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Evaluating effects of prenatal exposure to phthalate mixtures on birth weight: A comparison of three statistical approaches

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Abstract

Objectives—We applied three statistical approaches for evaluating associations between prenatal urinary concentrations of a mixture of phthalate metabolites and birth weight.

Methods—We included 300 women who provided 732 urine samples during pregnancy and delivered a singleton infant. We measured urinary concentrations of metabolites of di(2-ethylhexyl)-phthalate, di-*n*-butyl-, di-*n*-butyl-, butylbenzyl-, and diethyl phthalates. We applied 1) linear regressions; 2) classification methods [principal component analysis (PCA) and structural equation models (SEM)]; and 3) Bayesian Kernel Machine Regression (BKMR), to evaluate associations between phthalate metabolite mixtures and birth weight adjusting for potential

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confounders. Data were presented as mean differences (95%CI) in birth weight (grams) as each phthalate increased from the 10th to the 90th percentile.

Results—When analyzing individual phthalate metabolites using linear regressions, each metabolite demonstrated a modest inverse association with birth weight [from −93 (−206, 21) to −49 (−164, 65)]. When simultaneously including all metabolites in a multivariable model, inflation of the estimates and standard errors were noted. PCA identified two principal components, both inversely associated with birth weight [−23 (−68, 22), −27 (−71, 17), respectively]. These inverse associations were confirmed when applying SEM. BKMR further identified that monoethyl and mono(2-ethylhexyl) phthalate and phthalate concentrations were linearly related to lower birth weight [−51(−164, 63) and −122 (−311, 67), respectively], and suggested no evidence of interaction between metabolites.

Conclusions—While none of the methods produced significant results, we demonstrated the potential issues arising using linear regression models in the context of correlated exposures. Among the other selected approaches, classification techniques identified common sources of exposures with implications for interventions, while BKMR further identified specific contributions of individual metabolites.

Keywords

1. Introduction

General population exposure to potentially harmful environmental chemicals is widespread and varies throughout lifespan in terms of chemical and dose. Phthalates, for example, a class of known endocrine disrupting chemicals, are used in many consumer products including some plastics, personal care products, food, and medications, and have been associated with a variety of health outcomes (DiVall, 2013; Ejaredar et al., 2015; Hauser & Calafat, 2005; Mariana et al., 2016). Epidemiological studies have demonstrated associations of certain prenatal phthalate metabolite concentrations with lower birth weight and reduced fetal growth (Ferguson et al., 2016; Huo et al., 2015; Smarr et al., 2015; Veiga-Lopez et al., 2015). However, most of these studies analyzed one phthalate metabolite at a time, without considering chemical interactions, or effects of other chemicals that might confound the observed association. In fact, possible additive or multiplicative interaction effects between these metabolites have rarely been examined.

The National Institute of Environmental Health Sciences (NIEHS) has recently encouraged the field to move beyond the traditional "one chemical at a time" approach to evaluating effects of environmental chemical mixtures on health outcomes (Birnbaum, 2012). In 2015, the NIEHS conducted a workshop to identify and explore potential methods for analyzing chemical mixtures in epidemiological studies. These methods can be broadly classified as standard regression, classification and prediction [e.g., principle component analysis (PCA), structural equation modeling (SEM)], exposure-response surface estimation [e.g., Bayesian Kernel Machine Regression (BKMR), Exposure Surface Smoothing (ESS)] (Taylor et al.,

2016), and variable selection/shrinkage (e.g., least absolute shrinkage and selection operator). Nonetheless, few studies have applied these techniques to assess environmental chemical mixtures and health outcomes (Agier et al., 2016; Bobb et al., 2015; Lee et al., 2018; Lenters et al., 2015; Maresca et al., 2016; Park et al., 2017; Stafoggia et al., 2017; Valeri et al., 2017), and most of these studies that have been conducted on mixtures lack a comparison of the results using different methods, with a few exceptions (Agier et al., 2016; Sun et al., 2013; Valeri et al., 2017). In the present study we explored and compared three methods: 1) linear regression approaches, 2) classification methods (PCA and SEM), and 3) BKMR to evaluate mixtures of prenatal urinary phthalate metabolite concentrations in relation to birth weight in a prospective cohort study of pregnant women from a fertility clinic---a population with potential increased susceptibility to these chemicals (Messerlian et al., 2013).

2. Methods

2.1. Study population

The Environment and Reproductive Health (EARTH) Study is an ongoing, prospective cohort designed to identify environmental and dietary determinants of fertility and pregnancy outcomes among couples presenting to Massachusetts General Hospital (MGH) Fertility Center (Boston, MA). Women were eligible if they were age 18 to 45 years at enrollment. The details of the EARTH study have been described previously (Messerlian et al., 2018). The present analysis included women who contributed at least one urine sample during pregnancy for measurement of phthalates and delivered a singleton live born infant between 2005 and 2016. For women with more than one pregnancy during the study period (n=15), only their first infant was included in our analysis. Therefore, the present study consisted of 300 mother-infant pairs, for whom we collected a total of 732 urine samples (1 to 3 per woman) during the corresponding pregnancies. Trained research staff obtained informed consent and the study was approved by the Human Studies Institutional Review Boards of the Partners, Harvard T.H. Chan School of Public Health, and the Centers for Disease Control and Prevention (CDC).

2.2. Urinary phthalate metabolite measurements

Women collected spot urine samples during their first, second, or/and third trimesters of pregnancy in sterile polypropylene cups. Specific gravity(Christensen et al.) was measured at room temperature using a handheld refractometer within a several hours (typically within 1 hour) after urine collection (National Instrument Company, Inc., Baltimore, MD, USA). The urine was divided into aliquots and frozen at –80°C. Samples were shipped on dry ice overnight to the CDC (Atlanta, GA, USA).

We used on-line solid phase extraction coupled with high-performance liquid chromatography isotope dilution-tandem mass spectrometry to quantify the metabolites of di(2-ethylhexyl) phthalate (DEHP): mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), and the metabolites of di-isobutyl-, di-*n*-butyl-, butylbenzyl-, diethyl-phthalates: mono-isobutyl phthalate (MiBP), mono-n-butyl

phthalate (MBP), monobenzyl phthalate (MBzP) and monoethyl phthalate (MEP), respectively. The standard quality control procedures have been previously described (Silva et al., 2007; Ye et al., 2005) The limit of detection (LOD) was 0.5–1.2 µg/L for MEHP, 0.2–0.7 µg/L for MEHHP and MEOHP, 0.2–0.6 µg/L for MECPP, 0.1–0.2 µg/L for MCPP, 0.2–0.3 µg/L for MBzP, 0.4–0.8 µg/L for MEP, and 0.4–0.6 µg/L for MBP.

