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Urinary Phthalate Biomarker Concentrations and Postmenopausal Breast Cancer Risk

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Abstract

Background: Growing laboratory and animal model evidence supports the potentially carcinogenic effects of some phthalates, chemicals used as plasticizers in a wide variety of consumer products, including cosmetics, medications, and vinyl flooring. However, prospective data on whether phthalates are associated with human breast cancer risk are lacking.

Methods: We conducted a nested case-control study within the Women's Health Initiative (WHI) prospective cohort ($n = 419$ invasive case subjects and 838 control subjects). Control subjects were matched 2:1 to case subjects on age, enrollment date, follow-up time, and WHI study group. We quantified 13 phthalate metabolites and creatinine in two or three urine samples per participant over one to three years. Multivariable conditional logistic regression analysis was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for breast cancer risk associated with each phthalate biomarker up to 19 years of follow-up.

Results: Overall, we did not observe statistically significant positive associations between phthalate biomarkers and breast cancer risk in multivariable analyses (eg, 4th vs 1st quartile of diethylhexyl phthalate, OR = 1.03, 95% CI = 0.91 to 1.17). Results were generally similar in analyses restricted to disease subtypes, to nonusers of postmenopausal hormone therapy, stratified by body mass index, or to case subjects diagnosed within three, five, or ten years.

Conclusions: In the first prospective analysis of phthalates and postmenopausal breast cancer, phthalate biomarker concentrations did not result in an increased risk of developing invasive breast cancer.

Phthalates are present in many consumer products, including vinyl flooring, cosmetics, and medical supplies, and exposure is ubiquitous in the United States (1). Many phthalates interact with the estrogen receptor (ER), raising concerns for a potential impact on breast cancer (BC) risk. For example, butyl benzyl phthalate (BBP), di-n-butyl phthalate (DBP), and di(2-ethylhexyl) phthalate (DEHP) increase proliferation of ER+ (2–5) BC cell lines. In vitro screening assays demonstrate estrogenic effects of BBP, DBP, di-isobutyl phthalate (DiBP), and diethyl phthalate (DEP) (6,7). DEP also had estrogenic effects in MCF-7 cells and in

female rats (8); however, anti-estrogenic effects of BBP also have been reported (9).

Three retrospective breast cancer case-control studies report mixed findings. One reported increased BC risk with DEHP and DEP metabolites, but an inverse association with monobenzyl phthalate (MBzP) (10). Another reported increased BC risk associated with a different DEHP metabolite, but no association with other phthalate biomarkers (11). The most recent study reported statistically nonsignificant inverse associations between phthalate metabolites and BC risk (12). These prior studies are

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all subject to important limitations. Notably, the retrospective case-control study design is problematic because phthalate exposure may occur through contact with medical equipment during diagnosis and treatment. Further, because phthalates are rapidly metabolized and excreted (13), a single urine specimen does not reflect longer-term or earlier phthalate exposure, which are of greatest relevance to cancer risk.

Prospective studies with repeated measurements of multiple phthalate biomarkers can provide critical data to address the important question of whether phthalates impact breast health. We conducted a nested case-control study of phthalate biomarkers and BC risk within the Women's Health Initiative (WHI), including up to three replicate measurements of urinary phthalate biomarkers among 419 case subjects and 838 matched control subjects.

Methods

Study Population

The WHI recruited 161 808 postmenopausal women aged 50–79 years from 40 clinical centers nationwide between October 1, 1993, and December 21, 1998 (14). The WHI included three clinical trials (CT; $n=68\,132$) and an observational study (OS; $n=93\,676$). Three WHI clinical sites ($n=11\,020$) included a bone density substudy for all participants and were the only sites that collected urine samples. Participants provided first morning void urine samples at the baseline clinic screening visit, and additional samples were provided at year 1 (AV1; CT only) and year 3 (AV3; CT + OS) clinic visits. We included participants with no prior cancer history, other than nonmelanoma skin cancer, and who had at least two urine samples available. A total of 168 (40.1%) case and 336 (40.1%) control subjects had three urine samples available for analysis.

BC diagnoses were self-reported annually and subsequently adjudicated by trained physicians using medical records including pathology data. We included all adjudicated case subjects of primary invasive BC occurring among eligible women diagnosed after AV3 and during their WHI follow-up through 2013. Cases diagnosed prior to AV3 were excluded to ensure that urinary phthalate biomarker measurements reflected prediagnostic levels. Control subjects were selected using incidence density matching from among eligible participants who were not diagnosed with BC and were individually matched on enrollment date, length of follow-up, age at enrollment (± 3 years), and WHI study component (CT/OS) in a 2:1 ratio to case subjects. If more than two eligible control subjects existed as a potential match for a case subject, two were randomly selected.

All participants provided written informed consent upon enrollment into WHI. The WHI was approved by institutional review boards (IRBs) at each clinical center, with additional IRB approval for this analysis from the University of Massachusetts Amherst. The involvement of the Centers for Disease Control and Prevention (CDC) laboratory in the analysis of samples did not constitute engagement in human subjects research.

