Organochlorine pesticides, phthalate metabolites, and risk of endometriosis

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Abstract

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Background: Endometriosis is an estrogen-driven benign gynecologic condition that may be affected by exposure to environmental chemicals that mimic or alter endogenous hormonal activity, such as organochlorine pesticides (OCPs) and phthalates. However, the impact of these chemicals on endometriosis risk remains unclear, as prior epidemiologic studies have had conflicting results. Additionally, basic information is lacking on characteristics associated with increased phthalate body burden among premenopausal women. Methods: This research investigated the relationships between OCPs, phthalate metabolites, and endometriosis risk and predictors of phthalate exposure using data from a population-based case-control study of endometriosis, Women's Risk of Endometriosis (WREN). WREN was conducted among 18-49 year old female enrollees of a large healthcare system in the U.S. Pacific Northwest. OCP

concentrations were measured in the serum of 248 cases and 538 controls and urinary phthalate metabolites were quantified on 92 cases and 195 controls. To investigate the relationship between environmental chemicals and endometriosis risk, odds ratios (OR) and 95% confidence intervals (CI) were estimated using unconditional logistic regression. To examine predictors of phthalate exposure, ratios of median phthalate metabolite concentrations and 95% CI were estimated using linear regression. Results: Serum concentrations of β-hexachlorocyclohexane, particularly in analyses restricting cases to those with ovarian endometriosis, and mirex were associated with increased endometriosis risk. A strong inverse association was observed between urinary mono-(2-ethyl-5-hexyl) phthalate (MEHP) concentration and endometriosis risk. The data suggested an inverse association with other di-2-ethylhexyl phthalate (DEHP) metabolites and a positive association with mono-benzyl phthalate (MBzP) and mono-ethyl phthalate (MEP). Mono-isobutyl phthalate (MiBP) concentrations were inversely associated with waist circumference and waist-to-hip ratio. Trends across hours of the day (summed metabolites of DEHP), days of the week (MEP), and seasons of the year (MiBP) were observed. Conclusions: This research suggests that environmental exposure to OCPs and phthalates may alter endometriosis risk and that subgroups of premenopausal women may have increased phthalate exposure. Nearly ubiquitous exposure to phthalates as well as extensive past use of environmentally persistent OCPs in the United States or present use in other countries may impact the current health of reproductive-age women.

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Introduction

Endometriosis is characterized by the presence of endometrial glands and stroma outside of the uterus, usually within the peritoneal cavity. The symptoms associated with endometriosis vary, but symptoms typically include chronic pelvic pain, dysmenorrhea, dyspareunia, and infertility. For many women, the symptoms of endometriosis can be chronic and debilitating, negatively affecting health-related quality of life, personal relationships, and work productivity (Ballard et al. 2006; Denny and Mann 2007; Garry et al. 2000; Jones et al. 2004). Endometriosis is estimated to affect 6-10% of reproductive-age women in the United States, although the prevalence is difficult to estimate given that surgical evaluation is required for definitive disease diagnosis (Eskenazi and Warner 1997).

Despite the substantial impact of this condition on reproductive age women, the etiology of endometriosis is not well understood. The most widely accepted theory of disease pathogenesis is Sampson's theory of retrograde menstruation (Sampson 1927). Sampson postulated in 1927 that endometriosis develops from the reflux of shed endometrial tissue during menses, transporting endometrial cells through the uterine tubes and into the peritoneal cavity where viable cells implant and grow (Sampson 1927). Research since this time has revealed that the reflux of menstrual tissue is a common event and that additional factors are required for the establishment and persistence of ectopic disease (Halme et al. 1984). These factors include changes to the endometrium that allow refluxed endometrial cells to attach and invade the peritoneal mesothelium, establish a blood supply, proliferate, and survive; altered production of hormones by the ovaries to support the growth of implants in the peritoneal cavity; lack of immune surveillance to clear implants from the peritoneal surface; and the ability of ectopic implants to additionally biosynthesize estrogen for growth maintenance using unregulated

hormonal pathways (Bulun 2009; Giudice and Kao 2004; Nisolle and Donnez 1997; Ulukus et al. 2006). Thus, endometriosis is a complex disease involving genetic, immunologic, inflammatory, angiogenic, and anti-apoptotic factors, and hormonally-mediated mechanisms are central to disease onset and progression.

