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Phthalates and risk of endometriosis

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ABSTRACT

Background: Phthalates are ubiquitous environmental chemicals with endocrine disruptive properties. The impact of these chemicals on endocrine-related disease in reproductive-age women is not well understood.
Objective: To investigate the relationship between urinary phthalate metabolite concentrations and the risk of a hormonally-driven disease, endometriosis, in reproductive-age women.
Methods: We used data from a population-based case-control study of endometriosis, conducted among female enrollees of a large healthcare system in the U.S. Pacific Northwest. We measured urinary phthalate metabolite concentrations on incident, surgically-confirmed cases ($n=92$) diagnosed between 1996 and 2001 and population-based controls ($n=195$). Odds ratios (OR), and 95% confidence intervals (CI) were estimated using unconditional logistic regression, adjusting for urinary creatinine concentrations, age, and reference year.
Results: The majority of women in our study had detectable concentrations of phthalate metabolites. We observed a strong inverse association between urinary mono-(2-ethyl-5-hexyl) phthalate (MEHP) concentration and endometriosis risk, particularly when comparing the fourth and first MEHP quartiles (aOR 0.3, 95% CI: 0.1–0.7). Our data suggested an inverse association between endometriosis and urinary concentrations of other di-2-ethylhexyl phthalate (DEHP) metabolites (mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP)) and ∑DEHP, however, the confidence intervals include the null. Our data also suggested increased endometriosis risk with greater urinary concentrations of mono-benzyl phthalate (MBzP) and mono-ethyl phthalate (MEP), although the associations were not statistically significant.
Conclusions: Exposure to select phthalates is ubiquitous among female enrollees of a large healthcare system in the U.S. Pacific Northwest. The findings from our study suggest that phthalates may alter the risk of a hormonally-mediated disease among reproductive-age women.

1. Introduction

Phthalates (dialkyl or alkyl aryl esters of *o*-phthalic acid) are man-made chemicals used in numerous industrial and consumer products. These chemicals are of potential interest to human health as select phthalates have been shown *in vitro* and *in vivo* in animals to exhibit endocrine disruptive properties, or to mimic or alter endogenous hormone activity (ATSDR, 1995; ATSDR, 2001; ATSDR, 2002; CERHR, 2003). Adult human exposure to phthalates is primarily through ingestion of food contaminated from food processing machines and packaging materials and dermal application of personal

Abbreviations: DEHP, di-(2-ethylhexyl) phthalate; BzBP, benzyl butyl phthalate; DEP, diethyl phthalate; DBP, dibutyl phthalate; MEHP, mono-(2-ethyl-5-hexyl) phthalate; MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MBzP, mono-benzyl phthalate; MEP, mono-ethyl phthalate; MiBP, mono-iso-butyl phthalate; MnBP, mono-n-butyl phthalate; WREN, Women's Risk of Endometriosis study; GH, Group Health; POPs, Persistent Organic Pollutants and endometriosis risk study; LOQ, limit of quantitation; BMI, body mass index; OR, odds ratio; CI, confidence interval; GM, geometric mean; NHANES, National Health and Nutrition and Evaluation Survey; DAG, directed acyclic graph.

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care and cosmetic products (ECB, 2007; IPCS, 2003; Kavlock et al., 2002a; Kavlock et al., 2002b; Wittassek et al., 2011; Wormuth et al., 2006). Exposure is also possible through inhalation of indoor air contaminated from building materials, and parenteral exposure through medical equipment such as IV tubing and blood bags (ECB, 2007; IPCS, 2003; Kavlock et al., 2002a; Kavlock et al., 2002b; Wittassek et al., 2011; Wormuth et al., 2006). The detection of select phthalate metabolites in ≥78% of the U.S. population suggests that exposure to phthalates is widespread (Silva et al., 2004).