Data Ascertainment

Based on prior evidence of association with either phthalate exposure and/or BC, we included the following additional covariates, measured at WHI enrollment, as potential confounders: race/region; education level; neighborhood socioeconomic status (SES) index (15); body mass index (BMI); total physical

activity metabolic equivalent task (MET) hours per week; smoking status; alcohol use; Healthy Eating Index (HEI) score (16); total dietary energy intake; hormone therapy (HT) use at enrollment; age at menarche; parity; age at first birth; breastfeeding history; age at menopause; Gail score; diabetes; high cholesterol; hypertension; OS participant; and HT, dietary modification, and calcium and vitamin D trial assignments. Characteristics of BC case subjects, including ER and progesterone receptor (PR) status, were obtained from medical records during the adjudication process; ER and/or PR status was missing for 23 case subjects.

Quantification of Phthalate Biomarkers and Creatinine

WHI followed a standard urine collection, processing, and storage protocol. First morning void urine samples were collected at home and processed less than 30 minutes upon clinic arrival. Urine samples were centrifuged for 5 minutes at 1330 $\times$ g; 1.8 mL aliquots were frozen and shipped to McKesson Bioservices packed in dry ice via overnight FedEx then stored at -70°C .

Phthalate metabolites are quantified to ensure that measured concentrations relate to endogenous exposures. The CDC measured 13 phthalate metabolites in urine samples provided at baseline, AV1, and AV3 (mono-*n*-butyl phthalate [MBP], MBzP, mono-carboxynonyl phthalate [MCNP], mono-carboxyoctyl phthalate [MCOP], mono-3-carboxylpropyl phthalate [MCPP], mono(2-ethyl-5-carboxypentyl) phthalate [MECPP], mono-(2-ethyl-5-hydroxyhexyl) phthalate [MEHHP], mono-(2-ethylhexyl) phthalate [MEHP], mono-(2-ethyl-5-oxohexyl) phthalate [MEOHP], monoethyl phthalate [MEP], mono-hydroxybutyl phthalate [MHBP], mono-hydroxyisobutyl phthalate [MHiBP], and monoisobutyl phthalate [MiBP]). The glucuronidated phthalate monoester undergoes enzymatic deconjugation followed by online solid phase extraction and high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry. Samples were randomly distributed through the batches, with all replicates from case and matched control subjects analyzed together. A blinded 10% quality control sample was included and used to estimate coefficient of variations (CVs): MBP 5.4%, MBzP 6.1%, MCNP 4.7%, MCOP 6.3%, MCPP 5.8%, MECPP 4.3%, MEHHP 5.4%, MEHP 19.5%, MEOHP 6.0%, MEP 3.1%, MHBP 9.0%, MHiBP 21.9%, and MiBP 10.3%. Laboratory staff were masked to the identity, disease status, and demographic and risk factor characteristics of the samples. Creatinine was measured using a Roche Modular P Chemistry Analyzer (Indianapolis, IN) and an enzymatic assay. The limit of detection (LOD) for creatinine was 1 mg/dL and the CV was 2.5%.

Statistical Analysis

We imputed phthalate metabolite concentrations measured at less than LOD ($<1\%$ of observations) as the LOD/ $\sqrt{2}$ (17). We grouped phthalate biomarkers by their parent phthalate by dividing the concentrations of each metabolite of a single parent by its molecular weight and then summing concentrations across metabolites (18,19): Σ DEHP (MEHHP, MEHP, MECPP, MEOHP), Σ DBP (MBP, MHBP), and Σ DiBP (MiBP, MHiBP). Phthalate biomarker concentrations were natural log-transformed to improve normality. We calculated intraclass correlation coefficients (ICC) using a one-way random effects model on the creatinine corrected values to characterize the within- and between-participant variability across replicate samples and

Pearson correlation coefficients to evaluate correlations among the phthalate biomarker concentrations, using the individual replicate measures.

For further analyses, we averaged the creatinine-standardized phthalate biomarker concentrations across replicate measurements per participant and used the arithmetic average values in analyses. Geometric means for each phthalate biomarker were compared between BC case and control subjects and ER/PR status using the generalized linear models to appropriately account for the matched study design.

We fit conditional logistic regression models to calculate odds ratios (ORs) and 95% confidence intervals (CIs) to characterize associations between creatinine-standardized phthalate biomarkers and BC, both as continuous (natural log-transformed) variables and categorized into quartiles according to the control subject distribution. We evaluated the aforementioned variables as confounders by first fitting a multivariable model including these potential confounders, and then testing the statistical significance of each covariate using likelihood ratio tests, retaining all covariates with a *P* value less than .25 as a conservative approach given that a common set of covariates was to be identified for adjustment in separate models for each phthalate biomarker. The final set of adjustment factors was age, race/region, neighborhood SES index, BMI, alcohol use, smoking status, Gail score, HT use, HT trial assignment, dietary modification trial assignment, and calcium and vitamin D trial assignment. Tests of linear trend were conducted by testing the significance of an ordinal variable coding for quartiles of phthalate biomarkers.

