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A Possible Approach to Improving the Reproducibility of Urinary Concentrations of Phthalate Metabolites and Phenols during Pregnancy

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Abstract

In epidemiologic studies, classifying episodic exposures to chemicals with short half-lives, such as phthalates and phenols, is challenging. We assessed whether accounting for sources of variability unrelated to exposure pathways would improve the reproducibility of urine concentrations of select phthalate metabolites and phenols. In 2011, a subset of pregnant women (n=19) enrolled in a prospective study provided first morning urines every 3-4 weeks between 16 and 36 weeks gestation. At the time of collection, we identified potential contributors to variations in urinary concentrations: weight gain, gestational age, time slept, time since awoke, time since last food/drink, and time since last void. We estimated intraclass correlation coefficients (ICCs) among

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repeat urine concentrations with and without adjustment for sources of variability using a random intercept linear mixed model. Concentrations of monoethyl phthalate, butyl- and propyl parabens were the most reproducible (ICCs: 0.68, 0.56, and 0.56 respectively). However, adjustment for potential sources of variability unrelated to exposure pathways did not materially improve reproducibility nor the ability of a single sample to predict exposure based on average biomarker concentrations across pregnancy. Future studies should carefully consider the exposure timeframe and the reliability of using biomarker concentrations from a single time point to represent exposures over pregnancy.

Keywords

child exposure/health; endocrine disruptors; phenols; phthalates; pregnancy; prenatal exposure

INTRODUCTION

The potential for prenatal and childhood exposures to endocrine disrupting chemicals (EDCs) to adversely impact early life development is a growing public health concern. Phenols and phthalates are two classes of EDCs that are commonly used in a wide range of consumer and personal care products thereby resulting in pervasive human exposure as shown by regular detection of EDC biomarkers in urine[1, 2]. A growing research effort is focused on assessing the potential contribution of phenols and phthalates to adverse neurobehavioral and reproductive development in childhood[3].

Bisphenol A (BPA) is a widely studied EDC for which human exposure occurs largely through diet due to leaching from food packaging and polycarbonate containers, though transdermal absorption (e.g., contact with thermal receipts) and inhalation (e.g., cigarette filters) are also possible[1, 4]. Other commonly occurring phenols include triclosan and parabens both of which have antimicrobial properties resulting in their use in personal care products (e.g., soaps, toothpastes, deodorants) and/or as preservatives in cosmetics, pharmaceuticals, and foods[1, 5]. 2,4-Dichlorophenol is predominantly found in herbicides but can also be formed as a byproduct of manufacturing chlorinated chemicals[6]. 1,4-Dichlorobenzene, which metabolizes to 2,5-dichlorophenol, is used in moth balls and toilet deodorants[7]. Another phenolic compound, benzophenone-3 is used in sunscreen because of its capacity to block ultraviolet (UV) radiation[1]. Despite increasing use of substitutes for BPA and other common phenols in consumer products, these phenols are still detectable in human urine. Although typically short-lived in the body, a number of phenols, including benzophenone-3 and its metabolites, have been detected in lipid tissues supporting the possibility of bioaccummulation[8, 9].

Phthalates are a family of structurally related chemicals used commonly in many consumer and personal care products. For example, diethyl phthalate (DEP) is largely used in personal care products with perfume or fragrance while di(2-ethylhexyl) phthalate (DEHP) is used to produce flexible plastics (e.g., polyvinyl chloride) for a variety of household, garden, and medical products (e.g., intravenous tubing)[1].

The rapid growth and development that occurs prenatally can be particularly sensitive to perturbation by exposure to EDCs such as certain phthalates and phenols [2]. To date, urinary concentrations of phenols and phthalate metabolites are the best exposure biomarkers[1]. But these EDCs have short half-lives and individual exposures may be highly variable over the course of a day and from day to day, making human exposure assessment challenging. Consistent with their short half-life and the episodic nature of the exposure, repeat urinary concentrations of phenol or phthalate biomarkers have poor reproducibility over time. In addition, for some biomarkers, reproducibility is even poorer in pregnant than non-pregnant states [10-13]. As a result there is uncertainty regarding how well urinary biomarker concentrations from a limited number of urine specimens (often only one) reflect exposure that is relevant to the risk of adverse health outcomes, including measures of child development. However, because of logistical and cost constraints, most epidemiologic studies of phenols or phthalates exposures rely on a limited number of urine samples per participant.

Research that would allow investigators to optimize the utility of urinary measures for exposure assessment in epidemiologic studies is needed. The goal of the current study was to assess whether there are easily ascertained sources of variability in urinary concentrations of phthalate metabolites and phenols during pregnancy that are unrelated to exposure pathways and that, when accounted for, would improve the reproducibility of the measurements.

MATERIALS AND METHODS

Study Population

The formative (pilot) phase of the Illinois Kids Development Study (I-KIDS) enrolled 181 pregnant women between 2010 and 2012. I-KIDS is a prospective cohort study of the relation of prenatal exposures to phenols (or their precursors) and phthalates with fetal growth and sexual development as well as with subsequent infant and child cognition. The formative phase of I-KIDS ended in 2012 and an expanded version of the study is in progress. Women learned about the formative study at their first prenatal visit to a local obstetrics clinic in Urbana, Illinois and completed a reply card indicating their potential interest in participation. Of the 1,280 women who completed a reply card, 512 (40%) indicated interest in the study. Of these 512, 400 (78%) were reached in a follow-up phone call and 224 (56%) were identified as eligible, 181 (81%) of whom enrolled in the study. After enrollment, 24 (13%) women became ineligible or withdrew from the study during pregnancy and an additional eight (4%) became ineligible or withdrew from the study after delivery, resulting in 149 women in the final cohort. Eligible women were 18-40 years of age, fluent in English, not carrying multiples, and not taking prescription medication for a chronic health condition; they also resided within a 45 mile radius of the study clinic and planned to stay in the hospital for 48 hours after delivery and in the area for at least one year after the birth of their infant.

The study protocol was reviewed and approved by the Human Subjects Committees of the University of Illinois at Urbana-Champaign and Carle Foundation Hospital, Urbana, Illinois. Written informed consent was obtained from participants before study assessments were

performed. The analysis of blinded urine specimens by the Centers for Disease Control and Prevention (CDC) laboratory was determined not to constitute engagement in human subjects research.

