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Variability and longitudinal trajectories of phthalate and replacement biomarkers across pregnancy in the Human Placenta and Phthalates Study

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Abstract

Human exposure to phthalates is widespread, but assessment of variability across pregnancy has been hampered by short half-lives of phthalate biomarkers and few repeated measures in prior studies. We aimed to characterize variability and longitudinal profiles of phthalate and replacement biomarkers across pregnancy. Within the Human Placenta and Phthalates Study, 303 pregnant women provided urine samples at up to 8 visits across gestation. Concentrations of 14 metabolites of phthalates and 4 metabolites of replacements were quantified in each sample, and subject-specific averages within each trimester were calculated. We examined variability in individual biomarker concentrations across the 8 visits, within trimesters, and across trimester-

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Supporting Information: distribution of gestational weeks at each visit; participants contributing to pooled samples; detection frequency of metabolite by visit; fit statistics for GBTM models; Kappa statistics for agreement between GBTM models; correlation coefficients for exposure biomarkers

specific averages using intraclass correlation coefficients (ICCs). To explore longitudinal exposure biomarker profiles, we applied group-based trajectory modeling to trimester-specific averages over pregnancy. Pooling multiple visits into trimester-specific averages improved ICCs for all biomarkers. Most biomarkers generally showed stable concentrations across gestation, i.e., high, medium, and low concentration profiles, with small proportions of participants falling into the "high" exposure groups. Variability over pregnancy is likely attributable to random fluctuations around a baseline exposure, rather than true changes in concentrations over time.

Keywords

prenatal exposure; cohort study; exposure assessment; phthalates; pregnancy; epidemiology

Introduction

Phthalates are a class of non-persistent chemicals used in everyday products such as cosmetics, clothing, pharmaceuticals, and food packaging. Exposure to phthalates is a health concern, especially during pregnancy. Pregnancy is a vulnerable window for environmental insults, and phthalate exposure during this period has been associated with adverse outcomes such as preterm birth and fetal growth restriction.[1–5]

Accurately assessing environmental exposures is a critical component of correctly estimating associations with health effects but is rife with challenges. Biologically relevant exposure to non-persistent chemicals like phthalates can be especially difficult to assess, given the chemicals' short biological elimination half-lives, on the order of hours,[6,7] and episodic nature of exposure. It is an unstated assumption in many studies that the adverse effects of phthalate exposure are a consequence of long-term trends in exposure, rather than acute occurrences. Under this assumption, the goal of exposure characterization in studies evaluating associations with adverse pregnancy outcomes is to accurately capture average exposure across the course of gestation or within a potentially vulnerable window, such as an individual trimester. Yet, many studies examining phthalate exposure in pregnancy collect measurements from a single spot urine sample during gestation. Some studies measure phthalate metabolites in spot urine samples from multiple trimesters,[8–10] but few have greater than 3 measures during pregnancy.[11,12]

The paucity of data on repeated measures of phthalate exposure biomarkers during gestation means that knowledge on the variability and temporal patterning of concentrations and exposure concentration profiles over this highly sensitive period is limited. To fill this gap, the Human Placenta and Phthalates (HPP) study collected urine samples at up to 8 time points across pregnancy. Our goals were to 1) characterize multiple facets of variability of urinary biomarker concentrations of 14 phthalate metabolites and 4 replacement metabolites measured during pregnancy, examining intraclass correlation coefficients within each trimester in addition to across pregnancy, and 2) characterize temporal patterns of exposure biomarker concentrations over pregnancy with group-based trajectory models. The latter approach, which has been applied to other environmental chemicals[13] but never phthalate

exposure biomarkers, may provide insight into patterns of variability in the data and how exposure biomarker concentrations change over time among certain individuals.

Methods

Study population

Study participants were recruited from prenatal clinics at Eastern Virginia Medical School (EVMS) and University of Texas Medical Branch (UTMB) from 2016–2020. Participants were eligible if they were between 18–50 years old, carrying a singleton fetus, and had no detected fetal or placental abnormalities. Participants were enrolled in early pregnancy (<14 weeks' gestation) and followed through delivery. Information on demographics, smoking, and drug and alcohol use were self-reported at enrollment. Body mass index (BMI) was calculated using height and weight values from participants' medical records at enrollment.

The first three study visits occurred every 2 weeks, at median 13, 15, and 17 weeks' gestation (Supplemental Table 1) and the remaining five study visits occurred every 4 weeks, at median 21, 25, 29, 33, and 37 weeks' gestation. Additional detail on the study design has been previously described.[14] The study was funded by the National Institute for Child Health and Development Human Placenta Project (R01 HD086313). The study received IRB approval through EVMS and UTMB and participants signed informed consent forms prior to participating.

Beginning in 2017, study participants were asked to participate in the HPP study, a substudy that entailed additional urine specimen and questionnaire collection at each of the 8 study visits. A total of 303 women provided at least one urine sample during gestation and were included in the present analysis. Analysis of de-identified samples by the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research.

Quantification of phthalate and replacement metabolites

At each prenatal visit, women provided total urine voids in sterile specimen cups. Samples were shipped overnight on ice to a central processing location where specific gravity (SG) was measured after a 30 second vortex using a PAL-10S refractometer, and samples were aliquoted into cryovials. Samples were stored at −80°C until shipped frozen overnight to the National Center for Environmental Health at the CDC for analysis.

Briefly, quantification of the metabolites involved enzymatic hydrolysis from their conjugated (e.g., glucuronidated) form, automated online solid phase extraction, separation with high performance liquid chromatography, and detection using isotope-dilution tandem mass spectrometry.[15] Participant samples were randomly assigned to batches for each visit so that the same participants were not grouped together for every batch. The batches were randomly analyzed (i.e., not analyzed in the order of visit number). Coefficients of variation (CV) were assessed for pooled quality control samples, with 0 to 4 pooled aliquots analyzed per day. Overall CVs ranged from 0 to 13.1%. The following phthalate metabolites were measured: monoethyl phthalate (MEP), mono-n-butyl phthalate (MBP), mono-hydroxybutyl phthalate (MHBP), mono-isobutyl phthalate (MiBP), mono-hydroxy-isobutyl

phthalate (MHiBP), monobenzyl phthalate (MBzP), mono-3-carboxypropyl phthalate (MCPP), 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 oxononyl phthalate (MONP), mono carboxyisooctyl phthalate (MCOP), mono carboxyisononyl phthalate (MCNP). Additionally, four metabolites of replacements were measured: mono-2-ethyl-5-hydrohexyl terephthalate (MEHHTP), mono-2-ethyl-5-carboxypentyl terephthalate (MECPTP) and cyclohexane-1,2-dicarboxylic acid, monohydroxy isononyl ester (MHiNCH), and cyclohexane-1,2-dicarboxylic acid, monocarboxy isooctyl ester (MCOCH). MEHHTP and MECPTP are both metabolites of a terephthalate, di(2-ethylhexyl) terephthalate (DEHTP), while MHiNCH and MCOCH are metabolites of 2-cyclohexane dicarboxylic acid, diisononyl ester (DiNCH), a phthalate replacement that is not considered part of the phthalate chemical class.