2.3. Outcome assessment

We obtained infant birth weight in grams and gestational age at delivery in weeks from hospital medical records. For women who did not deliver at MGH (n=43), we estimated gestational age at delivery according to women's modes of conception. Specifically, for women who underwent in vitro fertilization (IVF) we used the formula: (date of delivery-date of the embryo transfer + day of transfer+14) in accordance with the American College of Obstetricians and Gynecologists (ACOG) guideline (Obstetricians & Gynecologists, 2014); for women who underwent intrauterine insemination (IUI) or conceived naturally, we calculated gestational age from the date of delivery minus the cycle start date.

2.4. Covariates Assessment

At enrollment, study staff administered a questionnaire regarding demographic factor, personal and family history, and lifestyle factors. Participants also completed a detailed take-home questionnaire on lifestyles, medical and reproductive history. Participants' weight and height were measured by trained research staff. Body mass index (BMI) at enrollment was calculated as weight (in kilograms) per height (in meters) squared. Infertility diagnosis by a physician was assigned to each patient based on the Society for Assisted Reproductive Technology criteria (Mok-Lin et al., 2010; Society for Assisted Reproductive Technologies (SART)), and classified as male factor, female factor or unexplained infertility. Women in this study achieved pregnancy by IVF, IUI, or naturally without medical intervention (naturally conceived without IVF or IUI procedures).

2.5. Statistical Analysis

Demographic characteristics of the study participants were reported using means and standard deviations (SDs) or counts with percentages. Phthalate metabolite concentrations were adjusted for urinary dilution using the following formula: $Pc = P[(1.014 - 1)/SG - 1]$, where Pc is the SG-adjusted phthalate metabolite concentration (µg/L), P is the measured phthalate metabolite concentration (µg/L), and 1.014 is median SG level in the study population (Smith et al., 2012; Teass et al., 1998). The very small percentage of non-detectable phthalate metabolite concentrations were replaced with a value equal to the LOD divided by square root of 2 prior to SG adjustment (Hornung & Reed, 1990). When multiple urine samples were available, we calculated the geometric mean of urinary metabolite concentrations for each individual phthalate for each woman. Due to right skewedness, urinary phthalate concentrations were $\log_e$-transformed for all statistical analyses.

Potential confounders were selected *a priori* for inclusion in adjusted models based on prior knowledge (Horton & Crump, 1958; Kawwass et al., 2013; Nelson & Lawlor, 2011) using a directed acyclic graph. We also adjusted for predictors of birth weight (infant sex, maternal height, parity, and gestational age at delivery) to help reduce random variability in the model

(Schisterman et al., 2009). All models included the following covariates: gestational age (weeks), gestational age square, infant sex (male/female), maternal age (years), pre-pregnancy BMI (kg/m^2), height (cm), education (college or higher/<college), smoking history (ever/never), infertility diagnosis (male factor/female factor/unexplained), parity (nulliparous/parous), season of conception (Jan–March/April–June/July–Sept/Oct–Dec), and method of conception (IVF/IUI/natural). Since gestational age may be on the causal pathway leading from phthalates exposure to birth weight (Polanska et al., 2016; Weinberger et al., 2014), we also replicated our final model (BKMR) in a sensitivity analysis when birth weight z-scores were considered. Gestational age-adjusted birth weight z-scores were calculated by taking the residuals of linear regression models of birth weight on gestational age, modeled with restricted cubic splines for additional smoothness (Hutcheon et al., 2013). Correlations between log_e-transformed urinary phthalate concentrations were assessed using Spearman correlation coefficients (Figure 1).

2.6. Statistical approaches for chemical mixtures

A wide variety of statistical approaches have been proposed to assess the health effects of environmental mixtures in epidemiological studies (Bobb et al., 2015; Sun et al., 2013; Taylor et al., 2016). As reported from a recent NIEHS workshop (Taylor et al., 2016), these techniques can generally be classified as falling into one of four categories: (1) linear regression approaches, (2) classification and prediction approaches, (3) exposure-surface estimation, (4) variable selection or/and variable shrinkage approaches. In this study, we compared three of these approaches including linear regression approaches, two classification approaches using PCA and SEM for data visualization, and BKMR, a recently proposed method for the estimation of the exposure-response surface (Bobb et al., 2015; Valeri et al., 2017). The estimates were presented as differences [95% confidence intervals (CI)] in birth weight (grams) as each individual phthalate was increased from the 10th to the 90th percentile.

Approach 1 – Linear regression approaches—We first evaluated the association between each chemical and the birth weight outcome in separate linear regression models. The main limitation of this approach is that it does not take into account the association with other exposures. Even if one could simultaneously evaluate the mixture of exposures in a mutually-adjusted regression model, given that exposures are highly correlated, multiple regression models can become unstable and this approach may provide unreliable results (Rosner, 2015). In addition, while two-way interactions between metabolites can be incorporated, model instability may still appear due to collinearity of exposures. Other statistical methods, as described below, can provide alternatives to take into account such high-dimensional correlation structures.

Approach 2 – Principal component analysis (PCA) and structural equation modeling (SEM)—PCA reduces a large number of correlated variables to a smaller number of uncorrelated components while retaining as much information as possible of the original variables. It is an unsupervised data reduction tool in that no outcome measure is taken into consideration. A set of scores (called loading factors) is calculated representing how closely the phthalate metabolites conform to identified principal components. In this

study, we used PCA with varimax rotation to identify the principal components with eigenvalue greater than one (O'Rourke & Hatcher, 2013). We then fitted a linear regression model using the principal component scores as the main exposure measures.

We also applied SEM to investigate the association between phthalate mixtures and birth weight. We conducted an exploratory factor analysis to reduce the eight urinary phthalate metabolite concentrations into two latent constructs. We then built an SEM to estimate the associations between the latent constructs and birth weight, and calculated standardized path coefficients. Overall SEM fit was assessed using the Comparative Fit Index (CFI), where CFI > 0.90 is generally considered as adequate fit (O'Rourke & Hatcher, 2013).