Although exposure is common, the impact of phthalates on endocrine-related disease in reproductive-age women is not well understood. One such disease is endometriosis, a serious condition characterized by the presence of endometrial-like tissue outside of the uterus, usually in the peritoneal cavity. Endometriosis affects 6–10% of reproductive-age women, often resulting in infertility and chronic, severe pelvic pain (Eskenazi and Warner, 1997). Results of investigations into the pathophysiology of endometriosis have suggested that disease onset and progression involve steroid-related mechanisms, including hormone-related changes of the endometrium and peritoneal cavity, excess estrogen production by ectopic endometriotic lesions, and alterations in ovarian steroidogenesis (Bulun, 2009; Giudice and Kao, 2004; Ulukus et al., 2006). Thus, it is plausible that endocrine-disrupting chemicals such as phthalates may affect endometriosis risk. Four prior studies that explored endometriosis in relation to phthalates were substantially limited by the measurement of serum phthalate diester concentrations as serum is highly prone to background phthalate contamination from the collection and storage of specimens and laboratory equipment and supplies (Cobellis et al., 2003; Kato et al., 2003; Kim et al., 2011; Koch and Calafat, 2009; Reddy et al., 2006a; Reddy et al., 2006b). Additionally, since phthalate diesters are rapidly metabolized after exposure, resulting in low or transient levels in serum, body burden of these chemicals is more accurately assessed by measuring phthalate metabolites in urine (Koch and Calafat, 2009). The three epidemiologic studies that have evaluated endometriosis risk in relation to phthalate metabolite concentrations quantified in urine were limited by inadequate case definition or control selection and have yielded contradictory results (Huang et al., 2010; Itoh et al., 2009; Weuve et al., 2010). The purpose of the current analyses was to further investigate the relationship between urinary phthalate metabolite concentrations and the risk of endometriosis in reproductive-age women, using data from a U.S. case-control study that employed a population-based sampling frame and surgically confirmed cases.

2. Material and methods

2.1. Study design and population

The parent study for the current analyses was the Women's Risk of Endometriosis (WREN), a five-year population-based case-control study of endometriosis conducted among 18–49 year old female enrollees of Group Health (GH), a large mixed-model healthcare system in western Washington State (Marino et al., 2008; Marino et al., 2009). As previously described, WREN study activities entailed participation in a structured, in-person interview covering a range of topics, including reproductive history and contraceptive use as well as medical and family history and lifestyle behaviors (Marino et al., 2008; Marino et al., 2009). The cases and controls who participated in WREN and completed the interview represented 73% of those invited to participate (Marino et al., 2008). Cases ($n=340$) were female GH enrollees diagnosed for the first time with endometriosis (International Classification of Disease Ninth Revision (ICD-9) diagnostic codes 617.0–617.5, 617.8–617.9, excluding adenomyosis) between April 1, 1996 and March 31, 2001. The diagnoses were confirmed by record review indicating the direct surgical visualization of endometriosis. Cases were assigned as a reference date the first visit for symptoms leading to endometriosis diagnosis. Female GH enrollees without endometriosis were identified as potential controls from computerized GH enrollment databases, frequency matched to cases on five year age groups. Controls ($n=741$) were assigned reference dates based on the distribution of reference dates among cases. Inclusion criteria for the WREN study included enrollment in GH for at least six months prior to the reference date, an intact uterus and at least one ovary. Menopausal or post-menopausal women were not eligible for the WREN study nor were women with a past history of surgically confirmed endometriosis, as the WREN study focused on first-time diagnosis of endometriosis. After enrollment, we discovered 12 cases and 14 controls with a past history of surgically confirmed endometriosis based on information collected during the WREN study interview and excluded these participants. We also excluded three cases whose endometriosis diagnoses were not confirmed surgically and 15 cases not meeting the definition of definite or possible endometriotic disease (Holt and Weiss, 2000). This definition focuses on progressive disease with evidence of tissue invasion or interference with normal physiologic processes.

A subset of WREN study participants also took part in a two-year ancillary study, the Persistent Organic Pollutants and Endometriosis Risk (POPs), in which serum and urine samples were collected to assess exposure to organochlorine pesticides and polychlorinated biphenyls (Trabert et al., 2010). Of the 340 cases and 741 controls interviewed in the WREN study, 169 cases and 343 controls were invited to provide a urine sample after the receipt of POPs study funding; 157 cases (92.9%) and 301 controls (87.8%) agreed. For the current analysis, urinary phthalate metabolites were quantified on all WREN/POPs participants with available urine samples that had not undergone a thaw-refreeze cycle (93 cases and 198 controls). Institutional review board approval was received from the Fred Hutchinson Cancer Research Center.

