Published in final edited form as:

Environ Int. 2014 September ; 70: 118–124. doi:10.1016/j.envint.2014.05.016.

Variability in urinary phthalate metabolite levels across pregnancy and sensitive windows of exposure for the risk of preterm birth

Kelly K. Ferguson^a, Thomas F. McElrath^b, Yi-An Ko^c, Bhramar Mukherjee^c, and John D. Meeker^a

^aDepartment of Environmental Health Sciences, University of Michigan School of Public Health, Ann Arbor, MI, United States

^bDivision of Maternal-Fetal Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, United States

^cDepartment of Biostatistics, University of Michigan School of Public Health, Ann Arbor, MI, United States

Abstract

Background—Preterm birth is a significant public health problem, affecting over 1 in 10 live births and contributing largely to infant mortality and morbidity. Everyday exposure to environmental chemicals such as phthalates could contribute, and may be modifiable. In the present study we examine variability in phthalate exposure across gestation and identify windows of susceptibility for the relationship with preterm birth.

Methods—Women were recruited early in pregnancy as part of a prospective, longitudinal birth cohort at the Brigham and Women's Hospital in Boston, Massachusetts. Urine samples were collected at up to 4 time points during gestation for phthalate measurement, and birth outcomes were recorded at delivery. From this population we selected all 130 cases of preterm birth, defined as delivery before 37 weeks completed gestation, as well as 352 random controls.

Results—Urinary phthalate metabolite levels were moderately variable over pregnancy, but levels measured at multiple time points were associated with increased odds of preterm birth. Adjusted odds ratios (aOR) for spontaneous preterm birth were strongest in association with phthalate metabolite concentrations measured at the beginning of the third trimester (aOR for summed di-2-ethylhexyl phthalate metabolites [DEHP]=1.33, 95% confidence interval [CI]=1.02, 1.73). Odds ratios for placental preterm birth, defined as delivery with presentation of preeclampsia or intrauterine growth restriction, were slightly elevated in the first trimester for DEHP metabolites (aOR for DEHP=1.33, 95% CI=0.99, 1.78).

© 2014 Elsevier Ltd. All rights reserved.

Corresponding author: John D. Meeker, University of Michigan School of Public Health, 1835 SPH I, 1415 Washington Heights, Ann Arbor, MI 48109-2029. Phone: 1-734-764-7184. Fax: 1-734-936-7283. meekerj@umich.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conclusions—Pregnant women with exposure to phthalates both early and late in pregnancy are at increased risk of delivering preterm, but mechanisms may differ based on etiology.

Keywords

phthalate; pregnancy; repeated measures; longitudinal; reproduction

1. Introduction

Phthalate diesters are produced in large quantities yearly in the US for use in everyday products such as polyvinyl flooring, shower curtains, food packaging plastics, and personal care products. Exposure occurs through contact with these products as well as the consumption of contaminated food and drinking water (ATSDR 1995; ATSDR 2001; ATSDR 2002). While phthalates may not persist or accumulate in the human body, even transient exposures, because of their high frequency, have been linked to an array of adverse health outcomes in humans, including altered thyroid and reproductive hormone levels (Meeker and Ferguson 2011; Mendiola et al. 2012), decreased semen quality in males (Hauser et al. 2006), and asthma and allergic symptoms (Bornehag and Nanberg 2010). Exposure to phthalates *in utero* has been linked to adverse birth outcomes as well, including altered reproductive tract development in male infants (Swan et al. 2005), neurodevelopment in both sexes (Engel et al. 2010; Engel et al. 2009), and both prematurity and small size at birth (Ferguson et al. 2014; Meeker 2012; Meeker et al. 2009; Whyatt et al. 2009).

Preterm birth, defined as delivery before 37 weeks completed gestation, is a particularly important endpoint of interest due to: 1) its contribution to neonatal mortality and morbidity and consequent cost to society; 2) the apparent increase in rates over the last three decades; and 3) poorly understood causes and lack of effective interventions (Behrman and Butler 2007). Research to uncover contributing causes, particularly those in connection with environmental contaminant exposures, is a public health priority (Ashton et al. 2009). We recently demonstrated clear associations between maternal exposure to phthalate levels averaged from multiple time points during pregnancy and increased odds of preterm birth in a nested case-control study of women who delivered at the Brigham and Women's Hospital in Boston (Ferguson et al. 2014).

Due to the short-half life of phthalates in the human body, and the consequent variability in exposure measures, a geometric mean of measures from multiple time points provides the most robust estimate of exposure over the course of pregnancy. However, the availability of multiple exposure measures additionally allows investigation of windows during gestation that may be particularly sensitive to the effects of phthalates. In the present analysis we examined variability in phthalate levels across pregnancy and attempted to identify any patterns in levels by gestational age. Also, we assessed associations between phthalate exposure at individual time points during pregnancy and preterm birth in order to identify windows of vulnerability.

2. Materials and methods

2.1 Study population

Participants were part of an ongoing prospective cohort study of pregnant women with initial prenatal visits at clinics in the Boston area. All women who wished to participate were included if they planned to deliver at the Brigham and Women's Hospital and if their initial visit was prior to 15 weeks gestation. Subjects were followed throughout the course of pregnancy and provided information (e.g., health status, weight) and urine samples at up to four visits. Urine samples were refrigerated (4 ° C) for a maximum of two hours before being processed and frozen (−80 ° C) for long-term storage. At delivery, birth outcome characteristics such as mode of delivery and fetal measurements were recorded. From 2006 to 2008 approximately 1600 women were recruited, and 1181 were followed until delivery and had live singleton infants. From this population, the present nested case-control study includes all 130 mothers who delivered preterm, as well as 352 controls selected randomly from subjects who had a urine sample from visit 1 and from at least one additional visit. Gestational ages at individual visits and at delivery were calculated based on last menstrual period (LMP) and confirmed by first trimester ultrasound. Study participants provided written informed consent and institutional review board approval was obtained from Brigham and Women's Hospital as well as the University of Michigan.