Urine Sample Collection

Women were enrolled in their second trimester of pregnancy, between 16 and 19 weeks gestation. They provided a first morning urine specimen at enrollment and a second one at the end of the third trimester, between 34 and 38 weeks gestation. For the current analysis, the 30 women living closest to the study clinic were asked to provide more frequent (every 3 to 4 weeks) first morning urines during pregnancy; 19 women agreed to do so. Each of the 19 women in this sub-study collected a total of 6 urine samples between 16 and 36 weeks gestation.

Once every 3-4 weeks for the last six months of pregnancy (at 16-18, 20-22, 23-26, 27-30, 32-33, and 35-36 weeks gestation), each of the 19 woman collected a first morning urine sample using a polypropylene urine collection cup with a high density polyethylene (HDPE) lid (Thermo Scientific Nalgene). Samples were kept refrigerated until processing, within approximately 24 hours of collection. For processing, samples were warmed to room temperature, mixed on a vortex mixer, and had specific gravity measured using a refractometer (TS400; Reichert). The urine was then aliquoted with disposable polyethylene transfer pipets (Fisherbrand) into polypropylene vials with HDPE lids (Thermo Scientific Nalgene) and stored at −20° C. Field blanks of purified water (Fisher Chemical W5SK-1 HPLC grade, submicron filtered) were collected periodically, transported to the lab, and processed and stored using the same procedures described for urine. Frozen urine samples and field blanks were shipped on dry ice by overnight courier to the CDC (Atlanta, GA).

Covariate Assessment

At enrollment, study participants completed a questionnaire to provide information on demographics, height, pre-pregnancy weight, occupation, lifestyle, reproductive and medical history, and exposure history. In addition, for the 24 hours before each urine collection, participants completed a diary recording use of products potentially containing phenols (or their precursors) or phthalates (e.g., personal care products, pre-packaged foods). The women also recorded the time of their last meal or drink and last void prior to collecting the urine samples. Finally, trained study staff reviewed medical records from prenatal visits to collect pregnancy weight. To calculate maternal weight gain at each urine collection, we identified the maternal weight in the medical record that was measured closest (within 14 days) to the urine collection date and subtracted it from the pre-pregnancy weight.

Urine Phthalate Metabolites and Phenol Measurements

Total (free plus conjugated) concentrations of eight phenols (BPA, triclosan, 2,4-dichlorophenol, 2,5-dichlorophenol, benzophenone-3 as well as butyl paraben (B-paraben), methyl paraben (M-paraben), and propyl paraben (P-paraben)) and 11 phthalate metabolites were quantified at CDC by online solid phase extraction coupled with high performance liquid chromatography-isotope dilution-tandem mass spectrometry[14-17]. The CDC

laboratory methods have excellent sensitivity and reproducibility for these urine analyses with coefficients of variation (CVs) ranging from 2.7-15%[16-18].

For this analysis, we examined the total concentrations of the eight phenols and the metabolites of two commonly used phthalates that accounted for the majority of phthalate urine biomarkers in our population: (1) monoethyl phthalate (MEP), the metabolite of diethyl phthalate (DEP); and (2) the micromolar sum (μmol/L) of four metabolites of di(2-ethylhexyl) phthalate (DEHP): mono (2-ethylhexyl) phthalate (MEHP), mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono (2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono (2-ethyl-5-carboxypentyl)phthalate (MECPP). We calculated the molar sum of the four DEHP metabolites (ΣDEHP) by dividing the concentration of each metabolite by its molar mass and summing the resulting measures. In the 149 women participating in the full formative center study, MEP accounted for approximately 38% and ΣDEHP metabolites accounted for 18% of the phthalate metabolites detected in urine.

Statistical Analysis

Concentrations of all urine biomarkers (including results below the limit of detection for which a signal was detected) were used as reported in the analyses after natural log transformation. Only two biomarkers had measured concentrations of zero (e.g., no signal detected) -- triclosan and B-paraben (12 urines from 8 women and 31 urines from 10 women, respectively). Prior to ln-transformation, these results were assigned 0.001 μg/L which was 1% of the lowest non-zero measured concentration. Non-reportable results that did not meet pre-established laboratory quality control criteria were set to missing. If more than 10% of measurements for a specific biomarker were missing, the biomarker was not included in the statistical analyses. To account for urine dilution, biomarker concentrations were adjusted for specific gravity using the following formula: $B_{SG} = B \times [(1.024 - 1)/(SG - 1)]$, where B_{SG} is the specific gravity adjusted biomarker concentration, B is the measured biomarker value, and SG is the urine specific gravity[19].

We identified covariates based on *a priori* considerations related to the short half-life of the biomarkers and the potential for time varying activities (e.g., time since last void) to impact urinary concentrations. We did not consider potential sources of exposure (e.g., personal care product use) as covariates in this analysis because our goal was to improve reproducibility over time by removing within person variation in urine measures that arise from differences in conditions at the time of urine collection unrelated to exposure itself. Our goal was not to predict biomarker concentrations nor to identify all sources of within person variability. For example, we wanted to retain variability related to suspected or known exposure pathways, as this is central to the utility of biomarker concentrations for health effect studies. Instead, we focused on individual time-varying behaviors that demonstrate substantial within person variability over time but are not related to differences in exposure itself. This is the primary reason we did not include covariates that are constant across a given person's urine collections (e.g., race, age, pre-pregnancy BMI). The chosen covariates included pregnancy weight gain (kg), gestational age (weeks), time since awoke (hours), time slept (hours), time since last food or drink (hours), and time since last void (hours) ascertained at the time of each urine collection. Covariate data were relatively

complete - of the 684 time-varying covariate measures (19 women observed 6 times with 6 time-varying covariates per observation), a total of 35 values were missing. The few covariates with missing values were imputed using multiple imputation methods; in SAS[20] we created 10 imputed datasets using Proc MI.

We used multivariable linear mixed models to model the relationship of the selected covariates with urinary concentrations of phenols or phthalate metabolites. We assessed the reproducibility of repeat urinary concentrations by estimating unadjusted intraclass correlation coefficients (ICCs) and then re-estimating the ICCs after adjustment for potential sources of variability (unrelated to exposure pathways) using our linear mixed models. ICCs and corresponding 95% confidence intervals (CIs) were estimated from a random intercept linear mixed model. The ICC is the ratio of the between-subject variability and the sum of the between- and within-subject variability[21]; an ICC of 0 indicates no reproducibility within a subject and 1 indicates perfect reproducibility.