2.2. Urinary phthalate measurements

Non-fasting spot urine samples were collected in person from WREN participants in 2001 and 2002 using a phthalate-free polypropylene container with a screw-top lid. Specimens were refrigerated immediately and processed by the Fred Hutchinson Cancer Research Center Specimen Processing Laboratory. Urine specimens were aliquoted into phthalate-free 30 mL flint glass vials with Teflon screw caps and stored at −20 °C until transport to the Environmental Health Laboratory at the University of Washington (UW). The UW laboratory analyzed the urine samples for eight phthalate metabolites using the method of direct injection followed by isotope-dilution high-performance liquid chromatography electrospray ionization-tandem mass spectrometry (HPLC-MS/MS) (Silva et al., 2007). The eight phthalate metabolites quantified were mono-(2-ethyl-5-hexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-benzyl phthalate (MBzP), mono-ethyl phthalate (MEP), mono-iso-butyl phthalate (MiBP), and mono-n-butyl phthalate (MnBP). These eight phthalate metabolites are hydrolytic or oxidative monoester metabolites of parent phthalate diesters, di-(2-ethylhexyl) phthalate (DEHP), benzylbutyl phthalate (BzBP), diethyl phthalate (DEP), and dibutyl phthalate (DBP) (Table 1). We selected these phthalate metabolites based on the frequency of detection in the U.S. population, endocrine disruptive properties exhibited in *in vitro* and *in vivo* animal studies, use in products specifically marketed to women, and investigation in prior studies of endometriosis. Process blanks and instrumental duplicates on 10% of samples were included in each analytic run as part of the internal laboratory control procedures. For external quality assessment of each phthalate metabolite, we included a pooled sample and a duplicate sample in each batch to monitor the interbatch and intrabatch reliability. The laboratory staff was blinded with regard to the case status of specimens and the inclusion of specimens for external quality assessment. The interbatch reliability among pooled samples was good, with a low percent of coefficient of variation (CV%) for phthalate metabolites: < 16% for MBzP and MiBP

Table 1
Parent phthalate diesters and corresponding urinary phthalate metabolites.

Parent phthalate diester	Phthalate metabolite
Di(2-ethylhexyl) phthalate (DEHP)	Mono-(2-ethyl-5-hexyl) phthalate (MEHP)[a]
	Mono- (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)[b]
	Mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP)[b]
	Mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP)[b]
Benzylbutyl phthalate (BzBP)	Mono-benzyl phthalate (MBzP)[a]
	Mono-n-butyl phthalate (MnBP)[a]
Diethyl phthalate (DEP)	Mono-ethyl phthalate (MEP)[a]
Dibutyl phthalate (DBP)	Mono-iso-butyl phthalate (MiBP)[a]
	Mono-n-butyl phthalate (MnBP)[a]

[a] Primary hydrolytic monoester metabolite.
[b] Secondary oxidative monoester metabolite.

and <5% for all other phthalate metabolites. There was strong agreement within batches, with intraclass correlation coefficient (ICC) >99% for all phthalate metabolites except MBzP (63%) and MEHP (82%).

Urinary creatinine concentrations were measured by the UW Department of Laboratory Medicine, Research Testing Services by means of the spectrophotometric Jaffe reaction rate method using the Beckman Coulter Synchron System (Beckman Coulter, Inc Brea, CA, USA). We excluded one case and two controls with creatinine concentrations >300 mg/dL, indicating dehydration which may alter renal elimination of phthalate metabolites (Barr et al., 2005). We also excluded one control with missing creatinine concentration data due to sample insufficiency, resulting in data on 92 cases and 195 controls for the current analyses.

2.3. Exposure coding

We categorized urinary phthalate metabolite concentrations into quartiles using the distribution of urinary phthalate metabolite concentrations in controls. We created three summary exposure variables among phthalate metabolites sharing a common parent phthalate diester, ΣDEHP (sum of MEHP, MEHHP, MEOHP, and MECPP), ∑BzBP (sum of MBzP and MnBP), and ΣDBP (sum of MiBP and MnBP), by imputing concentrations below the limit of quantitation (LOQ) and summing the molar concentrations of the individual phthalate metabolites in each group. The summary exposure variables were categorized by quartiles based on the distribution in controls.

We conducted single imputation for urinary phthalate metabolite and creatinine concentrations determined to be <LOQ, because of the small percent of missing data below the LOQ (4% for creatinine concentrations, <8% for all urinary phthalate metabolites except MEHP (16%)) (Table 2). The single imputation procedure was adapted from a distribution-based multiple imputation approach that entailed applying maximum likelihood to a bootstrap sample of the controls to fit a log-normal distribution to exposure, with a linear model for the mean which included age, reference year, smoking, alcohol, education, income, body mass index (BMI), and natural logarithm of imputed creatinine and assuming homogeneity of variance (Lubin et al., 2004). Values <LOQ were imputed by randomly sampling below the LOQ from the relevant fitted log-normal distributions. Urinary creatinine concentrations were imputed first, followed by separate imputations for each phthalate metabolite.

2.4. Statistical Analyses

Statistical analyses were conducted using STATA 12.0 (StataCorp, College Station, TX) and SAS version 9.3 (SAS Institute, Cary, NC). We summarized the distribution of urinary phthalate metabolites using the median and interquartile range. We conducted pairwise Spearman correlation among non-imputed urinary phthalate metabolite data to assess the degree of correlation. We compared the distribution of urinary phthalate metabolites from the WREN study to that from the nationally representative National Health and Nutrition Evaluation Survey (NHANES) (CDC, 2012) using the geometric mean (GM) and 95% confidence interval (CI). For this comparison only, we substituted missing observations below the LOQ with the value LOQ/$\sqrt{2}$, the procedure used with the NHANES data (CDC, 2009).