We repeated analyses stratifying by ER/PR status and BMI and testing for effect modification using likelihood ratio tests. In addition, we conducted a sensitivity analysis evaluating the role of phthalate biomarker concentration on BC risk among women without HT use (based on both self-report and HT trial assignment) between baseline and AV3. Finally, we repeated analyses restricting to case subjects diagnosed within three or five years of AV3 to evaluate short-term associations. *P* values less than .05 were considered as statistically significant. Statistical analyses were conducted using Stata v15.0 (Stata Corp, College Station, TX) and SAS v9.4 (SAS Institute, Cary, NC).

Because urinary biomarker concentrations exhibit within-person variability, we explored a statistical approach to adjust for the resultant attenuation in odds ratio (20–22). In brief, we used the phthalate-specific intraclass correlation coefficients to correct the calculated odds ratio, using publicly available SAS macros (23). These results were similar in direction of associations compared to our primary analysis without measurement error correction, although the confidence intervals were comparatively wide, as expected. We also evaluated choosing a single measurement of phthalate biomarkers (i.e. baseline or AV3), and results were as when using the average across replicates. Therefore, we present the uncorrected odds ratios and 95% confidence intervals, based on average urinary phthalate biomarker concentrations standardized by creatinine. All statistical tests were two-sided.

Results

The average (SD) age of case subjects and control subjects was 62.56 (6.93) and 62.46 (6.86) years, respectively (Table 1). Case subjects were more often obese, current smokers, and less physically active than control subjects, and Gail risk score was

higher among case subjects. The majority of case subjects had ER+/PR+ disease (74.0%).

Geometric mean concentrations of phthalate biomarkers were similar between case and control subjects (Table 2). ICCs were all no more than 0.12. Metabolite concentrations from the same parent phthalate were highly correlated (e.g. MHiBP and MiBP, $r = .95$), whereas others were moderately (e.g. MBzP and MHBP, $r = .74$) or not correlated (Supplementary Table 1, available online).

In general, phthalate biomarker concentrations were not associated with increased BC risk, either overall or by disease subtype (Tables 3 and 4; e.g. 4th vs 1st quartile of Σ DEHP: OR = 1.03, 95% CI = 0.91 to 1.17). There was a suggestive inverse association between MBzP and overall BC (4th vs 1st quartile: OR = 0.76, 95% CI = 0.52 to 1.09, $P_{\text{trend}} = .03$), which persisted among ER+/PR+ case subjects (4th vs 1st quartile: OR = 0.70, 95% CI = 0.44 to 1.12, $P_{\text{trend}} = .08$). Σ DiBP concentrations exhibited an inverse association with BC risk (e.g. 4th vs 1st quartile of Σ DiBP: OR = 0.69, 95% CI = 0.47 to 1.00, $P_{\text{trend}} = .07$).

We repeated analyses restricting to case subjects diagnosed within three ($n = 113$ case subjects, $n = 215$ control subjects) or five ($n = 183$ case subjects, $n = 348$ control subjects) years of AV3 (Supplementary Table 3, available online). We observed a statistically significant positive association with Σ DBP concentrations and ER+/PR+ BC within three years (4th vs 1st quartile: OR = 9.96, 95% CI = 1.93 to 51.27; $P_{\text{trend}} = .07$), which was not apparent within five years (4th vs 1st quartile: OR = 1.88, 95% CI = 0.82 to 4.29; $P_{\text{trend}} = .79$). In general, other associations were not statistically significant but were suggestive of increased risk within three years, although closer to the null within five years.

We observed generally similar results when restricting to participants with no HT use (89 case subjects; 113 control subjects; Supplementary Table 4, available online). However, Σ DBP, MCNP, MCOP, and MCPP were not statistically significantly associated with increased risk, whereas Σ DiBP, MBzP, and MEP were not statistically significantly associated with decreased risk. Results were consistent in analyses stratified on BMI category (Supplementary Table 5, available online).

Discussion

In this prospective study, we report no association between urinary phthalate biomarker concentrations and increased BC risk. There were suggestive inverse associations between MBzP concentrations and risk of invasive and ER+/PR+ disease and an inverse association between Σ DiBP and invasive BC (although not statistically significant). In general, however, the lack of statistically significant associations between urinary phthalate biomarker concentrations persisted in analyses restricted to disease subtypes, nonusers of HT, or case subjects diagnosed within three or five years of exposure assessment or when stratified on BMI.

