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Authors
Liang, Hai-Wei
Snyder, Nathaniel
Wang, Jiebiao
et al.

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A study on the association of placental and maternal urinary phthalate metabolites

Hai-Wei Liang[1], **Nathaniel Snyder**[2], **Jiebiao Wang**[3], **Xiaoshuang Xun**[1], **Qing Yin**[3], **Kaja LeWinn**[4], **Kecia N. Carroll**[5], **Nicole R. Bush**[4,6], **Kurunthachalam Kannan**[7], **Emily S. Barrett**[8], **Rod T. Mitchell**[9], **Fran Tylavsky**[10], **Jennifer J. Adibi**[1,11,✉]

[1]Department of Epidemiology, University of Pittsburgh School of Public Health, Pittsburgh, PA, USA

[2]Center for Metabolic Disease Research, Lewis Katz School of Medicine at Temple University, Philadelphia, PA, USA

[3]Department of Biostatistics, University of Pittsburgh School of Public Health, Pittsburgh, PA, USA

[4]Department of Psychiatry and Behavioral Sciences, University of California San Francisco, San Francisco, CA, USA

[5]Department of Pediatrics, Icahn School of Medicine at Mount Sinai, New York, NY, USA

[6]Department of Pediatrics, University of California San Francisco, San Francisco, CA, USA

[7]Department of Environmental Medicine, New York University Grossman School of Medicine, New York, NY, USA

[8]Department of Biostatistics and Epidemiology, Rutgers School of Public Health, Piscataway, NJ, USA

[9]MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, Scotland, UK

[10]Department of Preventive Medicine, University of Tennessee Health Science Center, Memphis, TN, USA

✉**Correspondence** and requests for materials should be addressed to Jennifer J. Adibi. adibij@pitt.edu.

AUTHOR CONTRIBUTIONS

HWL carried out the data analysis and helped to write the manuscript. NS developed the method for the analysis of placental tissue phthalates, and helped to write the manuscript. JW contributed the quantile normalization method to compare phthalate levels in the two tissue types, and reviewed the manuscript. XX and QY contributed R code and contributed to the data analysis for this manuscript. KLW, KC, NRB and FT were all part of the original conception of the project, and KLW served as a liaison between the Adibi and CANDLE research groups. KLW, NRB, and KC contributed key perspectives on reporting race differences in these associations. KK analyzed the urinary phthalate metabolites and contributed to the manuscript. EB and RTM offered edits and comments on the manuscript. FT is the original PI of the CANDLE study. JJA conceived of and initiated this research, mentored HWL, XX, and QY, and wrote the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL

Research was performed in accordance with the Declaration of Helsinki and approved by the Institutional Review Boards at the University of Tennessee Health Science Center and the University of Pittsburgh. The University of Pittsburgh declared this research to be exempt as Pitt investigators had access to de-identified data, and no contact with research participants.

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[11]Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, PA, USA

Abstract

BACKGROUND: Phthalate exposure in pregnancy is typically estimated using maternal urinary phthalate metabolite levels. Our aim was to evaluate the association of urinary and placental tissue phthalates, and to explore the role of maternal and pregnancy characteristics that may bias estimates.

METHODS: Fifty pregnancies were selected from the CANDLE Study, recruited from 2006 to 2011 in Tennessee. Linear models were used to estimate associations of urinary phthalates (2^{nd}, 3^{rd} trimesters) and placental tissue phthalates (birth). Potential confounders and modifiers were evaluated in categories: temporality (time between urine and placenta sample), fetal sex, demographics, social advantage, reproductive history, medication use, nutrition and adiposity. Molar and quantile normalized phthalates were calculated to facilitate comparison of placental and urinary levels.

RESULTS: Metabolites detectable in >80% of both urine and placental samples were MEP, MnBP, MBzP, MECPP, MEOHP, MEHHP, and MEHP. MEP was most abundant in urine (geometric mean [GM] 7.00 $\times 10^2$ nmol/l) and in placental tissue (GM 2.56 $\times 10^4$ nmol/l). MEHP was the least abundant in urine (GM 5.32 $\times 10^1$ nmol/l) and second most abundant in placental tissue (2.04 $\times 10^4$ nmol/l). In aggregate, MEHP differed the most between urine and placenta (2.21 log units), and MEHHP differed the least (0.07 log units). MECPP was positively associated between urine and placenta (regression coefficient: 0.31 95% CI 0.09, 0.53). Other urine-placenta metabolite associations were modified by measures of social advantage, reproductive history, medication use, and adiposity.

CONCLUSION: Phthalates were ubiquitous in 50 full-term placental samples, as has already been shown in maternal urine. MEP and MEHP were the most abundant. Measurement and comparison of urinary and placental phthalates can advance knowledge on phthalate toxicity in pregnancy and provide insight into the validity and accuracy of relying on maternal urinary concentrations to estimate placental exposures.