Instrumental-reading concentrations below the limit of detection (LOD) were retained and concentrations reported as 0 (i.e., absence of analytical signal) were imputed using LOD/$\sqrt{2}$.[16]

Molar sums were created by summing the molar concentrations (nmol/mL) of metabolites from the same parent compound:[12,14] MEHP, MEHHP, MEOHP, and MECPP for the sum of di(2-ethylhexyl) phthalate metabolites (∑DEHP); MCNP and MCOP for the sum of di-isononyl phthalate metabolites (∑DiNP); MBP and MHBP for the sum of di-n-butyl phthalate metabolites (∑DnBP); MiBP and MHiBP for the sum of di-iso-butyl phthalate metabolites (∑DiBP); MHiNCH and MCOCH for sum of 1,2-cyclohexane dicarboxylic acid, diisononyl ester (∑DiNCH); and MEHHTP and MECPTP for the sum of di(2-ethylhexyl) terephthalate metabolites (∑DEHTP). Molar concentrations (nmol/mL) were converted to ng/mL by multiplying the sum by the molecular weights of MECPP, MCOP, MBP, MiBP, MHiNCH, and MECPTP, respectively. Subsequently, all individual metabolites and summed metabolites are referred to as exposure biomarkers.

Exposure biomarker concentrations were corrected for urine dilution using covariate-adjusted standardization.[17,18] Natural log-transformed SG was modeled as a function of maternal age, gestational age, early pregnancy BMI, maternal education, and maternal race/ethnicity. Each exposure biomarker was divided by the ratio of observed SG to predicted SG, with predicted values generated from the above model.

In addition to exposure biomarker concentrations at each visit, we created subject-specific geometric means from repeated measures within approximate trimesters. SG correction was applied to biomarkers at each visit before creation of the trimester average. Visits 1 and 2 were considered first trimester (median gestational weeks at urine sample collection: 13 and 15), visits 3–5 were considered the second trimester (median gestational weeks: 17, 21, and 25), and visits 6–8 were considered third trimester (median gestational weeks: 29, 33, and 37). A trimester average could be an average of concentrations from two or three visits within a trimester, or simply the concentration from one visit if that was the only recorded exposure assessment measure in the timeframe. In each trimester, approximately 80% of the values were calculated using 2 or more values (Supplemental Table 2).

Statistical analysis

We examined demographic characteristics (N [%]) of participants in the HPP Study. To examine distributions of exposure biomarkers, we first assessed the percentage of samples with detectable concentrations of each individual metabolite across all visits. Second, we examined the median and interquartile range of trimester average concentrations for all biomarkers. These values were compared to those from the 2017–2018 National Health and Nutrition Examination Survey (NHANES) among women aged 18–49 years. Molar sums of NHANES exposure biomarkers were calculated and corrected for urine dilution with creatinine using the modified Boeniger formula[17] and weighted to account for NHANES sampling scheme. Third, Spearman correlations between trimester average exposure biomarkers were calculated using raw (i.e., not ln-transformed) concentrations.

Next, we examined intraclass correlation coefficients (ICCs) to assess temporal variability over pregnancy by participant. For each biomarker, the following ICCs were calculated: 1) across pregnancy using individual biomarker concentrations from visits 1–8; 2) across pregnancy using trimester averages; 3) within trimester using corresponding visits (1–2, 3–5, and 6–8). The ICCs were calculated using ln-transformed and SG-corrected biomarker concentrations.

To explore longitudinal exposure profiles across the trimesters of pregnancy, we utilized group based trajectory modeling (GBTM) to model ln-transformed trimester averages for each biomarker.[19,20] GBTM is a finite-mixture modeling approach that groups individuals who follow similar exposure biomarker profiles across pregnancy, creating latent classes of trajectories. Compared to other clustering methods, GBTM was selected as it clusters based on the individual rather than the exposure. Additionally, GBTM performs nearly identically to longitudinal k-means under most scenarios, and is preferred when data contains missing or nonaligned observations, as existed in our dataset.[21] A ln-transformation was implemented as the biomarker values were highly right skewed, which led to poor trajectories. GBTM can accommodate missingness and includes participants who have at least one value at any time point. Models were run using PROC TRAJ in SAS 9.4 and performed separately for each exposure biomarker. As recommended, we used multiple criteria to aid in our selection of optimal trajectory number and shape (linear, quadratic): comparison of Akaike information criterion (AIC), Bayesian information criterion (BIC), posterior probabilities, and group membership percentages.[22] In weighing various criteria to select the number and shape of trajectories, we prioritized identifying unique groups, with the goal of keeping all trajectory group sizes >5% of the total population and all median posterior probabilities >0.80. The group membership percentage criterion was relaxed if all other fit statistics pointed to the model with the group <5%. If fit statistics between two models were comparable, we then considered posterior probabilities.

We discuss results in the categories of low molecular weight (LMW) phthalate exposure biomarkers, consisting of MEP, ∑DnBP, and ∑DiBP, high molecular weight (HMW) phthalate biomarkers, consisting of MBzP, MCPP, ∑DEHP, ∑DiNP, and MCNP, and replacement biomarkers, consisting of ∑DiNCH and ∑DEHTP. The plotted trajectories and uncertainty bands represent 95% prediction intervals generated from the GBTM. Observed ln-transformed biomarker concentrations from spot urine samples are plotted at

the gestational age of sample collection among 50% of participants (selected via random sample) to visualize trends.

For comparison, we fit trajectories using the spot urine sample biomarker concentrations from all 8 visits and using one spot urine sample per trimester. Weighted Kappa statistics were computed to assess the agreement between trajectory group membership using these two alternative methods of exposure characterization (i.e., all spot-urine samples and 1 spot urine measurement per trimester) compared to our primary exposure characterization (i.e., trimester averages).

Lastly, we examined demographic characteristics among participants from each trajectory group, using the groups derived from the trimester averages. The biomarker concentration distribution within each trajectory was compared to the distribution in the overall population, measured as percent difference, and visualized with heat maps. We opted to use trajectories generated from the trimester average concentrations as these concentrations were found to be more stable estimates of exposure.

Results

Approximately 39% of the cohort self-identified as non-Hispanic White, 43% as non-Hispanic Black, and 16% as Hispanic (Table 1). The average maternal age in this population was 27 years old, and 45% of women had a BMI of 18.5–24.99 km/m^2 at enrollment. 36% of participants were nulliparous, approximately one third reported smoking in the 3 months prior to pregnancy, and 45% had only a high school education or less. Just over half reported employment at the time of questionnaire completion and over 70% used government-assisted health insurance. Missingness was <5% for all covariates.

