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Phthalate Exposure and Odds of Bacterial Vaginosis Among U.S. Reproductive-Aged Women, NHANES 2001-2004

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Abstract

Endocrine-disrupting chemicals, such as phthalates, are an unexamined potential risk factor for bacterial vaginosis (BV) and warrant investigation because hormones affect BV. We examined the association between phthalate exposure and BV in the National Health and Nutrition Examination Survey, 2001–2004. BV outcomes were defined as intermediate (Nugent score of 4–6) and positive (7–10). Phthalate metabolites, including monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MnBP), and di(2-ethylhexyl) phthalate (DEHP) metabolites, were measured in urine. Among 854 women with complete data, multinomial logistic regression revealed that concentrations of MnBP (Q4 vs. Q1 OR=3.01, 95% CI 1.76–5.15, *p*-trend<0.001) and ΣDEHP metabolites (Q4 OR=2.55, 95% CI 1.45–4.47, *p*-trend=0.03) were associated with Nugent-score BV, although only MnBP was significant after adjustment for confounders. Associations were null after adjustment for urinary creatinine (MnBP Q4 OR=1.11, 95% CI 0.63–1.96; ΣDEHP Q4 OR=0.72, 95% CI 0.37–1.39). Future work should further examine these relationships using direct measurements of intravaginal phthalates exposures.

Keywords

vaginal microbiota; endocrine disruptors; intravaginal exposure; NHANES; di-*n*-butyl phthalate; diethyl phthalate; di(2-ethylhexyl) phthalate; women's health

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1. INTRODUCTION

Bacterial vaginosis (BV) is a dysbiosis of the vaginal microbiota, characterized by low levels of lactic acid-producing bacterial species (primarily *Lactobacillus* species) and higher abundance of anaerobic bacteria [1]. BV is present in approximately 29% of U.S. reproductive-aged women [2] and symptomatic in approximately 50% of those affected [3]. BV is associated with increased risk of acquisition of sexually transmitted infections including HIV, [4–6] and adverse pregnancy outcomes including preterm delivery [7, 8]. Well-established risk factors for BV include vaginal douching, menses, and sexual behaviors [9–11], whereas hormonal contraceptive use and condom use are largely considered protective [12, 13]. However, the inciting factors which cause a woman to transition to BV or maintain more optimal *Lactobacillus*-dominated states remain poorly understood [1].

Exposures to endocrine-disrupting chemicals (EDCs) are a previously unexamined potential risk factor for BV. EDCs are substances that interfere with hormone biosynthesis, metabolism, or action [14]. Hormone action is mechanistically relevant to BV because estrogen affects the vaginal microbiome. Estrogen promotes a *Lactobacillus*-dominated microbiome by stimulating glycogen production by vaginal epithelial cells. *Lactobacillus* species can directly or indirectly metabolize glycogen to produce lactic acid, a protective component of the vaginal microbiome [15, 16]. Thus, any disruption to the endocrine system is likely to affect BV risk through modification of estrogen-mediated regulatory pathways.

Phthalates, a class of multifunctional industrial chemicals, warrant examination because many phthalates are known endocrine disruptors [17, 18] and U.S. reproductive-aged women encounter widespread exposures to multiple phthalates [19, 20]. In utero exposure to certain phthalates, such as di-*n*-butyl phthalate (DnBP) and di(2-ethylhexyl) phthalate (DEHP), results in anti-androgenic effects on male offspring [21, 22]. Additional work has shown that some phthalates, such as DnBP and diethyl phthalate (DEP), exhibit estrogenic activity [17, 18]. Furthermore, exposure to phthalates is associated with adverse effects on reproductive, endocrine, and developmental systems [14, 23–25]. Certain phthalates are commonly found in personal care products [26, 27], including those used in the genital area [28]. For example, low molecular weight phthalates, such as DEP, are commonly used in fragranced personal care products [27, 29], which are heavily used by women of reproductive age [30]. Vaginal douches have been implicated as a source of DEP among reproductive-aged women [31]. Other products used intravaginally or in the genital area, such as lubricant [26], ultrasound gel [32], and wipes [33], may also be sources of phthalate exposure. These exposures are widespread: in nationally representative surveys of U.S. women, 25% reported using vaginal lubricants in the past month [34], 62% reported ever using lubricant [34], and 18% reported douching in the past year [35]. In a recent study of 1,435 Canadian participants, 95% reported having used at least one personal care product in or around the vaginal area, including douche (21%), lubricant (41%), and wipes (42%) [36]. Thus, large numbers of reproductive-aged women are likely exposed to phthalates through an intravaginal route, and phthalate exposure may have localized effects on the vaginal microbiota.

To our knowledge, no prior study has examined the potential association of phthalate exposures and risk of BV. The objective of this study was to estimate the association of phthalate exposure with BV in a population-based sample of women of reproductive age. Additionally, as vaginal douching is a known risk factor for BV and douche products may contain low molecular weight phthalates, we sought to examine the contribution of phthalate exposure to the known association of vaginal douching with BV. Thus, as a secondary objective, we examined whether metabolites of low molecular weight phthalates (mono-ethyl phthalate [MEP] and mono-*n*-butyl phthalate [MnBP]) mediate the association of vaginal douching with BV.