We also assessed how well concentrations of phthalate metabolites or phenols in a single urine sample predicted average urine concentrations over the entire pregnancy. First, we obtained studentized residuals from unadjusted and adjusted linear mixed models predicting urine biomarker concentrations for each of the six collection times. The residuals were the differences between the observed concentration in each woman individually at a given time point and the predicted value for each woman at each time point. We then used these residuals to assess how well biomarker concentrations in a single sample predict the overall pregnancy mean concentration (which was calculated as the mean of all 6 urine concentration residuals). We refer to the latter as our “gold standard” measure, assuming that a biomarker’s average concentration across pregnancy is a more reliable measure of pregnancy exposure than the concentration at a single time point. This assessment included calculation of sensitivity, specificity and percent correct classification using adjusted and unadjusted residuals. We grouped the distribution of residuals for each biomarker concentration at each collection time into tertiles and, similarly, grouped the distribution of gold standard residuals into tertiles. We defined “high exposure” as the top concentration tertile and “low exposure” as the two lowest tertiles. We then estimated how well a single urine identified a woman as having high overall pregnancy exposure by calculating the sensitivity, specificity, and percent correctly classified comparing each urine collection time with the gold standard. Finally, we compared results from unadjusted and adjusted analyses to determine whether accounting for potential sources of variability unrelated to exposure pathways improved the reproducibility, sensitivity, specificity, or correct classification of urinary concentrations of phthalate metabolites or phenols during pregnancy.

All analyses were conducted in each of the 10 datasets for which missing covariate data were imputed. Results did not differ substantially among the 10 datasets for the reproducibility analysis; therefore, we present ICCs from the first imputed dataset for this analysis. The results did differ across the 10 datasets for the sensitivity and specificity analysis; therefore, we present the range of sensitivity, specificity, and percent correct classification that were identified across the 10 imputed datasets.

All analyses were performed using the Statistical Analysis Software version 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

Study Population

Participants in this sub-study (n=19) were similar to those in the full formative study (n=149). Consistent with the demographics of Urbana-Champaign which is a university community, they were mostly middle class (79% had household income >$30,000; vs. 82% in the full study), 90% were Caucasian (vs. 84% in the main study) and 74% were college educated (vs. 73% in the full study). All 19 women gave birth to full-term infants (mean 39.5 weeks) (Table 1). Mean age at enrollment was 29.5 years (range 23-34), most women (68%) were multiparous, and 37% reported ever smoking but only one (5%) smoked during pregnancy. For this sub-study, the majority of women (79%) reported a normal pre-pregnancy body mass index (18.5-24.9 kg/m^2) and the average weight gain in mid-pregnancy (20-22 weeks) and late pregnancy (25-36 weeks) was 5.9 kg (standard deviation (SD): 4.9 kg) and 12.9 kg (SD: 6.0 kg), respectively. Women reported an average of 8.4 (SD: 0.8) hours of sleep the night before their first morning urine collection. Prior to the urine collection, the average number of hours since a woman voided or consumed food/drinks was 6.2 (SD: 1.6) and 8.3 (SD: 2.4), respectively.

Potential Sources of Variability in Urinary Concentrations of Phthalate Metabolites and Phenols

Unadjusted urinary concentrations of phthalate metabolites and phenols at each time point, the number of samples with concentrations below the limit of detection for the target biomarkers, and the number of missing concentrations are presented in Table 2. Specific gravity adjusted urinary concentrations for the same biomarkers and time points are available in Supplementary Table S1. Benzophenone-3 and triclosan were not included in further analyses because of the number of missing concentrations, 14% and 10%, respectively. The univariate relationship between the time varying covariates and the phenols and phthalate metabolites are presented in Table 3. Associations were inconsistent across biomarkers and covariates. For example, later gestation was associated with lower 2,4-dichlorophenol concentrations (−2.5%, 95% CI: −4.4, −0.7) but higher 2,5-dichlorophenol concentrations (3.0%, 95% CI: 0.7, 5.4). Greater gestational weight gain was associated with higher BPA concentrations (2.5%, 95% CI: 0.4, 4.5) but did not have a significant impact on other study biomarkers. Although not all associations were significant, time slept prior to urine collection was generally associated with higher phenol but lower phthalate metabolite concentrations. Time since last void prior to urine collection was typically associated with higher biomarker concentrations but, again, not all associations were statistically significant.

Reproducibility of Urinary Phenol Concentrations

In our study population, urinary BPA concentrations were the least reproducible across 6 pregnancy samples (ICC: 0.24, 95% CI: 0.10, 0.49), while those of B-paraben and P-paraben were the most reproducible (ICC: 0.56, 95%CI: 0.36, 0.74 and ICC: 0.56, 95%CI: 0.36, 0.74, respectively). Adjustment for potential sources of variability in urinary concentrations

(unrelated to exposure pathways) did not improve reproducibility (Table 4). The largest, yet modest, improvement was observed for 2,5-dichlorophenol; after adjustment, the ICC increased from 0.34 to 0.39. Similarly, for 2,5-dichlorophenol as well as 2,4-dichlorophenol concentrations, the sensitivity, specificity, and percent correctly classified adjusted values typically reflected potential improvement relative to unadjusted measures (Table 5). For the other phenols, adjustment had an inconsistent impact on the percent correct classification, sensitivity, and specificity (Table 5). For example, adjustment improved the percent correctly classified for BPA in the 32-33 week urine samples (respective increases from 84% to a range of adjusted values from 89% to 100%) but ranged from poorer to similar classification accuracy for the 16-18 week urine (the unadjusted value was 74% and the adjusted values were between 58% and 74%).

Reproducibility of Urinary Concentrations of Phthalate Metabolites

Urinary MEP concentrations were the most reproducible over pregnancy (ICC: 0.68; 95% CI: 0.50, 0.82) and reproducibility was essentially unchanged by adjustment (ICC: 0. 66; 95% CI: 0. 46, 0. 81) (Table 4). Adjustment did not impact the sensitivity of urinary MEP concentrations which averaged 0.81 (range of unadjusted sensitivity values: 0.67 to 1.00) (Table 5).