We conducted unconditional logistic regression analyses to estimate the association between urinary concentration of phthalate metabolites and the risk of endometriosis, using odds ratios (OR) and 95% confidence intervals (CI). We modeled each phthalate metabolite or summary metric as a set of indicator variables, with the lowest quartile serving as the referent. A directed acyclic graph (DAG), informed by prior studies on sources of phthalate exposure and risk factors for endometriosis, was used to identify variables necessary for adjustment in the logistic regression model (Supplemental Materials, Fig. 1) (Greenland et al., 1999; Hernan et al., 2002). Based on the proposed DAG, we adjusted for natural logarithm-transformed urinary creatinine, age, and reference year. We considered each individual categorical phthalate metabolite or summary exposure variable in a separate logistic regression model, due to concern for unstable coefficient estimates or lack of model convergence when estimating multiple correlated exposure effects with maximum likelihood estimation. To test the trend across categories of an individual urinary phthalate metabolite or summary exposure variable, we included a continuous variable in the adjusted logistic regression model, assigning values equal to the median quartile concentration among controls to participants in each exposure category. Statistical significance was defined to be two-sided $P<0.05$ in all analyses.

Table 2
Laboratory measurement of urinary phthalate metabolite concentrations and distribution by case status, Group Health, 1996–2001.

Phthalate metabolite	LOQ (ng/mL)	Study samples ($n=287$) Measured ≥LOQ n (%)	Cases ($n=92$) Median (IQR)	Controls ($n=195$) Median (IQR)
MEHP	0.4	240 (83.6)	2.2 (0.6–4.6)	3.4 (1.0–11.1)
MEHHP	0.2	285 (99.3)	14.8 (5.3–31.0)	18.8 (6.3–56.5)
MEOHP	0.2	287 (100.0)	8.1 (3.5–18.0)	10.8 (3.5–29.1)
MECPP	0.3	286 (99.7)	14.4 (5.9–32.5)	18.0 (5.8–51.9)
MBzP	0.5	266 (92.7)	4.5 (2.2–9.9)	5.0 (2.0–11.5)
MEP	0.8	284 (99.0)	61.9 (23.5–155.9)	43.9 (16.8–144.4)
MiBP	0.2	270 (94.1)	1.3 (0.6–2.7)	1.5 (0.7–3.1)
MnBP	0.3	284 (99.0)	9.8 (5.0–20.9)	10.0 (4.9–23.5)

Abbreviations: LOQ=limit of quantitation; IQR=interquartile range; MEHP=mono-(2-ethyl-5-hexyl) phthalate; MEHHP=mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP=mono-(2-ethyl-5-oxohexyl) phthalate; MECPP=mono-(2-ethyl-5-carboxypentyl) phthalate; MBzP=mono-benzyl phthalate; MEP=mono-ethyl phthalate; MiBP=mono-iso-butyl phthalate; MnBP=mono-n-butyl phthalate.

3. Results

Among WREN participants with measured urinary phthalate metabolites, a greater percentage of cases than controls were 25–34 years old, Hispanic, and had a post graduate education (Table 3). Additionally, a greater percentage of cases than controls reported never smoking, being current consumers of alcohol, and nulliparity. The distribution of characteristics among WREN participants with measured urinary phthalate metabolites was generally similar to all WREN participants in the parent study with the exception that, in the parent study, a greater percentage of controls than cases reported never smoking (Supplemental Material, Table 1).

The majority of women in our study had detectable concentrations of phthalate metabolites (Table 2). The distribution of individual urinary phthalate concentrations was right-skewed (Table 2) and metabolites of DEHP were highly correlated ($r>0.80$) (Supplemental Material, Table 2). The geometric means of individual phthalate metabolite concentrations among controls in our study were generally comparable to those reported by the Centers for Disease Control and Prevention (CDC) using the nationally representative NHANES data (CDC, 2012) (Supplemental Material, Tables 3 and 4). The exceptions included lower creatinine-corrected MEP concentrations and higher creatinine-corrected concentrations of DEHP metabolites in the WREN study compared to the NHANES data.

Adjusting for age, reference year, and natural logarithm-transformed imputed creatinine concentrations, we observed a strong inverse association between urinary MEHP concentration and endometriosis risk, particularly when comparing the fourth and first quartiles (aOR 0.3, 95% CI: 0.1–0.7), and the test of trend across MEHP exposure categories was significant ($P=0.012$) (Table 4). Our data suggested an inverse association with greater urinary concentrations of other DEHP metabolites (MEHHP, MEOHP) and ∑DEHP, although the confidence intervals included the null. Our data also suggested increased endometriosis risk with greater urinary concentrations of MBzP and MEP, although the associations were not statistically significant. The magnitude of association generally increased by quartile of MEP, but was non-monotonic for MBzP. We repeated the analyses additionally adjusting for education, cigarette smoking and alcohol consumption, given the imbalance of these characteristics among cases and controls. Our findings were similar to those of the primary analysis with the exception of stronger associations between endometriosis risk with quartiles of urinary MEP metabolite concentrations and slightly stronger associations between DEHP metabolites and endometriosis risk (Table 4).