2. MATERIALS AND METHODS

2.1. Study population and BV measurement

We utilized data from the 2001–2002 and 2003–2004 survey cycles of the National Health and Nutrition Examination Survey (NHANES), a physical examination and survey representative of the civilian, non-institutionalized population of the U.S., conducted by the Centers for Disease Control and Prevention. Procedures for BV measurement are described in NHANES documentation [37, 38]. Briefly, participants aged 14–49 self-collected vaginal swabs in a private bathroom in the Mobile Examination Center after receiving oral and written instructions [2]. NHANES personnel applied the swab to pH paper and rolled the swab onto a glass slide. Slides were Gram-stained and assessed using Nugent's criteria by NHANES personnel in one central laboratory. BV outcomes were categorized as negative (Nugent score of 0–3), intermediate (score of 4–6) or positive BV (score of 7–10) [39]. Nugent score data were available for 2,814 (81%) of 3,465 women aged 18–49. (BV data on participants under 18 years old are not publicly available.) We excluded women who did not have Nugent results classified as positive, negative, or intermediate ($n=8$). Phthalate metabolites (further described below) were measured in a randomly selected one-third subsample of NHANES participants age 6 and above. Our study population consists of 940 women aged 18–49 who participated in NHANES, 2001–2004 and had complete data on Nugent score and phthalate metabolite concentrations. The sample for the complete case analysis consists of 854 women with complete data on BMI (n missing=11) and past six-month vaginal douching (n missing=79); data on age, race/ethnicity, and educational attainment were complete for all participants in the study population.

2.2. Phthalate metabolite and creatinine measurement.

Phthalate metabolites and creatinine were measured in spot urine samples. Urine samples were collected in the Mobile Examination Center and stored at -20°C prior to being shipped to the National Center for Environmental Health for analysis. Analytical methods are described in the NHANES documentation [40, 41]. Briefly, solid phase extraction-high performance liquid chromatography-isotope dilution-tandem mass spectrometry was used to quantify concentrations of phthalate metabolites [42]. Values below the limit of detection (LOD) were replaced with the LOD divided by the square root of two [40, 41]. Phthalate metabolites measured include MEP, a metabolite of DEP; MnBP, a metabolite of DnBP; and several metabolites of DEHP (mono(2-ethylhexyl) phthalate [MEHP], mono(2-ethyl-5-hydroxy-hexyl) phthalate [MEHHP], mono(2-ethyl-5-oxohexyl) phthalate [MEOHP], and

mono(2-ethyl-5-carboxypentyl) phthalate [MECPP; 2003–2004 only]). To obtain a summary measure of Σ DEHP metabolites, we used the method described by Zota *et al.* [20]. First, we calculated the molar sum of MEHP, MEHHP, and MEOHP. (MECPP was not included because it was not measured in 2001–2002.) Concentrations of each metabolite were divided by their respective molar weight (MW) to obtain the molar equivalent. The molar equivalents were summed to obtain total micromoles of DEHP metabolites per liter. Finally, the total micromoles per liter were multiplied by the average MW of the DEHP metabolites (288 $\mu\text{g}/\mu\text{mol}$) to obtain Σ DEHP metabolites in nanograms per liter [20]. Urinary creatinine was analyzed with a modified Jaffe reaction [43], using a Beckman Synchron CX3 clinical analyzer (Beckman Instruments, Inc., Brea, CA) [44, 45].

2.3. Measurement of covariates.

Covariates were identified based on known associations with phthalates exposures [31, 46] and Nugent-score BV [2]. Demographic data including age, race/ethnicity, and educational attainment were obtained via computer-assisted personal interviewing (CAPI). Body mass index (BMI) was calculated based on weight and height measured in the physical examination. Frequency of vaginal douching in the past six months and number of lifetime sex partners were obtained via face-to-face interview in 2001–2002 and via CAPI in 2003–2004. Douching was defined in the questionnaire as “putting a substance into your vagina either for routine cleansing or for vaginal irritation or signs of infection.” Sex was defined in the questionnaire as “vaginal, oral, or anal sex,” and the number of lifetime male sex partners and lifetime female sex partners were collected separately.

2.4. Statistical methods.

Statistical analyses were conducted using Stata 13.1 software (StataCorp, College Station, TX). Analyses were weighted to account for the complex survey design of NHANES. Sample weights are provided in the NHANES laboratory files; we divided participants’ two-year phthalate subsample weights by two to account for combining two survey cycles. A significance level of $\alpha=0.05$ was used.

Because urinary phthalate metabolite concentrations can vary by an individual’s hydration status, we used creatinine measurements to account for variations in urine dilutions. Creatinine adjustments are one common way to adjust spot urine samples for variations in hydration status. Creatinine adjustment was carried out by covariate-adjusted standardization with covariate adjustment, as recommended by O’Brien *et al.* [47]. This method was developed to minimize bias that can result from between-individual variations in urine dilution. For each participant, predicted urinary creatinine was calculated as a function of age, race/ethnicity, and BMI, as these variables are associated with creatinine [47, 48]. The ratio of observed to predicted urinary creatinine was calculated for each participant. Phthalate exposure was modeled as metabolite concentration divided by the ratio of observed to predicted creatinine (i.e., covariate-adjusted standardization) [47]. This variable was used to calculate quartiles of phthalate exposure based on the distribution in the weighted study population. Creatinine concentration was also added separately as a covariate in creatinine-adjusted regression analyses (i.e., covariate adjustment) [47]. Quartiles of creatinine-adjusted phthalate exposure were modeled as nominal categorical

variables in order to avoid assuming linear relationships. Creatinine-adjusted phthalate exposures were also modeled as ordinal variables (using the median value within each quartile) to calculate p-trends.

To compare the distributions of demographic and behavioral characteristics, we calculated survey-weighted percentages by Nugent-score BV results. Additionally, we calculated the geometric mean (GM) and 95% confidence interval (CI) of phthalate metabolites and creatinine concentrations by Nugent-score BV results. The generalizability of the complete case sample to all women aged 18–49 in the environmental subsample (N=1,125) was assessed using descriptive statistics. Using multinomial logistic regression models [49], we estimated associations of phthalate exposures with intermediate and Nugent-score BV, respectively, compared to women with no BV, for quartiles of each phthalate metabolite individually. Multinomial logistic regression estimates a ratio of relative risks; we will refer to the estimator as a prevalence odds ratio (OR). Adjusted models controlled for age (continuous), BMI (continuous), race/ethnicity (non-Hispanic White, non-Hispanic Black, or Other), educational attainment (less than high school, high school graduate or equivalent, or some college or more), and self-reported past six-month vaginal douching (yes or no). Finally, we further adjusted our multinomial models for urine dilution with covariate-adjusted standardization and the inclusion of creatinine as a covariate [47]. Complete case analysis (with respect to variables in the fully-adjusted models) was used in all regression models. We assessed statistical significance using Wald tests and additionally calculated the p-trend for each phthalate.