Phthalate biomarker concentrations exhibited high within-person variability over a three-year period, which is consistent with prior investigations (19,24–28) and suggests that a single urinary measurement of phthalate biomarkers insufficiently characterizes exposure over a three-year period. We addressed this concern by incorporating up to three repeated measurements of phthalate biomarkers per participant. We explored various approaches to analyzing these data, including application of statistical measurement error correction methods (20,21). Regardless of the analytic technique utilized, null results persisted. We conclude that any large increases in BC risk

Table 1. Descriptive characteristics of study group at enrollment, n = 1257

Characteristic	Case subjects n = 419	Control subjects n = 838	P*
Age, mean (SD), y	62.56 (6.93)	62.46 (6.86)	.82
Race/region, No. (%)			.17
White/Northeast	149 (35.6)	318 (37.9)	
White/South	77 (18.4)	138 (16.5)	
White/West	117 (27.9)	246 (29.4)	
Non-white/Northeast	10 (2.4)	18 (2.1)	
Non-white/South	45 (10.7)	59 (7.0)	
Non-white/West	21 (5.0)	59 (7.0)	
Education level; No. (%)			.65
Less than high school degree	111 (26.7)	234 (28.0)	
Post-high school/some college	148 (35.6)	308 (36.9)	
College degree or higher	157 (37.7)	293 (35.1)	
Neighborhood SES index, mean (SD)	72.31 (9.47)	73.48 (8.33)	.03
Body mass index, mean (SD), kg/m ²	29.03 (5.94)	27.65 (5.60)	<.001
Body mass index category, No. (%)			<.001
Underweight/normal, <25 kg/m ²	120 (28.8)	299 (35.9)	
Overweight, 25–<30 kg/m ²	144 (34.5)	305 (36.7)	
Obese, ≥30 kg/m ²	153 (36.7)	228 (27.4)	
Alcohol intake, No. (%)			.29
0 drinks/wk	137 (32.9)	275 (33.1)	
<1 drink/wk	136 (32.7)	295 (35.5)	
1–6 drinks/wk	95 (22.8)	193 (23.2)	
≥7 drinks/wk	48 (11.5)	69 (8.3)	
Smoking status, No. (%)			<.001
Never smoked	209 (50.1)	489 (59.6)	
Past smoker	173 (41.5)	288 (35.1)	
Current smoker	35 (8.4)	43 (5.2)	
Total physical activity, MET-h/wk, mean (SD)	10.25 (12.04)	12.89 (15.42)	<.001
Healthy Eating Index score, mean (SD)	66.03 (11.40)	67.13 (10.65)	.09
Gail risk score, mean (SD)	1.83 (1.12)	1.65 (0.89)	<.001
Any postmenopausal hormone therapy use, No. (%)			.40
Never use	197 (47.1)	387 (46.2)	
Past user	54 (12.9)	132 (15.8)	
Current user	167 (40.0)	318 (38.0)	
OS participation, No. (%)	221 (52.7)	441 (52.6)	.97
Hormone therapy trial assignment, No. (%)			.24
Not randomly assigned	349 (83.3)	683 (81.5)	
E-alone intervention	13 (3.1)	31 (3.7)	
E-alone control	17 (4.1)	41 (4.9)	
E + P intervention	28 (6.7)	41 (4.9)	
E + P control	12 (2.9)	42 (5.0)	
Dietary modification trial assignment, No. (%)			.74
Not randomly assigned	275 (65.6)	547 (65.3)	
Intervention	51 (12.2)	114 (13.6)	
Control	93 (22.2)	177 (21.1)	
Calcium and vitamin D trial assignment, No. (%)			.19
Not randomly assigned	326 (77.8)	613 (73.2)	
Intervention	49 (11.7)	123 (14.7)	
Control	44 (10.5)	102 (12.2)	

(continued)

Table 1. (continued)

Characteristic	Case subjects n = 419	Control subjects n = 838	P*
ER/PR status, No. (%)			–
ER+/PR+	288 (74.0)	–	
ER+/PR–	40 (10.3)	–	
ER–/PR–	61 (15.7)	–	

*P values calculated from χ^2 tests or t tests for categorical and continuous variables, respectively. All tests were two-sided. E = estrogen; ER = estrogen receptor; MET = metabolic equivalent task; OS = observational study; P = progesterin; PR = progesterone receptor; SES = socioeconomic status.

Table 2. Geometric mean values and intraclass correlation coefficients (ICCs) of phthalate biomarker concentrations by case/control subject status (n = 1257)