4. Discussion

In the current analysis using data from a population-based case-control study of endometriosis, we found a strong inverse association between endometriosis risk and urinary concentration

Table 3
Characteristics of study participants, Group Health, 1996–2001.

Characteristic	Cases ($n=92$) n (%)	Controls ($n=195$) n (%)
Age (years)		
17–24	6 (6.5)	13 (6.7)
25–34	22 (23.9)	30 (15.4)
35–44	41 (44.6)	95 (48.7)
45–49	23 (25.0)	57 (29.2)
Race		
White	81 (88.0)	168 (86.2)
Black	2 (2.2)	11 (5.6)
Asian/Pacific Islander	7 (7.6)	12 (6.2)
American Indian	1 (1.1)	1 (0.5)
More than one race	1 (1.1)	3 (1.5)
Ethnicity[a]		
Hispanic	6 (6.5)	4 (2.1)
Non-Hispanic	86 (93.5)	190 (97.9)
Income[a] (US$)		
$<35,000$	26 (28.6)	50 (26.5)
35,000–69,999	42 (46.2)	82 (43.4)
$\geq 70,000$	23 (25.3)	57 (30.2)
Education		
$<$ HS	3 (3.3)	5 (2.6)
HS graduate	17 (18.5)	38 (19.5)
Some college	28 (30.4)	80 (41.0)
College graduate	24 (26.1)	45 (23.1)
Post graduate	20 (21.7)	27 (13.9)
Cigarette smoking		
Never	55 (59.8)	105 (53.9)
Former	17 (18.5)	51 (26.2)
Current	20 (21.7)	39 (20.0)
Alcohol use		
Never	29 (31.5)	60 (30.8)
Former	11 (12.0)	45 (23.1)
Current	52 (56.5)	90 (46.2)
BMI (kg/m^2)[a]		
<18.5	1 (1.1)	6 (3.1)
18.5– <25.0	48 (52.2)	101 (52.6)
25.0– <30.0	18 (19.6)	42 (21.9)
≥ 30.0	25 (27.2)	43 (22.4)
Parity		
Nulliparous	45 (48.9)	63 (32.3)
Parous	47 (51.1)	132 (67.7)
Urinary creatinine (mg/dL) median (IQR)	54.5 (27–106)	62.0 (26–111)

Abbreviations: HS=high school; BMI=body mass index; IQR=interquartile range.
[a] Numbers do not add to column total due to missing data.

of the DEHP metabolite, MEHP, accompanied by the suggestion of weaker inverse associations with urinary concentrations of other DEHP metabolites, MEHHP and MEOHP, and ∑DEHP. DEHP is a high molecular weight phthalate commonly used as a plasticizer, or compound added for softening and flexibility in polymer products such as polyvinyl chloride (PVC) (ECB, 2008; Kavlock et al., 2002b). DEHP is found in a range of products, from building materials (such as flooring, wall covering, upholstery, and cables), home furnishings, and car interiors, to clothing articles (such as rainwear, gloves and footwear), medical devices, and food contact materials (ECB, 2008). In limited female animal studies, adult exposure to DEHP has been associated with ovarian toxicity, including increased estrous cycle length, decreased ovulation, and histopathologic ovarian changes (Davis et al., 1994; Li et al., 2012; Takai et al., 2009). Both DEHP and MEHP have been found *in vivo* and *in vitro* to alter ovarian steroidogenesis including decreased estradiol and progesterone production (Davis et al., 1994; Gupta et al., 2010; Li et al., 2012; Reinsberg et al., 2009). These endocrine disruptive actions, if similarly exhibited in women, may explain the inverse association we observed between the DEHP metabolites and endometriosis risk.