To assess the mediation hypothesis, we estimated the OR of Nugent-score BV associated with past six-month vaginal douching, adjusted for age, race/ethnicity, BMI, educational attainment, and creatinine. We then added MEP and MnBP in separate models and compared the magnitude and significance of the douching-BV association before and after adjustment for each phthalate metabolite [50].

2.6. Sensitivity analyses.

We conducted several sensitivity analyses to evaluate the robustness of our findings. (1) We compared multiple methods of creatinine adjustment: no adjustment, standardization (dividing phthalate metabolite concentration by creatinine concentration), covariate adjustment (adjusting for creatinine as a covariate) [51], covariate-adjusted standardization, and covariate-adjusted standardization with covariate adjustment. All models were adjusted for demographic and behavioral covariates. (2) We calculated unadjusted and adjusted ORs of intermediate and high Nugent-score BV modeling phthalate exposure as a continuous variable (calculated by natural log transformation of the covariate-standardized exposures). (3) In a logistic regression, we combined intermediate and high Nugent score in a binary outcome for BV to assess whether phthalates were associated with any dysbiosis of the vaginal bacteria. (4) As women may douche in response to BV symptoms [9, 52], adjustment for past six-month douching may be inappropriate. Therefore, we ran the adjusted models without adjusting for past six-month douching. Additional sensitivity analyses included adjustment for current hormonal contraceptive use (defined as current use of oral contraceptives or injectable contraceptives; the questionnaires did not ask about

contraceptive patch, ring, or hormonal intrauterine device) (n=96), exclusion of peri- and post-menopausal women (defined as women who reported not having a regular period in the past 12 months due to menopause, or who reported current use of hormone replacement therapy) (n=60), adjustment for current pregnancy (n=127), and exclusion of participants with urinary creatinine concentrations <30 mg/dL or >300 mg/dL (n=65) [48].

3. RESULTS

The weighted prevalence of intermediate and high Nugent-score BV results was 27.1% and 31.5%, respectively (Table 1). High Nugent-score BV was more prevalent among women who were non-Hispanic Black, had lower educational attainment, had BMI ≥ 30 kg/m², or reported frequent or occasional vaginal douching in the past six months. Concentrations of MnBP, Σ DEHP metabolites, and urinary creatinine differed by Nugent category and were highest among women with BV. There was evidence of a positive, dose response relationship between creatinine concentration and Nugent score category ($p < 0.001$). The complete case sample was representative of all women aged 18–49 in the environmental subsample in terms of phthalate metabolites, age, race/ethnicity, BMI, educational attainment, and past six-month douching (not shown). Similar to previous research in NHANES 2001–2004 [31], we observed a positive dose-response relationship between past six-month frequency of douching and MEP concentration, and to a lesser extent with MnBP concentration (not shown).

In unadjusted models, MnBP and EDEHP exposure were positively and significantly associated with high Nugent-score BV (p-trend <0.001 and 0.03, respectively) (Table 2). For example, the highest quartile of MnBP exposure was associated with 3-fold greater odds of BV (OR 3.01, 95% CI 1.76, 5.15). After adjustment for demographic and behavioral risk factors, MnBP concentrations remained positively and significantly associated with BV (p-trend = 0.02); the highest quartile of MnBP exposure was associated with a significant 2-fold increased odds of BV (OR 2.28, 95% CI 1.24, 4.20). Although the linear association of Σ DEHP with BV was not significant after adjustment (p-trend = 0.20), there were significantly higher odds of BV in the second, third, and fourth quartiles of Σ DEHP exposure. However, further adjustment for creatinine attenuated all observed associations between phthalate exposures and Nugent-score BV. For example, the highest quartile of creatinine-adjusted MnBP exposure was not associated with BV (OR 1.11, 95% CI 0.63, 1.96, p-trend = 0.55). Similarly, phthalate metabolite concentrations were not associated with intermediate BV in any models.

In the mediation analysis, adjustment for MEP and MnBP did not attenuate the association between vaginal douching and BV (Table 3). Douching in the past six months was associated with 2.53 (95% CI 1.40, 4.59) times the odds of Nugent-score BV without adjustment for phthalates; the association remained virtually unchanged after separately adjusting for MEP (OR 2.69, 95% CI 1.45, 4.97) and MnBP (OR 2.50, 95% CI 1.37, 4.56). These results suggest the association between douching and BV is not mediated by phthalates exposures.

Comparison of creatinine adjustment methods did not reveal consistent trends across phthalate biomarkers. Creatinine-adjusted results for MEP consistently showed non-significant inverse associations with Nugent-score BV in the third and fourth exposure quartiles (Figure 1). For MnBP, non-significant positive associations with Nugent-score BV were observed in the second, third, and fourth quartiles using standardization, covariate adjustment, and to a lesser extent, covariate-adjusted standardization (Figure 2). For Σ DEHP metabolites, standardization, covariate adjustment, and covariate-adjusted standardization yielded disparate results (Figure 3).