IMPACT STATEMENT: This is the first report of correlations/associations of urinary and placental tissue phthalates in human pregnancy. Epidemiologists have relied exclusively on maternal urinary phthalate metabolite concentrations to assess exposures in pregnant women and risk to their fetuses. Even though it has not yet been confirmed empirically, it is widely assumed that urinary concentrations are strongly and positively correlated with placental and fetal levels. Our data suggest that may not be the case, and these associations may vary by phthalate metabolite and associations may be modified by measures of social advantage, reproductive history, medication use, and adiposity.

Keywords

Endocrine Disruptors; Epidemiology; Exposure Modeling; Phthalates; Vulnerable Populations

INTRODUCTION

Pregnant women in the U.S. experience widespread exposure to phthalates, a class of endocrine-disrupting chemicals [1]. Exposure primarily occurs through diet [2, 3], but may also occur by way of inhalation [4, 5], and dermal exposure [6–8]. Phthalates have been associated with changes in inflammatory [9, 10], steroidogenic [11, 12], immune [13], adipogenesis [14–16] and oxidative stress [17, 18] pathways. Outcomes associated with prenatal phthalate exposure include: duration of labor, [19–21] altered reproductive tract development, [22–24] body composition changes in the children, [25–27] asthma, [28–31] and altered child brain development. [32–35] Aside from the mother and the fetus as a target of phthalate exposure, the placenta itself is a site of phthalate toxicity which can indirectly affect child and maternal health outcomes [36–39].

Conventionally, phthalate exposure in pregnancy is determined by measuring phthalate metabolites in maternal urine. [40, 41] Urinary metabolites are the measure of choice given their high concentrations, longer half-life as compared to blood and low potential for contamination by the phthalate diester [40–42]. They are desirable for ease of collection and acceptability to participants.

Phthalate metabolites have been detected in multiple compartments within the maternal-placental-fetal unit including maternal urine [4], amniotic fluid [43], placental tissue [44], umbilical cord blood [45] and meconium [46]. The degree to which urinary metabolites are representative of concentrations within the placental-fetal unit (i.e., target tissue) is not well-studied. There are few studies where prenatal phthalates were measured in two biologic matrices simultaneously [44, 47, 48].

It is generally accepted that phthalates can either actively or passively be transported from maternal circulation, into the intervillous space, and through the trophoblast layer of the placenta and into fetal circulation [44, 49], however the actual mechanism of transport has not been established. In the absence of this type of knowledge, correlations of matched phthalate concentrations in multiple matrices can improve understanding.

The aim was to quantitate phthalate concentrations in placental tissue matched to maternal urine, and to measure their associations. Because this is a previously unstudied relationship that likely involves complex pharmacokinetics and pregnancy-specific physiology, another aim was to gain insight by exploratory analyses of effect modification by measures of social advantage, self-reported race, reproductive history, medication use, and adiposity. This can generate hypotheses on mechanisms and variables to be studied in larger, well-powered studies. A final goal was to compare phthalate distributions in the two tissue types in terms of relative ranking and difference.

METHODS

Study subjects

A pilot sample of 50 pregnancies was selected from the longitudinal CANDLE Study (Conditions Affecting Neurocognitive Development and Learning in Early childhood)

pregnancy cohort. The CANDLE study at the University of Tennessee Health Science Center was designed to examine factors in the pregnancy and early childhood environment that influence child neurodevelopmental outcomes and was extended to include a broad range of child health outcomes (e.g., cardiometabolic and airway health). Pregnant women were recruited from Shelby County/Memphis, TN, from 2006 to 2011 during the second trimester of pregnancy. Primary recruitment sites included an urban hospital obstetric clinic and community obstetric practices. Inclusion criteria were: planning to deliver at one of five hospitals in Shelby County; maternal age 16–40 years; residence in Shelby County; having a low medical risk pregnancy, singleton pregnancy; and being able to speak and understand English. A total of 1503 pregnant mothers were enrolled, and mother-child dyads were followed up at regular intervals with between 912 and 1157 participants attending each follow-up visit [50–52]. The institutional review boards (IRB) at the University of Tennessee Health Sciences Center (UTHSC) and the University of Pittsburgh approved the study. All subjects provided informed written consent before enrolling. Prenatal maternal urine samples were collected at two visits roughly corresponding to the second (16–29 weeks) and third trimesters (22–39 weeks). Placental villous tissue was biopsied at birth and stored in RNALater (Qiagen). From the full cohort of 1457 women, for this pilot study, 50 pregnant women were chosen based on an equal proportion of male and female babies, full-term birth (>37 weeks), fetal birth weight ≥2500 g, and an oversampling of child asthma cases to address priorities of another study using the same samples.

Phthalate analysis, urine and placenta

The phthalate metabolites, their parent compounds, their abbreviations, full names, and molecular weights [53, 54] are provided in Supplementary Table 1. Methods for the analysis of placental and urinary phthalates are provided in the Supplement. Urine and placenta samples were analyzed in different labs. Placental tissue concentrations were converted to molar concentrations by dimension analysis: ng/mg to ng/ml to moles/liter. Placental tissue and urine were reported to be roughly the same density (~1.018 g/ml) [55].