In contrast to MEP, concentrations of ΣDEHP metabolites were not as reproducible (ICC: 0.32; 95% CI: 0.15, 0.55) and adjustment for potential sources of variation did not improve the ICC (Table 4). Additionally, the sensitivity, specificity, and percent correct classification for ΣDEHP concentrations were not improved by adjustment and, instead, often were worse (Table 5).

DISCUSSION

Developing cost effective approaches for accurately characterizing exposure to phthalates or phenols during prenatal development is important for conducting epidemiologic studies. Our goal was to improve reproducibility of these measures over time by minimizing potential sources of time-varying within person variation in these biomarkers unrelated to exposure. We hoped to thereby enhance their utility in studies of human health impacts as well as in studies identifying key exposure risk factors. We hypothesized that we could achieve this goal by implementing an analytic strategy to decrease random temporal variability. To the best of our knowledge, this goal has not been addressed in previous studies which have characterized variability in urine EDC measures [12, 22] but have not attempted to minimize that variability. The one exception is an analysis adjusting for time-varying sampling conditions (e.g. hour of random urine collection) as well as urine handling (e.g., storage time prior to freezing) but reproducibility was not improved by this approach and the analysis did not consider the role of individual time-varying behaviors [23].

In addition, our study is notable for having first morning urine samples available for 6 time points across pregnancy whereas previous studies in pregnancy have typically collected urine at no more than 2-4 time points[10-12]. Our design optimized the ability to characterize long term pregnancy exposure in ways not done previously. In addition, because the study population’s sociodemographic characteristics were relatively homogeneous, potential

variability related to socio-demographics was minimized. Lastly, first morning voids were used to optimize the reproducibility of our measures as timed collections are more likely to be comparable across participants vis-a-vis proximity to meal time, personal grooming activities, and last void than random urine collections. For example, most, if not all, previous studies of the reproducibility of MEP in pregnant women have not used first morning urine samples[12, 23-27]. Our relatively strong ICCs for phthalates (e.g., MEP and ΣDEHP metabolites) compared to other studies may, in part, reflect the use of a first morning timed urine collection (Table 4). Despite these design strengths, adjustment for *a priori* potential sources of variability unrelated to exposure pathways had minimal impact on the reproducibility of urinary concentrations of phthalate metabolites or phenols during pregnancy (Table 4). Similarly, the specificity, sensitivity, and probability of correctly classifying urinary concentrations across pregnancy using a single sample were not materially improved by adjustment for these potential sources of variability. This pattern of findings applied to the biomarkers of both phthalates (MEP, ΣDEHP metabolites) and phenols (n=6) we assessed (Table 5). One possible explanation for our findings is that identifying correlates of urinary concentrations of these compounds may be particularly difficult in pregnant women for whom changes in xenobiotic metabolism, body composition, nutritional status, and even health behaviors may contribute to enhanced variability in exposure to, absorption, distribution, metabolism and elimination of phenols and phthalates[12, 28]. Although use of first morning voids (rather than random collections) may have improved reproducibility by decreasing variability in urine collection circumstances, a timed collection also could limit the variability of key covariates in our analysis (e.g., time since last void) and thus minimize the impact of our covariate adjustments. However, despite collection of first morning voids, there was variability in measures related to urine sampling time. For example, across the 114 urine collections, time since last void ranged from 1.3 to 12.8 hours with a mean (SD) of 6.2 (2.5) hours; and time since last food/drink ranged from 10 minutes to 15.0 hours with a mean (SD) of 8.3 (3.2) hours (data not shown), consistent with a pregnant population where awakenings to void or eat (followed by return to sleep) are not uncommon.

More fundamentally, our inability to improve the value of a urinary concentration from a single sample for predicting long term exposure to the target biomarkers may reflect, in part, the uncertainty in identifying sources of variation unrelated to exposure regardless of pregnancy status. In addition, by design, we did not adjust for personal behaviors related to likely exposure pathways (e.g., personal care product use, diet). These behaviors may account for both within and between person variation in biomarker levels over time and thus could explain persistence of limited reproducibility of individual urine measures. Instead, our choice of adjustment factors was based on *a priori* considerations unrelated to exposure including the short half-life of the target biomarkers (e.g., time since last void would thereby impact urine concentrations) and the likely correlation of urinary concentrations with daily activities. For example, meal time may represent a period of potentially high exposure but is not specific enough to capture actual exposure. Despite these considerations, none of the *a priori* factors we considered consistently improved the predictive utility of a single urinary concentration of any of the target biomarkers. This result is consistent with the relatively

modest associations observed between the urine EDC biomarker concentrations and many of our time-varying covariates (Table 3).

It is possible that sources of variation (unrelated to exposure pathways) may differ by exposure and population characteristics. However, our findings are in a population with urinary concentrations of phthalate metabolites and phenols generally comparable to other population-based studies of pregnant women (Table 2)[10, 11, 24, 29-44]. For example, our average urinary concentrations across pregnancy for BPA, 2,4-dichlorophenol, M-paraben, P-paraben, triclosan, ΣDEHP metabolites, and MEP were within range of previously published studies of pregnant women (Table 2). Our urinary concentrations of 2,5-dichlorophenol were slightly lower whereas benzophenone-3 concentrations were slightly higher (Table 2) suggesting that use of products with sunscreen containing benzophenone-3 may be more common in our largely non-Hispanic white population than observed in other studies. More generally, our findings are consistent with lower 2,5-dichlorophenol and higher benzophenone-3 concentrations observed in white non-Hispanic populations as compared to other racial or ethnic groups[45, 46]. Although we were unable to improve the value of predicting longer term exposure from a single sample for urinary concentration of phthalate metabolites or phenols, we demonstrated good reproducibility of urinary MEP concentrations (ICC = 0.68) and reasonable reproducibility of paraben concentrations (ICC's = 0.44-0.56). In fact, concentrations of MEP as well as ΣDEHP metabolites typically had better reproducibility (unadjusted ICCs of 0.68 and 0.32, respectively) than has been observed in other studies (ICCs ranging from 0.21 to 0.50 and 0.08 to 0.31, respectively)[12, 24-27]. For other biomarkers, reproducibility, whether adjusted or not, was similar to values reported elsewhere[4, 10-13, 26, 47-49]. For example, the ICC for M-paraben in our study was 0.44 and in other populations of pregnant women the ICC for M-paraben was between 0.24 and 0.61 (Table 4)[10, 11, 13, 49]. In contrast, our urinary 2,5-dichlorophenol concentrations had poorer reproducibility than observed elsewhere (unadjusted ICC of 0.34 versus 0.49 to 0.61) (Table 4)[10,11].