Creatinine-adjusted phthalate exposures modeled as continuous variables were not associated with odds of intermediate or high Nugent-score BV (Table 4). Creatinine-adjusted phthalate exposures were not associated with BV when intermediate and high Nugent-score were combined into one binary dysbiotic outcome (Table 5) or when the past six-month douching variable was left out of regression models (Table 6). Other sensitivity analyses (adjustment for current hormonal contraceptive use, exclusion of peri- and post-menopausal women, adjustment for current pregnancy, and exclusion of participants with urinary creatinine concentrations <30 mg/dL or >300 mg/dL) did not meaningfully change the null results between creatinine-adjusted phthalate exposures and BV (not shown).

4. DISCUSSION

In this cross-sectional, population-based analysis of U.S. women aged 18–49, we observed null associations between BV outcomes and creatinine-adjusted urinary concentrations of several phthalate metabolites. We also observed no indication of mediation between vaginal douching and BV by creatinine-adjusted phthalate exposures. However, creatinine-unadjusted concentrations of MnBP and Σ DEHP metabolites showed significant bivariate associations with BV, and the linear association of MnBP with BV persisted after adjustment for important demographic and behavioral risk factors for BV. To our knowledge, this is the first evaluation of the potential role of EDCs in BV prevalence in an epidemiologic study.

Debate is ongoing with respect to the optimal methods for urine dilution standardization in large epidemiologic studies. Although not optimal [53], creatinine adjustment is an acceptable method of adjustment for urine dilution in measurement of non-persistent chemicals [48]. We used creatinine adjustment because no other surrogates for hydration status were measured in NHANES 2001–2004 (the years in which BV was assessed). More optimal methods for urine dilution standardization include calculating the average excretion rate of a biomarker by multiplying the concentration in a spot urine sample by urinary flow rate [54], or adjusting for the specific gravity [55] or osmolality of the urine sample [56, 57]. Future studies of BV and environmental chemicals measured in urine should consider including more direct measures of hydration status, such as urine flow rate or 24-hour urine samples, to reduce measurement error in exposure assessment.

Additionally, a range of statistical approaches for urine dilution adjustment exist. In the context of creatinine adjustment, the most traditional approach is to divide the biomarker concentration by the urinary creatinine concentration (referred to as standardization) [47, 48]. In addition to hydration status, average urinary creatinine concentration varies by

demographic and biologic characteristics including age, sex, race/ethnicity, BMI, and lean muscle mass.[48] Barr et al. [48] asserted that standardization does not account for between-individual variability in average urinary creatinine concentration, and proposed modeling the urinary biomarker concentration and creatinine concentration as separate independent variables in the regression model. This approach adjusts the environmental chemical for creatinine while also adjusting other covariates in the model (e.g., age, race/ethnicity, BMI) for creatinine [48]. However, this method may induce collider stratification bias [47, 58] and inadequately control measurement error [47]. O'Brien et al. recently proposed the use of covariate-adjusted standardization, which controls for the multiplicative effect of hydration on creatinine, after accounting for differences in creatinine levels between subpopulations [47, 59]. Covariate-adjusted standardization has since been used in several environmental epidemiologic studies, with some variations in statistical implementation [60–62]. Other proposed standardization methods include creatinine regression normalization and modified specific-gravity-adjusted-creatinine ratio-normalization [63]. In general, researchers recommend that the choice of standardization method consider the causal network and the chemical-specific renal excretion mechanism [47, 54, 63].

We found that creatinine adjustment changed our results substantially. Urinary creatinine concentration was highly associated with Nugent-score BV in this study, even in the final adjusted models. This finding was unexpected; to our knowledge, no previous study has assessed the potential for association between urinary creatinine and measures of the vaginal microbiota. Based on environmental epidemiologic literature [47, 48], we know that substantial measurement error may be present without urine dilution adjustment. However, it seems unlikely that large discrepancy between creatinine-adjusted and -unadjusted models is due to dilution-related measurement error alone. As such, we believe that a physiological association between creatinine and BV may also be impacting our results. For example, phthalate exposure may increase obesity risk [64–66], and dehydration is more prevalent among obese individuals [67, 68]. Mild dehydration and consequent infrequent urination increase risk of urinary tract infection [69], which in turn may be related to risk of BV [70]. In this way, dehydration could be an intermediary between long-term phthalates exposure and BV. As such, creatinine adjustment (or other adjustment for urine dilution) may not be optimal for studies of BV, as it may constitute either overadjustment or adjustment for a proxy of disease. Alternatively, as urinary creatinine levels primarily reflect muscle mass [71], women with higher creatinine may also have higher levels of endogenous androgens. The effects of androgens on the vaginal microbiota have not been studied. In summary, we are concerned about the appropriateness of adjustment for urinary creatinine in studies of the vaginal microbiota. Other common surrogates of urine dilution, such as specific gravity, were not available, so we were unable to compare whether other measures were similarly associated with BV.

Although covariate-adjusted standardization improves upon previous statistical methods for creatinine adjustment [47], there may be model misspecification as much remains unknown about the underlying causal network. Our interpretation of the creatinine-adjusted results is limited because existing creatinine adjustment methods do not account for causal scenarios in which creatinine is associated with our vaginal health outcome. A related but separate concern is that we currently have a poor understanding of the extent to which potential

absorption of phthalates from consumer products used in the vaginal area would be reflected in spot urine samples. Thus, future studies of phthalate exposures and vaginal microbiota should include more direct measures of intravaginal phthalate exposures as well as urine samples before and after product use to advance this field of research.