Approach 3 - Bayesian Kernel Machine Regression (BKMR)—BKMR was recently proposed as a novel method for investigating environmental mixtures(Bobb; Bobb et al., 2015; Valeri et al., 2017). BKMR utilizes a non-parametric approach to evaluate dose-response relationships, allowing for possible non-linearity and interactions in exposure-outcome associations, which can often occur in the context of endocrine disrupting chemicals (National Research Council, 2014).

In this study, for each subject i=1,…,n, the BKMR model is given by $Y_i = h(MEP_i, MBP_i, MiBP_i, MBzP_i, MEHP_i, MEHHP_i, MEOHP_i, MECPP_i) + \beta^T Z_i + e_i$, where the function h() is an exposure-response function that accommodates nonlinearity and/or interaction among the mixture components, and $Z = Z_1,.., Z_p$ are p potential confounders. There are several possible choices for specifying the kernel function, and we used the Gaussian kernel, which has been applied in simulation studies and real-life scenarios (Bobb et al., 2015; Valeri et al., 2017). There are two major approaches for variable selection: component-wise variable selection and hierarchical variable selection. In the scenario where phthalate mixture components are highly correlated, a hierarchical variable selection will allow us to estimate the effect of highly correlated components that would not be identifiable in regression models. After fitting the model, BKMR produces estimates of the exposure-response function h() and point wise 95% credible intervals, which incorporate the uncertainty due to estimation of high dimensional exposures and multiple-testing penalty. Details (Bobb et al., 2015) and examples of how to implement the method using the R package are well described elsewhere(Bobb).

We used a hierarchical variable selection approach (i.e., we pre-specified DEHP and non-DEHP groups based on the PCA results) to estimate the exposure-response surface of the relationship between eight phthalate metabolites and birth weight. While h(.) is a high dimensional response surface, BKMR allows visualization of different cross-sectional views of this surface. Specifically, we plotted the relationships of each phthalate metabolite with birth weight while fixing the remaining phthalate metabolites at their median levels (Figure 3). In addition, we also summarized the joint effects of the two phthalate metabolites by plotting a dose-response relationship of a single metabolite at various quantiles (e.g., 10th, 50th, 90th quantiles) of the 2nd metabolite and fixing the remaining six metabolites at their median values (Figure 5). Lastly, BKMR also allows one to summarize the effects of an individual phthalate metabolite on birth weight. For example, we calculated the mean

difference in birth weight when each metabolite was increased from the 10th to the 90th percentile while setting the other metabolites at their median concentrations (Figure 4).

The SEM and PCA approaches were implemented using SAS 9.4 (SAS Institute Inc, Cary, NC). The remaining statistical analyses were conducted using R (version 3.1.0; R Foundation for Statistical Computing) (Bobb et al., 2015).

3. Results

The study consisted of 300 mother-child pairs. A total of 732 prenatal urine samples were collected: 59% of women provided three samples, 26% provided two samples and 15% provided one sample. The study population was predominantly white (86%), never smokers (74%), and college educated (87%). The mean age at enrollment was 34.6 years (SD: 3.8), and mean pre-pregnancy BMI was 24.1 (SD: 4.2) kg/m^2 (Table 1). Approximately 33% of women had a female factor fertility diagnosis at enrollment. 54 % of women conceived via IVF, 21% via IUI, while 25% of women conceived naturally without medical intervention. The mean birth weight (SD) was 3340 (512) grams; the mean (SD) gestational age at delivery was 39.4 (1.7) weeks; and 3.7% of infants were of low birth weight (<2500 grams) (World Health Organization, 2014).

The urinary concentrations of phthalate metabolites were within the ranges to those found in U.S. females, with high detection frequencies ranging from 94% for MBzP to 100% for MEP (Supplemental Table S1). Figure 1 shows the correlation matrix between urinary concentrations of the eight phthalate metabolites. Globally, metabolites of DEHP highly correlated with each other (r ranging from 0.80 to 0.98), while metabolites of non-DEHP phthalates were weakly associated with all other metabolites (r<0.35) except for MBP.

None of the individual phthalates or phthalate mixtures produced a statistically significant association with birth weight using the three selected approaches. We therefore focused on the interpretation of effect estimates in the following sections.

3.1. Linear regression approaches

Table 2 displays associations between maternal urinary concentrations of phthalate metabolites and birth weight using basic linear regression models. When we analyzed one metabolite at a time, all metabolites had negative but not statistically significant associations with birth weight, with adjusted differences in mean birth weight ranging from −93 (CI: −206, 21) to −49 (95%CI: −164,65) grams from 10th to 90th percentiles increase in metabolite concentrations. When mutually adjusting for other phthalate metabolites in a multivariable model, inflation of the estimates and standard errors were noted. Specifically, in the mutually adjusted multivariable regression model, the association of MEOHP with birth weight became positive, although not significant, with a change in the adjusted mean difference increase from −49 (95% CI: −165, 66) grams to 651 (95%CI: −57, 1360) grams, likely due to multicollinearity. All remaining phthalate metabolites remained negatively associated with birth weight, with beta coefficients ranging from −16 (95%CI: −148, 116) grams for MBP to −351 (95%CI: −1041, 340) grams for MEHHP.

3.2. Principal Component Analysis and Structural Equation Model

Application of PCA yielded an identification of two main components accounting for 53% and 18%, respectively, of the variance in urinary metabolite concentrations. Loading factors for each metabolite on each of the components are presented in Supplemental Table S2. The first principal component, which we named "DEHP component" had high loading factors for MEHP, MEHHP, MEOHP and MECPP, while the second principal component, which we named "non-DEHP component" had high loading factors for MEP, MBP, MiBP, and MBzP. In the multivariable-adjusted models where these two components were included, higher DEHP and non-DEHP component scores were related to lower birth weight [adjusted mean difference =−23 (95%CI: −68, 22) and −27 (95%CI: −71, 17) grams, respectively (Figure 2)]. When applying SEM using two latent constructs, the conceptual model provided an acceptable fit (comparative fit index=0.96). Results were consistent with PCA, with standardized regression coefficients for the DEHP and non-DEHP latent constructs of −0.05 (95%CI: −0.18, 0.08) and −0.03 (95%CI: −0.14, 0.09), respectively (Supplemental Figure S1).