Covariates

We used available questionnaire and medical record data to screen for possible categories of confounding and effect modification of pregnancy pharmacokinetics [56, 57]. The selection of categories of variables is based on substantive knowledge of factors that are predictive of placental function, and which may also influence phthalate exposure and metabolism: temporality (time between urine and placenta sample), biology (fetal sex), demographics (age), social advantage (income, marital status, insurance status, education), self-reported maternal race, reproductive history (previous spontaneous abortion, parity, previous term or preterm pregnancy, previous induced labor), substance use (alcohol, tobacco), medication use by way of drug-drug interactions (analgesic, antacid, nausea medication) [58], nutrition (vitamin supplementation), inflammation (asthma ever), adiposity (body mass index or BMI) and infection (abnormal vaginal discharge, herpes simplex virus, human papilloma virus). Variables were coded as: maternal race (Black, White), fetal sex, education (<high school, high school or general education development test [GED], >high school), income (<$24999, $25000–54999, >$55000), companion status (partnered, unpartnered), and parity (1, 2, ≥3). The following variables were coded as yes/no: public insurance, alcohol in pregnancy,

tobacco in pregnancy, previous term pregnancy, previous preterm pregnancy, previous spontaneous abortion, asthma-current, asthma-ever, analgesic use in pregnancy, anti-acid in pregnancy, nausea medication in pregnancy, vitamin supplementation in pregnancy, allergy medication in pregnancy, vaginal discharge, Herpes simplex virus (HSV) in pregnancy, and HSV ever. We assume that the medications (analgesics, anti-acids, allergy) used were over the counter vs. prescription given that women with chronic conditions that required medication at baseline were excluded from the study. Screening was conducted as univariate analyses. A narrow set of variables that met a *p*-value cut-off of 0.2 were identified and applied to the analysis of the urine-placenta association.

Statistical analysis

We analyzed seven phthalate metabolites (MEP, MnBP, MBzP, MECPP, MEOHP, MEHHP, MEHP) measured in paired urine and placental samples. Samples below the limit of detection (LOD) were imputed as the LOD divided by the square root of two. To adjust for urinary dilution at the time of the sample, individual phthalate metabolite levels were adjusted by the formula: P (observed phthalate concentration (μg/L) × [(mean specific gravity – 1)/(specific gravity – 1)] [59]. Phthalate metabolite levels were natural log transformed to normalize their distributions for use in regression models.

Spearman's rank correlations were used to determine the correlation of specific gravity adjusted urinary phthalate metabolites at each time point and placental phthalate metabolites. To screen for potential confounders and effect modifiers (see Covariate section above), Pearson's correlation was used for continuous variables and a t-test/ANOVA was used for categorical variables. An α cut-off of 0.20 was applied to identify candidate covariates correlated with phthalate levels in both matrices. Linear regression models were fit to calculate urine-placental phthalate associations, adjusted for covariates. A minimal set of covariates (maternal age, BMI, race, fetal sex, asthma, education) were forced into all models. Models were fit separately for second and third trimester urinary phthalates. All variables identified in the screen as associated with urinary and placental phthalate levels (p-value ≤ 0.2) were considered as potential confounders and effect modifiers. Effect modification was evaluated by including a product term of the urinary phthalate × covariate. A linear contrast statement was used to calculate the stratum specific beta coefficients, confidence intervals, and p-values. Data were not stratified due to the small sample size.

To evaluate concordance in the phthalate distributions in urine and in placental tissue, we used a quantile normalization approach [60, 61]. Quantile normalization is commonly used in microarray data analysis to make distributions identical in statistical properties, and to compare mean values. In this case, we compared phthalate metabolites in two tissue matrices (urine, placenta) with different methods of sample extraction and measurement, and differences in overall abundance given the complex pharmacokinetics of pregnancy. Each phthalate measure was first log-transformed and sorted from low to high. For each individual, a rank from 1–7 was assigned for the 7 metabolites in their urine (second or third trimester separately), and the 7 corresponding metabolites in their placentas. The arithmetic mean for the kth largest value (k = 1,…7) across the 50 subjects was calculated and assigned to each individual according to their 1–7 ranking. In the end, each individual had the same

7 values for urinary metabolites and the same 7 values for the placental metabolites, but in different orders according to the ranking of the original values of those metabolites in that individual. Using the normalized values, we calculated a mean difference between the urinary and placental concentrations for each metabolite. This analysis was done using the normalize.quantile function in the prepocessCore R package.

P-values ≤ 0.10 are highlighted. This cut-off was chosen given the small sample size and exploratory nature of this study. All statistical analyses were performed using R (Version 1.3.1093).

RESULTS

Subject characteristics were similar in our pilot study of 50 CANDLE participants compared to the overall CANDLE study, which has been reported previously [62]. In brief, the average age was 26.9 years (95% CI: 25.3, 28.6) and BMI was 28.5 (95% CI: 26.2, 30.8). About half had higher than high school education, 68% were Black, 76% had income less than $54,999, 66% were partnered, and half had 3 or more previous livebirths. Urine samples were collected at 23 weeks on average in the second trimester and 32 weeks in the third trimester. Placentas were sampled at 39 weeks on average (Table 1).