Within this study, visit 1 urine samples were taken at median 9.71 weeks gestation (range 4.71 to 16.1 weeks), visit 2 at median 17.9 weeks (range 14.9 to 21.9 weeks), visit 3 at median 26.0 weeks (range 22.9 to 29.3 weeks), and visit 4 at median 35.1 weeks (range 33.1 to 38.3 weeks). The number of subjects with samples available decreased slightly with increasing visit, with the fourth visit having the smallest number of samples. Visit 4 also had a smaller proportion of cases with urine samples, since some had delivered by this time point.

Overall preterm birth was defined using the clinical definition of birth before 37 weeks postmenstrual gestation. However, further classifying preterm birth by clinical presentation may provide cleaner subpopulations with more homogenous etiologies. A study of placental histology of women who delivered very preterm (<32 weeks gestation) showed that women who presented with spontaneous preterm labor or preterm premature rupture of the membranes (PPROM) had distinct patterns of placental inflammation (McElrath et al. 2008). On the other hand, women who delivered preterm as a result of preeclampsia or intrauterine growth restriction (IUGR) showed placental aberrations. Because these differences may be indicative of different mechanistic precursors of preterm birth, we additionally examined prematurity by categories of clinical presentation including 1) spontaneous preterm birth, defined as delivery with presentation of spontaneous preterm labor or PPRM (N=52), and 2) preterm birth of placental origin (placental preterm birth), defined as preterm birth following preeclampsia or IUGR (N=35). A third group were considered protocol driven preterm births, with non-spontaneous delivery or C-section preterm due to maternal complications (e.g., prior C-section, placental abruption, cervical insufficiency, etc.; N=38). These cases were not analyzed separately, as they have no known unifying etiology. Five cases had characteristics of both spontaneous and placental preterm birth, and were

examined more carefully to determine the root of prematurity. Four were determined to be the result of spontaneous preterm labor, and one resulted from placental previa (final N for spontaneous preterm=56, final N for placental preterm=35, final N for protocol driven=39).

2.2 Phthalate exposure

Nine phthalate metabolites were measured in each available urine sample (N=1,693) by NSF International in Ann Arbor, MI, following methods developed by the Centers for Disease Control (CDC), described in detail elsewhere (Silva et al. 2007). Two urine samples (both from control subjects) from visit 1 had insufficient volume for analysis. The final number of samples analyzed for all phthalate metabolites were as follows by visit (cases, controls): Visit 1 (129, 350); Visit 2 (118, 304); Visit 3 (111, 301); and Visit 4 (66, 314). Phthalate measurements below the limit of detection (LOD) were replaced with the LOD divided by 2 (Hornung and Reed 1990).

To adjust for urinary dilution, specific gravity (SG) levels were also measured in each urine sample using a digital handheld refractometer (ATAGO Company Ltd., Tokyo, Japan). For univariate analyses phthalate levels were corrected for urinary SG using the following formula: $P_c = P[(1.015 - 1)/SG - 1]$, where P_c represents the SG-corrected phthalate concentration (micrograms per liter), P represents the measured concentration in urine, 1.015 is the median SG of all samples measured, and SG represents the SG of the individual sample (Meeker et al. 2009).

For regression models, unadjusted phthalate levels were used and urinary SG was included as a covariate, since modeling adjusted phthalate levels may incur bias (Barr et al. 2005). In addition to analysis of individual phthalate metabolites, a summed measure of di(2-ethylhexyl) phthalate (DEHP) metabolites (DEHP; nanomoles/liter) was also examined. All individual metabolites and DEHP were log-normally distributed and ln-transformed for analysis.

2.3 Statistical analysis

Analysis was performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) and R version 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria). To assess variability in phthalate levels across pregnancy, we examined changes in levels by visit both in the population overall and in cases and controls separately. Geometric means and standard deviations of phthalates at individual visits were calculated and differences in SG-corrected concentrations from visits 2–4 compared to visit 1 were tested using linear mixed models (LMM) with random intercepts to adjust for intra-individual correlation. Spearman correlations between measures of the same phthalate metabolite at each visit were calculated using SG-corrected values.

To examine temporal variability in phthalate levels by subject, intraclass correlation coefficients (ICC) and 95% confidence intervals were calculated using uncorrected and SG-corrected phthalate levels, both in the overall population and in cases and controls separately (Hankinson et al. 1995). The ICC measure represents the ratio of intra-individual variability

to intra plus inter-individual variability, and ranges from zero to one with a value of one indicating no intra-individual variability (Rosner 2011).

As a final way of examining variability in urinary phthalate metabolites across pregnancy, we fit generalized additive mixed models (GAMMs) using the R *mgcv* package to examine each phthalate metabolite in relation to gestational age at sample collection (Wood 2011). GAMMs incorporated repeated measures of phthalate levels across pregnancy and regressed these levels on a smooth function of gestational age. This allowed inspection of potentially non-linear associations between phthalate concentrations and gestational age at sample collection, after accounting for within-subject correlation in the repeated phthalate measures. Additional models were created with an interaction term between gestational age at sample collection and preterm birth, to assess whether the patterns of phthalate levels across gestation depended on case-control status. Predicted phthalate metabolite concentrations from GAMMs were plotted to present trends in concentrations across pregnancy.