Strengths of the study include the use of a population-based sample. BV was assessed using Nugent's Gram stain score which has been widely used and validated in clinical studies [72]. However, there are limitations of using Nugent's criteria to assess BV as it is based on morphology [5, 73]. Molecular methods, including 16S rRNA gene amplicon sequencing, could offer higher resolution information on the bacterial composition [1]. Due to the cross-sectional study design, we cannot establish temporality in the observed associations. There may be potential for reverse causality if phthalates are present in vaginal douches, and women may douche in response to BV symptoms [9, 52]. There may be exposure misclassification in the phthalate measurements due to the use of a single spot urine sample [74, 75]. Phthalate metabolites have short half-lives in the body (3–18 hours); repeated-measure studies have found weak to moderate intraclass correlations of phthalate metabolites across periods ranging from weeks to months [76]. Outcome misclassification is also possible as there are temporal dynamics in Nugent scores within individuals [77]. Prospective studies are needed to assess the relationships between fluctuations in both environmental exposures and microbiota outcomes.

This study reflects a novel area of inquiry and a growing interest in the contributions of environmental chemicals to the human microbiota [78]. The extent to which creatinine adjustment changed the results was unexpected and presents challenges to interpretation. Using an accepted yet sub-optimal method of adjustment for urine dilution, associations between phthalate exposures and prevalence of BV were null. However, our findings are limited by substantial measurement concerns, including the fact that our proxy for hydration, urinary creatinine, was independently associated with the outcome. Therefore, we believe there is a possibility that the strong signal observed in creatinine-unadjusted models may reflect a true association and warrants further investigation using alternative phthalate or hydration biomarkers. Recommendations for future studies include the use of molecular methods to assess the vaginal microbiota [1, 79], direct measurement of phthalates in the vagina, and longitudinal study designs that collect repeated measurements of environmental chemical exposures and the vaginal microbiota. Future studies should also consider investigating other EDCs as risk factors for BV. For example, parabens, which are commonly found in consumer products used intravaginally, are weakly estrogenic [80, 81] and have not been evaluated in the context of BV.

5. CONCLUSIONS

Exposure to phthalates is widespread among reproductive-aged women. Given the rising interest of health outcomes associated with environmental chemical toxicity, effects of phthalate exposures on the vaginal microbiome should be further investigated in experimental and human studies. Because adjustment for urinary creatinine or other measures of urine dilution may not be optimal, novel methods to directly measure intravaginal exposures to environmental chemicals are needed.

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Highlights

- Endocrine-disrupting chemicals are present in products used in the vaginal area.
- Effects of endocrine-disrupting chemicals on the vaginal microbiome are unknown.
- Some phthalate metabolites were bivariately associated with bacterial vaginosis.
- Associations were attenuated after adjustment for urinary creatinine.
- Novel methods of directly measuring intravaginal chemical exposures are needed.

Figure 1.

Associations of MEP Quartiles with Odds of Nugent-Score BV Results Among U.S. Women Aged 18–49, 2001–2004: Comparison of Creatinine Adjustment Methods.^{a,b} Abbreviations: BV, bacterial vaginosis; OR, odds ratio; CI, confidence interval; MEP, monoethyl phthalate.

^a All models are adjusted for age (continuous), body mass index (continuous), race/ethnicity (non-Hispanic White, non-Hispanic Black, or Other), educational attainment (less than high school [HS], HS graduate or equivalent, or some college or more), and self-report of vaginal douching in the past six months (yes or no). N=854 (all models). ^b Creatinine adjustment methods are: no creatinine adjustment, standardization (phthalate concentration divided by creatinine concentration), covariate adjustment (creatinine included as a covariate), covariate-adjusted standardization (MEP concentration divided by ratio of observed to predicted creatinine), and covariate-adjusted standardization with covariate adjustment.

Figure 2.

Associations of MnBP Quartiles with Odds of Nugent-Score BV Results Among U.S. Women Aged 18–49, 2001–2004: Comparison of Creatinine Adjustment Methods.^{a,b} Abbreviations: BV, bacterial vaginosis; OR, odds ratio; CI, confidence interval; MnBP, mono-*n*-butyl phthalate. ^a All models are adjusted for age (continuous), body mass index (continuous), race/ethnicity (non-Hispanic White, non-Hispanic Black, or Other), educational attainment (less than high school [HS], HS graduate or equivalent, or some college or more), and self-report of vaginal douching in the past six months (yes or no). N=854 (all models). ^b Creatinine adjustment methods are: no creatinine adjustment, standardization (phthalate concentration divided by creatinine concentration), covariate adjustment (creatinine included as a covariate), covariate-adjusted standardization (MnBP concentration divided by ratio of observed to predicted creatinine), and covariate-adjusted standardization with covariate adjustment.

Figure 3.

Associations of Σ DEHP Quartiles with Odds of Nugent-Score BV Results Among U.S. Women Aged 18–49, 2001–2004: Comparison of Creatinine Adjustment Methods.^{a,b} Abbreviations: BV, bacterial vaginosis; OR, odds ratio; CI, confidence interval; DEHP, di(2-ethylhexyl) phthalate. ^a All models are adjusted for age (continuous), body mass index (continuous), race/ethnicity (non-Hispanic White, non-Hispanic Black, or Other), educational attainment (less than high school [HS], HS graduate or equivalent, or some college or more), and self-report of vaginal douching in the past six months (yes or no). N=854 (all models). ^b Creatinine adjustment methods are: no creatinine adjustment, standardization (phthalate concentration divided by creatinine concentration), covariate adjustment (creatinine included as a covariate), covariate-adjusted standardization (Σ DEHP concentration divided by ratio of observed to predicted creatinine), and covariate-adjusted standardization with covariate adjustment.

Demographic Characteristics and Phthalate Metabolite Concentrations by Nugent-Score BV Results Among U.S. Women Aged 18–49, 2001–2004.

Table 1.