In placentas sampled at birth, phthalate detection rates were 92–100%, similar to those of the urinary phthalates (Table 2). Within the placental tissue, mono-2-ethylhexyl phthalate (MEHP) and monoethyl phthalate (MEP) were the highest and roughly the same in abundance (~5 ng/mg or 20–25 μmol/l). MnBP and MBzP concentrations in placental tissue were in a similar range (12–14 μmol/l); and metabolites of MEHP (MEOHP, MEHHP, MECPP) were similar and in the range of 6–8 μmol/l. Urinary concentrations were generally lower by 2 orders of magnitude as compared to placental tissue. Within the urine, phthalate concentrations were lower in the third vs. second trimester as has been reported previously in the full CANDLE cohort [63]. Quantile normalization facilitates comparison of the means between the tissue types in the sample overall (Table 3). As the difference between the urinary and placental normalized means becomes greater in either the positive or negative direction, there is a higher discordance between the two matrices in characterizing mean phthalate levels. At the aggregate level, MEHP was the most discordant (2.21 log units) and MEHHP was the least discordant (0.07 log units).

Correlations of MnBP, MBzP, and MECPP were positive and generally in the magnitude of 0.2–0.3 between the two matrices (third trimester urine, placenta at birth) (Supplemental Table 2). There was no correlation detected between phthalates in second trimester urine and placental tissue at birth (data not shown). The screen for potential confounders and effect modifiers was a post hoc analysis to generate hypotheses to provide insight into the lack of stronger or more consistent correlations of urine and placental phthalates.

Race differences were observed in MECPP (hydroxylated metabolite of MEHP) such that it was higher in the placentas of Black vs. White women (Table 4) but lower in the urine of Black vs. White women (data not shown). Women with a previous preterm labor had higher mono-n-butyl phthalate (MnBP) in the placenta and lower levels in the urine. This was also

true for a history of induced labor and placental and urinary MEP. A history of asthma was associated with higher MnBP and MEHP in the placenta and higher levels in the urine (data not shown). These are all factors previously studied in relation to urinary phthalates in pregnancy [52, 64–66].

After adjustment for confounders, associations of second trimester urine and placental tissue phthalates at birth were null (Supplementary Table 3) Third trimester urinary and placental tissue MECPP was associated (β: 0.31 95% CI 0.09, 0.53). MECPP was slightly higher in male vs. female placentas (Table 4). Placental phthalates were not associated with BMI, age, education or asthma in adjusted models.

Urine-placental tissue associations were modified by variables related to social advantage, maternal race, reproductive history, medication use, and adiposity (Table 5). Associations were not modified by time elapsed between urine and placental tissue collection, fetal sex, maternal age, parity, or vitamin supplementation. Of the phthalate metabolites, MECPP was the most consistently associated between urine and placental tissue across the variables examined (Table 5).

After the discovery of effect modification by self-reported race, we further explored by fitting models in the unstratified dataset and including a product term, urinary phthalate metabolite × maternal race, co-adjusted for confounding variables related to social conditions and other exposures (reproductive history, alcohol use, vitamin use, analgesic use, income, education, insurance, marital status, BMI, asthma) (12 models total, data not shown). Each model was adjusted for maternal age, fetal sex, and one additional variable from the list. The p-value for the product term urinary MEHP × race remained consistently below 0.07 (mean $p = 0.05$) and the mean beta coefficient for MEHP in White women was 0.15 (SD: 0.01), and null in Black women. From this analysis, we concluded that the finding was stable to adjustment for socioeconomic status. However, these variables (education, income, marital status) are not ‘causes’ of a person’s race and therefore are not operating here as confounders of this association [67, 68]. More work in a larger sample is necessary to identify the specific and manipulatable factors that are driving the observed difference in association by race.

Consistent with the MEHP finding, urinary and placental MEHHP and MEOHP were also positively associated in White women only. Urinary and placental MECPP was positively associated in all women.

In summary, levels of detectable phthalates in urine and placental tissue were positively associated among women self-identifying as White, with private insurance, and a history of spontaneous abortion (MBzP). Associations were negative in the case of women not taking analgesics (MEP), women with a BMI > 30 (MEP), and women with a history of spontaneous abortion (MEHP).

Correlations between identical and non-identical placental and urinary phthalates give a different perspective of correlation structure between molecules in the placenta tissue and in maternal urine (Supplementary Table 2). Placental MEP was the only placental monoester metabolite not correlated with third trimester urinary metabolites.

DISCUSSION

This was a first investigation in 50 paired urine-placental samples to examine the utility of placental tissue as an exposure matrix to assess phthalate metabolite levels. MECPP (a metabolite of MEHP) was broadly and positively associated between the two matrices. Associations of the other metabolites were null. Modifiers of the association reveal factors in the maternal environment that may impact phthalate transfer and/or phthalate pharmacokinetics in pregnancy. There was statistical evidence for effect modification of urine-placental associations by self-reported race (MEHP), private vs. public insurance (MEHHP), spontaneous abortion history (MEHP, MBzP), analgesic use (MEP, MEHP), and obesity (MEP).