Windows of vulnerability for preterm birth were assessed in two ways. First, we fit logistic regression models with preterm birth as the outcome and obtained odds ratios corresponding to phthalate levels from individual visits (i.e., cross-sectional analysis). SG-corrected models only included urinary SG from the corresponding visit as a covariate. In full models, maternal age, race/ethnicity, and education level were included *a priori*, and additional covariates were added in a forward step-wise procedure with inclusion in final models if they altered effect estimates by greater than 10 percent. Variables that were considered included health insurance category, pre-pregnancy body mass index (BMI), smoking status, alcohol use during pregnancy, parity, gender of infant, use of assisted reproductive technology (ART), and time of day of urine sample collection (before vs. after 1pm, dichotomized based on largest differences in phthalate metabolite concentrations observed between those two groups). The same sets of covariates were included in models for each visit for consistency.

The second method we used to assess windows of sensitivity to phthalate exposure involved modeling subject-specific patterns of exposure in relation to preterm birth (Sanchez et al. 2011). Note that this is not a standard repeated measures outcome analysis, as we have a single binary outcome (preterm birth) and repeated measures of the exposure. To assess if change in phthalate level over pregnancy (i.e., individual exposure curve) was significantly associated with preterm birth, we created two-step models. In the first step we fit linear mixed models with phthalate levels regressed on gestational age at sample collection, with random intercepts and slopes for each subject. These models were adjusted for exposure-specific covariates including SG and time of day of sample collection (before vs. after 1pm). In the second step, the best linear unbiased predictors of the slopes for each subject obtained from this model were used as predictors of preterm birth with adjustment for the additional confounders used in cross-sectional models. The two-step strategy allows one to extract features of the exposure trajectory over gestation (in this case the estimated random slopes) to use as a single summary predictor in the subsequent case-control analysis using logistic regression.

Finally, we repeated the above steps for subtypes of preterm birth, including placental and spontaneous preterm birth. For these models, preterm cases that did not fit into the subtype were excluded from analysis as opposed to being recoded as controls. Covariates for full models were kept the same as for models of overall preterm birth for comparison.

3. Results

Population characteristics are described in detail elsewhere (Ferguson, et al., 2014). As expected, phthalate levels were highly detectable in our population. All metabolites were detected in over 99% of samples with the exceptions of mono(2-ethylhexyl) phthalate (MEHP; 95.3%) and mono(2-carboxypropyl) phthalate (MCP; 96.8%).

3.1 Variability in phthalate levels across pregnancy

Total population phthalate metabolite geometric means and standard deviations by individual visit are presented in Table 1 (corrected for urinary SG). Significantly decreased levels compared to Visit 1 were detected with LMM for all DEHP metabolites, including MEHP, mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), and DEHP as well as MCP. Significantly increased levels of mono-benzyl phthalate (MBzP), mono-*n*-butyl phthalate (MBP), and mono-iso-butyl phthalate (MBP) were observed at Visit 4. Levels of urinary SG were relatively constant across pregnancy with means (standard deviations) as follows for each visit: Visit 1: 1.017 (0.008); Visit 2: 1.014 (0.008); Visit 3: 1.014 (0.008); and Visit 4: 1.015 (0.007).

Spearman correlations of individual phthalate metabolites within visit (e.g., between visit 1 MEHP, visit 2 MEHP, visit 3 MEHP, and visit 4 MEHP) were low to moderate (Supplementary data, Table S1). ICC for SG-corrected phthalate metabolites showed the lowest temporal reliability for DEHP metabolites and MCP, and the highest reliability for MBzP and MBP (Table 1). SG levels showed moderate reproducibility across pregnancy (ICC=0.38, 95% CI=0.33 to 0.43). When ICC were examined in cases and controls separately, coefficients were consistently, although slightly higher for all phthalate metabolites within mothers who delivered preterm, except for MiBP (Supplementary data, Table S2).

Predicted values from GAMMs were calculated using reference levels of covariates, including SG (centered), maternal age (centered), race/ethnicity, education level, and category of health insurance provider. Smoothed plots of predicted phthalate levels across gestation in the total population showed slight decreases in DEHP metabolites and MCP as pregnancy progressed and slight increases in other phthalate metabolites, similar to the patterns demonstrated in Table 1 (data not shown). Stratification by case status showed that trajectories of some phthalate metabolites differed in mothers who delivered preterm compared to term. Early in pregnancy urinary concentrations of MEHP, MECPP, MBzP and MBP were similar in spontaneous preterm cases compared to controls, but levels diverged as pregnancy progressed (Figure 1). Interaction terms between gestational age and spontaneous preterm birth were significant ($p<0.05$) or marginally significant ($p<0.10$) for these metabolites. In the analysis of placental preterm births alone, the only significant interaction

detected in GAMMs was for MECPP (Figure 2). Predicted values of MECPP in cases of placental preterm birth were higher in samples collected early in pregnancy but as gestation progressed levels were more similar to those observed in controls. For other metabolites, no significant interactions were observed between gestational age at sample collection and placental preterm birth, and levels were relatively flat across pregnancy.

3.2 Windows of susceptibility for preterm birth

Odds ratios (OR) from individual visits indicated that for most phthalate metabolites levels from visit 3 had the strongest associations with overall preterm birth (Supplementary data, Table S3). Models created for visit 4 may be biased because many preterm cases had already delivered by that time point, however results are presented for completeness. SG-corrected models showed significantly increased OR in association with MECPP and DEHP levels at visits 1 and 3. Adjusted models for DEHP metabolites included urinary SG, maternal age at visit 1, race/ethnicity, education level, and time of day of urine sample collection (before vs. after 1pm) as covariates. Adjusted models for all other metabolites included urinary SG, maternal age, race/ethnicity, education level, and category of health insurance provider. OR from adjusted models were slightly attenuated compared to estimates from models adjusting for SG only. In both models OR for phthalate metabolites measured at visits 2 and 4 were generally null, although several OR for visit 4 were less than one.