Our finding of an inverse association between urinary MEHP concentration and endometriosis risk contrasts with two small case-control studies conducted among women undergoing laparoscopy at a university obstetrics and gynecology department. Those studies reported adjusted ORs of 1.57 (95% CI: 0.74–3.30) (Itoh et al., 2009) and 1.42 (95% CI: 0.45–4.50) (Huang et al., 2010), comparing MEHP concentrations above and below the median. However, our observation is consistent with a population-based cross-sectional study conducted using NHANES data. Among a subset of women ($n=1227$) who participated in the mobile exam component and completed a reproductive health questionnaire, Weuve et al. (2010) reported an OR of 0.39 (95% CI: 0.16–0.95) when comparing the fourth and first quartile of MEHP concentrations. Just as in our study, the investigators reported odds ratios suggesting a decreased risk of endometriosis with greater concentrations of oxidative metabolites of DEHP–MEHHP, MEOHP and MECPP–although not statistically significant (Weuve et al., 2010). It is possible that the population-based sampling framework in both studies minimized selection bias and allowed for more accurate risk estimates than those obtained in studies sampling from laparoscopic patients. In the WREN study, controls were randomly sampled directly from the source population giving rise to endometriosis cases. With this sampling design, controls were likely to represent the underlying population's distribution of phthalate exposure. In contrast, among studies sampling from laparoscopic patients, the indication warranting surgical evaluation among controls may be associated with phthalate exposure, resulting in a biased risk estimate.

Our findings also suggested that urinary concentrations of the BzBP metabolite MBzP and the DEP metabolite MEP may be associated with increased risk of endometriosis. BzBP is used in the production of home interior products such as vinyl floor tile and carpet backing, and conveyor belts in food manufacturing (2003; IPCS, 1999). DEP is commonly used as a solvent or fragrance fixative in personal care products (such as bath preparations, perfume, cosmetics, and nail polish), insecticide sprays and mosquito repellants, in plastic packaging including plastic films and blister packaging, and aspirin coating (IPCS, 2003). Published findings of female reproductive system toxicity from adult exposure to BzBP and DEP in animal and *in vitro* studies are limited and inconsistent, unable to offer a clear biologic rationale for the possible associations observed in our study. Some *in vitro* studies have suggested that BzBP may have estrogenic effects (Coldham et al., 1997; Harris et al., 1997; Jobling et al., 1995; Okubo et al., 2003; Soto et al., 1995; Zacharewski et al., 1998), while a lack of estrogenic response was reported with adult exposure to BzBP in an *in vivo* adult female rodent study (Zacharewski et al., 1998). DEP did not exhibit or weakly exhibited estrogenic effects in limited *in vitro* studies (Harris et al., 1997; Okubo et al., 2003) and was not found to impact reproductive performance in one *in vivo* adult female rodent study (Lamb et al., 1987).

Two prior case-control studies that restricted the study population to laparoscopic patients reported divergent adjusted odds ratios for the associations between MBzP and MEP and endometriosis risk. For the same reason previously mentioned, the divergent results may be due to bias from the selection of laparoscopic controls with conditions associated with phthalate exposure. In contrast to our study, Weuve et al. (2010) reported no appreciable association between endometriosis risk and concentrations of MBzP (aOR 1.16, 95% CI: 0.58–2.33) and MEP (aOR 1.12, 95% CI: 0.58–2.17). In that population-based cross-sectional study, endometriosis diagnosis was ascertained by self-report ($n=87$) and diagnoses occurred <1 year to 34 years (median 9 years) prior to collection of urine samples for quantification of phthalate metabolites (Weuve et al., 2010). The investigators acknowledged the possibility that non-differential misclassification of disease

Table 4
Odds ratios and 95% confidence intervals for the relationship between individual urinary phthalate metabolites and risk of endometriosis, Group Health, 1996–2001.