3.3. Bayesian Kernel Machine Regression

We used results from PCA and SEM to assign metabolites into two groups and used a hierarchical variable selection within these two groups to estimate the kernel function. Although all results from this model yielded wide confidence intervals, BKMR identified two specific metabolites, namely MEP and MEHP, which had stronger associations with lower birth weight, both with linear relationships (Figure 3). Figure 4 quantifies the magnitude of these associations by presenting the mean differences (95%CI) in birth weight for a change in concentrations of individual phthalate metabolites from their 10th to 90th percentiles [−51 (95%CI: −164, 63) grams for MEP, and −122 (95%CI: −311, 67) grams for MEHP]. There was a positive yet non-significant association between MEOHP and birth weight [77 (95%CI: −173, 328)]. Other individual phthalate metabolites had no association with birth weight. Lastly, we examined the potential interaction between MEP and MEHP based on examination of the empirically estimated response surface. Figure 5 shows differences in birth weight as a function of MEP, by moving MEHP concentrations from 10th to 50th and to 90th percentile (while fixing all other phthalate metabolites to their 50th percentile), and vice versa. The parallel exposure-response relationships suggested no evidence of interaction between MEP and MEHP.

Results were consistent when evaluating gestational age-adjusted birth weight z-scores. This analysis is presented in Supplemental Figure S2, which shows the dose-response associations of MEP and MEHP concentration with birth weight z-scores based on BKMR.

4. Discussion

We examined the results of three different statistical approaches to analyzing phthalate mixtures in relation to birth weight in the context of a prospective study among women seeking fertility treatment. Neither of the applied approaches produced statistically significant results between individual phthalates or phthalate mixture and birth weight. Despite these non-significant associations, we recognize that such null findings may due to

small samples, high within-person variability of urinary biomarkers, survival bias, and unmeasured or/and residual confounding. Interpreting the estimates and providing pros and cons of different approaches was the primary objective of this study. Using linear regression approaches with each individual metabolite analyzed in a separate linear model, there were moderate inverse relationships between individual phthalate metabolites and birth weight. However, after mutually adjusting for all other metabolites in the same statistical model, inflation of the estimates and standard errors were noted, likely due to collinearity. We presented two approaches among those proposed (Taylor et al., 2016) to address this problem. Classification approaches (PCA and SEM) identified two principal components that can largely be interpreted as the DEHP and non-DEHP components, both of which had moderate inverse associations with birth weight. BKMR further identified MEP (a component of non-DEHP), as well as MEHP (a component of DEHP) as the most important contributors to these associations. There was no suggestive evidence of synergistic effects between these phthalates based on visualization of the bivariate exposure-response functions in BKMR.

Some prior studies have examined the association between urinary phthalate metabolites and birth weight, but the findings have been inconsistent. For example, in a birth cohort of 482 mother-child pairs, Ferguson et al. reported that the sum of maternal urinary DEHP phthalate metabolites during pregnancy was inversely associated with estimated or actual fetal weight, and this association was strongest for MECPP (Ferguson et al., 2016). In a population-based birth cohort study in Spain (n=488 mother-child pairs), Casas et al. showed that prenatal urinary MBzP concentrations were positively associated with birth weight among boys but not in girls. By contrast, three studies, including a prospective multiethnic cohort in New York city (n=404)(Wolff et al., 2008), a case-control study (n=191) in France, (Philippat et al., 2012) and a recent prospective study from the EARTH cohort (Messerlian et al., 2017) (n=321) reported that neither the individual prenatal phthalate metabolites nor the grouping of phthalate metabolites based on molecular weight were associated with birth weight. Of note, however, in the latter study (Messerlian et al., 2017), Messerlian et al. reported that specific phthalate metabolites were associated with lower birth weight among IVF-conceived singletons (n=208), but these associations were no longer significant after adjusting for paternal phthalate metabolite concentration (Messerlian et al., 2017). Further, Messerlian et al. found that paternal urinary concentration of the sum of DEHP metabolites was associated with lower birth weight among IVF singletons regardless of adjustment for maternal prenatal DEHP concentrations (Messerlian et al., 2017). It is important to point out that these studies performed statistical analysis using one phthalate metabolite at a time, without accounting for other metabolites that are possibly associated with each other and with the outcome. The specific phthalate metabolite identified by individual analysis could be potentially confounded by other phthalate metabolites or other correlated chemicals. In the present study, when simultaneously including all phthalate metabolites in a single regression model, the effect estimates changed substantially, with certain metabolites (e.g. MEOHP) switching the sign of the association. As such, our results of the regression methods underscore the need to apply appropriate statistical methods that take into account the correlation structure of the evaluated biomarkers.

Classification approaches such as PCA and SEM have been used to investigate the mixture of environmental chemicals or pollutants while taking into account multicollinearity. PCA has been used to assess urinary phthalate metabolites in the literature (Maresca et al., 2016). As metabolites that come from a single parent compound (e.g., MEHP, MEHHP, MEOHP, MECPP come from the parent compound, DEHP) and metabolites that have similar exposure sources (e.g, MEP and MBP, metabolites of diethyl phthalate and dibutyl phthalate respectively are both used in personal care and beauty products)(Hauser & Calafat, 2005) are often correlated, the identified principal components (based on the observed variance-covariance structure of the data) are likely to reflect such exposure patterns. Notably, PCA converts a set of observed variables into principal components based on the collinearity between the exposure variables rather than the underlying biological effects of a given mixture on the outcomes. Therefore, the associations would be masked if phthalates within the component have opposite effects on birth weight. In addition, the derived component scores are known to be data dependent, which may limit its generalizability across studies. However, data from earlier studies including the Columbia Center for Children's Environmental Health study and the U.S. National Health and Nutrition Examination Survey have identified similar components (namely DEHP and non-DEHP components) as well as component loadings for phthalate exposure (Maresca et al., 2016), suggesting that the correlation structure between phthalate metabolite concentrations is similar to some extent with the general U.S. population.