Phthalate biomarker, $\mu\text{g/g}$ creatinine	Geometric mean (SD)		P*	ICC (95% CI)†
	Case subjects (n = 419)	Control subjects (n = 838)		
MEP	131.56 (2.85)	140.52 (2.82)	.28	0.07 (0.04 to 0.13)
MBP	39.35 (2.15)	38.66 (2.08)	.69	0.05 (0.02 to 0.12)
MHBP	3.15 (2.24)	3.22 (2.19)	.61	0.08 (0.05 to 0.13)
ΣDBP , $\mu\text{mol/g}$ creatinine	0.19 (2.14)	0.19 (2.07)	.77	0.05 (0.02 to 0.12)
MiBP	3.14 (2.31)	3.30 (2.23)	.32	0.06 (0.03 to 0.11)
MHiBP	1.32 (2.02)	1.42 (1.94)	.10	0.02 (0.003 to 0.10)
ΣDiBP , $\mu\text{mol/g}$ creatinine	0.02 (2.14)	0.02 (2.06)	.21	0.04 (0.02 to 0.10)
MBzP	17.42 (2.01)	18.82 (2.02)	.06	0.06 (0.03 to 0.14)
MCPP	4.94 (1.85)	4.92 (1.81)	.90	0.12 (0.09 to 0.17)
MEHP	3.22 (2.39)	3.40 (2.32)	.28	0.03 (0.01 to 0.09)
MEHHP	29.63 (2.07)	29.36 (2.03)	.82	0.01 (0.0002 to 0.29)
MEOHP	18.22 (2.05)	18.15 (2.00)	.92	0.01 (0.0003 to 0.27)
MECPP	38.99 (1.93)	38.55 (1.91)	.77	0.01 (0.001 to 0.20)
ΣDEHP , $\mu\text{mol/g}$ creatinine	0.31 (1.96)	0.30 (1.93)	.86	0.01 (0.001 to 0.19)
MCOP	6.15 (1.94)	6.34 (1.96)	.42	0.07 (0.04 to 0.12)
MCNP	4.74 (2.05)	4.78 (2.06)	.82	0.01 (0.001 to 0.15)

*P values calculated using generalized linear models to account for matching in comparing geometric means between cases and controls. All tests are two-sided. CI = confidence interval; DBP = di-n-butyl phthalate; DEHP = di(2-ethyl-hexyl) phthalate; DiBP = di-isobutyl phthalate; MBP = mono-n-butyl phthalate; MBzP = monobenzyl phthalate; MCNP = mono-carboxynonyl phthalate; MCOP = mono-carboxyoctyl phthalate; MCPP = mono-3-carboxylpropyl phthalate; MECPP = mono(2-ethyl-5-carboxypentyl) phthalate; MEHP = mono-(2-ethyl-hexyl) phthalate; MEHHP = mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP = mono-(2-ethyl-5-oxohexyl) phthalate; MEP = monoethyl phthalate; MHBP = mono-hydroxybutyl phthalate; MHiBP = mono-hydroxyisobutyl phthalate; MiBP = monoisobutyl phthalate.

†ICC calculated using up to three replicate samples per person as the proportion of total variability that is due to between-subject variability.

associated with phthalate exposure are unlikely, although smaller biological effects, if they exist, could have been missed because of measurement error.

Given the strong possibility that measurement error may have biased our results toward null findings, it is useful to consider the direction of effect estimates even if not statistically significant. For example, we observed statistically nonsignificant positive associations with ΣDBP , MCPP, MCOP, and MCNP

Table 3. Associations between urinary phthalate biomarker concentrations modeled as continuous variables and breast cancer risk (n = 1172)*

Phthalate biomarker	Full sample (404 case subjects, 768 control subjects)		ER+/PR+ (277 case subjects, 527 control subjects)	ER-/PR- (58 case subjects, 111 control subjects)
	Unadjusted OR (95% CI)	Adjusted† OR (95% CI)	Adjusted† OR (95% CI)	Adjusted† OR (95% CI)
ΣDBP	1.03 (0.92 to 1.16)	1.04 (0.92 to 1.17)	1.12 (0.96 to 1.31)	1.71 (0.97 to 3.00)
ΣDiBP	1.05 (0.93 to 1.18)	1.10 (0.97 to 1.24)	0.98 (0.81 to 1.17)	0.70 (0.42 to 1.18)
MBzP	0.72 (0.28 to 1.88)	0.85 (0.38 to 1.91)	0.67 (0.13 to 3.44)	0.87 (0.54 to 1.40)
MCNP	1.02 (0.91 to 1.15)	1.03 (0.91 to 1.17)	0.98 (0.84 to 1.15)	0.84 (0.53 to 1.33)
MCOP	1.03 (0.91 to 1.16)	1.02 (0.90 to 1.16)	0.99 (0.84 to 1.16)	0.67 (0.27 to 1.68)
MCPP	1.03 (0.91 to 1.16)	1.02 (0.90 to 1.16)	0.98 (0.84 to 1.15)	1.56 (0.99 to 2.46)
MEP	0.99 (0.87 to 1.12)	0.93 (0.82 to 1.06)	0.93 (0.79 to 1.09)	1.02 (0.65 to 1.60)
ΣDEHP	1.01 (0.90 to 1.14)	1.03 (0.91 to 1.17)	1.07 (0.92 to 1.24)	0.62 (0.18 to 2.19)

*Odds ratios (OR) for each phthalate biomarker are for a 1 standard deviation increase in the respective biomarker. CI = confidence interval; DBP = di-n-butyl phthalate; DEHP = di(2-ethylhexyl) phthalate; DiBP = di-isobutyl phthalate; MBzP = monobenzyl phthalate; MCNP = mono-carboxynonyl phthalate; MCOP = mono-carboxyoctyl phthalate; MCPP = mono-3-carboxylpropyl phthalate; MEP = monoethyl phthalate.