	Negative	Intermediate	BV	Total
	N (weighted prevalence %)			
	363 (41.3%)	256 (27.1%)	321 (31.5%)	940 (100.0%)
	Geometric mean (95% CI)			
Phthalate metabolite concentrations (ng/mL)				
MEP	139.7 (113.2, 172.4)	140.4 (112.2, 175.7)	187.2 (142.2, 246.5)	153.4 (134.0, 175.7)
MnBP	18.6 (15.7, 22.0)	19.3 (15.3, 24.3)	27.8 (25.4, 30.4)	21.3 (19.6, 23.2)
ΣDEHP metabolites	36.2 (28.6, 45.8)	36.0 (28.1, 46.2)	45.6 (40.0, 51.9)	38.9 (33.6, 44.9)
Creatinine concentration (mg/dL)	90.2 (80.9, 100.5)	96.5 (84.2, 110.7)	118.0 (107.2, 129.8)	100.0 (93.8, 106.5)
	Column percent (95% CI)			
Age				
18–19	5.9 (3.3, 10.2)	5.3 (3.1, 9.0)	4.7 (3.1, 7.1)	5.4 (4.1, 7.1)
20–29	30.2 (25.5, 35.4)	29.1 (21.7, 37.9)	31.6 (26.2, 37.6)	30.4 (27.0, 34.0)
30–39	35.0 (29.1, 41.5)	28.5 (22.1, 35.9)	26.4 (21.6, 31.8)	30.5 (27.5, 33.8)
40–49	28.9 (23.7, 34.7)	37.1 (27.9, 47.3)	37.3 (31.6, 43.2)	33.8 (29.5, 38.3)
Race/ethnicity				
Non-Hispanic White	74.8 (69.2, 79.7)	69.3 (62.5, 75.3)	56.1 (46.9, 64.9)	67.4 (62.2, 72.2)
Non-Hispanic Black	8.5 (5.9, 12.1)	11.2 (7.2, 17.0)	21.9 (16.7, 28.2)	13.5 (10.8, 16.7)
Other	16.7 (12.3, 22.2)	19.5 (13.2, 27.9)	22.0 (15.4, 30.4)	19.1 (14.5, 24.7)
Educational attainment				
Did not complete HS	14.2 (9.9, 20.0)	9.6 (6.9, 13.3)	22.6 (16.5, 30.0)	15.6 (12.9, 18.8)
HS graduate or equivalent	17.6 (13.3, 23.0)	22.6 (15.0, 32.5)	27.8 (21.3, 35.4)	22.2 (19.0, 25.7)
Some college or more	68.2 (62.3, 73.5)	67.8 (58.4, 76.0)	49.7 (41.4, 58.0)	62.2 (57.8, 66.5)
BMI				
<18.5 kg/m ²	1.9 (0.7, 4.9)	4.9 (2.2, 10.6)	1.8 (1.1, 3.0)	2.7 (1.8, 4.0)
18.5–24.9 kg/m ²	42.4 (35.8, 49.4)	36.9 (31.1, 43.1)	31.0 (24.2, 38.7)	37.3 (34.0, 40.7)
25.0–29.9 kg/m ²	30.7 (24.8, 37.3)	26.5 (20.4, 33.6)	26.7 (21.1, 33.2)	28.3 (24.6, 32.3)

	Negative	Intermediate	BV	Total
30.0 kg/m ²	23.6 (18.7, 29.4)	31.8 (25.5, 38.8)	39.0 (31.8, 46.7)	30.7 (28.0, 33.6)
Missing	1.3 (0.4, 4.1)	0.0	1.4 (0.5, 4.0)	1.0 (0.4, 2.4)
Frequency of vaginal douching, past six months				
None	80.0 (74.9, 84.3)	74.1 (68.7, 78.9)	58.4 (50.7, 65.7)	71.6 (67.6, 75.3)
Occasional	9.1 (5.8, 13.9)	11.8 (7.3, 18.4)	19.7 (15.2, 25.2)	13.2 (10.5, 16.3)
Frequent	4.0 (2.0, 7.8)	5.8 (3.6, 9.3)	12.9 (9.0, 18.2)	7.3 (5.7, 9.4)
Missing	6.9 (4.5, 10.5)	8.3 (4.9, 13.6)	9.0 (5.4, 14.5)	7.9 (6.2, 10.1)
Number of lifetime sex partners				
0	3.7 (2.0, 6.6)	1.2 (0.3, 4.1)	0.6 (0.1, 2.6)	2.0 (1.1, 3.8)
1	17.7 (12.0, 25.4)	14.0 (9.5, 20.1)	13.6 (8.7, 20.8)	15.4 (12.0, 19.5)
2	11.3 (8.1, 15.7)	8.8 (4.9, 15.3)	8.7 (5.7, 13.0)	9.8 (7.3, 13.1)
3–5	21.9 (16.3, 28.7)	24.1 (17.2, 32.7)	21.2 (16.3, 27.1)	22.3 (18.8, 26.2)
6	29.8 (24.6, 35.5)	37.4 (30.9, 44.3)	41.0 (33.0, 49.5)	35.4 (31.6, 39.4)
Missing	15.6 (10.5, 22.5)	14.5 (10.0, 20.6)	14.8 (10.1, 21.3)	15.0 (13.1, 17.2)

Abbreviations: BV, bacterial vaginosis; CI, confidence interval; MEP, monoethyl phthalate; MnBP, mono-*n*-butyl phthalate; DEHP, di(2-ethylhexyl) phthalate; HS, high school; BMI, body mass index.

Table 2.