Another similar source apportionment method is SEM, which is often used to analyze the structural relationship between measured variables and latent constructs. In this study, both PCA and SEM suggested inverse associations of DEHP components and of non-DEHP component with birth weight. An important distinction between PCA and SEM is that the latter assumes the latent constructs "DEHP group" and "non-DEHP group" exert directional influence on the measured phthalate metabolite concentrations (i.e., measured biomarker concentrations are modeled as a function of the latent construct) presented in Figure 3. In addition, SEM accounts for the measurement errors of these latent constructs when assessing their associations with birth weight (O'Rourke & Hatcher, 2013), while PCA simply groups the measured variables based on the total variance of the observed variables without considering uncertainty of principal components in the second step of the regression model (O'Rourke & Hatcher, 2013). Further, compared with PCA approach, SEM tends to make broader assumptions about linearity and normality distributions for all the paths present in the entire SEM (VanderWeele, 2012). Therefore, SEM constitutes as a more powerful tool when we attempt to capture a wide range of exposures or simultaneously evaluate numerous pathways for different health outcomes of a given population (VanderWeele, 2012).

From the perspective of policy makers and regulatory agencies, PCA and SEM methods are attractive as they help to identify common sources of exposure to phthalate mixtures that are responsible for adverse health outcomes for regulatory intervention. However, these methods have some important limitations. First, both SEM and a regression model incorporating principal components as "exposures" assume a linear function between two components (or latent constructs) and birth weight. Second, as PCA is agnostic approach to reduce the dimension of exposure without considering the correlations with the outcome measure, it is unclear whether the group of phthalate metabolites or specific phthalate metabolites within

the group was responsible for the inverse association with birth weight. Third, PCA and SEM cannot account for interactions among individual phthalate metabolites. In an *in vivo* study, combinations of benzylbutylphalate (BBP) and dibutyl phthalate (DBP) and of DBP and diethyl phthalate (DEP) demonstrated synergistic anti-androgenic activity at high doses and antagonistic activity at low concentrations (Christen et al., 2012). While it is unclear whether phthalate mixtures also exert similar synergistic effects on oxidative stress (Holland et al., 2016; Tetz et al., 2013) or epigenetic modifications (LaRocca et al., 2014; Zhao et al., 2015)(some possible mechanisms affecting fetal growth), such possibility cannot be ruled out and cannot be tested using PCA and SEM methods.

Recently, BKMR was introduced as a new approach to study mixtures, in which non-linear effect and non-additive relationship with health outcomes can be captured based on a flexible kernel function. By using this method we identified two specific metabolites, MEP and MEHP, which were associated, in a linear fashion, with lower birth weight. On the other hand, MEOHP was positively associated with birth weight, though all confidence intervals were somewhat wide. Other individual phthalate metabolites had no association with birth weight. Of note is that, as MEHP and MEOHP are grouped as DEHP components (shown in the classification analyses), but exert opposite effects on birth weight (shown in the BKMR analysis), the associations between DEHP components and birth weight based on PCA and SEM approaches may potentially be diluted. Furthermore, while we found no evidence of nonlinearity or interactions among phthalate metabolites, given the small sample size, it may be difficult to pick up interactions that were in fact present (Coull & Parket, 2015). Currently, formal statistical tests for interactions among phthalate in the mixture in BKMR are not readily available.

Importantly, although we compared pros and cons of each method, these statistical approaches may complement each other and help to optimize the analysis strategies for the study of interests. Moreover, while we herein focused on selected approaches, other available methods may be more suitable for specific settings. For example, when the effect is linear and interactions are not present, a method such as weighted quantile regression could represent a more powerful approach (Bello et al., 2017). In addition, there is limited knowledge regarding the sensitive window of environmental chemical mixtures. Novel methods have recently been proposed such as a distributed lag model (Wilson et al., 2017) and lagged kernel machine regression(Liu et al., 2017), which allow researchers to explore the effect of mixture across different exposure window. In the specific field of environmental reproductive epidemiology, future advances are also necessary to model correlated mixtures of exposure between couples for couple-dependent outcomes given the increasing recognition of importance of male partners for offspring's health (Messerlian et al., 2017; Sundaram et al., 2017). Lastly, in this paper we investigated different approaches in characterizing exposure to phthalate mixtures in relation to a continuous measure. The extension of R packages for running BKMR in the cases of non-normal outcomes is currently under development.

5. Conclusions

Humans are exposed to many chemicals simultaneously. In this paper we demonstrated potential issues that can arise when using linear regression analyses (either one chemical at a time or a mutually adjustment approach) in the context of evaluating a mixture of correlated phthalate metabolites. Our example showed that there was a general consistency between classification methods (PCA, SEM) and BKMR. Among the selected analytic strategies, BKMR represents a flexible approach that allows identifying the specific contribution of each included predictors, while also incorporating possible non-linear and interaction effects. Classification methods (PCA, SEM), on the other hand, help to identify common sources of exposure contributing to health effects. Importantly, optimal analytic strategies may depend on study question and underlying exposure-response relationship; applying different techniques that complement each other may improve the robustness and interpretation of the findings. As modern statistical tools are now available to capture a high degree of correlations amongst explanatory variables, future studies should move from one chemical at a time approach to conducting a comprehensive risk assessment with attempt to capture chemical mixtures on human diseases and well-being.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Abbreviations

BKMR	Bayesian Kernel Machine Regression
PCA	principal component analysis
SEM	structural equation modeling

References

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Highlights

- We applied and compared three statistical approaches for evaluating the associations between a mixture of prenatal urinary phthalate metabolites and birth weight.

- We demonstrated potential issues arising using linear regression models (either one chemical at a time approach or a mutual adjustment approach) in the context of correlated exposures.

- Principal component analysis and structuring equation modeling identified common sources of exposures with implications for intervention.