MEHP was a relatively high abundant metabolite in placental tissue sampled at birth, but in relatively low abundance in maternal urine. MEHP was measured in micromolar concentrations in the placenta and in nanomolar concentrations in the urine. MEP was the most abundant in the two tissues. In aggregate, there was the highest level of discordance (i.e., potential measurement error) in MEHP and the least amount in MEHHP when using maternal urine to rank metabolites by abundance.

These results suggest a minimal to moderate level of transfer of phthalates from maternal blood, into the intervillous space, and across the trophoblast layer. Notably higher molar concentrations in the placental tissue vs. the urine suggest that there may be a process of passive accumulation of phthalates in the fetal placenta. The placenta is more proximal to the fetus than maternal urine, nevertheless, we cannot infer fetal exposure from these data.

MEHP is generally difficult to quantify in urine and has a lower detection frequency as compared to its more stable oxidative metabolites [69–71]. These data suggest that MEHP may be more stably detected in the placenta than in urine, either because it is more persistent in the placenta and/or has a longer half-life within the placental-fetal unit.

MEHP levels may also be high in the placental tissue due to the use of medical devices (i.e., intravenous tubing, bags containing saline solutions, other PVC products) during labor and delivery [72]. This could occur while the subject is still pregnant or during the handling of the placenta after delivery. It is likely that esterases in the placenta remain active after delivery, which means DEHP contamination during tissue processing and sampling could also contribute to high MEHP in the placenta specifically. This type of contamination would be systematic and bias associations towards the null. Placental tissue samples were treated with a reagent to stabilize total RNA at room temperature. To our knowledge, there are no phthalates in this reagent, but there may be compounds that interfered with the placental tissue phthalate analysis.

The gap in time between second and third trimester urine and placenta collection may account for the weak and null correlations. Phthalates are non-persistent with a half-life of 12–48 h in a non-pregnant body without a placenta [69]. For two reasons, we postulate that the associations of third trimester urinary phthalates reported here were meaningful even with the time gap. First, phthalates in the second trimester urine samples were not correlated with placental tissue levels whereas the third trimester urinary concentrations

were, indicating a temporal correspondence. Second, prior evidence suggests that urinary phthalate metabolite levels are moderately stable within women in the third trimester (intraclass correlation coefficients range from 0.3–0.6) [47, 73]. The levels we measured in the 3rd trimester may be reasonable proxies for concentrations at the time of placental collection. Additionally, phthalate pharmacokinetics in pregnancy are likely different as compared to a non-pregnant body, which could result in a longer half-life.

Potential effect modifiers explored in this study might reflect sources of variability in pharmacokinetics. The specific types of effect modification identified here are not robust due to small sample size and should not be interpreted as causal in nature. This is a road map for the types of information to be collected and considered in future, well-powered studies. This can include processes such as competition for receptors or co-regulation of xenobiotic metabolizing enzymes in the liver and/or the placenta [56, 57]. When urine and placenta are sampled at time points that are several weeks apart as was done here, sources of confounding and effect modification could also relate to longer-term sources of variability in exposure and in liver and placental function. Fetal sex was also suspected to be a modifier of this relationship as it has been demonstrated that some other xenobiotics preferentially accumulate in male placentas [57, 74, 75], however in this case, fetal sex did not modify associations.

The association of urinary and placental DEHP metabolites were positive in White women and generally null in Black women. The exception was MECPP which was positively associated in both White and Black women, and was 0.2 log units higher in the placentas of Black vs. White women. From these data, it is not possible to interpret if the positive or negative associations of urinary-placental phthalates reflect toxicity vs. resilience or adaptation of the body to more effectively excrete the phthalates. The authors acknowledge that race is a political and social construct, and not biological. For this reason, effect modification by maternal race reported here should be followed up in future studies in which birthing people report their experiences of stress and discrimination according to well-validated instruments. Effect modification seen here might be explained by group differences in diet [2, 76–79], personal care product use [80], and experiences of race and discrimination that are associated with placental function. Stress-related neuroendocrine pathways are active in the placenta [81] and some genes and proteins involved in glucocorticoid metabolism are also involved in xenobiotic metabolism [82, 83]. These may differ over a woman’s lifetime depending on her race/ethnicity, and they also might change during pregnancy.

Over the counter analgesic use in pregnancy could correlate with circulating levels of paracetamol, aspirin, or ibuprofen. Diethyl and di-n-butyl phthalate are intentionally used as inactive ingredients in analgesics for purposes of localized availability and timed release [58, 84]. The finding of reported analgesic use in 38% of subjects, and it being a modifier of the urinary-placental MEP association might indicate a possible drug-drug interaction [85]. A limitation is the lack of information on timing of the analgesic or what type of analgesic.