We detected 13 of the 18 metabolites measured in >90% of samples collected across all visits (n=1866 total, Table 2).

Visit-specific frequency per metabolite is displayed in Supplemental Table 3. Detection frequency was the lowest for MEHP (73%) and for the two DiNCH metabolites (MHiNCH: 75%, MCOCH: 43%). Median exposure biomarker concentrations were similar across trimesters (Table 3).

MEP, ∑DEHTP, and MCPP, however, were all highest in the 1st trimester and then declined in the 2nd and 3rd trimesters. Exposure biomarker concentrations in this population were comparable to concentrations measured in NHANES, apart from MEP and ∑DEHTP. HPP participants had first trimester MEP concentrations that were higher than those observed in NHANES participants, although concentrations were more similar for HPP MEP concentrations from the 2nd or 3rd trimesters. Concentrations of ∑DEHTP were consistently higher in HPP participants compared to NHANES participants. Compared to our population, the women in NHANES were slightly older, more highly educated, with a higher proportion identifying as Hispanic.

Bivariate Spearman correlations between exposure biomarker concentrations ranged from −0.04 (∑DiNCH/MBzP in 3rd trimester) to 0.95 (MCPP/∑DiNP in 1st trimester) and most

correlations were low to moderate (Supplemental Figure 1). The correlation between 1st trimester ∑DiNP and MCPP was an outlier and was lower in the following trimesters (rho: 0.77, 0.73). ∑DnBP was moderately correlated with MEP, MBzP, and MCPP (range: 0.23–0.58). Most correlations were not meaningfully different across trimesters.

ICCs for exposure biomarkers across the 8 study visits ranged from 0.17 to 0.69 for ∑DiNCH to MBzP, respectively (Table 4). ICCs for trimester averages across pregnancy were higher, ranging from 0.28 (∑DINCH) to 0.80 (MBzP). Within trimester, ICCs were only slightly higher compared to ICCs across the entirety of gestation. For example, the ICC for MEP across the 8 study visits was 0.61, and for repeated measurements within trimester the ICCs were 0.69, 0.62, and 0.63. Some within-trimester ICCs were the same or lower than the ICC generated from the 8 study visits. For example, the ICC for ∑DEHP across all visits was 0.34, while the ICCs for visits within trimesters were 0.35, 0.32, and 0.45 for the first, second, and third trimester, respectively.

For the trajectory analyses, the best fitting models contained 4 classes for MEP and MBzP, 3 classes for ∑DnBP, ∑DiBP, ∑DEHP, ∑DiNP, MCNP, ∑DiNCH, and 2 classes for MCPP and ∑DEHTP (Figure 1). Fit statistics are displayed in Supplemental Table 4. In general, the “medium” exposure biomarker concentration trajectories contained the most women, followed by the “low” concentration trajectories. Among exposures with at least 3 trajectories, the groups with highest exposure biomarker concentrations consistently contained the fewest women (4–14%). Generally, trajectories showed that most group averages of exposure biomarker concentrations were consistent across gestation (i.e., consistently low, medium, or high concentrations). For ∑DiNP and ∑DiNCH, however, some groups of participants had temporal trends in concentration profiles across pregnancy. Both ∑DiNP and ∑DiNCH showed groups of women with low and medium exposure biomarker concentrations, but there was a third group of participants who had the highest concentrations of exposure biomarkers in early pregnancy, and then demonstrated a decline in concentrations across pregnancy, resulting in concentrations in the 3rd trimester that were lower than women in the “medium” group. In both cases, these groups were small, containing 5 and 4% of the population, respectively.

The number and shape of best-fitting trajectories was generally consistent when comparing results from models with trimester averages to individual spot samples from all 8 visits, with the exceptions of ∑DiNP and ∑DiNCH. These trajectories had slightly different shapes when all spot urine concentrations were included in the model (Supplemental Table 5). Kappa statistics nevertheless showed good agreement across the two approaches for ∑DiNP and ∑DiNCH (0.68 and 0.79, respectively). Kappa statistics for all other compounds indicated excellent agreement (mean: 0.89, range: 0.81–0.95).

When using a single spot urine sample per trimester to generate trajectories, the number of trajectories differed for five of the ten exposure biomarkers. Among the exposure biomarkers with the same number of trajectories across the two methods (i.e., one spot urine sample per trimester vs. trimester averages), weighted Kappa statistics were poor (average: 0.60, ranging from 0.26 for MCNP to 0.85 for MBzP). For the five exposure biomarkers with discrepant numbers of trajectories across methods, there was no pattern regarding whether

the single visit trajectories contained more or fewer trajectories compared to the trimester averages, but posterior probabilities were generally lower with models generated from 3 spot urine measurements.

There were notable demographic differences in the women across trajectory groups (Figure 2). Regarding LMW phthalate biomarkers, women in the highest exposure biomarker groups for each biomarker were much more likely to be non-Hispanic Black compared to other race and ethnicity groups. Other trends were not as consistent, but some differences were notable for individual LMW biomarkers. For example, women in the “very high” MEP group were more likely to smoke and to have a lower early pregnancy BMI compared to the rest of the population.

For the HMW phthalate biomarkers, trajectory groups showed fewer striking differences in terms of race and ethnicity, though non-Hispanic Black women were overrepresented in the “high” ∑DEHP group. Women in the “very high” MBzP group were more likely to have a 4-year degree or higher education level and more likely to be multiparous (3+ children) compared to women from the rest of the study population. Across biomarkers, women in the highest concentration groups were more likely to have an early pregnancy BMI >30 kg/m^2. This was true for women in the “high declining” ∑DiNP group as well. This group also had higher representation from women ages 27–30 and who were non-Hispanic White compared to the overall population.

Finally, for replacement biomarkers, we observed that women in the highest concentration groups were also more likely to be non-Hispanic Black compared to the rest of the population, although the magnitude of the differences was not as great as what was observed for LMW phthalate biomarkers. For the “high declining” trajectory of ∑DiNCH, we observed similar findings as for the “high declining” trajectory of for ∑DiNP, such that women with BMI>30 kg/m^2 were overrepresented. Fewer differences were observed overall for ∑DEHTP compared with ∑DiNCH.