Associations between phthalate exposure and odds of placental or spontaneous preterm births are presented in Tables 2 and 3, respectively. For placental preterm birth, the only relationship observed was in association with MECPP levels at visit 1, although OR for most other metabolites were somewhat higher at visit 1 compared to visits later in pregnancy. For spontaneous preterm birth, higher OR were observed for phthalate metabolite levels measured at visit 3 compared to other visits. OR for MECPP and DEHP were elevated at visit 3, which was consistent with our observation for overall preterm birth, and relationships with spontaneous preterm birth emerged for MBzP and MBP at visit 3.

We additionally examined whether individual exposure curves across gestation were associated with preterm birth. Linear mixed models with random slopes and intercepts by subject were created, predicting phthalate level by gestational age at sample collection and adjusting for SG and time of day of sample collection (before vs. after 1pm) as covariates. Subject-specific random slopes from these models were then examined in association with preterm birth, with and without adjustment for the covariates listed above. However, individual slopes were all close to zero and no meaningful associations were apparent (data not shown).

4. Discussion

In a nested case-control study from a large prospective birth cohort, we analyzed variability in urinary phthalate metabolite levels across pregnancy and identified vulnerable windows for the relationship between exposure and preterm birth. Furthermore, to address the mechanism for this relationship, we examined these associations within subsets of preterm birth which were classified based on clinical presentation.

4.1 Variability in phthalate levels across pregnancy

Levels of urinary phthalate metabolites were similar in our study population compared to those observed in the National Health and Nutrition Examination Survey, although DEHP metabolites, particularly MEHP, were somewhat higher in our study population (Zota et al. 2014). This may be due to truly higher concentrations in Boston women compared to other women in the US, which has been observed previously (Braun et al. 2012), or due to differences in standards used between laboratories. Neither explanation would be expected to bias results. Urinary phthalate metabolite levels showed moderate individual and population-wide variability during pregnancy. DEHP metabolites and MCP showed the most intra-individual variability; MBzP, MBP, MiBP, and MEP, were more stable. Additionally, most phthalate metabolites and particularly DEHP metabolites demonstrated a slightly downward sloping trend across gestation. Variability over pregnancy within women who later delivered prematurely was similar compared to women who carried to term, but trends in metabolite levels across pregnancy between these groups were different. In women who had a spontaneous preterm birth, metabolite levels decreased over the first half of pregnancy but began to slope upward at approximately 20 weeks gestation and continued to rise until parturition.

These conclusions support findings from smaller studies examining variability in urinary phthalate metabolites across the course of pregnancy. ICC for uncorrected DEHP metabolites were similar to those from a population of women from New York City with two to four urinary phthalate measurements taken between 33 weeks gestation and delivery (N=28 women) (Adibi et al. 2008). ICC for MBzP, MBP, and MiBP were somewhat lower in our population, which may have been due to the increased time in our study between sample collections or collection of samples earlier in gestation. In another study of Boston women with at least two (median three) phthalate measures per woman from a wider window (3–38 weeks gestation), ICC for SG-corrected DEHP metabolites, MBzP, MBP, and MiBP were lower than in the present study but still conveyed the same trend, with low molecular weight metabolite measures being much more reliable over time compared to DEHP metabolite measures (N=113 women) (Braun et al. 2012).

The ICC for MEP levels in our study (ICC for SG-corrected MEP=0.47) was slightly higher compared to the ICC observed in a population of pregnant women in New York City (ICC=0.30) (Adibi et al. 2008). However our findings are consistent with the study in Boston women, where the highest ICC of all metabolites was observed for MEP (ICC for SG-corrected MEP=0.50), and with other studies of phthalate variability in adults over longer time periods, although those studies were in men (Frederiksen et al. 2013; Hauser et al. 2004).

In controls, DEHP metabolite and MCP levels decreased slightly over the course of pregnancy, while levels of other metabolites showed little change. These findings are consistent with results from the study of pregnant women from Boston, where DEHP metabolite levels showed a significant decline across pregnancy (Braun et al. 2012). Such a decline may indicate that women are altering behavioral patterns during gestation that result in lower phthalate levels, or that pharmacokinetic changes occur during pregnancy that results in decreased excretion of metabolites (Braun et al. 2012). The latter seems less likely

given the present data, as levels of non-DEHP metabolites remain largely unchanged for the duration of pregnancy.

Differences in variability by metabolites across pregnancy may be due to changes in exposure sources. Exposure to high molecular-weight phthalates may occur more prominently through ingestion of contaminated food and drinking water, and it seems highly likely that diet would change considerably across the course of pregnancy. Exposure to low molecular-weight phthalates, on the other hand, may be occurring more through use of personal care products, which could be more consistent across pregnancy. Trends in phthalate metabolites across gestation may also be due to alterations in behavioral patterns (dietary, personal care product use, medications) or from individual changes in metabolism. Sources and contributors to phthalate variability during pregnancy deserve more attention in future studies.

4.2 Windows of susceptibility for preterm birth

We previously demonstrated that average exposures to MEHP, MECPP, and DEHP metabolites across the duration of pregnancy are associated with increased odds of preterm birth (Ferguson et al. 2014). In the present analysis, we expanded on these results, showing that odds ratios for overall preterm birth were strongest at visit 3, at approximately the beginning of the third trimester, for MECPP and DEHP. Notably, odds ratios were not as strong as with average measurements, suggesting that a geometric average of multiple phthalate measurements over pregnancy may provide a more robust measure of exposure for assessing relationships with health outcomes.