†Adjusted models include the following covariates: age (continuous); race/region (white/Northeast, non-white/Northeast, white/South, non-white/South, white/West, non-white/West); neighborhood socioeconomic status index (continuous); body mass index (continuous); alcohol use (0 drinks/wk, <1 drink/wk, 1–6 drinks/wk, ≥7 drinks/wk); smoking status (never, past, current); Gail risk score (continuous); postmenopausal hormone therapy use at enrollment (never, past, current); hormone therapy trial assignment (not randomized, estrogen [E]-alone intervention, E-alone control, estrogen + progestin [E + P] intervention, E + P control); dietary modification trial assignment (not randomized, intervention, control); and calcium and vitamin D trial assignment (not randomized, intervention, control).

and invasive BC risk among women not using HT. Because HT use was very common in this study population, the numbers of non-HT users were very small for this analysis (89 case subjects and 114 control subjects), and therefore, reduced statistical power also may have contributed to the lack of statistical significance despite some strong effect estimates (e.g. 4th vs 1st quartile of MCOP: OR = 4.21, 95% CI = 0.96 to 18.54). Because phthalates are far less estrogenic than HT formulations, it is possible that HT use may mask any true effect of phthalate exposure on BC risk. It is thus noteworthy that we did observe some potential positive, but not statistically significant, associations between phthalate biomarker concentrations and BC risk among this subgroup of women. Future studies with larger numbers of non-HT users will be informative in understanding whether phthalate exposure is associated with postmenopausal BC risk among women not using HT. Such studies are especially important given the recent population decline in postmenopausal HT use (29).

Although not entirely consistent, we observed some positive effect estimates between phthalate biomarker concentrations and BCs diagnosed within three years of the last biomarker measurement, some of which were statistically significant for ER+/PR+ disease (e.g. 4th vs 1st quartile of ΣDBP: OR = 9.96, 95% CI = 1.93 to 51.27, $P_{\text{trend}} = .07$). Interestingly, effect estimates were closer to the null and not statistically significant when analyses were extended to include case subjects diagnosed within five years or among the full study population. However, these results may reflect random variation rather than true associations. Additional investigations will be useful for understanding whether phthalate biomarker concentrations predict short-term, but not long-term, BC risk.

Although we report the first prospective analysis of phthalate biomarker concentrations in relation to BC risk, a few retrospective studies have been conducted. Our observed inverse association with MBzP concentrations, overall and in ER+/PR+ subtypes, is consistent with similar reports in case-control studies of Mexican (10) and northeastern US women (12), but no association was observed among Alaskan Native women (11). Many laboratory and animal studies report estrogenic effects of BBP (4,5,7,9,30–35), the precursor of MBzP. However,

anti-estrogenic effects of BBP also have been reported (9,36), which support our findings.

We report no association between urinary concentrations of ΣDEHP, MCOP, and MCNP and inverse associations with ΣDiBP/MiBP, consistent with prior studies (10–12). In the case-control studies that measured MCPP, both reported inverse associations with BC risk (10,12) that were not supported by our observed null association. Likewise, we did not observe the inverse associations with ΣDBP/MBP and ΣDEHP/MEOHP reported by Lopez-Carillo et al. (10). Positive associations with MEP (10) and MEHP (11) have been reported, but our study and others are not consistent with these findings. Differences in observed associations across studies may reflect differences in population exposure resulting in different phthalate biomarker distributions, differences in study design, the potential impact of recent exposure to phthalates via medical equipment and medications among case subjects, or random variation given the relatively low reproducibility of urinary concentrations of phthalate biomarkers.

This study must be considered in the context of some additional limitations. First, our participants were postmenopausal at the time of sample donation, and this timing may not be relevant for the initiation of BC. The importance of early life exposures to later BC risk is increasingly appreciated. Some (37–42), but not all (43–49), studies observed associations between phthalate biomarkers and breast development and earlier puberty. Exposure to endocrine-disrupting chemicals may be important at critical periods earlier in life, such as during adolescence or prior to first pregnancy; however, we were unable to evaluate exposure at these time periods. Future studies taking a life-course approach would be useful and may identify associations that were not apparent given exposure assessed after menopause. Second, we performed numerous statistical tests and did not adjust for multiple comparisons to avoid being overly conservative given the measurement error issues previously noted. It is possible, therefore, that the statistically significant associations we observed might reflect type I error. Finally, although our study population was multi-ethnic, the number of BC case subjects among racial and ethnic minority groups was insufficient to conduct stratified analyses.