Pilot studies of this nature are necessary to validate novel measures and to generate insights that can motivate larger studies. The robustness and generalizeability of these findings are

limited by small sample size. Urinary and placental phthalates were measured in different laboratories that used well-validated, standard methods; yet they have not cross-validated their methods which would further increase confidence in these comparisons across matrices and across studies. Future studies can be strengthened by measuring and comparing urinary and phthalate metabolites closer in time (12–48 h), and by measuring mRNA and protein biomarkers of placental transfer and/or metabolism in the placental tissue. These can be used to evaluate and adjust more specifically for inter-individual differences in transport and metabolism.

These findings provide a framework for well-powered studies to evaluate placental exposure to phthalates and sources of variability in phthalate pharmacokinetics in pregnancy. When sampled in the third vs. second trimester, urinary phthalates were more representative of placental tissue levels. Differences in correlations between phthalate metabolites likely reflect differences in their pharmacokinetics and half-lives in the pregnant body. There is evidence that MEHP may accumulate preferentially in the placenta as compared to undergoing maternal excretion. The major modifiers of urine-placenta associations relate to social advantage, race/ethnicity, reproductive history, medication use, and obesity. These can point to specific hypotheses to be studied in future work. Placental biomarkers of phthalate exposure hold promise in allowing us to ask questions on phthalates and placental mechanisms, which connect molecular measures of exposure with measures of gene and protein expression, morphology, vascularization, and size. Placental phthalate biomarkers may prove useful to accurately quantify placental and fetal toxicity, and to directly study molecular mechanisms in the placenta affected by phthalates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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DATA AVAILABILITY

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided. CANDLE is a participating cohort in the National

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Table 1.

Characteristics of the CANDLE sample ($N = 50$).

Variable	Mean (SD)
Age, years	26.9 (5.75)
BMI, kg/m^2	28.5 (8.14)
Gestational age, second trimester urine sample	23.2 (3.34)
Gestational age, third trimester urine sample	31.6 (1.67)
Gestational age, placental tissue sample	38.9 (1.01)
Baby sex	N (%)
Male	25 (50)
Female	25 (50)
Education	
Less than high school	3 (6)
High school/GED	23 (46)
Higher than high school	24 (48)
Race	
Black	34 (68)
White	16 (32)
Annual household income, dollars	
<$24,999	21 (43)
$25,000–$54,999	16 (33)
$55,000 and over	12 (24)
Public insurance	30 (60)
Marital status	
Partnered	33 (66)
Single/previously married	17 (34)
Parity	
1 livebirth	14 (28)
2 livebirths	11 (22)
≥3 livebirths	25 (50)
No	41 (82)
History of abortion (spontaneous)	
Yes	13 (26)
No	37 (74)
Asthma history	9 (18)
Vitamin consumption (any)	
Yes	46 (92)
No	4 (8)
Alcohol consumption (any)	
Yes	3 (6)

Variable	Mean (SD)
No	47 (94)
Analgesic use in pregnancy	
Yes	19 (38)
No	31 (62)
Tobacco	
Yes	2 (4)
No	48 (96)

Table 2.

Concentrations of urinary and placental tissue phthalate metabolites, expressed as geometric means (GM).

		Maternal urine, second trimester		Maternal urine, third trimester		Placenta, birth		
Metabolite	**Molecular weight, gr/mole**	**GM, nmol/l**	**%>LOD**	**GM, nmol/l**	**%>LOD**	**GM, nmol/l**	**%>LOD**	**GM (95% CI) ng/mg**
MnBP	222.24	1.82×10^2	100%	9.18×10^1	100%	1.19×10^4	100%	2.45 (2.09, 2.87)
MBzP	256.25	1.01×10^2	100%	4.41×10^1	96%	1.41×10^4	100%	3.34 (2.83, 3.93)
MEP	194.18	7.98×10^2	100%	7.00×10^2	100%	2.56×10^4	92%	4.60 (2.21, 9.60)
MEHP	278.34	3.70×10^1	100%	1.07×10^1	82%	2.04×10^4	100%	5.25 (4.67, 5.89)
MEOHP	292.33	6.80×10^1	100%	2.60×10^1	100%	5.84×10^3	100%	7.46 (6.32, 8.88)
MEHHP	294.35	1.40×10^2	100%	2.60×10^1	100%	8.37×10^3	100%	10.5 (8.79, 12.4)
MECPP	308.33	9.02×10^1	100%	5.32×10^1	100%	5.82×10^3	100%	1.66 (1.46, 1.88)

LOD limit of detection.

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Table 3.

Comparison of phthalate metabolite distributions in the two tissue matrices (urine, placenta) using quantile normalization.

Third trimester urine[a]		Placental tissue at birth[a]		Urine-placenta[b]	
Metabolite	**Mean**	**Metabolite**	**Mean**	**Metabolite**	**Difference[c]**
MEP	3.6	MEHP	2.9	MEHP	−2.21
MnBP	2.2	MEP	2.5	MEP	1.10
MECPP	2.0	MBzP	2.1	MECPP	0.97
MBzP	1.7	MEHHP	1.7	MnBP	0.49
MEHHP	1.6	MnBP	1.7	MBzP	−0.40
MEOHP	1.1	MECPP	1.0	MEOHP	0.12
MEHP	0.6	MEOHP	1.0	MEHHP	−0.07

[a] Phthalate mean after quantile normalization in each matrix.