Other studies examining the association between single measures of phthalate exposure during pregnancy and preterm birth or length of gestation have reported conflicting results, potentially due to the use of only one urine or blood sample for assessing exposure. An early study observed a lower average gestational age in newborns who had detectable MEHP levels in cord blood compared to newborns who had undetectable levels (Latini et al. 2003). In studies that measured urinary phthalate metabolites once during the third trimester, two observed associations between urinary phthalate metabolites and decreased gestational age at delivery or increased odds of preterm birth (Meeker et al. 2009; Whyatt et al. 2009), while two other studies observed associations with increased gestational age at delivery or decreased odds of preterm birth (Adibi et al. 2009; Wolff et al. 2008). Finally, one study in which maternal urinary phthalate metabolites were measured once at any time between 9 and 40 weeks gestation observed no association with gestational age or preterm birth (Suzuki et al. 2010).

The evidence from the present analysis strongly supports the conclusion that phthalate exposure at the beginning of the third trimester is related to increased odds of preterm birth. However, some metabolites measured at our visit 4 (median 35.1 weeks) were associated with reduced odds of preterm birth. As other studies may have observed similar effects due to sample collection later in pregnancy, these results may not be spurious. One possible explanation is that in those studies and in our subset with visit 4 measures available a statistical phenomenon similar to what is sometimes referred to as “harvesting” in air pollution epidemiology exists (Schwartz 2001). Under this scenario, women already at

elevated risk of delivering prematurely are pushed to do so earlier by phthalate exposure, thereby decreasing the number of women in the high risk pool later in pregnancy. In this way, the already protective effect of survival until visit 4 against prematurity would be related to phthalate exposure. An alternative explanation is that women who carry until visit 4 or to sample collection in these other studies may be more likely to have a genetic polymorphism related to phthalate and other toxicant metabolism that is protective against preterm birth.

An advantage to the present analysis was our ability to stratify preterm cases based on clinical presentation at delivery, creating more homogenous subsets based on etiology of preterm birth. For placental preterm birth, odds ratios were generally null but were slightly elevated for MECPP measured at visit 1. Contrastingly, for spontaneous preterm birth, odds ratios for nearly all metabolites were highest at visit 3, and the relationships with MECPP, DEHP, MBzP, and MBP were particularly strong.

Two distinct mechanisms could explain the relationship between phthalate exposure and preterm birth. First, phthalate exposure early in pregnancy could cause impaired placentation early in pregnancy via induction of oxidative stress. MEHP and other phthalate metabolites have been shown to create oxidative stress in cellular studies (Fan et al. 2010; Tetz et al. 2013) and have been associated with oxidative stress biomarkers in cross-sectional studies of human populations (Ferguson et al. 2012; Hong et al. 2009). The intrauterine environment in early stages of placentation is highly sensitive. Increases in circulating maternal levels of reactive oxygen species can cause apoptosis and alter cytotrophoblast turnover rate in the developing placenta, leading to impaired invasion (Burton et al. 2009; Heazell and Crocker 2008). This impaired placentation can cause preeclampsia or IUGR which are characteristic of placental preterm birth. We observed some evidence to support this hypothesis, as MECPP measured during the first trimester was associated with increased odds of placental preterm birth. However, as one of many comparisons in this analysis, the association could have been spurious.

Spontaneous preterm birth results primarily from inappropriate initiation of an intrauterine inflammatory cascade that leads to a sequence of events, encompassing preterm rupture of membranes, cervical ripening, and parturition (Challis et al. 2009). Some phthalates have also been shown to induce proinflammatory cytokine release in cell lines (Jepsen et al. 2004; Nishioka et al. 2012) and have been linked to increased systemic levels of inflammatory markers such as C-reactive protein in humans (Ferguson et al. 2011). Our data support this mechanism, as for spontaneous preterm births we saw the strongest associations with phthalate metabolites measured in third trimester urine samples. Alternative pathways, for example via phthalate disruption of reproductive hormones during development and maintenance of pregnancy, are plausible as well, and further investigation will be necessary before drawing any firm conclusions.

5. Conclusions

The primary strengths of our study were the large number of subjects and repeated time points from which we collected phthalate measurements. This enabled us to examine the

relationship between phthalate exposures at more than one time point during pregnancy in relation to preterm birth, which has not been done previously. When phthalate metabolite levels from individual time points during pregnancy were modeled in relation to subtypes of preterm birth, we observed that MECPP exposure early in pregnancy may be related to a modest but significant increase in odds of placental preterm birth. MECPP, DEHP, MBzP, and MBP levels measured near the beginning of the third trimester were associated with increased odds of spontaneous preterm birth. These data support previous evidence of a relationship between phthalate exposure and prematurity, and highlight potential mechanisms that deserve further exploration in toxicology and population studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Kurtis Kneen, Scott Clipper, Gerry Pace, David Weller, and Jennifer Bell of NSF International in Ann Arbor, MI, USA for urine phthalate analysis, and Dr. Russ Hauser of Harvard School of Public Health for guidance on study design. Funding for this work was supported by grants R01ES018872, P42ES017198, R21 ES020811, and P30ES017885 from the National Institute of Environmental Health Sciences.