Epidemiologic studies of risk factors for endometriosis have contributed strong circumstantial evidence that the condition is estrogen-driven. Endometriosis is rarely seen before the onset of menses or after menopause (Houston 1984) and factors such as greater lifetime ovulatory cycles, shorter menstrual cycles, and early onset of menarche are associated with an increased risk of disease (Missmer et al. 2004). Conversely, lifestyle factors that lower circulating estrogen levels, such as physical activity and cigarette smoking, are associated with decreased disease risk (Dhillon and Holt 2003; Sangi-Haghpeykar and Poindexter 1995; Vitonis et al. 2010). Additionally, pain symptoms are reduced by medical therapy that suppresses ovarian hormone production, such as combined oral contraceptives, progestins and gonadotropin-relating hormone agonist therapy (Giudice 2010).

Given that endometriosis is a hormonally-driven condition, it is plausible that environmental chemicals that are endocrine disruptive, or mimic or interfere with endogenous hormonal activity may alter the risk of the endometriosis. It is also plausible to consider the menstrual cycle during the reproductive years as the window for disease susceptibility from exposure to environmental chemicals. I hypothesize that two specific classes of environmental chemicals, organochlorine pesticides and phthalates, may alter the risk of endometriosis. Specifically, I hypothesize that organochlorine pesticides and phthalates may affect the normal dynamic structural changes of hormonally-responsive endometrial tissue during the menstrual cycle, disrupting the regulation of proliferation and apoptosis and facilitating the implantation

and persistence of endometrial tissue after displacement to ectopic sites, and that these chemicals may alter the endogenous hormonal milieu. My hypothesis is based on evidence that 1) organochlorine pesticides generally have demonstrated estrogenic properties in *in vitro* studies (Andersen et al. 2002; Shelby et al. 1996; Soto et al. 1995) and exhibited adverse reproductive system effects in laboratory animal studies, altering uterine and ovarian function and endogenous hormone production (Alvarez et al. 2000; Foster et al. 1995; Shelby et al. 1996; Van Velsen et al. 1986) and 2) select phthalates have been associated with ovarian toxicity, including increased estrous cycle length, decreased ovulation, histopathologic ovarian changes, and altered ovarian steroidogenesis such as decreased estradiol and progesterone production (Davis et al. 1994; Li et al. 2012; Takai et al. 2009) while other phthalates have exhibited estrogenic effects in some *in vitro* studies (Coldham et al. 1997; Harris et al. 1997; Jobling et al. 1995; Soto et al. 1995; Zacharewski et al. 1998).

Organochlorine pesticides and phthalates or their metabolites are detected in the blood and urine of the U.S. general population. However, the relationship between exposure to these chemicals and the risk of endometriosis among reproductive age women remains unclear, as prior epidemiologic studies have had inconsistent results. The majority of these studies have been case-control studies conducted among women undergoing laparoscopic evaluation for infertility, pelvic pain, benign gynecologic conditions, and tubal sterilization, with cases and controls identified by the presence or absence of endometriosis during surgery. The selected laparoscopic controls may not represent a random sample of the source population that gave rise to the endometriosis cases and may have conditions associated with atypical concentrations of organochlorine pesticides and phthalate metabolites (Holt and Weiss 2000; Zondervan et al. 2002). Thus, these studies of laparoscopic patients may be subject to unpredictable bias.