[b] The mean difference between phthalate in urine and in the placenta after quantile normalization.

[c] A negative value indicates that the phthalate mean was higher in the placenta vs. the urine. A positive value indicates that the phthalate mean value was higher in the third trimester urine. The magnitude of the difference is a measure of discordance in the distributions of that metabolite in the two matrices.

Table 4.

Linear associations of third trimester urinary phthalate metabolite (log, specific gravity adjusted), fetal sex, maternal race, maternal age, education, and asthma with placental tissue phthalates (log).

	MEP	MnBP	MBzP	MECPP	MEOHP	MEHHP	MEHP
Urinary phthalate	−0.61 (−1.5, 0.3)	0.21 (−0.1, 0.5)	0.10 (−0.1, 0.3)	0.31 (0.1, 0.5)	0.13 (−0.1, 0.4)	0.18 (0.0, 0.4)	0.00 (−0.1, 0.1)
Fetal sex (ref: Male)	−0.49 (−2.3, 1.3)	0.07 (−0.3, 0.4)	−0.06 (−0.4, 0.3)	−0.24 (−0.5, 0.0)	−0.09 (−0.4, 0.2)	−0.18 (−0.5, 0.1)	0.16 (−0.1, 0.4)
Maternal race (ref: Black)	0.37 (−1.6, 2.3)	0.20 (−0.2, 0.6)	−0.09 (−0.5, 0.3)	−0.31 (−0.6, 0.0)	−0.01 (−0.3, 0.3)	−0.16 (−0.5, 0.2)	−0.02 (−0.3, 0.3)
BMI	0.10 (0.0, 0.2)	0.01 (0.0, 0.0)	0.01 (0.0, 0.0)	−0.01 (0.0, 0.0)	−0.01 (0.0, 0.0)	0.00 (0.0, 0.0)	−0.01 (0.0, 0.0)
Maternal age	−0.11 (−0.3, 0.1)	0.00 (0.0, 0.0)	0.00 (0.0, 0.0)	−0.02 (0.0, 0.0)	0.00 (0.0, 0.0)	0.00 (0.0, 0.0)	−0.01 (0.0, 0.0)
Education (ref: <HS)							
High school	0.14 (−3.3, 3.6)	−0.10 (−0.8, 0.6)	−0.42 (−1.2. 0.3)	−0.26 (−0.8, 0.3)	−0.03 (−0.6, 0.5)	−0.08 (−0.6, 0.5)	−0.10 (−0.7, 0.5)
≥ High school	0.50 (−2.8, 3.8)	−0.20 (−0.9, 0.5)	−0.51 (−1.2, 0.2)	0.01 (−0.5, 0.5)	0.01 (−0.5, 0.5)	0.11 (−0.4, 0.7)	−0.14 (−0.7, 0.4)
Asthma	1.55 (−0.6, 3.7)	0.34 (−0.1, 0.8)	0.24 (−0.3, 0.7)	0.03 (−0.3, 0.4)	0.10 (−0.3, 0.5)	0.03 (−0.3, 0.4)	0.19 (−0.2, 0.6)

All models are adjusted for maternal age, pre-pregnancy BMI, asthma diagnosis ever, fetal sex, and maternal race, and education. Gray shading indicates *p*-value ≤ 0.10.

Table 5.

Linear associations of third trimester urinary phthalate metabolite (log, specific gravity adjusted) and placental tissue phthalates (log), reported by levels of effect modifiers.