- Bayesian Kernel Machine Regression further identified specific contributions of individual metabolites to reduced birthweight.
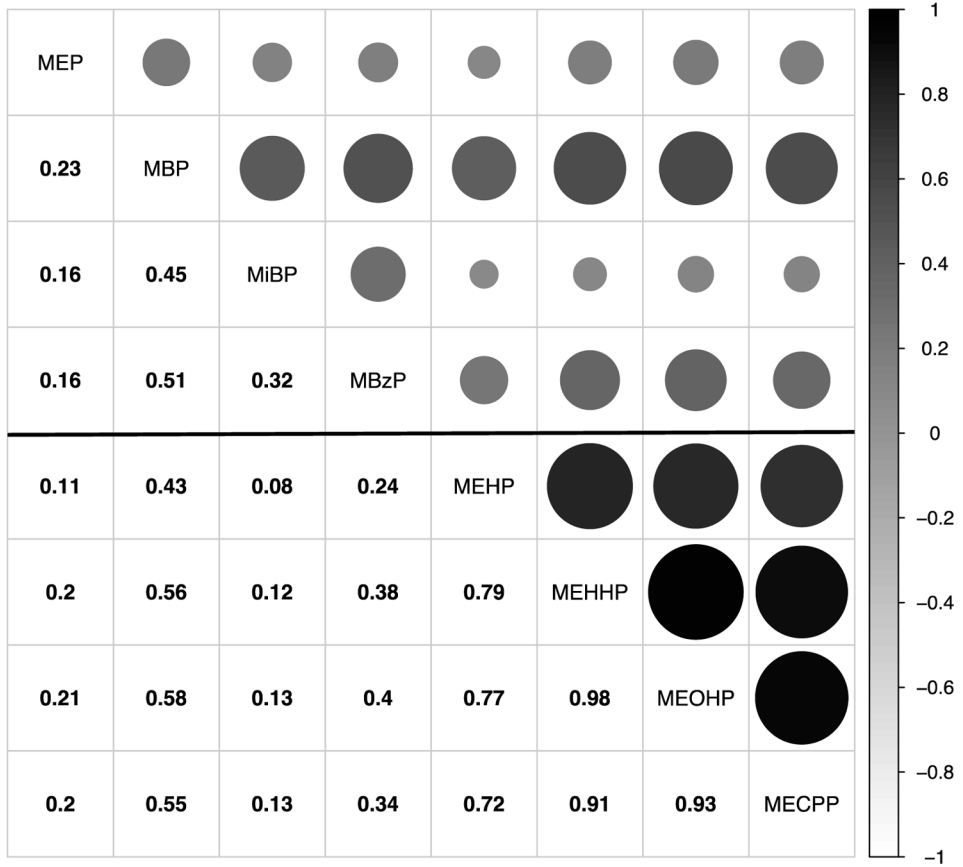
Figure 1.
Correlation plot of the urinary concentrations of eight phthalate metabolites
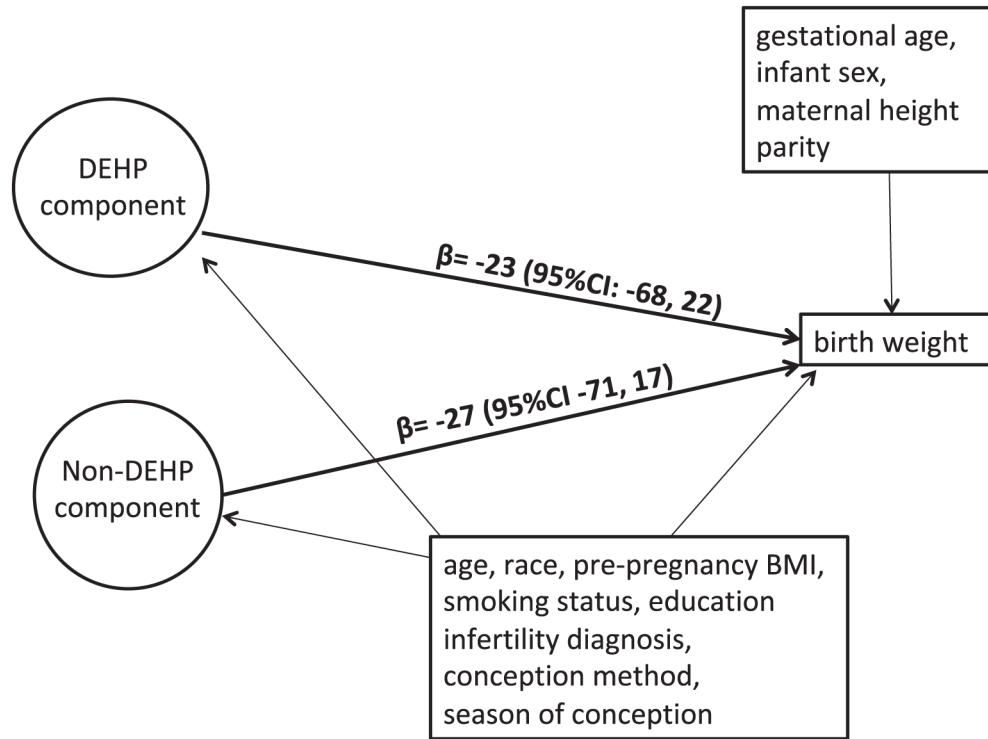Dot size is proportional to the magnitude of Spearman correlation coefficients.