There are few studies assessing the sensitivity or specificity of biomarker concentrations from a single urine specimen for classifying high versus low exposure across pregnancy (a.k.a., "surrogate analyses") and, to the best of our knowledge, there are no published reports in pregnant or non-pregnant adults assessing 2,4-dichlorophenol or 2,5-dichlorophenol. In our study population, the unadjusted specificity and percent correctly classified for these two phenols were relatively high (for 2,4-dichlorophenol: 0.75-0.92 and 63%-84% and for 2,5-dichlorophenol: 0.75-1.0 and 63-95%, respectively) thereby providing a point of comparison for future studies in other populations. In contrast to other phenols examined, likely exposure sources for 2,4-dichlorophenol and 2,5-dichlorophenol or their precursors are not predominantly food or personal care products but rather from herbicides (2,4-dichlorophenol), byproducts of chlorinated chemical manufacture (2,4-dichlorophenol), or consumer goods such as mothballs and toilet bowl deodorants (2,5-dichlorophenol)[45]. These specific exposure risk factors may impact the reproducibility and predictive value of urine 2,4-dichlorophenol and 2,5-dichlorophenol as biomarkers across populations.

To the best of our knowledge, there are only four published reports of surrogate analyses in pregnant women on the other biomarkers we studied. These include women attending a

fertility clinic in Massachusetts[12, 49], a pregnancy cohort in New York City[24], and a study of personal-care product use in pregnant women in Ottawa, Canada[35]. Depending on the study, high versus low exposures for surrogate analyses have been variously defined either based on quantiles of the observed urinary concentrations or on reference population data (e.g., National Health and Nutrition Examination Survey)[24]. For BPA, M-paraben, P-paraben, B-paraben, MEP and individual DEHP metabolites or their sum, previously reported sensitivities and specificities among pregnant women are similar to ours[12, 24, 35, 49]. For example, in our analysis the (unadjusted) sensitivity and specificity for BPA were between 0.57 and 0.71 and 0.83 and 0.92, respectively, which is comparable to other populations of pregnant women (sensitivity range: 0.60-0.70; specificity range: 0.66-0.85)[12, 35]. Similarly, in our study, the range of sensitivity values for MEP (0.67-1.0) and P-paraben (0.57-0.71) were similar to previous studies (MEP: 0.62-0.81; P-paraben: 0.63-0.73), while specificity values (MEP: 0.85-1.0; P-paraben: 0.83-0.92) were on the higher end of previous reports (MEP 0.43-0.90; P-paraben: 0.80-0.86)[12, 24, 35].

In our surrogate analysis we used the mean of 6 urinary concentrations as the ‘gold standard’ measure, assuming it represented exposure over the entire pregnancy; however, it is possible there was variability in exposures over pregnancy that may not have been captured even with 6 samples. Additionally, when assessing how well a single urinary concentration predicted overall pregnancy concentrations (using the 6 samples), the single urinary concentration was included in the mean concentration of the 6 samples. We chose this approach to optimize our ability to characterize exposure across pregnancy. However, this approach also means the single urinary concentration is not completely independent of the “gold standard.” Moreover, with a modest sample size of 19, only one woman with discordant high/low exposure ranking based on the single compared to the mean of six urine samples, could have a substantial impact on our calculated sensitivity and specificity. For example, seven women had BPA concentrations in the highest tertile when using the mean of six samples (the ‘gold standard’), among these seven women, four spot urinary concentrations at 16-18 weeks were correctly classified as ‘high’ and three were incorrectly classified as ‘low/medium’, resulting in a sensitivity of 57%. If one woman moved from the ‘high’ to the ‘low/medium’ group, the sensitivity would be reduced to 43%. Thus our findings are limited by sample size and the resultant sensitivity to changes in ranking for individual observations. That said, our findings are remarkably consistent with the existing literature and, in general, the range of possible values did not alter our conclusion that adjustment for variability in individual behavior over time did not substantially improve sensitivity or specificity.

In summary, we optimized our ability to assess long-term exposure over pregnancy with multiple (n=6) timed urine collections in a relatively homogeneous population of women. Collection of detailed diaries at each time period allowed us to assess and adjust for potential sources of variability related to time-varying behaviors (e.g., hours slept, last void). Despite these design strengths, adjustment for variability (unrelated to exposure) had minimal impact on the reproducibility, sensitivity, specificity, or percent correct classification of urinary concentrations of phenol or phthalate metabolites during pregnancy. Thus, despite using a demographically homogeneous study population, this approach does not appear to enhance the utility of a single urine measure for assessing exposure in pregnancy. Certain urinary biomarkers had higher ICCs (e.g., MEP), suggesting they may be

more reliably measured with just one sample than other biomarkers. Future studies need to carefully consider the exposure of interest and whether it is appropriate to use biomarker concentrations from a single spot urine sample to represent exposures over pregnancy given the exposure timeframe and the reproducibility of the biomarker.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

BPA	bisphenol A
B-PB	Butyl paraben
CDC	Centers for Disease Control and Prevention
EDC	endocrine disrupting chemical
DEHP	di-2-ethylhexyl phthalate
DEP	diethyl phthalate
ICC	intraclass correlation coefficient
MEP	monoethyl phthalate
M-PB	methyl paraben
P-PB	Propyl paraben

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Table 1

Characteristics of I-KIDS mothers who provided six pregnancy urine samples (n=19) compared to the full cohort participating in the I-KIDS formative study (n=149)