Phthalate metabolite Quartiles (ng/ml urine)	Cases ($n=92$) n (%)	Controls ($n=195$) n (%)	aOR[a] (95% CI)	aOR[b] (95% CI)
MEHP				
≤1.0	33 (35.9)	50 (25.6)	1.0	1.0
> 1.0–3.4	21 (22.8)	47 (24.1)	0.6 (0.3–1.3)	0.5 (0.2–1.2)
> 3.4–11.1	26 (28.3)	49 (25.1)	0.7 (0.3–1.5)	0.6 (0.3–1.4)
> 11.1	12 (13.0)	49 (25.1)	0.3 (0.1–0.7)	0.2 (0.08–0.6)
P_{trend}[c]			$P=0.012$	$P=0.007$
MEHHP				
≤6.3	25 (27.2)	48 (24.6)	1.0	1.0
> 6.3–18.8	31 (33.7)	51 (26.2)	1.1 (0.5–2.4)	1.1 (0.5–2.4)
> 18.8–56.5	22 (23.9)	48 (24.6)	0.8 (0.3–2.0)	0.7 (0.3–1.8)
> 56.5	14 (15.2)	48 (24.6)	0.5 (0.2–1.5)	0.5 (0.1–1.4)
P_{trend}[c]			$P=0.085$	$P=0.083$
MEOHP				
≤3.5	23 (25.0)	49 (25.1)	1.0	1.0
> 3.5–10.8	33 (35.9)	47 (24.1)	1.4 (0.6–2.9)	1.2 (0.5–2.6)
> 10.8–29.1	21 (22.8)	50 (25.6)	0.8 (0.3–2.1)	0.6 (0.2–1.7)
> 29.1	15 (16.3)	49 (25.1)	0.6 (0.2–1.7)	0.5 (0.1–1.5)
P_{trend}[c]			$P=0.097$	$P=0.093$
MECPP				
≤5.8	22 (23.9)	48 (24.6)	1.0	1.0
> 5.8–18.0	30 (32.6)	50 (25.6)	1.3 (0.6–2.9)	1.0 (0.4–2.4)
> 18.0–51.9	23 (25.0)	48 (24.6)	1.2 (0.5–3.0)	1.0 (0.4–2.6)
> 51.9	17 (18.5)	49 (25.1)	0.8 (0.3-2.3)	0.6 (0.2–2.0)
P_{trend}[c]			$P=0.225$	$P=0.223$
∑DEHP (nmol/ml)				
≤0.06	23 (25.0)	48 (24.6)	1.0	1.0
> 0.06–0.18	34 (37.0)	49 (25.1)	1.3 (0.6–2.8)	1.1 (0.5–2.5)
> 0.18–0.50	21 (22.8)	50 (25.6)	0.8 (0.3–2.1)	0.7 (0.3–1.8)
> 0.50	14 (15.2)	48 (24.6)	0.5 (0.2–1.5)	0.4 (0.1–1.3)
P_{trend}[c]			$P=0.066$	$P=0.053$
MBzP				
≤2.0	21 (22.8)	51 (26.2)	1.0	1.0
> 2.0–5.0	29 (31.5)	47 (24.1)	1.7 (0.8–3.8)	1.6 (0.7–3.8)
> 5.0–11.5	22 (23.9)	49 (25.1)	1.5 (0.6–4.0)	1.5 (0.5–4.2)
> 11.5	20 (21.7)	48 (24.6)	1.3 (0.4–4.0)	1.3 (0.4–4.0)
P_{trend}[c]			$P=0.799$	$P=0.701$
∑BzBP (nmol/ml)				
≤0.03	21 (22.8)	48 (24.6)	1.0	1.0
> 0.03–0.07	34 (37.0)	50 (25.6)	1.6 (0.7–3.7)	1.7 (0.7–4.2)
> 0.07–0.16	17 (18.5)	49 (25.1)	0.9 (0.3-2.6)	0.9 (0.3–2.8)
> 0.16	20 (21.7)	48 (24.6)	1.2 (0.4–3.9)	1.2 (0.3-4.2)
P_{trend}[c]			$P=0.673$	$P=0.559$
MEP				
≤16.8	19 (20.7)	49 (25.1)	1.0	1.0
> 16.8–43.9	20 (21.7)	48 (24.6)	1.1 (0.5–2.4)	1.3 (0.6–2.9)
> 43.9–144.4	30 (32.6)	50 (25.6)	1.8 (0.8–3.8)	2.2 (1.0–5.0)
> 144.4	23 (25.0)	48 (24.6)	1.7 (0.7–4.1)	2.2 (0.9–5.5)
P_{trend}[c]			$P=0.350$	$P=0.248$
MiBP				
≤0.7	26 (28.3)	50 (25.6)	1.0	1.0
> 0.7–1.5	27 (29.4)	53 (27.2)	0.9 (0.4–2.0)	0.8 (0.3–1.8)
> 1.5–3.1	20 (21.7)	44 (22.6)	0.8 (0.3–2.2)	0.9 (0.3–2.3)
> 3.1	19 (20.7)	48 (24.6)	0.8 (0.3–2.6)	0.8 (0.3–2.5)
P_{trend}[c]			$P=0.836$	$P=0.897$
MnBP				
≤4.9	22 (23.9)	48 (24.6)	1.0	1.0
> 4.9–10.0	25 (27.2)	50 (25.6)	1.2 (0.5–2.8)	1.2 (0.5–2.9)
> 10.0–23.5	25 (27.2)	49 (25.1)	1.5 (0.6–3.9)	1.5 (0.6–4.0)
> 23.5	20 (21.7)	48 (24.6)	1.3 (0.4–3.9)	1.1 (0.3–3.7)
P_{trend}[c]			$P=0.957$	$P=0.793$
∑DBP (nmol/ml)				
≤0.03	22 (23.9)	48 (24.6)	1.0	1.0
> 0.03–0.06	28 (30.4)	51 (26.2)	1.2 (0.5–2.8)	1.3 (0.5–3.1)
> 0.06–0.12	21 (22.8)	47 (24.1)	1.2 (0.5–3.2)	1.3 (0.5–3.5)