Table 4. Associations between urinary phthalate biomarker concentrations and breast cancer risk (n = 1172)

Phthalate biomarker, µg/g creatinine	Full sample		ER+/PR+		ER-/PR-	
	No. case subjects/ control subjects	404 case subjects, 768 control subjects	No. case subjects/ control subjects	277 case subjects, 527 control subjects Adjusted* OR (95% CI)	No. case subjects/ control subjects	58 case subjects, 111 control subjects Adjusted* OR (95% CI)
		OR (95% CI)				
ΣDBP†						
0.01–0.12	102/197	1.00 (Referent)	69/132	1.00 (Referent)	17/36	1.00 (Referent)
0.12–0.18	112/183	1.21 (0.86 to 1.70)	80/126	1.40 (0.88 to 2.22)	16/31	0.86 (0.25 to 2.92)
0.18–0.29	79/210	0.73 (0.52 to 1.04)	50/145	0.70 (0.43 to 1.13)	12/27	0.70 (0.20 to 2.46)
0.29–11.81	111/178	1.24 (0.88 to 1.73)	78/124	1.28 (0.82 to 2.01)	13/17	1.53 (0.41 to 5.73)
P _{trend} †		.79		.91		.66
ΣDiBP†						
0.00–0.01	108/191	1.00 (Referent)	75/133	1.00 (Referent)	17/24	1.00 (Referent)
0.01–0.02	106/188	1.01 (0.72 to 1.41)	73/128	1.06 (0.67 to 1.67)	15/30	1.54 (0.39 to 6.06)
0.02–0.03	107/188	1.00 (0.72 to 1.41)	72/126	0.99 (0.63 to 1.55)	14/29	0.57 (0.14 to 2.22)
0.03–1.55	83/201	0.73 (0.52 to 1.04)	57/140	0.64 (0.40 to 1.02)	12/28	0.48 (0.12 to 1.93)
P _{trend} †		.11		.07		.15
MBzP						
1.82–11.93	122/171	1.00 (Referent)	83/113	1.00 (Referent)	185/24	1.00 (Referent)
11.98–18.01	105/186	0.79 (0.56 to 1.11)	73/141	0.83 (0.52 to 1.31)	14/25	0.34 (0.08 to 1.51)
18.03–27.42	78/215	0.52 (0.37 to 0.74)	57/137	0.65 (0.41 to 1.03)	10/34	0.23 (0.05 to 0.97)
27.48–5767.02	99/196	0.72 (0.51 to 1.01)	64/136	0.70 (0.44 to 1.12)	16/28	0.51 (0.15 to 1.68)
P _{trend} †		.01		.08		.19
MCNP						
0.78–2.98	96/194	1.00 (Referent)	68/129	1.00 (Referent)	16/24	1.00 (Referent)
2.98–4.29	106/187	1.14 (0.82 to 1.59)	75/134	1.06 (0.68 to 1.64)	13/26	0.66 (0.15 to 2.95)
4.29–6.42	97/193	1.02 (0.72 to 1.45)	60/131	0.80 (0.49 to 1.30)	19/32	0.97 (0.23 to 4.01)
6.42–227.60	105/194	1.09 (0.78 to 1.53)	74/133	1.13 (0.70 to 1.80)	10/29	0.27 (0.06 to 1.33)
P _{trend} †		.78		.92		.20
MCOP						
1.27–4.03	109/182	1.00 (Referent)	74/133	1.00 (Referent)	17/22	1.00 (Referent)
4.04–5.76	97/195	0.83 (0.58 to 1.17)	69/144	0.88 (0.55 to 1.40)	5/23	0.06 (0.01 to 0.64)
5.77–8.90	101/192	0.87 (0.62 to 1.22)	67/119	1.06 (0.66 to 1.69)	22/37	1.00 (0.21 to 4.78)
8.90–558.83	97/199	0.81 (0.57 to 1.16)	67/131	1.02 (0.62 to 1.65)	14/29	0.81 (0.17 to 3.85)
P _{trend} †		.32		.73		.84

(continued)

Table 4. (continued)