Maternal Characteristics	Full Cohort (n=149)	Subgroup Analysis (n=19)	P-value*
Age (yrs); mean ± SD and n(%)	29.5 ± 4.1	29.5 ± 3.5	0.99
<20	3 (2.0)	0 (0.0)	
20-24	15 (10.1)	1 (5.3)	
25-29	59 (39.6)	7 (36.8)	
30-34	55 (36.9)	11 (57.9)	
≥35	17 (11.4)	0 (0.0)	
Race/ethnicity; n(%)			
Non-Hispanic White	125 (83.9)	17 (89.5)	0.53
Non-Hispanic Black	8 (5.4)	1 (5.3)	
Hispanic	3 (2.0)	1 (5.3)	
Asian	7 (4.7)	0 (0.0)	
Multiracial or other race/ethnicity	6 (4.0)	0 (0.0)	
Education; n(%)			
≤ 12 years	6 (4.0)	0 (0.0)	0.82
Some college	35 (23.5)	5 (26.3)	
College graduate	108 (72.5)	14 (73.7)	
Income; n(%)			
< $30,000	24 (16.1)	4 (21.1)	0.72
$30,000-$59,999	33 (22.2)	4 (21.1)	
$60,000-$89,999	57 (38.3)	7 (36.8)	
≥ $90,000	32 (21.5)	4 (21.1)	
Missing	3 (2.0)	0 (0.0)	
Marital status; n(%)			
Married	127 (85.2)	18 (94.7)	0.41
Co-habitating as married	14 (9.4)	1 (5.3)	
Single	8 (5.4)	0 (0.0)	
Number of previous live born children; n(%)			
0	54 (36.2)	6 (31.6)	0.50
1	58 (38.9)	7 (36.8)	
≥ 2	37 (24.8)	6 (31.6)	
Smoking during pregnancy; n(%)			
Yes	5 (3.4)	1 (5.3)	0.62
No	144 (96.6)	18 (94.7)	
Pre-pregnancy BMI (kg/m^2); n(%)			
Underweight (<18.5)	1 (0.7)	0 (0.0)	0.10
Normal (18.5-24.9)	93 (62.4)	15 (79.0)	
Overweight (25-29.9)	30 (20.1)	4 (21.1)	
Obese (≥30)	23 (15.4)	0 (0.0)	

Maternal Characteristics	Full Cohort (n=149)	Subgroup Analysis (n=19)	P-value*
Missing	2 (1.3)	0 (0.0)	
Gestational age at birth (wks); mean ± SD	39.3 ± 1.0	39.5 ± 1.3	0.58

Abbreviations: BMI, body mass index; SD, standard deviations

* p-value comparing n=19 in this analysis to the n=130 excluded from this analysis. The Wilcoxon-Mann-Whitney test was used for ordinal variables and chi-square test for the other categorical variables.

Table 2

Median (range) of pregnancy urinary concentrations of phenols and phthalate metabolites among I-KIDS participants providing 6 pregnancy urines (n=19 women)[1]

Biomarker [LOD]	Pregnancy urine collection time in I-KIDS													Previous literature in pregnant women (range of median values, unadjusted for urine dilution)
	16-18 wks		20-22 wks		23-26 wks		27-30 wks		32-33 wks		35-36 wks		Total[3] Median (range)	
	N <LOD[2]/NR	Median (range)	N <LOD[2]/NR	Median (range)	N <LOD[2]/NR	Median (range)	N <LOD[2]/NR	Median (range)	N <LOD[2]/NR	Median (range)	N <LOD[2]/NR	Median (range)		
Bisphenol A (µg/L) [0.4]	1 / 0	1.3 (0.2-6.4)	1 / 0	1.2 (0.3-7.0)	1 / 0	1.3 (0.4-4.4)	0 / 0	1.6 (0.6-8.4)	0 / 0	1.2 (0.4-8.6)	1 / 0	1.4 (0.2-6.8)	1.5 (0.7-4.8)	0.6 - 2.7[10, 11, 29, 31-33, 35, 36, 44]
2,4-Dichlorophenol (µg/L) [0.2]	0 / 0	0.9 (0.5-6.0)	0 / 0	0.8 (0.4-2.9)	0 / 0	0.9 (0.4-3.1)	0 / 0	0.7 (0.4-2.4)	0 / 0	0.7 (0.2-2.5)	1 / 0	0.8 (0.2-2.8)	0.9 (0.6-3.1)	0.5 - 2.1[10, 11, 29-33]
2,5-Dichlorophenol (µg/L) [0.2]	0 / 0	0.7 (0.3-5.6)	0 / 0	1.1 (0.3-3.1)	0 / 0	1.6 (0.2-6.6)	0 / 0	1.3 (0.4-10.8)	0 / 0	1.4 (0.5-10.7)	1 / 0	1.4 (0.2-6.9)	1.5 (0.6-4.0)	2.7 – 53[10,11, 29-33]
Benzophenone-3 (µg/L) [0.4]	0 / 1	80.1 (3.0-809)	0 / 1	49.0 (4.0-849)	0 / 3	46.3 (1.6-861)	0 / 3	30.8 (7.3-995)	0 / 5	72.9 (4.1-883)	0 / 3	102.0 (4.9-904)	91.5 (19.1-761.5)	1.7 - 77[10, 11, 29, 31-33, 36]
Butyl Paraben (µg/L) [0.2]	10 / 0	0.2 (0-22.6)	8 / 0	0.3 (0 -3.1)	9 / 0	0.2 (0 -24.4)	10 / 0	0.1 (0-15.6)	12 / 0	0.1 (0-170)	12 / 0	0.1 (0.001-5.8)	0.4 (0-39.4)	0.4 -2.4[10, 11, 29, 32]
Methyl Paraben (µg/L) [1.0]	0 / 1	108.5 (7.4-566)	0 / 0	65.1 (7.5-233)	0 / 0	85.0 (3.5-355)	0 / 0	83.2 (8.7-880)	0 / 1	129.5 (10.4-833)	0 / 0	76.6 (2.2-445)	111.1 (14.8-317.9)	84.7 - 272[10, 11, 29, 31, 32]
Propyl Paraben (µg/L) [0.2]	0 / 0	16.6 (0.3-279)	0 / 0	16.3 (0.8-115)	0 / 0	21.8 (0.3-84.3)	1 / 0	12.0 (0.1-97.1)	0 / 0	24.3 (0.5-849)	0 / 0	6.2 (0.4-124)	31.0 (0.4-212.4)	12.5 - 45.6[10, 11, 29, 31, 32]
Triclosan (µg/L) [2.3]	7 / 0	5.3 (0-309)	10 / 0	0.9 (0 -178)	4 / 0	6.1 (0-161)	3 / 0	4.6 (1.1-286)	3 / 11	19.5 (0.1-124)	3 / 0	9.4 (1.2-187)	14.8 (1.1-202.7)	<LOD - 26.2[10, 11, 29, 31-34, 36, 44]
Monoethyl phthalate (µg/L) [0.6]	0 / 0	31.5 (5.7-709)	0 / 0	26.6 (2.7-870)	0 / 0	21.2 (4.9-349)	0 / 0	32.3 (8.4-258)	0 / 0	23.8 (3.1-202)	0 / 0	30.8 (5.2-103)	33.2 (5.8-356.3)	21.5 - 380[24, 29, 32, 33, 35-42]
ΣDEHP metabolites (µmol/L)[4]	3*/ 0	0.1 (0.02-4.2)	4*/ 0	0.1 (0.02-1.3)	4*/ 0	0.1 (0.01-6.3)	2*/ 0	0.1 (0.03-0.3)	0 / 0	0.1 (0.03-0.9)	7*/ 0	0.1 (0.02-1.1)	0.1 (0.03-1.8)	0.072 - 0.31**[33, 38, 40, 43]