References

- Adibi JJ, Hauser R, Williams PL, Whyatt RM, Calafat AM, Nelson H, Herrick R, Swan SH. Maternal urinary metabolites of Di-(2-Ethylhexyl) phthalate in relation to the timing of labor in a US multicenter pregnancy cohort study. *Am J Epidemiol*. 2009; 169:1015–1024. [PubMed: 19251754]
- Adibi JJ, Whyatt RM, Williams PL, Calafat AM, Camann D, Herrick R, Nelson H, Bhat HK, Perera FP, Silva MJ, Hauser R. Characterization of phthalate exposure among pregnant women assessed by repeat air and urine samples. *Environ Health Perspect*. 2008; 116:467–473. [PubMed: 18414628]
- Ashton DM, Lawrence HC, Adams NL 3rd, Fleischman AR 3rd. Surgeon General's Conference on the prevention of preterm birth. *Obstet Gynecol*. 2009; 113:925–930. [PubMed: 19305340]
- ATSDR. Toxicological Profile for Diethyl phthalate (DEP). 1995
- ATSDR. Toxicological Profile for Di-n-butyl phthalate (DBP). 2001
- ATSDR. Toxicological Profile for Di(2-ethylhexyl)phthalate (DEHP). Agency for Toxic Substances and Disease Registry. 2002
- 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; 113:192–200. [PubMed: 15687057]
- Behrman, RE.; Butler, AS. Preterm birth: causes, consequences, and prevention. Washington, DC: The National Academies Press; 2007.
- Bornehag CG, Nanberg E. Phthalate exposure and asthma in children. *International journal of andrology*. 2010; 33:333–345. [PubMed: 20059582]
- Braun JM, Smith KW, Williams PL, Calafat AM, Berry K, Ehrlich S, Hauser R. Variability of urinary phthalate metabolite and bisphenol A concentrations before and during pregnancy. *Environ Health Perspect*. 2012; 120:739–745. [PubMed: 22262702]
- Burton GJ, Charnock-Jones DS, Jauniaux E. Regulation of vascular growth and function in the human placenta. *Reproduction*. 2009; 138:895–902. [PubMed: 19470597]
- Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss JF, Petraglia F 3rd. Inflammation and pregnancy. *Reprod Sci*. 2009; 16:206–215. [PubMed: 19208789]
- Engel SM, Miodovnik A, Canfield RL, Zhu C, Silva MJ, Calafat AM, Wolff MS. Prenatal phthalate exposure is associated with childhood behavior and executive functioning. *Environmental health perspectives*. 2010; 118:565–571. [PubMed: 20106747]

- Engel SM, Zhu C, Berkowitz GS, Calafat AM, Silva MJ, Miodovnik A, Wolff MS. Prenatal phthalate exposure and performance on the Neonatal Behavioral Assessment Scale in a multiethnic birth cohort. *Neurotoxicology*. 2009; 30:522–528. [PubMed: 19375452]
- Fan J, Traore K, Li W, Amri H, Huang H, Wu C, Chen H, Zirkin B, Papadopoulos V. Molecular mechanisms mediating the effect of mono-(2-ethylhexyl) phthalate on hormone-stimulated steroidogenesis in MA-10 mouse tumor Leydig cells. *Endocrinology*. 2010; 151:3348–3362. [PubMed: 20463053]
- Ferguson KK, Loch-Caruso R, Meeker JD. Urinary phthalate metabolites in relation to biomarkers of inflammation and oxidative stress: NHANES 1999–2006. *Environ Res*. 2011; 111:718–726. [PubMed: 21349512]
- Ferguson KK, Loch-Caruso R, Meeker JD. Exploration of oxidative stress and inflammatory markers in relation to urinary phthalate metabolites: NHANES 1999–2006. *Environ Sci Technol*. 2012; 46:477–485. [PubMed: 22085025]
- Ferguson KK, McElrath TF, Meeker JD. Environmental phthalate exposure and preterm birth. *JAMA Pediatr*. 2014; 168:61–67. [PubMed: 24247736]
- Frederiksen H, Kranich SK, Jorgensen N, Taboureaux O, Petersen JH, Andersson AM. Temporal variability in urinary phthalate metabolite excretion based on spot, morning, and 24-h urine samples: considerations for epidemiological studies. *Environ Sci Technol*. 2013; 47:958–967. [PubMed: 23234290]
- Hankinson SE, Manson JE, Spiegelman D, Willett WC, Longcope C, Speizer FE. Reproducibility of plasma hormone levels in postmenopausal women over a 2-3-year period. *Cancer Epidemiol Biomarkers Prev*. 1995; 4:649–654. [PubMed: 8547832]
- Hauser R, Meeker JD, Duty S, Silva MJ, Calafat AM. Altered semen quality in relation to urinary concentrations of phthalate monoester and oxidative metabolites. *Epidemiology*. 2006; 17:682–691. [PubMed: 17003688]
- Hauser R, Meeker JD, Park S, Silva MJ, Calafat AM. Temporal variability of urinary phthalate metabolite levels in men of reproductive age. *Environ Health Perspect*. 2004; 112:1734–1740. [PubMed: 15579421]
- Heazell AE, Crocker IP. Live and let die - regulation of villous trophoblast apoptosis in normal and abnormal pregnancies. *Placenta*. 2008; 29:772–783. [PubMed: 18706691]
- Hong YC, Park EY, Park MS, Ko JA, Oh SY, Kim H, Lee KH, Leem JH, Ha EH. Community level exposure to chemicals and oxidative stress in adult population. *Toxicol Lett*. 2009; 184:139–144. [PubMed: 19049859]
- Hornung RW, Reed LD. Estimation of average concentration in the presence of nondetectable values. *Appl Occup Environ Hyg*. 1990; 5:46–51.
- Jepsen KF, Abildtrup A, Larsen ST. Monophthalates promote IL-6 and IL-8 production in the human epithelial cell line A549. *Toxicol In Vitro*. 2004; 18:265–269. [PubMed: 15046772]
- Latini G, De Felice C, Presta G, Del Vecchio A, Paris I, Ruggieri F, Mazzeo P. In utero exposure to di-(2-ethylhexyl)phthalate and duration of human pregnancy. *Environ Health Perspect*. 2003; 111:1783–1785. [PubMed: 14594632]
- McElrath TF, Hecht JL, Dammann O, Boggess K, Onderdonk A, Markenson G, Harper M, Delpapa E, Allred EN, Leviton A, Investigators ES. Pregnancy disorders that lead to delivery before the 28th week of gestation: an epidemiologic approach to classification. *Am J Epidemiol*. 2008; 168:980–989. [PubMed: 18756014]
- Meeker JD. Exposure to environmental endocrine disruptors and child development. *Arch Pediatr Adolesc Med*. 2012; 166:952–958. [PubMed: 23367522]
- Meeker JD, Ferguson KK. Relationship between urinary phthalate bisphenol A concentrations serum thyroid measures in U.S adults and adolescents from the National Health and Nutrition Examination Survey (NHANES) 2007–2008. *Environ Health Perspect*. 2011; 119:1396–1402. [PubMed: 21749963]
- Meeker JD, Hu H, Cantonwine DE, Lamadrid-Figueroa H, Calafat AM, Ettinger AS, Hernandez-Avila M, Loch-Caruso R, Tellez-Rojo MM. Urinary phthalate metabolites in relation to preterm birth in Mexico city. *Environ Health Perspect*. 2009; 117:1587–1592. [PubMed: 20019910]