Associations of Phthalate Quartiles with Odds of Nugent-Score BV Results Among U.S. Women Aged 18–49, 2001–2004.^a

	OR of Intermediate Nugent Score (95% CI)		OR of High Nugent-Score BV (95% CI)	
	Unadjusted ^b	Adjusted for Covariates ^{b,c}	Unadjusted ^b	Adjusted for Covariates ^{b,c}
				Adjusted for Creatinine ^{c,d}
MEP				
Q1	ref	ref	ref	ref
Q2	0.68 (0.34, 1.35)	0.67 (0.33, 1.38)	1.01 (0.58, 1.75)	0.82 (0.44, 1.53)
Q3	1.41 (0.72, 2.77)	1.35 (0.67, 2.68)	1.48 (0.75, 2.93)	0.76 (0.42, 1.39)
Q4	0.94 (0.52, 1.69)	0.83 (0.44, 1.57)	1.58 (0.87, 2.87)	0.65 (0.30, 1.38)
Wald p-value	0.17	0.15	0.21	0.77
p-trend	0.77	0.85	0.12	0.83
MnBP				
Q1	ref	ref	ref	ref
Q2	1.15 (0.48, 2.76)	1.12 (0.45, 2.78)	1.71 (0.94, 3.13)	1.62 (0.88, 3.00)
Q3	0.87 (0.43, 1.75)	0.85 (0.41, 1.75)	1.88 (0.95, 3.71)	1.65 (0.82, 3.31)
Q4	1.11 (0.51, 2.41)	1.05 (0.46, 2.41)	3.01 (1.76, 5.15)	2.28 (1.24, 4.20)
Wald p-value	0.76	0.78	0.002	0.08
p-trend	0.88	0.99	<0.001	0.02
ΣDEHP metabolites				
Q1	ref	ref	ref	ref
Q2	1.68 (0.90, 3.15)	1.63 (0.84, 3.17)	2.49 (1.28, 4.86)	2.22 (1.13, 4.34)
Q3	1.15 (0.57, 2.35)	1.12 (0.53, 2.40)	2.41 (1.28, 4.56)	2.10 (1.05, 4.24)
Q4	1.19 (0.57, 2.48)	1.13 (0.51, 2.48)	2.55 (1.45, 4.47)	2.06 (1.07, 3.97)
Wald p-value	0.35	0.42	0.02	0.09
p-trend	0.98	0.87	0.03	0.20

Abbreviations: BV, bacterial vaginosis; OR, odds ratio; CI, confidence interval; MEP, monoethyl phthalate; MnBP, mono-*n*-butyl phthalate; DEHP, di(2-ethylhexyl) phthalate.

^aN=854 (all models).

^bExposures are phthalate metabolite concentrations modeled as quartiles.

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Covariates include age (continuous), body mass index (continuous), race/ethnicity (non-Hispanic White, non-Hispanic Black, or Other), educational attainment (less than high school [HS], HS graduate or equivalent, or some college or more), and self-report of vaginal douching in the past six months (yes or no).

^dExposures are phthalate metabolite concentrations divided by the ratio of observed to predicted creatinine, modeled as quartiles. Models are additionally adjusted for urinary creatinine concentration as a covariate, modeled continuously [47].

Table 3.

Association of Past Six-Month Vaginal Douching with Odds of Nugent-Score BV Among U.S. Women Aged 18–49, 2001–2004, with and Without Adjustment for MEP and MnBP Exposure.^a

	OR of Intermediate Nugent Score (95% CI)	OR of High Nugent- Score BV (95% CI)
<i>Model 1^b</i>		
Past six-month vaginal douching		
No	ref	ref
Yes	1.40 (0.73, 2.68)	2.53 (1.40, 4.59)
p-value	0.30	0.003
<i>Model 2 (with MEP)^c</i>		
Past six-month vaginal douching		
No	ref	ref
Yes	1.43 (0.75, 2.73)	2.69 (1.45, 4.97)
p-value	0.27	0.003
<i>Model 3 (with MnBP)^d</i>		
Past six-month vaginal douching		
No	ref	ref
Yes	1.41 (0.73, 2.71)	2.50 (1.37, 4.56)
p-value	0.30	0.004

Abbreviations: BV, bacterial vaginosis; CI, confidence interval; MEP, monoethyl phthalate; MnBP, mono-*n*-butyl phthalate.

^aN=854 (all models).

^bAdjusted for age (continuous), body mass index (BMI; continuous), race/ethnicity (non-Hispanic White, non-Hispanic Black, or Other), educational attainment (less than high school [HS], HS graduate or equivalent, or some college or more), and creatinine concentration (continuous).

^cAdjusted for MEP concentration (divided by ratio of observed to predicted creatinine, modeled as quartiles), age, BMI, race/ethnicity, educational attainment, and creatinine concentration.

^dAdjusted for MnBP concentration (divided by ratio of observed to predicted creatinine, modeled as quartiles), age, BMI, race/ethnicity, educational attainment, and creatinine concentration.

Table 4.

Associations of Phthalate Exposures (Modeled Continuously) with Odds of Nugent-Score BV Results Among U.S. Women Aged 18–49, 2001–2004.^{a,b}

	OR of Intermediate Nugent Score (95% CI)		OR of High Nugent-Score BV (95% CI)	
	Unadjusted ^c	Adjusted ^d	Unadjusted ^c	Adjusted ^d
MEP	0.96 (0.82, 1.13)	0.93 (0.79, 1.10)	1.03 (0.84, 1.25)	0.90 (0.72, 1.12)
p-value	0.63	0.38	0.79	0.32
MnBP	0.87 (0.65, 1.16)	0.85 (0.63, 1.14)	1.17 (0.92, 1.48)	1.06 (0.82, 1.36)
p-value	0.32	0.27	0.18	0.65
ΣDEHP metabolites	0.94 (0.77, 1.15)	0.92 (0.74, 1.16)	1.00 (0.80, 1.25)	0.97 (0.75, 1.25)
p-value	0.54	0.48	0.99	0.80

Abbreviations: BV, bacterial vaginosis; OR, odds ratio; CI, confidence interval; MEP, monoethyl phthalate; MnBP, mono-*n*-butyl phthalate; DEHP, di(2-ethylhexyl) phthalate.

^aN=854 (all models).

^bExposures are phthalate concentrations divided by ratio of observed to predicted creatinine, modeled as natural log-transformed continuous variables.