Discussion

In this study, we sought to characterize temporal variation of phthalate and replacement biomarkers in a cohort of diverse pregnant women with frequent urine sampling. We observed high detection of most biomarkers evaluated, including near universal detection for metabolites of DEHTP and moderate detection of metabolites of DiNCH, both of which are considered replacements. DEHTP is both a phthalate – specifically, a terephthalate – and a replacement for traditional phthalates. Urinary biomarker concentrations of trimester averages pooled from multiple visits showed less variability over pregnancy compared to concentrations from individual visits, as expected. However, variability of spot urine sample metabolite concentrations within trimester was similar to that observed across pregnancy, which suggests that a spot urine sample may reflect exposure in that trimester and across pregnancy equally well. Exploration of exposure biomarker profiles across pregnancy showed that for most biomarkers, individuals had consistently low, medium, or high exposure biomarker concentrations across pregnancy. Exceptions were noted for

∑DiNP and ∑DiNCH, where there were subgroups of women who experienced marked declines in concentrations from the 1st to the 3rd trimesters, though both groups were small.

Exposure to phthalates and their replacements is an important public health problem. Studying the effects of exposure on health outcomes is complex in part because of difficulties in exposure assessment. Phthalates and their replacements are rapidly metabolized within the body, usually in less than 24 hours, and their metabolites are excreted primarily via urine.[6,23] Increased renal glomerular filtration rate in pregnancy, a physiologic adaptation, may also result in increased urine excretion.[24] Accordingly, urinary biomarker concentrations only reflect exposure immediately prior to sample collection, which is of limited utility if the goal is to capture long-term exposure. Additionally, because of the nature of the exposure sources (e.g., diet, personal care products, medications), exposure tends to be episodic. The short biological persistence coupled with the likely irregularity in frequency, intensity, and duration of exposure contributes to urinary metabolite concentration variability. Studies have found that phthalate biomarkers have high within-subject temporal variability, as indicated by low to moderate ICCs, across the course of pregnancy, and collecting too few samples leads to a phenomenon similar to classical exposure measurement error and attenuation of exposure-outcome associations relative to what might be observed with exposures derived by averaging over several measurements.[28] It has been demonstrated that collecting multiple samples from participants can mitigate this bias and provide measures that more accurately reflect average exposure over pregnancy.[28]

ICCs are commonly used to assess variability of an exposure across a certain time period, such as pregnancy. The ICCs we observed using spot urine samples were largely comparable to those noted in prior literature for spot samples collected across gestation.[29–35] Additionally, in this study, we demonstrated that pooling multiple samples within a short time window (i.e., trimester), leads to a higher ICC across pregnancy compared to using individual spot samples. This reaffirms findings from other studies that pooling samples within small exposure windows results in exposure estimates that are more stable across larger periods of time.[28,30,36,37] It also suggests that accurately assessing exposure in a short time window through pooling may enable approximation of biomarker concentrations for the duration of pregnancy. This finding may have implications for selection of timing and frequency of sample collections in future studies.

We also examined within-trimester ICCs for phthalate biomarkers, which, to our knowledge, has not been done previously. We found that the ICCs within each trimester were not meaningfully different from the ICCs across all of pregnancy using the individual spot samples. Thus, biomarker concentrations pooled within a short time period (i.e., trimester) may reflect exposures in that trimester and across pregnancy equally well (or equally poorly).

To our knowledge, no previous studies provided ICCs for the replacement biomarkers ∑DiNCH or ∑DEHTP. We observed that both ∑DiNCH and ∑DEHTP had relatively low ICCs, similar to those of the HMW phthalates that DiNCH and DEHTP replace. This was the case for ICCs both across pregnancy and within trimester. Diet is a major exposure

source of HMW and their replacements[38] and thus the lower consistency may relate to normal variability in diet.

Use and detection of DiNCH and DEHTP is increasing. Two recent pregnancy studies measured biomarkers of both DiNCH and DEHTP, while an additional study measured only DiNCH. These studies noted detection frequencies that were similar to what we observed in the HPP study population.[12,39,40] Across studies, LODs were generally similar, though slightly higher in our laboratory analyses. For DiNCH metabolites, detection in previous studies ranged from 50–66% for MCOCH and 58–77% for MHiNCH, compared to 43% and 75% in our study, respectively. Both DEHTP metabolites, MEHHTP and MECPTP, were detected in 100% of samples in both studies, similar to our detection frequency.[12,40] Furthermore, biomarker concentrations in our population were generally similar to those from reproductive-aged women in a similar time period in NHANES, suggesting that exposures in our population are similar to those within the general U.S. population.

Our trajectory analysis of trimester-specific averages showed results that were consistent with ICC of these same exposure measures. For most exposure biomarkers, study participants mapped onto “low”, “medium”, and “high” trajectories. This could support a hypothesis that variability in urinary concentrations of phthalate and replacement biomarkers over pregnancy is attributable, at least in part, to random variability around an average exposure, rather than true variation in concentrations over time. However, it could also reflect the fact that the best fitting models group people into consistent categories over time. Nonetheless, the identification of a “high” exposure group may be useful for future exposure-outcome analyses. This group of individuals may experience consistently high exposures across the course of pregnancy that are more relevant to an adverse outcome than exposures identified with an arbitrary cutoff (e.g., quantile). Additionally, prior studies on endocrine disrupting chemicals have found that they can exhibit non-monotonic dose-response associations, such that low concentrations may also have toxic effects.[41] As such, identification of low exposure groups may also be important. Of note, however, we observed that trajectories created from one spot urine measurement per trimester compared poorly to trajectories derived from trimester averages. Thus, our advanced exposure assessment approach was essential for identifying these patterns.

For a small proportion of women, ∑DiNP and ∑DiNCH concentrations showed a changing pattern across the course of pregnancy, where exposure biomarker concentrations started off higher in the first trimester and declined across the course of pregnancy. These biomarkers had two of the lowest ICCs for capturing exposure across pregnancy using either all 8 visits (0.21 and 0.17, respectively) or the trimester average values (0.35 and 0.28, respectively). The low ICCs and variability in trajectories may reflect temporal patterns in exposures experienced over pregnancy.

We took particular note of women in the “high declining” trajectories for ∑DiNP and ∑DiNCH, which could reflect individuals who make changes to behaviors in pregnancy that would reduce phthalate or replacement exposure. However, it is important to note that these groups were small and somewhat unstable to different exposure specifications (i.e., trimester vs. visit). This may be attributable to the fact that exposure to DiNCH and DINP

is not as frequent or intense because of their uses, such as in floorings, textile coatings, and wire and cable insulation.[42,43] There were few demographic similarities between women in those two groups. For ∑DiNP, the "high declining" group had a larger proportion of non-Hispanic White women and a larger proportion of women with the highest early-pregnancy BMI compared to the overall population. For ∑DiNCH, non-Hispanic Black women were overrepresented in the "high declining" trajectory, but, as with the ∑DiNP group, there was a larger proportion of women with the highest early pregnancy BMI compared to the rest of the study population. Both DiNP and DiNCH are known to have been used in polyvinylchloride (PVC) materials like food packaging, with DiNCH commonly used as a replacement for DiNP.[44,45] The declining concentrations of ∑DiNP and ∑DiNCH among these women may thus relate to diet changes during pregnancy. Unfortunately, we had limited information on diet in this cohort to examine potential changes. It is possible that declining groups may reflect seasonal trends, however compounds that are known to have seasonality due to use in bug sprays and sunscreens (e.g., MEP, DnBP)[46–48] did not exhibit trajectories with changing patterns so we did not explore seasonality further.