- Mendiola J, Meeker JD, Jorgensen N, Andersson AM, Liu F, Calafat AM, Redmon JB, Drobnis EZ, Sparks AE, Wang C, Hauser R, Swan SH. Urinary concentrations of di(2-ethylhexyl) phthalate metabolites and serum reproductive hormones: pooled analysis of fertile and infertile men. *Journal of andrology*. 2012; 33:488–498. [PubMed: 21597090]
- Nishioka J, Iwahara C, Kawasaki M, Yoshizaki F, Nakayama H, Takamori K, Ogawa H, Iwabuchi K. Di-(2-ethylhexyl) phthalate induces production of inflammatory molecules in human macrophages. *Inflamm Res*. 2012; 61:69–78. [PubMed: 22005928]
- Rosner B. *Fundamentals of biostatistics*. Boston, MA: Brooks/Cole. 2011
- Sanchez BN, Hu H, Litman HJ, Tellez-Rojo MM. Statistical methods to study timing of vulnerability with sparsely sampled data on environmental toxicants. *Environ Health Perspect*. 2011; 119:409–415. [PubMed: 21362588]
- Schwartz J. Is there harvesting in the association of airborne particles with daily deaths and hospital admissions? *Epidemiology*. 2001; 12:55–61. [PubMed: 11138820]
- Silva MJ, Samandar E, Preau JL Jr, Reidy JA, Needham LL, Calafat AM. Quantification of 22 phthalate metabolites in human urine. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2007; 860:106–112.
- Suzuki Y, Niwa M, Yoshinaga J, Mizumoto Y, Serizawa S, Shiraishi H. Prenatal exposure to phthalate esters and PAHs and birth outcomes. *Environ Int*. 2010; 36:699–704. [PubMed: 20605637]
- Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, Mao CS, Redmon JB, Ternand CL, Sullivan S, Teague JL. Study for Future Families Research T Decrease in anogenital distance among male infants with prenatal phthalate exposure. *Environ Health Perspect*. 2005; 113:1056–1061. [PubMed: 16079079]
- Tetz LM, Cheng AA, Korte CS, Giese RW, Wang P, Harris C, Meeker JD, Loch-Carus R. Mono-2-ethylhexyl phthalate induces oxidative stress responses in human placental cells in vitro. *Toxicol Appl Pharmacol*. 2013; 268:47–54. [PubMed: 23360888]
- Whyatt RM, Adibi JJ, Calafat AM, Camann DE, Rauh V, Bhat HK, Perera FP, Andrews H, Just AC, Hoepner L, Tang D, Hauser R. Prenatal di(2-ethylhexyl)phthalate exposure and length of gestation among an inner-city cohort. *Pediatrics*. 2009; 124:e1213–1220. [PubMed: 19948620]
- Wolff MS, Engel SM, Berkowitz GS, Ye X, Silva MJ, Zhu C, Wetmur J, Calafat AM. Prenatal phenol and phthalate exposures and birth outcomes. *Environ Health Perspect*. 2008; 116:1092–1097. [PubMed: 18709157]
- Wood SN. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. *Journal of the Royal Statistical Society (B)*. 2011; 73:3–36.
- Zota AR, Calafat AM, Woodruff TJ. Temporal trends in phthalate exposures: findings from the National Health and Nutrition Examination Survey, 2001–2010. *Environ Health Perspect*. 2014; 122:235–241. [PubMed: 24425099]

Highlights

- We examine changes in maternal urinary phthalate metabolites over pregnancy.
- We modeled trajectories of metabolites in mothers who delivered preterm vs. term.
- We calculated odds of prematurity with phthalates measured at each time point.
- Spontaneous preterm birth was associated with 3rd trimester phthalate levels.
- Placental preterm birth was associated with 1st trimester phthalate levels.

Figure 1.

Predicted urinary phthalate metabolite concentrations (95% confidence intervals) from generalized additive mixed models (GAMM) in mothers with spontaneous preterm (dashed) compared to term (solid) births. N=180 observations for cases, N=1211 observations for controls.

Figure 2. Predicted urinary phthalate metabolite concentrations (95% confidence intervals) from generalized additive mixed models (GAMM) in mothers with placental preterm (dashed) compared to term (solid) births. N=104 observations for cases, N=1211 observations for controls.

Table 1

Specific gravity-corrected urinary phthalate metabolite geometric means (geometric standard deviations) by study visit.