Phthalate biomarker, µg/g creatinine	No. case subjects/ control subjects	Full sample		ER+/PR+		ER-/PR-	
		404 case subjects, 768 control subjects		No. case subjects/ control subjects	277 case subjects, 527 control subjects Adjusted* OR (95% CI)	No. case subjects/ control subjects	58 case subjects, 111 control subjects Adjusted* OR (95% CI)
		OR (95% CI)	Adjusted* OR (95% CI)				
MCPP							
0.87–3.32	111/185	1.00 (Referent)	1.00 (Referent)	84/122	1.00 (Referent)	12/27	1.00 (Referent)
3.32–4.65	92/197	0.79 (0.56 to 1.11)	0.80 (0.55 to 1.16)	63/133	0.69 (0.44 to 1.08)	11/30	0.96 (0.22 to 4.14)
4.66–6.70	93/199	0.79 (0.55 to 1.12)	0.92 (0.62 to 1.35)	56/134	0.64 (0.40 to 1.04)	19/34	2.89 (0.69 to 12.14)
6.72–101.32	108/187	1.00 (0.70 to 1.41)	1.07 (0.73 to 1.57)	74/138	0.79 (0.50 to 1.25)	16/20	6.71 (1.09 to 41.37)
<i>P</i> _{trend} †		.92	.61		.28		.02
MEP							
9.28–67.58	110/185	1.00 (Referent)	1.00 (Referent)	73/130	1.00 (Referent)	13/26	1.00 (Referent)
67.66–120.78	98/201	0.81 (0.58 to 1.14)	0.84 (0.58 to 1.21)	72/137	0.99 (0.63 to 1.56)	14/30	0.61 (0.16 to 2.24)
120.85–237.42	105/189	0.91 (0.65 to 1.27)	0.88 (0.61 to 1.26)	74/134	0.93 (0.60 to 1.44)	18/24	1.62 (0.42 to 6.23)
238.74–14226.57	91/193	0.79 (0.55 to 1.12)	0.70 (0.47 to 1.03)	58/126	0.77 (0.47 to 1.25)	13/31	0.38 (0.07 to 1.99)
<i>P</i> _{trend} †		.31	.11		.27		.64
ΣDEHP†							
0.05–0.20	107/190	1.00 (Referent)	1.00 (Referent)	74/132	1.00 (Referent)	16/25	1.00 (Referent)
0.20–0.29	98/192	0.94 (0.67 to 1.31)	0.99 (0.69 to 1.42)	69/131	0.92 (0.59 to 1.43)	12/30	0.48 (0.10 to 2.33)
0.29–0.43	85/210	0.73 (0.51 to 1.04)	0.76 (0.52 to 1.10)	59/150	0.64 (0.40 to 1.03)	12/29	1.21 (0.31 to 4.76)
0.43–11.31	114/176	1.16 (0.83 to 1.62)	1.18 (0.81 to 1.70)	75/114	1.07 (0.67 to 1.70)	18/27	0.50 (0.12 to 2.13)
<i>P</i> _{trend} †		.65	.64		.87		.66

*Adjusted models include the following covariates: age (continuous); race/region (white/Northeast, non-white/Northeast, white/South, non-white/South, white/West, non-white/West); neighborhood socioeconomic status index (continuous); body mass index (continuous); alcohol use (0 drinks/wk, <1 drink/wk, 1–6 drinks/wk, ≥7 drinks/wk); smoking status (never, past, current); Gail risk score (continuous); postmenopausal hormone therapy use at enrollment (never, past, current); hormone therapy trial assignment (not randomly assigned, estrogen [E]-alone intervention, E-alone control, estrogen+progestin [E+P] intervention, E+P control); dietary modification trial assignment (not randomly assigned, intervention, control); and calcium and vitamin D trial assignment (not randomly assigned, intervention, control). CI = confidence interval; DBP = di-n-butyl phthalate; DEHP = di(2-ethylhexyl) phthalate; DiBP = di-isobutyl phthalate; MBzP = monobenzyl phthalate; MCNP = mono-carboxynonyl phthalate; MCOP = mono-carboxyoctyl phthalate; MEP = monoethyl phthalate; OR = odds ratio.

†ΣDBP, ΣDiBP, and ΣDEHP reported as µmol/g creatinine.

#P values calculated from a test of statistical significance of an ordinal variable for the phthalate biomarker in quartiles. All tests were two-sided.

Our results are strengthened by the prospective study design, which is a notable improvement over prior retrospective studies. Also, we utilized a large number of adjudicated, invasive BC case subjects and matched control subjects on factors including age at enrollment, enrollment date, and length of follow-up, which aided in adjusting for potential confounding in our analyses, along with the adjustments permitted by the extensive covariate data available through the WHI. Measured concentrations of urinary phthalate metabolites were generally similar to those measured among the US general population during the same time period (50). Importantly, we incorporated repeated measurements of a broad panel of phthalate metabolites, which allowed for better characterization of phthalate exposure than prior work. The ICC increases with the number of replicates (k) used according to the following equation: $ICC_{adj} = ICC/[ICC + (1-ICC)/k]$. Assuming an observed ICC of 0.10, six replicates would be required to achieve a moderate ICC of 0.40, whereas 21 would be needed to achieve an ICC of 0.70. Such estimates will be useful for planning future epidemiologic studies of phthalates and other nonpersistent endocrine-disrupting chemicals.

In summary, our results suggest that phthalate biomarker concentrations are unlikely to be related to large increases in BC risk, although smaller associations are possible. Some phthalate biomarker concentrations exhibited suggestive associations with decreased BC risk, although confirmation of these findings in future studies is needed. Additionally, prior work has reported positive associations between phthalate biomarkers and conditions that may themselves affect breast cancer risk, including elevated BMI (51,52), weight gain (53), diabetes (54), and insulin resistance (55); therefore, phthalate exposures could indirectly affect postmenopausal breast cancer risk. Although our study is the most comprehensive and methodologically rigorous evaluation to date, we focused only on postmenopausal exposure to phthalates in relation to risk of postmenopausal BC. Given the continued ubiquity of these chemicals in consumer products, additional exploration of the potential impact of phthalate exposure at various periods during the life course in relation to later BC risk will be useful to fully understand the risk profile of these chemicals.

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