Figure 2.
The associations of principal components of phthalate metabolites and birth weight among 300 mother-child pairs in the Environment and Reproductive Health (EARTH) Study.
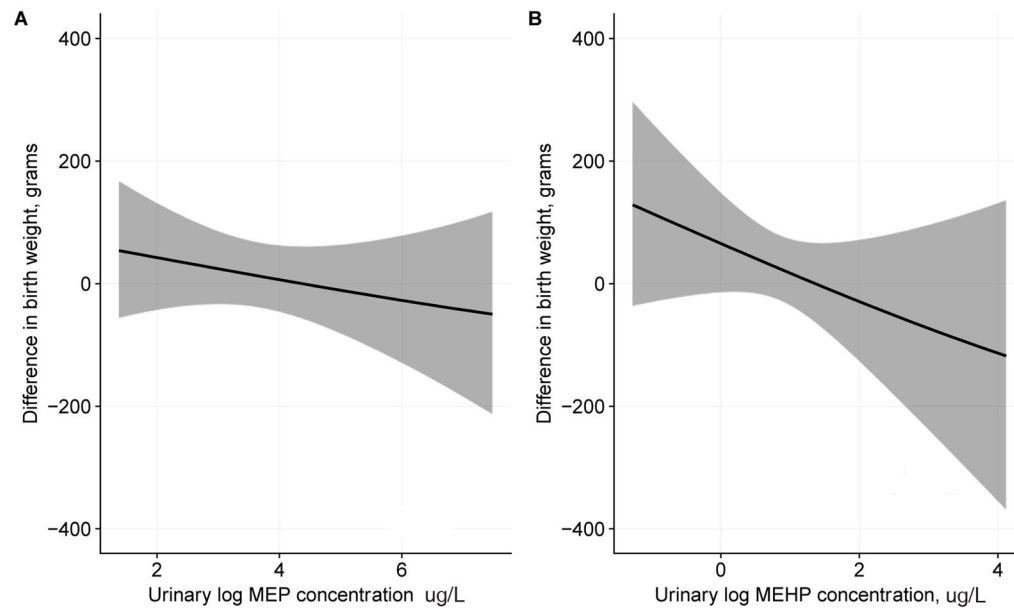
Figure 3.
Dose-response function (95% credible intervals) between selected metabolite concentrations (i.e., A) MEP and B) MEHP) and birth weight while fixing other phthalate metabolite concentrations at median values. The results were estimated by Bayesian Kernel Machine Regression, adjusting for gestational age, gestational age square, maternal age, BMI, height, education, smoking, infertility diagnosis, parity, method of conception, season of conception, and infant sex.
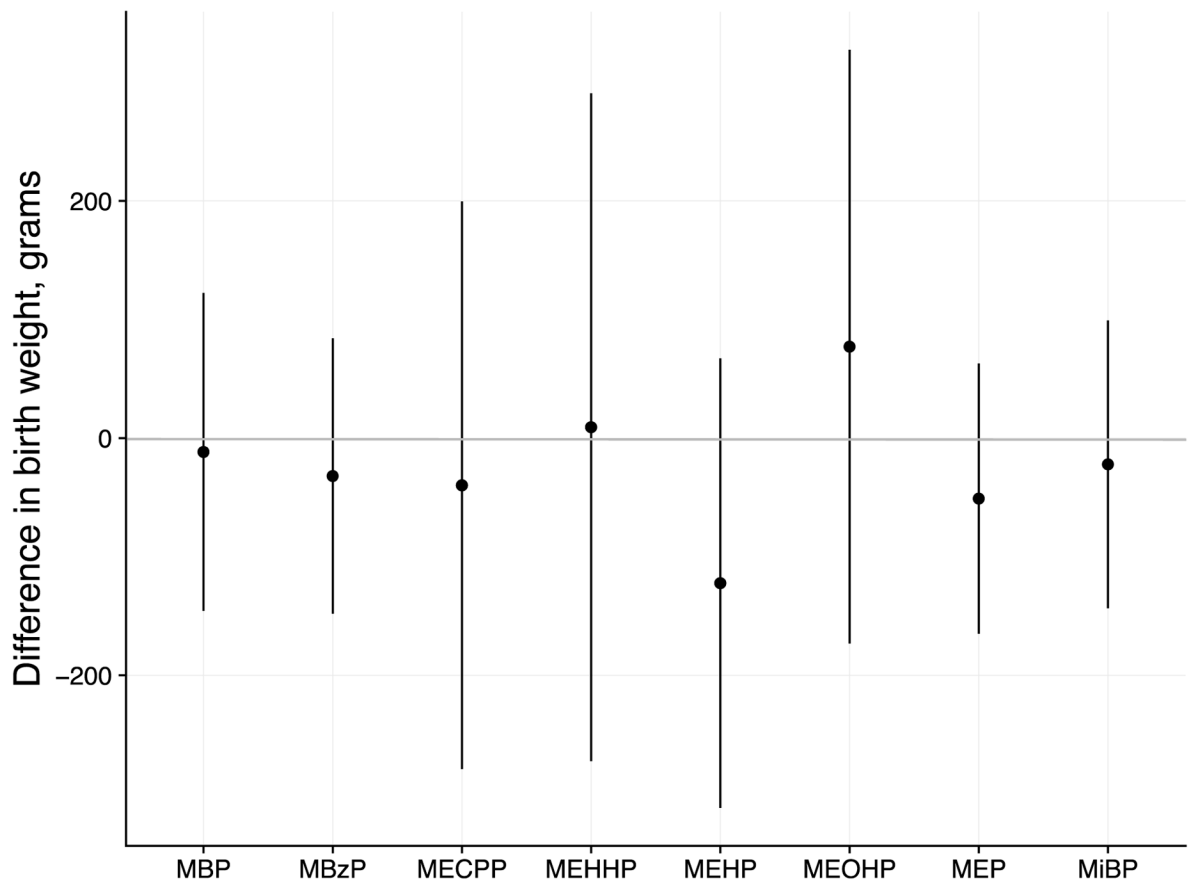
Figure 4.
Mean differences in birth weight (estimates and 95% confidence intervals) as a function of phthalate metabolite concentrations in the EARTH study. Point estimates show the difference in mean birth weight when each phthalate metabolite was increased from the 10th to the 90th percentile of its distribution, while fixing other phthalate metabolite concentrations at their median concentrations. The results were estimated by Bayesian Kernel Machine Regression, adjusting for gestational age, gestational age square, maternal age, BMI, height, education, smoking, infertility diagnosis, parity, method of conception, season of conception, and infant sex.
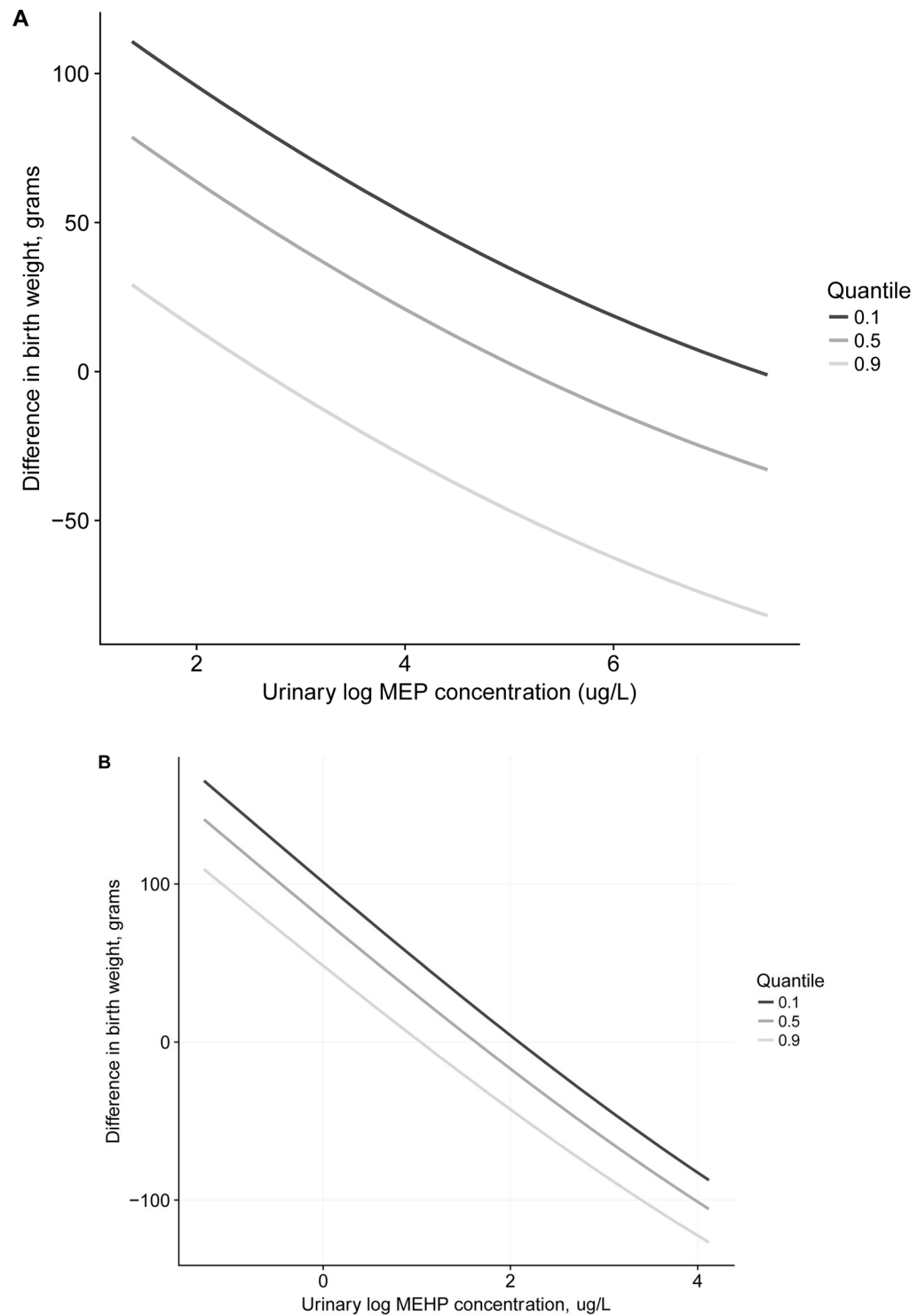
Figure 5.
Mean differences in birth weight as a bivariate exposure-response function of A) MEP at 10th, 50th, and 90th percentile of MEHP B) MEHP at 10th, 50th, and 90th percentile of MEP, while other phthalate metabolite concentration are fixed at the median values. The results