Additionally, women in the "high" trajectory of MCNP had slightly increasing concentrations over pregnancy. MCNP is used in many products, primarily in PVC and flexible plastics. The increase in MCNP concentrations may reflect behavior or product use changes, but these findings should be interpreted with caution since the confidence intervals were wide.

We also observed that few women fell into the highest exposure biomarker groups for most biomarkers (range: 3.6–13.5%). This highlights an important limitation in how we typically examine exposure biomarkers in studies of phthalates in pregnancy. Dividing study participants into exposure concentration quantiles does not adequately demonstrate the distribution of exposure biomarkers in the way that trajectories do. Quantiles are not able to identify groups of individuals with exposure patterns that diverge from the overall trend and also force approximately equivalent numbers of women in each group. Thus, investigating these exposure biomarker profiles in association with health outcomes may provide effect estimates with less measurement error than other approaches.

Many prior studies have examined the relationship between phthalate metabolite concentrations and demographic characteristics. Here, using the results from our trajectory analysis, we focus on characterizing individuals who fell into the profiles with the highest concentrations of individual biomarkers. Regarding race and ethnicity, non-Hispanic Black women were overrepresented in "high" groups for MEP, ∑DnBP, MBzP, and ∑DEHP. This is consistent with findings from other studies.[49–51] Differences in phthalate biomarker concentrations by race are frequently attributed to different patterns of consumer product use, namely personal care products.[11,27,35,52]

Limitations of our study include small overall sample size, hindering our ability to draw conclusions about subgroups. Additionally, for trajectories that contain a large proportion of the population (e.g., "high" ∑DEHTP, "low" ∑DiNCH and "low" MCPP), we may be grouping heterogeneous women together and missing patterns, as exposure in these groups frequently encapsulates a wide concentration range. However, we relied on model

fit statistics to guide our analytic decisions and thus trajectories among these exposure biomarkers may be less evident. Furthermore, GBTM may be subject to misclassification given that true group membership is not known. It is additionally an approach in which results reflect the specified parameters (e.g., trajectory shape and number). This is less of a concern in the present analysis, however, as the high posterior probabilities indicated good model fit and sensitivity analyses produced similar results.

The main strength of our study was the availability of phthalate and novel replacement biomarker measurements at 8 time points across gestation. Additionally, our study was comprised of a racially, ethnically, and socioeconomically diverse population, thus allowing inference to often understudied populations. We also examined replacement biomarkers, including ∑DiNCH and ∑DEHTP, for which data on temporal variation are sparse. Lastly, this is the first study to use longitudinal trajectory modeling to assess phthalate and replacement biomarker concentrations over time, allowing for identification of highly exposed subgroups that would not be captured by examining population means or quantiles.

This study provides a fresh perspective on investigating variability in urinary concentrations of phthalate and replacement exposure biomarkers over the course of pregnancy with implications for future work. As in previous studies, we show that trimester averages capture long-term exposure better than single spot urine measurements. More originally, we show that the average of several measurements within a short time window (e.g., trimester) may be an accurate measure of average exposure across the course of pregnancy. In addition, trajectories based on trimester averages or large numbers of repeated samples may be useful for identifying small groups of pregnant women with consistently “high” or “very high” biomarker concentrations for studies linking exposure to health effects. These findings may help inform decision-making when balancing the need for accurate exposure measurement with the burden that repeated sampling creates for both study participants and resources.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Synopsis:

Assessment of temporal variability of phthalates over pregnancy is not well-established and is critical for properly estimating exposure in epidemiological studies. Here, we characterize variability and temporal trends in metabolites of phthalates and phthalate replacements.