^cAdjusted for urinary creatinine concentration (continuous) as a covariate.

^dAdjusted for age (continuous), body mass index (continuous), race/ethnicity (non-Hispanic White, non-Hispanic Black, or Other), educational attainment (less than high school [HS], HS graduate or equivalent, or some college or more), self-report of vaginal douching in the past six months (yes or no), and creatinine concentration (continuous).

Table 5.

Associations of Phthalate Quartiles with Odds of Intermediate or High Nugent-Score BV Results (Modeled as a Single Outcome) Among U.S. Women Aged 18–49, 2001–2004.^{a,b}

	Unadjusted OR ^c (95% CI)	Adjusted OR ^d (95% CI)
MEP		
Q1	ref	ref
Q2	1.02 (0.56, 1.86)	0.96 (0.52, 1.79)
Q3	1.08 (0.61, 1.90)	0.91 (0.50, 1.65)
Q4	0.90 (0.51, 1.60)	0.72 (0.39, 1.32)
Wald p-value	0.92	0.56
p-trend	0.58	0.16
MnBP		
Q1	ref	ref
Q2	0.84 (0.48, 1.47)	0.86 (0.48, 1.53)
Q3	1.05 (0.54, 2.03)	0.96 (0.47, 1.93)
Q4	0.99 (0.59, 1.64)	0.86 (0.49, 1.51)
Wald p-value	0.77	0.93
p-trend	0.79	0.68
ΣDEHP metabolites		
Q1	ref	ref
Q2	0.97 (0.62, 1.50)	1.00 (0.64, 1.58)
Q3	1.45 (0.81, 2.60)	1.36 (0.76, 2.45)
Q4	0.90 (0.52, 1.56)	0.83 (0.45, 1.55)
Wald p-value	0.51	0.45
p-trend	0.57	0.39

Abbreviations: BV, bacterial vaginosis; OR, odds ratio; CI, confidence interval; MEP, monoethyl phthalate; MnBP, mono-*n*-butyl phthalate; DEHP, di(2-ethylhexyl) phthalate.

^aN=854 (all models).

^bExposures are phthalate concentrations divided by the ratio of observed to predicted creatinine, modeled as quartiles.

^cAdjusted for urinary creatinine concentration as a covariate.

^dAdjusted for age (continuous), body mass index (continuous), race/ethnicity (non-Hispanic White, non-Hispanic Black, or Other), educational attainment (less than high school [HS], HS graduate or equivalent, or some college or more), self-report of vaginal douching in the past six months (yes or no), and creatinine concentration (continuous).

Table 6.

Associations of Phthalate Quartiles with Odds of Nugent-Score BV Results Among U.S. Women Aged 18–49, 2001–2004 (Unadjusted for Past Six-Month Vaginal Douching).^a

	OR of Intermediate Nugent Score (95% CI)		OR of High Nugent-Score BV (95% CI)	
	Adjusted for Covariates ^{b,c}	Adjusted for Covariates and Creatinine ^{b,d}	Adjusted for Covariates ^{b,c}	Adjusted for Covariates and Creatinine ^{b,d}
MEP				
Q1	ref	ref	ref	ref
Q2	0.68 (0.33, 1.40)	0.97 (0.43, 2.17)	0.88 (0.48, 1.62)	1.02 (0.54, 1.90)
Q3	1.38 (0.70, 2.74)	1.09 (0.52, 2.27)	1.25 (0.65, 2.42)	0.85 (0.48, 1.53)
Q4	0.87 (0.46, 1.64)	0.82 (0.41, 1.67)	1.15 (0.58, 2.28)	0.75 (0.35, 1.58)
Wald p-value	0.15	0.74	0.61	0.72
p-trend	1.00	0.41	0.55	0.34
MnBP				
Q1	ref	ref	ref	ref
Q2	1.13 (0.46, 2.80)	0.83 (0.46, 1.49)	1.71 (0.91, 3.20)	0.86 (0.41, 1.80)
Q3	0.85 (0.41, 1.76)	0.96 (0.44, 2.08)	1.71 (0.85, 3.43)	1.05 (0.55, 2.01)
Q4	1.09 (0.47, 2.51)	0.64 (0.31, 1.34)	2.53 (1.35, 4.75)	1.17 (0.68, 2.04)
Wald p-value	0.76	0.65	0.05	0.83
p-trend	0.92	0.23	0.01	0.39
ΣDEHP metabolites				
Q1	ref	ref	ref	ref
Q2	1.62 (0.84, 3.14)	1.33 (0.72, 2.45)	2.24 (1.15, 4.34)	0.71 (0.40, 1.26)
Q3	1.12 (0.53, 2.39)	1.40 (0.71, 2.76)	2.11 (1.07, 4.15)	1.37 (0.72, 2.60)
Q4	1.14 (0.52, 2.47)	0.98 (0.48, 2.02)	2.11 (1.14, 3.89)	0.68 (0.38, 1.23)
Wald p-value	0.42	0.37	0.07	0.25
p-trend	0.89	0.52	0.15	0.25

Abbreviations: BV, bacterial vaginosis; OR, odds ratio; CI, confidence interval; MEP, monoethyl phthalate; MnBP, mono-*n*-butyl phthalate; DEHP, di(2-ethylhexyl) phthalate.

^aN=854 (all models).

^bCovariates include age (continuous), body mass index (continuous), race/ethnicity (non-Hispanic White, non-Hispanic Black, or Other), and educational attainment (less than high school [HS], HS graduate or equivalent, or some college or more).

^cExposures are phthalate metabolite concentrations modeled as quartiles.

^dExposures are phthalate metabolite concentrations divided by the ratio of observed to predicted creatinine, modeled as quartiles. Models are additionally adjusted for urinary creatinine concentration as a covariate, modeled continuously.