were estimated by Bayesian Kernel Machine Regression, adjusting for gestational age, gestational age square, maternal age, BMI, height, education, smoking, infertility diagnosis, parity, method of conception, season of conception, and infant sex.

Table 1

Baseline characteristics among 300 pregnant women with a live singleton birth in the Environment and Reproductive Health (EARTH) Study.

	Mean ± SD or N (%)			
Characteristic	Overall	Providing 1 sample	Providing 2 samples	Providing 3 samples
N	300	44	79	177
Age (years)	34.6 ± 3.8	34.3 ± 4.2	34.8 ± 3.5	34.6 ± 3.9
Pre-pregnancy BMI (kg/m^2)	24.1 ± 4.2	24.1 ± 3.9	24.4 ± 4.2	23.9 ± 4.3
Ever smokers	78 (26%)			
Race				
Caucasian	259 (86%)	37 (84%)	68 (86%)	154 (87%)
Black/African American	7 (2%)	2 (5%)	1 (1%)	4 (2%)
Asian	23 (8%)	3 (7%)	7 (9%)	13 (7%)
Other	11 (4%)	2 (5%)	3 (4%)	6 (3%)
Education				
High school graduate or less	25 (8%)	5 (11%)	6 (8%)	14 (8%)
Some college	14 (5%)	6 (14%)	2 (3%)	6 (3%)
College graduate or higher	261 (87%)	33 (75%)	71 (90%)	157 (89%)
Infertility diagnosis				
Male factor	78 (26%)	11 (25%)	16 (20%)	51 (29%)
Female factor	99 (33%)	17 (39%)	28 (35%)	54 (31%)
Unexplained	123 (41%)	16 (36%)	35 (44%)	72 (41%)
Methods of conception				
IVF	163 (54%)	23 (52%)	45 (57%)	95 (54%)
IUI	63 (21%)	10 (23%)	11 (14%)	42 (24%)
Natural	74 (25%)	11 (25%)	23 (29%)	40 (23%)
Gestational age (weeks)	39.4 ±1.6	39.2 ± 2.1	39.4 ±1.6	39.4 ±1.6
Birth weight (grams)	3341 ±512	3355 ± 630	3362 ± 449	3328 ±508
Male Infant gender	156 (52%)	21 (48%)	43 (54%)	92 (52%)

Table 2

The association between individual phthalate metabolites and birth weight (grams) among 300 mother-child pair based on linear regression models

Urinary phthalate metabolite	One at a time	Mutually Adjusted for other Phthalate Metabolites
	β (95%CI)[1]	β (95%CI)[1,2]
MEP	−60 (−172, 52)	−49 (−165, 66)
MBP	−55 (−154, 44)	−16 (−148, 116)
MiBP	−51 (−160, 57)	−31 (−153, 91)
MBzP	−55 (−160, 50)	−42 (−162, 79)
MEHP	−93 (−206, 21)	−163 (−374, 48)
MEHHP	−67 (−187, 54)	−351 (−1041, 340)
MEOHP	−49 (−164, 65)	651 (−57, 1360)
MECPP	−71 (−188, 47)	−214 (−586, 157)

CI=confidence interval, β estimates represent the mean differences in birth weight (grams) when each metabolite was increased from the 10th to the 90th percentiles

[1] Adjusted for gestational age, gestational age square, maternal age, pre-pregnancy BMI, height, education, smoking, infertility diagnosis, parity, method of conception, season of conception, and infant sex.

[2] Additionally adjusted for all the other phthalate metabolites.