Abbreviations: LOD, limit of detection; NR, no result; ΣDEHP metabolites, sum of metabolites of di(2-ethylhexyl) phthalate

[1] Values are not adjusted for urinary dilution (e.g., they are not adjusted for specific gravity).

[2] Measured concentrations <LOD for which a signal was detected, as well as those for which no signal was detected (i.e., concentration = 0), are reported in summary statistics

[3] Total = the mean biomarker concentration for each woman's 6 urine samples

[4] LODs (in µg/L) for the 4 DEHP metabolites were 0.5 for mono-2-ethylhexyl phthalate (MEHP), and 0.2 for mono-2-ethyl-5-carboxypentyl phthalate, mono-2-ethyl-5-hydroxyhe phthalate, and mono-2-ethyl-5-oxohexyl phthalate.

* For ΣDEHP, the detection frequency of all metabolites, except MEHP, was 100%. Where MEHP was <LOD, ΣDEHP was assigned as <LOD.

** Includes mean in addition to median values

Table 3

Univariate determinants of urinary concentrations of phenols and phthalate metabolites (SG adjusted) during pregnancy, % change (95% CI)

	Bisphenol A (n=114)	2,4-dichlorophenol (n=114)	2,5-dichlorophenol (n=114)	Butyl Paraben (n=114)	Methyl Paraben (n=112)	Propyl Paraben (n=114)	Monoethyl phthalate (n=114)	ΣDEHP metabolites (n=114)
Determinants	**% change (95% CI)**	**% change (95% CI)**	**% change (95% CI)**	**% change (95% CI)**	**% change (95% CI)**	**% change (95% CI)**	**% change (95% CI)**	**% change (95% CI)**
Gestational age (wks)	1.3 (−0.3, 2.9)	−2.5 (−4.4, −0.7)	3.0 (0.7, 5.4)	−5.2 (−13.4, 3.0)	0.1 (−2.3, 2.5)	−2.4 (−6.3, 1.5)	−0.6 (−2.9, 1.7)	0.5 (−1.8, 2.7)
Weight gain (kg)	2.5 (0.4, 4.7)	−1.6 (−3.9, 0.7)	2.0 (−1.2, 5.2)	0.0 (−11.1, 11.1)	0.8 (−3.0, 4.5)	−0.7 (−6.8, 5.3)	1.1 (−2.0, 4.2)	−0.1 (−2.9, 2.7)
Time since awoke (hrs)	−5.6 (−29.8, 18.6)	−25.2 (−40.3, −10.0)	−18.0 (−41.4, 5.5)	−38.6 (−112.8, 35.5)	−7.2 (−37.7, 23.4)	0.7 (−46.8, 48.1)	−0.2 (−16.4, 16.0)	13.1 (−11.8, 38.0)
Time slept (hrs)	8.9 (−2.1, 19.8)	13.6 (5.7, 21.5)	6.8 (−4.4, 18.0)	7.4 (−29.1, 43.9)	−2.9 (−18.4, 12.6)	−6.7 (−28.2,14.7)	−0.8 (−11.5, 10.0)	−3.3 (−13.6, 7.0)
Time since last food/drink (hrs)	2.8 (−0.5, 6.0)	−1.0 (−3.4, 1.4)	0.8 (−2.2, 3.7)	−6.7 (−17.1, 3.6)	−2.4 (−7.2, 2.4)	−4.5 (−10.7, 1.6)	0.2 (−3.0, 3.3)	1.4 (−1.6, 4.4)
Time since last void (hrs)	8.6 (4.0, 13.2)	3.6 (0.3, 6.9)	4.1 (−0.4, 8.6)	0.0 (−15.2, 15.2)	3.8 (−2.7, 10.2)	8.5 (−0.5, 17.6)	2.9 (−1.8, 7.5)	4.9 (0.4, 9.5)

Abbreviations: ΣDEHP metabolites, sum of metabolites of di(2-ethylhexyl) phthalate

Maximum number of observations is 114 (19 women × 6 samples each)

Table 4

Intraclass correlation coefficients for repeat urinary concentrations of phenols and phthalate metabolites (SG adjusted) before and after adjustment for potential sources of variability unrelated to exposure pathways

Biomarker	Unadjusted ICC (95% CI)	Adjusted[1] ICC (95% CI)	Previous literature in pregnant women
			ICCs adjusted for urinary concentration[2]
Bisphenol A	0.24 (0.10, 0.49)	0.20 (0.06, 0.47)	0.04 – 0.31 [4, 10-13, 26, 47, 48]
2,4-dichlorophenol	0.43 (0.24, 0.64)	0.46 (0.25, 0.68)	0.38 – 0.60 [10, 11]
2,5-dichlorophenol	0.34 (0.16, 0.57)	0.39 (0.20, 0.62)	0.49 – 0.61 [10, 11]
Butyl Paraben	0.56 (0.36, 0.74)	0.59 (0.38, 0.76)	0.38 – 0.56 [10, 11, 13, 49]
Methyl Paraben	0.44 (0.25, 0.65)	0.42 (0.22, 0.64)	0.24 – 0.61 [10, 11, 13, 49]
Propyl Paraben	0.56 (0.36, 0.74)	0.54 (0.34, 0.73)	0.32 – 0.62 [10, 11, 13, 49]
Monoethyl phthalate	0.68 (0.50, 0.82)	0.66 (0.46, 0.81)	0.21 – 0.50 [12, 24-27]
ΣDEHP metabolites	0.32 (0.15, 0.55)	0.27 (0.10, 0.57)	0.08 – 0.31 [12, 24-27]

Abbreviations: ΣDEHP metabolites, sum of metabolites of di(2-ethylhexyl) phthalate; ICC, intraclass correlation coefficient

[1]Adjusted for gestational age, weight gain, time since awoke, time slept, time since last food/drink, and time since last void.