	Visit 1	Visit 2	Visit 3	Visit 4	ICC (95% CI)
N (cases, controls)	129, 350	118, 304	111, 301	66, 314	129, 349
MEHP (ug/L)	12.7 (3.78)	11.3 (3.33)	9.83 (3.27) ^a	9.94 (3.44) ^a	0.30 (0.25, 0.35)
MEHHP (ug/L)	40.8 (3.69)	34.1 (3.13) ^a	27.1 (3.42) ^a	34.9 (3.37) ^a	0.21 (0.17, 0.27)
MEOHP (ug/L)	20.1 (3.62)	18.2 (3.05)	15.8 (3.38) ^a	20.1 (3.27)	0.19 (0.15, 0.25)
MECPP (ug/L)	51.8 (3.53)	43.0 (3.25) ^a	38.5 (3.47) ^a	48.7 (3.39)	0.31 (0.26, 0.36)
DEHP (nmol/L)	45.7 (3.38)	38.6 (3.00) ^a	33.3 (3.13) ^a	41.3 (3.17)	0.23 (0.18, 0.28)
MBzP (ug/L)	6.95 (3.19)	6.95 (3.05)	6.89 (2.95)	7.86 (2.97) ^a	0.61 (0.56, 0.65)
MBP (ug/L)	17.9 (2.57)	18.3 (2.62)	17.4 (2.75)	19.9 (2.33) ^a	0.57 (0.53, 0.62)
MIBP (ug/L)	7.28 (2.25)	7.17 (2.33)	7.30 (2.35)	9.04 (2.21) ^a	0.52 (0.48, 0.57)
MEP (ug/L)	140 (4.42)	147 (4.85)	140 (4.67)	147 (5.00)	0.47 (0.42, 0.52)
MCPP (ug/L)	2.27 (3/46)	2.30 (3.35)	1.95 (3.02) ^a	2.11 (2.89)	0.36 (0.31, 0.41)

^a Significant difference ($p < 0.05$) in urinary phthalate metabolite concentration compared to Visit 1 (reference).

Table 2

Adjusted^a odds ratios (95% CI) of placental preterm birth in association with ln-unit increase in urinary phthalate metabolite.

	Visit 1	Visit 2	Visit 3	Visit 4
N (cases, controls)	35, 336	31, 297	32, 289	15, 303
MEHP	1.12 (0.85, 1.48)	1.03 (0.75, 1.41)	1.07 (0.78, 1.48)	1.02 (0.64, 1.63)
MEHHP	1.14 (0.86, 1.52)	0.99 (0.71, 1.39)	1.19 (0.86, 1.63)	0.74 (0.42, 1.29)
MEOHP	1.18 (0.88, 1.57)	1.03 (0.74, 1.45)	1.20 (0.87, 1.66)	0.91 (0.52, 1.56)
MECPP	1.46 (1.10, 1.95)	1.22 (0.90, 1.67)	1.32 (0.98, 1.78)	1.29 (0.79, 2.11)
DEHP	1.33 (0.99, 1.78)	1.14 (0.82, 1.60)	1.26 (0.91, 1.74)	1.04 (0.61, 1.78)
N (cases, controls)	33, 334	29, 295	30, 288	13, 300
MBzP	1.02 (0.73, 1.43)	1.07 (0.73, 1.55)	1.00 (0.68, 1.48)	1.02 (0.57, 1.84)
MBP	0.97 (0.62, 1.50)	1.23 (0.79, 1.93)	1.15 (0.77, 1.72)	0.94 (0.40, 2.22)
MiBP	0.92 (0.57, 1.47)	0.88 (0.54, 1.41)	0.75 (0.50, 1.13)	0.66 (0.28, 1.55)
MEP	0.92 (0.71, 1.19)	0.96 (0.74, 1.26)	0.99 (0.77, 1.27)	0.88 (0.60, 1.29)
MCCP	1.20 (0.90, 1.60)	0.91 (0.64, 1.28)	1.18 (0.83, 1.69)	1.24 (0.74, 2.09)

^a Adjusted models include specific gravity of respective visit, maternal age, Race/Ethnicity, and education level. DEHP metabolite models additionally adjusted for time of day of sample collection (before vs. after 1pm). Other metabolite models additionally adjusted for health insurance category.

Table 3

Adjusted^a odds ratios (95% CI) of spontaneous preterm birth in association with ln-unit increase in phthalate metabolite

	Visit 1	Visit 2	Visit 3	Visit 4
N (cases, controls)	56, 336	52, 297	47, 289	25, 303
MEHP	1.17 (0.94, 1.46)	1.12 (0.88, 1.43)	1.28 (0.99, 1.66)	1.25 (0.90, 1.74)
MEHHP	1.09 (0.87, 1.36)	0.89 (0.68, 1.16)	1.19 (0.92, 1.54)	1.06 (0.76, 1.48)
MEOHP	1.14 (0.90, 1.45)	0.94 (0.71, 1.23)	1.28 (0.98, 1.66)	1.12 (0.79, 1.58)
MECPP	1.26 (0.99, 1.60)	1.01 (0.79, 1.30)	1.33 (1.04, 1.70)	1.27 (0.90, 1.78)
DEHP	1.21 (0.95, 1.55)	0.99 (0.75, 1.29)	1.33 (1.02, 1.73)	1.22 (0.85, 1.73)
N (cases, controls)	56, 334	52, 295	47, 288	25, 300
MBzP	1.16 (0.88, 1.52)	1.20 (0.90, 1.60)	1.43 (1.05, 1.95)	1.24 (0.83, 1.85)
MBP	1.32 (0.99, 1.77)	1.19 (0.87, 1.63)	1.45 (1.08, 1.96)	1.56 (0.99, 2.46)
MiBP	1.14 (0.78, 1.68)	1.36 (0.94, 1.97)	1.28 (0.88, 1.86)	1.26 (0.76, 2.10)
MEP	1.11 (0.91, 1.35)	1.20 (0.98, 1.46)	1.16 (0.94, 1.42)	0.95 (0.73, 1.24)
MCPP	1.18 (0.96, 1.47)	1.15 (0.91, 1.44)	1.25 (0.96, 1.64)	1.33 (0.93, 1.90)

^a Adjusted models include specific gravity of respective visit, maternal age, Race/Ethnicity, and education level. DEHP metabolite models additionally adjusted for time of day of sample collection (before vs. after 1pm). Other metabolite models additionally adjusted for health insurance category.