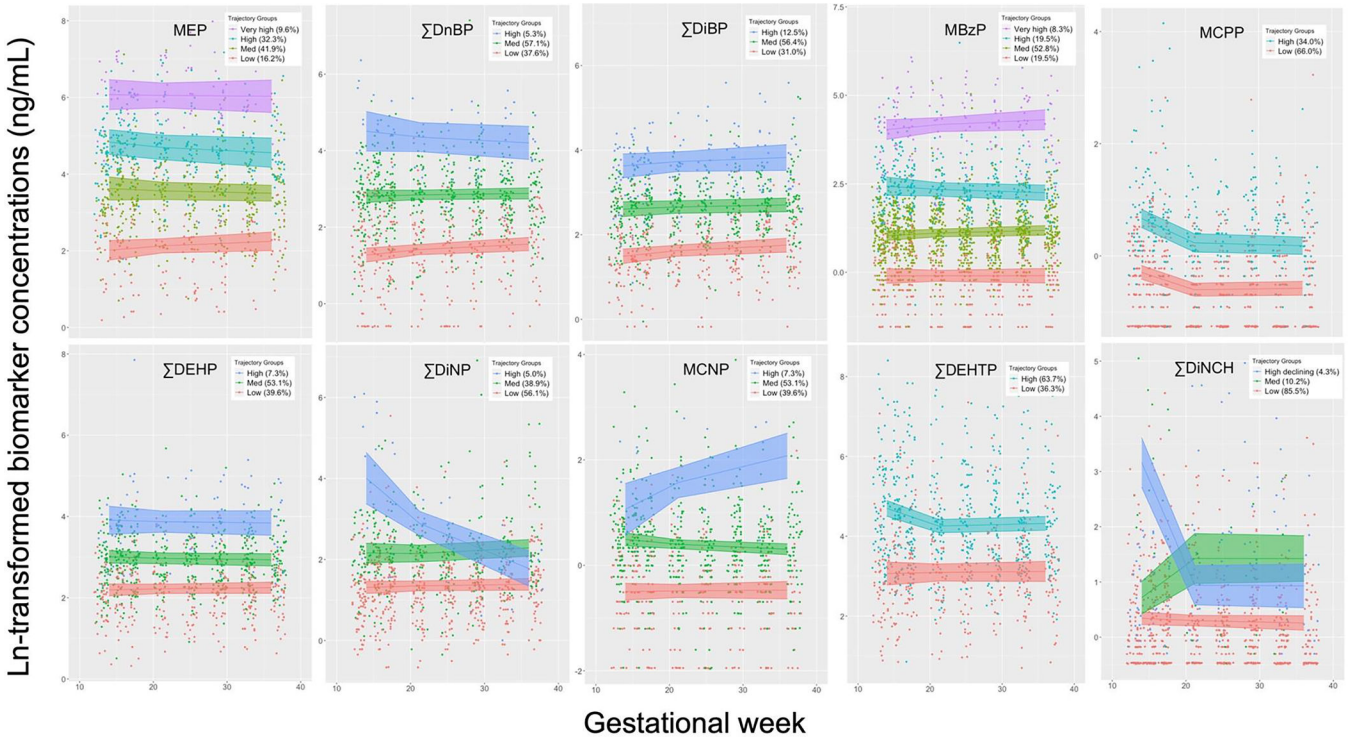
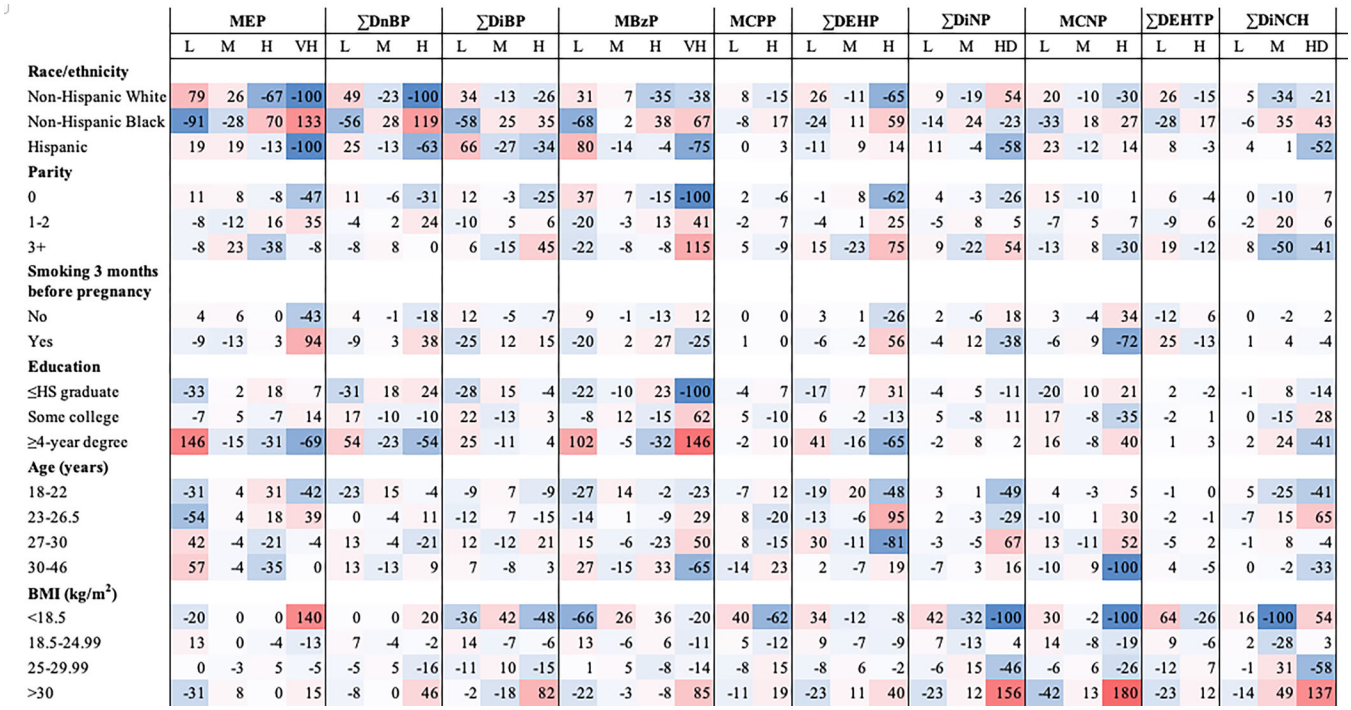


Figure 1.

Mean predicted exposure biomarker trajectories (95% confidence intervals) and observed spot urine concentrations for phthalate and replacement biomarker concentrations[1] (ln-ng/mL)

1. Biomarker concentrations were corrected for SG.

Note: Data points on each plot are a random subset of urine samples (50% of total) to better visualize trends. The trajectories were developed using the full sample. The points reflect observed participant biomarker concentrations plotted by gestational week at which they were collected. The trajectories were derived from group-based trajectory modeling using trimester averaged biomarker concentrations. MEP, ∑DnBP, and ∑DiBP are LMW phthalates, while MBzP, MCPP, ∑DEHP, ∑DiNP, and MCNP are HMW phthalates. ∑DEHTP and ∑DiNCH are replacements. ***Abbreviations***: monoethyl phthalate (MEP); sum of di-n-butyl phthalate metabolites (∑DnBP); sum of di-iso-butyl phthalate metabolites (∑DiBP); monobenzyl phthalate (MBzP); mono-3-carboxypropyl phthalate (MCPP); sum of di(2-ethylhexyl) phthalate metabolites (∑DEHP); sum of di-isononyl phthalate metabolites (∑DiNP); mono carboxyisononyl phthalate (MCNP); sum of 1,2-cyclohexane dicarboxylic acid, diisononyl ester metabolites (∑DiNCH); sum of di(2-ethylhexyl) terephthalate metabolites (∑DEHTP).



