 112 (28) | | 70 (27) | |
| Hispanic | | 200 (50) | | 129 (50) | |
| Other | | 6 (1) | | 3 (1) | |
| Maternal Education | | | | | |
| < High school | | 118 (29) | | 74 (29) | 0.9 |
| High school | | 83 (21) | | 53 (21) | |
| Some college | | 103 (26) | | 65 (25) | |
| > College degree | | 98 (24) | | 66 (26) | |
| Marital status at enrollment | | | | | |
| Married | | 117 (29) | | 73 (28) | 0.4 |
| Living with father of child | | 98 (24) | | 58 (22) | |
| Single | | 189 (47) | | 127 (49) | |
| Smoke during pregnancy | | | | | |
| No | | 337 (83) | | 215 (83) | 1.0 |
| Yes | | 67 (17) | | 43 (17) | |
| Alcohol during pregnancy | | | | | |
| No | | 337 (85) | | 217 (86) | 0.5 |
| Yes | | 59 (15) | | 35 (14) | |
| Child's sex | | | | | |
| Boy | | 220 (54) | | 140 (54) | 0.9 |
| Girl | | 184 (46) | | 118 (46) | |
| Breastfeeding duration (months) | - | | 1.5 (0-6) | | |
| HOME score (total) | - | | 39 (36-41) | | |

Abbreviations: IQR, Interquartile range; HOME, Home Observation for Measurement of the Environment.

[a] p-Value for comparison between full cohort and analysis population: t-tests were used for comparisons of continuous measures; Chi-Square tests were used for comparisons of categorical measures.

**Table 2**

Statistical descriptions of phthalate metabolite concentrations (μg/L) measured in third trimester maternal urine samples collected in 1998–2002 from mothers included in the present analysis (n=258).

| Metabolite | LOD | % < LOD | GM (SE) | 50th Percentile | 95th Percentile |
|---|---|---|---|---|---|
| MEP | 0.40 | 0.4 | 200 (4.4) | 172 | 3372 |
| MnBP | 0.40 | 0.0 | 33 (3.7) | 35 | 202 |
| MiBP | 0.26 | 2.7 | 5.6 (3.3) | 6.2 | 35 |
| MCPP | 0.16 | 2.3 | 2.9 (3.2) | 2.9 | 17 |
| MBzP | 0.11 | 0.4 | 14 (4.0) | 15 | 124 |
| DEHP (μmol/L) | NA | NA | 0.28 (3.7) | 0.29 | 3.2 |
| MEHP | 0.90 | 8.5 | 6.2 (3.8) | 6.1 | 85 |
| MEHHP | 0.32 | 0.8 | 20 (4.0) | 20 | 293 |
| MEOHP | 0.45 | 1.1 | 18 (3.9) | 17 | 215 |
| MECPP | 0.25 | 0.4 | 35 (3.7) | 37 | 318 |

Abbreviations: LOD, Limit of Detection; GM, Geometric Mean; SE, Standard Error; NA, Not Applicable.

**Table 3**

Distributions of MDI and PDI scores within strata of select demographic characteristics of the Mount Sinai Children's Environmental Health Study.

| | MDI (*n* = 247) | | PDI (*n* = 250) | |
|---|---|---|---|---|
| **Characteristic** | **Median (IQR)** | **p-Value[a]** | **Median (IQR)** | **p-Value** |
| Sex | | | | |
| Boy | 86 (78-96) | Ref | 96 (88-103) | Ref |
| Girl | 89 (80-99) | 0.02 | 94 (88-103) | 0.9 |
| Race | | | | |
| White | 102 (96-107) | Ref | 96 (88-103) | Ref |
| Black | 84 (76-94) | <0.0001 | 97 (87-103) | 0.9 |
| Hispanic | 84 (76-94) | <0.0001 | 92 (85-100) | 0.03 |
| Other | 109 (82-112) | 1 | 100 (99-107) | 0.09 |
| Maternal Education | | | | |
| Less than High school | 83 (76-94) | Ref | 93 (85-103) | Ref |
| High school | 83 (76-92) | 0.9 | 92 (84-100) | 0.5 |
| Some college | 84 (75-96) | 0.8 | 96 (88-103) | 0.6 |
| College graduate or more | 100 (90-106) | <0.0001 | 96 (88-103) | 0.6 |

Abbreviations: MDI, Mental Development Index; PDI, Psychomotor Development Index; IQR, Interquartile Range; REF, Referent.