	MEP	MnBP	MBzP	MECPP	MEOHP	MEHHP	MEHP
Phthalate × race *p*-value	0.19	0.69	0.80	0.76	0.16	0.20	0.03
Black	−0.96 (−2.0, 0.0)	0.26 (0.0, 0.6)	0.10 (−0.1, 0.3)	0.38 (0.1, 0.7)	0.00 (−0.3, 0.3)	0.05 (−0.2, 0.3)	−0.07 (−0.2, 0.1)
White	0.06 (−1.2, 1.3)	0.15 (−0.3, 0.6)	0.04 (−0.3, 0.4)	0.31 (0.0, 0.7)	0.29 (0.0, 0.6)	0.34 (0.0, 0.7)	0.14 (0.0, 0.3)
Phthalate × fetal sex *p*-value	0.99	0.65	0.88	0.48	0.87	0.44	0.36
Male	−0.57 (−1.6, 0.5)	0.29 (−0.1, 0.7)	0.10 (−0.1, 0.3)	0.22 (−0.2, 0.7)	0.11 (−0.2, 0.4)	0.08 (−0.3, 0.4)	0.05 (−0.1, 0.2)
Female	−0.56 (−1.7, 0.6)	0.18 (−0.2, 0.5)	0.08 (−0.1, 0.3)	0.39 (0.1, 0.7)	0.15 (−0.1, 0.4)	0.26 (0.0, 0.6)	−0.04 (−0.2, 0.1)
Phthalate × time *p*-value	0.61	0.73	0.34	0.97	0.91	0.89	0.31
≤6 weeks (urine-placenta)	0.26 (−1.4, 1.9)	0.36 (−0.2, 0.9)	0.03 (−0.2, 0.3)	0.27 (−0.1, 0.7)	0.15 (−0.2, 0.5)	0.17 (−0.2, 0.5)	0.04 (−0.2, 0.3)
6–8 weeks (urine-placenta)	−0.48 (−1.9, 0.9)	0.15 (−0.2, 0.5)	0.19(0.0, 0.4)	0.34 (0.0, 0.6)	0.04 (0.0, 0.4)	0.06 (−0.3, 05)	−0.07 (−0.2, 0.1)
>8 weeks (urine-placenta)	−0.7 (−1.9, 0.5)	0.33 (−0.2, 0.9)	−0.23 (−0.8,0.4)	0.35 (−0.4, 1.1)	0.1 (−0.5, 0.7)	0.07 (−0.5, 0.6)	0.12 (−0.1, 0.3)
Phthalate × vitamin *p*-value	0.91	0.94	0.68	0.68	0.19	0.15	0.75
None	−0.69 (−2.9, 1.5)	0.26 (−0.7, 1.2)	0.14 (−0.2, 0.5)	0.97 (−2.1, 4.1)	−0.43 (−1.3, 0.4)	−0.34 (−1.1, 0.4)	−0.06 (−0.5, 0.4)
Vitamin use	−0.54 (−1.5, 0.4)	0.23 (0.0, 0.5)	0.07 (−0.1, 0.3)	0.34 (0.1, 0.6)	0.17 (−0.1, 0.4)	0.22 (0.0, 0.4)	0.01 (−0.1, 0.1)
Phthalate × analgesic *p*-value	<0.01	0.91	0.25	0.98	0.88	0.95	0.05
None	−1.65 (−2.7, −0.7)	0.25 (−0.1, 0.6)	0.15 (0.0, 0.4)	0.34 (0.1, 0.6)	0.14 (−0.2, 0.5)	0.15 (−0.2, 0.5)	−0.07 (−0.2, 0.1)
Analgesic use	0.37 (−0.7, 1.4)	0.22 (−0.2, 0.6)	−0.02 (−0.3, 0.2)	0.34 (0.0, 0.7)	0.1 (−0.2, 0.4)	0.17 (−0.1, 0.4)	0.14 (0.0, 0.3)
Phthalate × insurance *p*-value	0.38	0.87	0.14	0.83	0.23	0.10	0.28
Public	−0.9 (−2.0, 0.2)	0.23 (−0.1, 0.6)	0.02 (−0.2, 0.2)	0.33 (0.1, 0.6)	0.05 (−0.2, 0.3)	0.06 (−0.2, 0.3)	−0.04 (−0.2, 0.1)
Private	−0.2 (−1.4, 1.0)	0.28 (−0.2, 0.7)	0.32 (0.0, 0.7)	0.38 (0.1, 0.7)	0.29 (0.0, 0.6)	0.40 (0.1, 0.7)	0.07 (−0.1, 0.2)
Phthalate × BMI *p*-value	0.09	0.18	1.00	0.27	0.95	0.70	0.72
<25	−0.54 (−1.9, 0.8)	0.42 (0.0, 0.8)	0.09 (−0.1, 0.3)	0.05 (−0.4, 0.5)	0.14 (−0.2, 0.5)	0.06 (−0.3, 0.4)	0.02 (−0.1, 0.2)
25–29.9	1.25 (−0.5, 2.9)	0.33 (−0.2, 0.8)	0.11 (−0.3, 0.6)	0.40 (0.0, 0.8)	0.08 (−0.3, 0.5)	0.21 (−0.1, 0.6)	−0.17 (−0.6, 0.3)
>30	−0.99 (−2.1, 0.2)	−0.04 (−0.4, 0.3)	0.09 (−0.1, 0.3)	0.46 (0.2, 0.8)	0.16 (−0.2, 0.5)	0.27 (−0.1, 0.6)	0.03 (−0.1, 0.2)
Phthalate × spontaneous abortion *p*-value	0.76	0.37	0.03	0.54	0.97	0.47	0.01
No	−0.32 (−1.2, 0.6)	0.28 (0.0, 0.6)	0.03 (−0.1, 0.2)	0.41 (0.1, 0.7)	0.14 (−0.1, 0.4)	0.16 (−0.1, 0.4)	0.14 (0.0, 0.3)
Yes	−0.62 (−2.4, 1.2)	0.05 (−0.4, 0.5)	0.55 (0.1, 1.0)	0.28 (0.0, 0.6)	0.13 (−0.3, 0.5)	0.35 (−0.1, 0.8)	−0.13 (−0.3, 0.0)

All models are adjusted for maternal age, pre-pregnancy BMI, asthma diagnosis ever, fetal sex, maternal race, and education. Gray shading indicates *p*-value ≤ 0.10.