	MEP				∑DnBP			∑DiBP			MBzP				MCPP		∑DEHP			∑DiNP			MCNP			∑DEHTP		∑DiNCH		
	L	M	H	VH	L	M	H	L	M	H	L	M	H	VH	L	H	L	M	H	L	M	HD	L	M	H	L	H	L	M	HD
Race/ethnicity																														
Non-Hispanic White	79	26	-67	-100	49	-23	-100	34	-13	-26	31	7	-35	-38	8	-15	26	-11	-65	9	-19	54	20	-10	-30	26	-15	5	-34	-21
Non-Hispanic Black	-91	-28	70	133	-56	28	119	-58	25	35	-68	2	38	67	-8	17	-24	11	59	-14	24	-23	-33	18	27	-28	17	-6	35	43
Hispanic	19	19	-13	-100	25	-13	-63	66	-27	-34	80	-14	-4	-75	0	3	-11	9	14	11	-4	-58	23	-12	14	8	-3	4	1	-52
Parity																														
0	11	8	-8	-47	11	-6	-31	12	-3	-25	37	7	-15	-100	2	-6	-1	8	-62	4	-3	-26	15	-10	1	6	-4	0	-10	7
1-2	-8	-12	16	35	-4	2	24	-10	5	6	-20	-3	13	41	-2	7	-4	1	25	-5	8	5	-7	5	7	-9	6	-2	20	6
3+	-8	23	-38	-8	-8	8	0	6	-15	45	-22	-8	-8	115	5	-9	15	-23	75	9	-22	54	-13	8	-30	19	-12	8	-50	-41
Smoking 3 months before pregnancy																														
No	4	6	0	-43	4	-1	-18	12	-5	-7	9	-1	-13	12	0	0	3	1	-26	2	-6	18	3	-4	34	-12	6	0	-2	2
Yes	-9	-13	3	94	-9	3	38	-25	12	15	-20	2	27	-25	1	0	-6	-2	56	-4	12	-38	-6	9	-72	25	-13	1	4	-4
Education																														
≤HS graduate	-33	2	18	7	-31	18	24	-28	15	-4	-22	-10	23	-100	-4	7	-17	7	31	-4	5	-11	-20	10	21	2	-2	-1	8	-14
Some college	-7	5	-7	14	17	-10	-10	22	-13	3	-8	12	-15	62	5	-10	6	-2	-13	5	-8	11	17	-8	-35	-2	1	0	-15	28
≥4-year degree	146	-15	-31	-69	54	-23	-54	25	-11	4	102	-5	-32	146	-2	10	41	-16	-65	-2	8	2	16	-8	40	1	3	2	24	-41
Age (years)																														
18-22	-31	4	31	-42	-23	15	-4	-9	7	-9	-27	14	-2	-23	-7	12	-19	20	-48	3	1	-49	4	-3	5	-1	0	5	-25	-41
23-26.5	-54	4	18	39	0	-4	11	-12	7	-15	-14	1	-9	29	8	-20	-13	-6	95	2	-3	-29	-10	1	30	-2	-1	-7	15	65
27-30	42	-4	-21	-4	13	-4	-21	12	-12	21	15	-6	-23	50	8	-15	30	-11	-81	-3	-5	67	13	-11	52	-5	2	-1	8	-4
30-46	57	-4	-35	0	13	-13	9	7	-8	3	27	-15	33	-65	-14	23	2	-7	19	-7	3	16	-10	9	-100	4	-5	0	-2	-33
BMI (kg/m^2)																														
<18.5	-20	0	0	140	0	0	20	-36	42	-48	-66	26	36	-20	40	-62	34	-12	-8	42	-32	-100	30	-2	-100	64	-26	16	-100	54
18.5-24.99	13	0	-4	-13	7	-4	-2	14	-7	-6	13	-6	6	-11	5	-12	9	-7	-9	7	-13	4	14	-8	-19	9	-6	2	-28	3
25-29.99	0	-3	5	-5	-5	5	-16	-11	10	-15	1	5	-8	-14	-8	15	-8	6	-2	-6	15	-46	-6	6	-26	-12	7	-1	31	-58
>30	-31	8	0	15	-8	0	46	-2	-18	82	-22	-3	-8	85	-11	19	-23	11	40	-23	12	156	-42	13	180	-23	12	-14	49	137

Figure 2.

Percent difference in demographic characteristics within exposure biomarker trajectory compared to the overall population

Note: Blue cells reflect a lower percentage of participants in the specified trajectory group relative to the distribution in the overall population. Red cells reflect a higher percentage in the specified trajectory group relative to the distribution overall population. Women of "other" race/ethnicity not displayed due to low sample size.

MEP, ∑DnBP, and ∑DiBP are LMW phthalates, while MBzP, MCPP, ∑DEHP, ∑DiNP, and MCNP are HMW phthalates. ∑DEHTP and ∑DiNCH are replacements.

Abbreviations: low trajectory (L); medium trajectory (M); high trajectory (H); very high trajectory (VH); high declining trajectory (HD); monoethyl phthalate (MEP); sum of di-n-butyl phthalate metabolites (∑DnBP); sum of di-iso-butyl phthalate metabolites (∑DiBP); monobenzyl phthalate (MBzP); mono-3-carboxypropyl phthalate (MCPP); sum of di(2-ethylhexyl) phthalate metabolites (∑DEHP); sum of di-isononyl phthalate metabolites (∑DiNP); mono carboxyisononyl phthalate (MCNP); sum of 1,2-cyclohexane dicarboxylic acid, diisononyl ester metabolites (∑DiNCH); sum of di(2-ethylhexyl) terephthalate metabolites (∑DEHTP).

Table 1.

Demographic characteristics of participants in the Human Placenta and Phthalates Study (N=303)

	N (%)
Race/ethnicity	
Non-Hispanic White	118 (38.9)
Non-Hispanic Black	131 (43.2)
Hispanic	49 (16.2)
Other[1]	5 (1.7)
Clinic Site	
EVMS	218 (71.9)
UTMB	85 (28.1)
Marital status	
Single[2]	131 (44.1)
Married or living with partner	166 (55.9)
Missing	6
Current employment	
None	139 (48.1)
Any	150 (51.9)
Missing	14
Health insurance [3]	
Private	82 (27.5)
Government-assisted	216 (72.5)
Missing	5
Parity	
0	108 (35.8)
1–2	155 (51.3)
3+	39 (12.9)
Missing	**1**
Smoking in 3 months prior to pregnancy	
No	204 (67.8)
Yes	97 (32.2)
Missing	2
Education	
High school graduate or below	131 (44.7)
Some college, technical school, or associates degree	123 (42.0)
4-year college degree	39 (13.3)
Missing	10
Age (years)	
18–22	78 (25.9)
23–26.5	83 (27.6)
27–30	72 (23.9)

	N (%)
30–46	68 (22.6)
Missing	2
Early pregnancy BMI (kg/m^2)	
<18.5	16 (5.3)
18.5–24.99	135 (44.7)
25–29.99	112 (37.1)
>30	39 (12.9)
Missing	1

1. Includes Asian, Hawaiian/Pacific Islander, multiracial

2. Includes never married, divorced, separated, widowed

3. One participant who reported “self-pay/uninsured” was grouped with government-assisted.

Table 2.

Detection frequency of phthalate and replacement metabolites in all urine samples analyzed (n=1866) from the Human Placenta and Phthalates Study (N=303).

Phthalate metabolites	Biomarker abbreviation	LOD (ng/mL)	% Detection
Monoethyl phthalate	MEP	1.2	99.6
Mono-n-butyl phthalate	MBP	0.4	96.8
Mono-hydroxybutyl phthalate	MHBP	0.4	76.7
Mono-iso-butyl phthalate	MiBP	0.8	98.9
Mono-hydroxy-isobutyl phthalate	MHiBP	0.4	98.5
Monobenzyl phthalate	MBzP	0.3	98.0
Mono-3-carboxypropyl phthalate	MCPP	0.4	73.2
Mono-2-ethylhexyl phthalate	MEHP	0.8	61.5
Mono-2-ethyl-5-hydroxyhexyl phthalate	MEHHP	0.4	99.1
Mono-2-ethyl-5-oxohexyl phthalate	MEOHP	0.2	99.8
Mono-2-ethyl-5-carboxypentyl phthalate	MECPP	0.4	99.9
Mono oxononyl phthalate	MONP	0.4	91.0
Mono carboxyisooctyl phthalate	MCOP	0.3	99.7
Mono carboxyisononyl phthalate	MCNP	0.2	95.2
Replacement metabolites			
Mono-2-ethyl-5-hydrohexyl terephthalate	MEHHTP	0.4	98.9
Mono-2-ethyl-5-carboxypentyl terephthalate	MECPTP	0.2	100
Cyclohexane-1,2-dicarboxylic acid, monohydroxy isononyl ester	MHiNCH	0.4	74.8
Cyclohexane-1,2-dicarboxylic acid, monocarboxy isooctyl ester	MCOCH	0.5	42.7

Abbreviations: Limit of detection (LOD)

Table 3.