Table 4 (*continued*)

Phthalate metabolite Quartiles (ng/ml urine)	Cases ($n=92$) n (%)	Controls ($n=195$) n (%)	aOR[a] (95% CI)	aOR[b] (95% CI)
>0.12	21 (22.8)	49 (25.1)	1.3 (0.4–4.1)	1.3 (0.4–4.3)
P_{trend}[c]			$P=0.820$	$P=0.986$

Abbreviations: OR=odds ratio; CI=confidence interval; MEHP=mono-(2-ethyl-5-hexyl) phthalate; MEHHP=mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP=mono-(2-ethyl-5-oxohexyl) phthalate; MECPP=mono-(2-ethyl-5-carboxypentyl) phthalate; DEHP=di(2-ethylhexyl) phthalate; MBzP=mono-benzyl phthalate; BzBP=benzyl butyl phthalate; MEP=mono-ethyl phthalate; MiBP=mono-iso-butyl phthalate; MnBP=mono-n-butyl phthalate; DBP=dibutyl phthalate.

[a] Odds ratio adjusted for age, reference year, and natural logarithm-transformed imputed urinary creatinine concentrations.

[b] Odds ratio adjusted for age, reference year, natural logarithm-transformed imputed urinary creatinine concentrations, education, smoking status, and alcohol consumption.

[c] *P*-value for test of trend across quartiles.

may have attenuated the true associations for phthalate metabolites (Weuve et al., 2010). In WREN, incident endometriosis diagnosis was confirmed by record review indicating surgical visualization of endometriosis and the date of diagnosis transpired 6 months to 5.8 years (median 3 years) prior to urine collection. However, it is also possible that the associations we observed were chance findings given the multiple comparisons carried out in assessing endometriosis risk across urinary phthalate metabolites and summary exposures.

There are two main limitations with our study. Single, spot urine samples, collected after the onset of symptoms among cases, were used for the quantification of phthalate metabolites. Since phthalates are non-persistent chemicals that are rapidly metabolized and excreted (Wittassek et al., 2011), phthalate metabolite concentrations detected in a single urine sample represent only recent exposure to the parent phthalate diester. However, creatinine-corrected urinary concentrations of MEP have been moderately reproducible (ICC >0.48) across 2–4 week sampling intervals among studies of reproductive age women (Baird et al., 2010; Peck et al., 2010) and creatinine-corrected urinary concentrations of MBzP have demonstrated high temporal reliability (ICC >0.53) across studies of various populations, evaluating time intervals ranging from eight days to six months with some of these studies using spot urine samples (Adibi et al., 2008; Baird et al., 2010; Fromme et al., 2007; Peck et al., 2010; Teitelbaum et al., 2008). Despite generally poorer temporal reliability for urinary DEHP metabolites compared to other metabolites, these studies suggest that a single sample may be representative of exposure levels over time, particularly if exposure is consistent. Although our samples were collected after diagnosis in cases, the potential for differential exposure misclassification was minimized by collecting the urine samples during the WREN interview and not during laparoscopy or other endometriosis care-related procedures at which phthalate exposure from medical devices among cases is possible (FDA, 2001). Additionally, samples were collected in 2001 and 2002, before the first publication of studies investigating the relationship between phthalates and endometriosis risk (Cobellis et al., 2003), making it less likely that a case, after being diagnosed, would have modified dietary intake or consumer and personal care product use to limit phthalate exposure.

A second limitation of our study is the possibility of undiagnosed endometriosis among controls. Laparoscopic-confirmation of the disease absence was not feasible among WREN controls given the population-based sampling framework. However, the prevalence of undiagnosed disease meeting the endometriotic disease definition is likely to be small, <2% among controls (Holt and Weiss, 2000), which would cause minimal conservative bias in our results. Moreover, our study benefitted from the selection of population-based controls who likely represented the frequency of phthalate exposure among women enrolled in a large healthcare system in the Pacific Northwest. Thus, we were able to avoid the unpredictable bias that may occur in studies restricting controls to women undergoing laparoscopy, who may have atypical concentrations of phthalates.

5. Conclusions

In this study of enrollees of a large healthcare system in the U.S. Pacific Northwest, we found that the majority of women were exposed to phthalates based on detectable urinary concentrations of phthalate metabolites, confirming the ubiquitous nature of these chemicals. The findings from our study suggest that phthalates may alter risk of a hormonally-mediated disease among reproductive-age women.

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Protection of Human subjects

Institutional review board approval for study procedures in the Women's Risk of Endometriosis study was received from the Fred Hutchinson Cancer Research Center (FHCRC Institutional Review File #6997). Informed consent was obtained from study subjects prior to the initiation of research activities.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.envres.2013.07.003.

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