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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.