[a]p-Value for t-test comparing the means of the index group with the referent group.

**Table 4a**

Estimated change in MDI score per unit change in ln-transformed standardized phthalate metabolite concentration.

| | All (n = 247) | Boys (n = 131) | Girls (n = 116) | Interaction |
|---|---|---|---|---|
| **Metabolite** | **Estimate (95% CI)** | **Estimate (95% CI)** | **Estimate (95% CI)** | ***p*-Value** |
| MEP | 0.56 (−0.52, 1.64) | 0.97 (−0.46, 2.41) | −0.11 (−1.71, 1.49) | 0.3 |
| MnBP | 0.67 (−0.67, 2.01) | 1.71 (0.08, 3.34) | −2.78 (−5.03, −0.54) | 0.002 |
| MiBP | 0.11 (−1.35, 1.57) | 1.55 (−0.39, 3.48) | −2.28 (−4.33, −0.22) | 0.01 |
| MCPP | 0.36 (−1.24, 1.96) | 2.03 (−0.02, 4.08) | −2.39 (−4.72, −0.05) | 0.007 |
| MBzP | 0.73 (−0.51, 1.97) | 1.83 (0.07, 3.58) | −0.58 (−2.25, 1.09) | 0.05 |
| ΣDEHP | 0.64 (−0.59, 1.87) | 0.10 (−1.48, 1.68) | 1.77 (−0.13, 3.67) | 0.2 |

Abbreviations: MDI, Mental Development Index; CI, Confidence Interval.

Beta coefficients (95% Confidence Intervals) per natural log increase in creatinine-standardized phthalate biomarker concentrations estimated in general linear models adjusted for ln-transformed urinary creatinine concentration (mg/dL), pre-pregnancy body mass index (kg/m$^2$), maternal race (Hispanic/White/Black/other), maternal education (less than high school/high school graduate/some college/college graduate), HOME Score, duration of breastfeeding in months, maternal age in years, child age at testing in months, child's sex (male/female), and maternal marital status (single/married/living with father).

**Table 4b**

Estimated change in PDI score per unit change in ln-transformed standardized phthalate metabolite concentration.

| | All (n = 250) | Boys (n=134) | Girls (n=116) | Interaction |
|---|---|---|---|---|
| **Metabolite** | **Estimate (95% CI)** | **Estimate (95% CI)** | **Estimate (95% CI)** | ***p*-Value** |
| MEP | 0.40 (−0.67, 1.48) | 0.19 (−1.25, 1.63) | 0.63 (−1.08, 2.34) | 0.7 |
| MnBP | 0.55 (−0.76, 1.87) | 1.92 (0.31, 3.54) | −2.29 (−4.63, 0.05) | 0.005 |
| MiBP | 0.13 (−1.30, 1.57) | 0.68 (−1.24, 2.6) | −0.65 (−2.87, 1.58) | 0.4 |
| MCPP | −0.12 (−1.68, 1.44) | 1.61 (−0.42, 3.64) | −2.93 (−5.35, −0.51) | 0.006 |
| MBzP | −0.60 (−1.81, 0.61) | 0.55 (−1.21, 2.31) | −2.08 (−3.77, −0.38) | 0.04 |
| ΣDEHP | 0.18 (−1.03, 1.39) | 0.22 (−1.33, 1.77) | 0.26 (−1.71, 2.24) | 1 |

Abbreviations: PDI, Psychomotor Development Index; CI, Confidence Interval.

Beta coefficients (95% Confidence Intervals) per natural log increase in creatinine-standardized phthalate biomarker concentrations estimated in general linear models adjusted for ln-transformed urinary creatinine concentration (mg/dL), pre-pregnancy body mass index ($kg/m^2$), maternal race (Hispanic/White/Black/other), maternal education (less than high school/high school graduate/some college/college graduate), HOME Score, duration of breastfeeding in months, maternal age in years, child age at testing in months, child's sex (male/female), and maternal marital status (single/married/living with father).