[2]When ICCs for ΣDEHP metabolites were not available, the mean ICC for the four individual metabolites was reported

Table 5

Sensitivity, specificity, and percent correct classified (as high vs. low average exposure across pregnancy[1]) using biomarker concentrations in a single pregnancy urine

		16-18 wks		20-22 wks		23-26 wks		27-30 wks		32-33 wks		35-36 wks	
		Unadjusted	Adjusted[2]	Unadjusted	Adjusted[2]	Unadjusted	Adjusted[2]	Unadjusted	Adjusted[2]	Unadjusted	Adjusted[2]	Unadjusted	Adjusted[2]
Bisphenol A (μg/L)	**% Crct**	74%	58%-74%	74%	63%-74%	74%	58%-79%	74%	79%-89%	84%	89%-100%	74%	58%-74%
	Sens/Spec	0.57/0.83	0.33-0.57/0.69-0.83	0.57/0.83	0.43-0.57/0.75-0.83	0.57/0.83	0.33-0.67/0.69-0.85	0.57/0.83	0.67-0.83/0.85-0.92	0.71/0.92	0.83-1.0/0.92-1.0	0.57/0.83	0.33-0.57/0.69-0.83
2 4-Dichlorophenol (μg/L)	**% Crct**	84%	84%-89%	84%	63%-79%	84%	79%-100%	84%	89%-95%	63%	68%-84%	74%	63%-84%
	Sens/Spec	0.71/0.92	0.71-0.83/0.92-0.92	0.71/0.92	0.43-0.67/0.75-0.85	0.71/0.92	0.67-1.0/0.85-1.0	0.71/0.92	0.83-0.86/0.92-1.0	0.43/0.75	0.50-0.71/0.77-0.92	0.57/0.83	0.43-0.71/0.75-0.92
2 5-Dichlorophenol (μg/L)	**% Crct**	84%	68%-89%	84%	89%-100%	95%	79%-95%	84%	74%-89%	63%	58%-79%	74%	68%-79%
	Sens/Spec	0.71/0.92	0.50-0.83/0.77-0.92	0.71/0.92	0.83-1.0/0.92-1.0	0.86/1.00	0.67-0.86/0.85-1.0	0.71/0.92	0.57-0.83/0.83-0.92	0.43/0.75	0.33-0.67/0.69-0.85	0.57/0.83	0.50-0.67/0.77-0.85
Butyl Paraben (μg/L)	**% Crct**	74%	58%-74%	84%	63%-89%	74%	63%-89%	84%	68%-84%	84%	79%-100%	84%	79%-100%
	Sens/Spec	0.57/0.83	0.33-0.57/0.69-0.83	0.71/0.92	0.43-0.83/0.75-0.92	0.57/0.83	0.43-0.83/0.75-0.92	0.71/0.92	0.50-0.71/0.77-0.92	0.71/0.92	0.67-1.0/0.85-1.0	0.71/0.92	0.67-1.0/0.85-1.0
Methyl Paraben (μg/L)	**% Crct**	83%	61%-83%	79%	74%-89%	79%	68%-84%	79%	74%-89%	56%	44%-56%	89%	68%-84%
	Sens/Spec	0.67/0.92	0.33-0.67/0.75-0.92	0.67/0.85	0.57-0.83/0.83-0.92	0.67/0.85	0.50-0.71/0.77-0.92	0.67/0.85	0.57-0.83/0.83-0.92	0.20/0.69	0.00-0.29/0.62-0.73	0.83/0.92	0.50-0.71/0.77-0.92
Propyl Paraben (μg/L)	**% Crct**	84%	63%-79%	84%	79%-95%	74%	68%-89%	74%	74%-95%	74%	58%-79%	74%	68%-89%
	Sens/Spec	0.71/0.92	0.43-0.67/0.75-0.85	0.71/0.92	0.67-0.86/0.85-1.0	0.57/0.83	0.50-0.83/0.77-0.92	0.57/0.83	0.57-0.86/0.83-1.0	0.57/0.83	0.33-0.67/0.69-0.85	0.57/0.83	0.50-0.83/0.77-0.92
Monoethyl phthalate (μg/L)	**% Crct**	79%	68%-79%	79%	74%-79%	100%	89%-100%	89%	84%-95%	89%	74%-95%	89%	79%-95%
	Sens/Spec	0.67/0.85	0.50-0.67/0.77-0.85	0.67/0.85	0.57-0.67/0.83-0.85	1.00/1.00	0.83-1.0/0.92-1.0	0.83/0.92	0.71-0.86/0.92-1.0	0.83/0.92	0.57-0.86/0.83-1.0	0.83/0.92	0.67-0.86/0.85-1.0
ΣDEHP metabolites (μmol/L)	**% Crct**	79%	63%-79%	79%	68%-74%	89%	79%-89%	79%	58%-74%	79%	68%-74%	79%	63%-89%
	Sens/Spec	0.67/0.85	0.43-0.67/0.75-0.85	0.67/0.85	0.50-0.57/0.77-0.83	0.83/0.92	0.67-0.83/0.85-0.92	0.67/0.85	0.33-0.57/0.69-0.83	0.67/0.85	0.50-0.57/0.77-0.83	0.67/0.85	0.43-0.83/0.75-0.92

Abbreviations: ΣDEHP metabolites: sum of metabolites of di(2-ethylhexyl) phthalate; % Crct: percent correctly classified; Sens: sensitivity; Spec: Specificity

[1]Estimated using the mean of the residuals for the 6 urines collected across pregnancy.

[2]Adjusted analyses use imputed variables and results presented are the range of values across the 10 imputed datasets. Estimates are adjusted for gestational age, weight gain, time since awoke, time slept, time since last food/drink, and time since last void.