Median (interquartile range) of phthalate and replacement biomarker concentrations[1] (ng/mL) by trimester and in comparison to 2017–2018 NHANES concentrations.

	NHANES 2017–2018[2]	Trimester 1 (n=260)	Trimester 2 (n=268)	Trimester 3 (n=274)
Phthalate biomarkers				
MEP	36.9 (18.6, 80.0)	58.5 (20.9, 129.1)	42.5 (17.3, 106.6)	44.5 (17.7, 99.5)
∑DnBP	11.3 (7.1, 17.1)	12.0 (5.1, 24.8)	11.6 (4.6, 21.4)	11.7 (6.5, 23.9)
∑DiBP	11.3 (7.3, 17.8)	11.2 (5.2, 21.0)	11.2 (6.5, 18.5)	11.8 (7.1, 23.2)
MBzP	3.5 (1.7, 7.8)	3.2 (1.7, 7.4)	3.4 (1.7, 7.0)	3.6 (1.8, 7.4)
MCPP	1.2 (0.8, 1.8)	1.1 (0.6, 1.7)	0.7 (0.4, 1.2)	0.7 (0.4, 1.2)
∑DEHP	17.1 (11.9, 25.1)	15.5 (8.8, 28.4)	14.6 (9.6, 24.2)	15.3 (9.6, 22.9)
∑DiNP	6.6 (4.3, 11.5)	5.5 (3.3, 9.8)	5.5 (3.3, 9.7)	5.5 (3.6, 9.0)
MCNP	1.3 (0.9, 2.0)	1.2 (0.7, 2.1)	1.1 (0.7, 1.7)	1.0 (0.7, 1.8)
Replacement biomarkers				
∑DEHTP	35.6 (17.0, 101.1)	63.9 (25.4, 136.9)	46.2 (21.7, 89.2)	44.5 (23, 89.5)
∑DiNCH	1.5 (0.8, 3.1)	1.4 (0.8, 2.7)	1.5 (0.9, 2.6)	1.2 (0.9, 2.3)

1. Biomarker concentrations displayed are corrected for specific gravity and averaged within trimesters.

2. NHANES concentrations are presented for women aged 18–49 and are creatinine-corrected (μg/g creatinine) and weighted to account for sampling scheme.

Abbreviations: National Health and Nutrition Examination Survey (NHANES); monoethyl phthalate (MEP); sum of di-n-butyl phthalate metabolites (∑DnBP); sum of di-iso-butyl phthalate metabolites (∑DiBP); monobenzyl phthalate (MBzP); mono-3-carboxypropyl phthalate (MCPP); sum of di(2-ethylhexyl) phthalate metabolites (∑DEHP); sum of di-isononyl phthalate metabolites (∑DiNP); mono carboxyisononyl phthalate (MCNP); sum of di(2-ethylhexyl) terephthalate metabolites (∑DEHTP); sum of 1,2-cyclohexane dicarboxylic acid, diisononyl ester metabolites (∑DiNCH).

Table 4.

Intraclass correlation coefficients (95% confidence intervals) for phthalate and replacement biomarkers across pregnancy and within trimester.

	Across pregnancy		Within trimester		
	Visits 1–8 (303 subjects, 1866 observations)	**Trimesters 1–3 (303 subjects, 802 observations)**	**Visits 1–2 (260 subjects, 472 observations)**	**Visits 3–5 (268 subjects, 705 observations)**	**Visits 6–8 (274 subjects, 691 observations)**
Phthalate biomarkers					
MEP	0.61 (0.56, 0.65)	0.73 (0.68, 0.77)	0.69 (0.62, 0.76)	0.62 (0.56, 0.68)	0.63 (0.56, 0.69)
∑DnBP	0.52 (0.47, 0.57)	0.65 (0.59, 0.70)	0.58 (0.49, 0.66)	0.52 (0.44, 0.59)	0.62 (0.55, 0.68)
∑DiBP	0.49 (0.44, 0.54)	0.61 (0.55, 0.67)	0.61 (0.53, 0.69)	0.50 (0.42, 0.57)	0.57 (0.50, 0.64)
MBzP	0.69 (0.65, 0.73)	0.80 (0.76, 0.83)	0.74 (0.68, 0.80)	0.67 (0.61, 0.72)	0.75 (0.70, 0.79)
MCPP	0.25 (0.20, 0.30)	0.34 (0.26, 0.42)	0.28 (0.17, 0.42)	0.31 (0.23, 0.40)	0.31 (0.23, 0.40)
∑DEHP	0.34 (0.29, 0.39)	0.49 (0.41, 0.56)	0.35 (0.25, 0.48)	0.32 (0.24, 0.41)	0.45 (0.37, 0.53)
∑DiNP	0.21 (0.17, 0.26)	0.35 (0.27, 0.43)	0.17 (0.07, 0.35)	0.23 (0.16, 0.33)	0.29 (0.21, 0.38)
MCNP	0.27 (0.22, 0.32)	0.42 (0.35, 0.50)	0.32 (0.21, 0.45)	0.29 (0.21, 0.38)	0.27 (0.19, 0.37)
Replacement biomarkers					
∑DEHTP	0.27 (0.22, 0.32)	0.39 (0.31, 0.46)	0.29 (0.18, 0.43)	0.27 (0.19, 0.37)	0.36 (0.28, 0.45)
∑DiNCH	0.17 (0.13, 0.22)	0.28 (0.21, 0.37)	0.38 (0.27, 0.50)	0.11 (0.05, 0.23)	0.22 (0.15, 0.32)

Abbreviations: monoethyl phthalate (MEP); sum of di-n-butyl phthalate metabolites (∑DnBP); sum of di-iso-butyl phthalate metabolites (∑DiBP); monobenzyl phthalate (MBzP); mono-3-carboxypropyl phthalate (MCPP); sum of di(2-ethylhexyl) phthalate metabolites (∑DEHP); sum of di-isononyl phthalate metabolites (∑DiNP); mono carboxyisononyl phthalate (MCNP); sum of di(2-ethylhexyl) terephthalate metabolites (∑DEHTP); sum of 1,2-cyclohexane dicarboxylic acid, diisononyl ester metabolites (∑DiNCH)