The purpose of this dissertation research was to investigate endometriosis risk in relation to organochlorine pesticides and phthalate metabolites using data from a U.S. case-control study of endometriosis that employed a population-based sampling frame and surgically confirmed cases. In Chapter 1, I evaluate the relationship between serum organochlorine pesticide analytes and endometriosis risk and, in Chapter 2, I investigate the association between urinary phthalate metabolites and endometriosis risk. Given the extremely limited data available on demographic, temporal, and anthropometric characteristics associated with phthalate body burden among premenopausal women, in Chapter 3, I explore these characteristics in relation to urinary phthalate metabolite concentrations using data on controls from the case-control study of phthalate metabolites and endometriosis risk.

Chapter 1: Organochlorine pesticides and risk of endometriosis: Findings from a population-based case-control study

Abstract

Background: Endometriosis is considered an estrogen-dependent disease. Persistent environmental chemicals that exhibit hormonal properties, such as organochlorine pesticides (OCPs), may affect endometriosis risk.

Objectives: We investigated endometriosis risk in relation to environmental exposure to OCPs.

Methods: We conducted the current analyses using data from the Women's Risk of Endometriosis (WREN) study, a population-based case-control study of endometriosis conducted among 18-49 year old female enrollees of a large health plan in western Washington State. OCP concentrations were measured in sera from incident, surgically confirmed endometriosis cases (n=248) first diagnosed between 1996 and 2001 and population-based controls (n=538). We estimated odds ratios (OR) and 95% confidence intervals (CI) using unconditional logistic regression, adjusting for age, reference year, serum lipids, education, race, smoking, and alcohol intake.

Results: Our data suggested increased endometriosis risk associated with serum concentrations of β-hexachlorocyclohexane (HCH) (third vs. lowest quartile: OR 1.7, 95% CI: 1.0-2.8; highest vs. lowest quartile OR 1.3, 95% CI: 0.8-2.4) and mirex (highest vs. lowest category: OR 1.5, 95% CI: 1.0-2.2). The association between serum β-HCH concentrations and endometriosis was stronger in analyses restricting cases to those with ovarian endometriosis (third vs. lowest quartile: OR 2.5, 95% CI: 1.5-5.2; highest vs. lowest quartile: OR 2.5, 95% CI: 1.1-5.3).

Conclusions: Among enrollees of a large healthcare system in the U.S. Pacific Northwest, select pesticides were associated with a possible increased endometriosis risk. Extensive past use of environmentally persistent OCPs in the United States or present use in other countries may impact the current health of reproductive-age women.

Introduction

Endometriosis, characterized by the presence of endometrial glands and stroma outside of the uterus, is associated with substantial morbidity, including severe, chronic pelvic pain, heavy menstrual bleeding, and infertility (Eskenazi and Warner 1997; Nisolle and Donnez 1997). This serious, chronic condition is estimated to affect 6-10% of reproductive-age women in the United States (Eskenazi and Warner 1997). Although its etiology is not fully understood, endometriosis is considered an estrogen-dependent disease. Endometriosis rarely is seen before the onset of menses or after menopause (Houston 1984) and suppression of ovarian hormone production, such as with combined oral contraceptives, progestins and gonadotropin-releasing hormone agonist therapy, reduces pain symptoms (Giudice 2010). Investigations into the pathophysiology of endometriosis suggest that disease onset and progression involve steroid-related alterations of the endometrium and peritoneal cavity, excess estrogen production by ectopic endometriotic lesions, and changes in ovarian steroidogenesis (Bulun 2009; Giudice and Kao 2004; Ulukus et al. 2006). Thus, environmental chemicals that are endocrine disruptive, or mimic or alter endogenous hormonal activity, may plausibly affect endometriosis risk.

Organochlorine pesticides (OCPs) are synthetic pesticides that were widely used in the latter half of the 20th century. Despite bans and restrictions on OCP use in the United States over the past several decades, the U.S. population still has detectable serum concentrations of OCPs,

due to the environmental persistence of the chemicals and their bioaccumulation within organisms and up the food chain (CDC 2009). Currently, U.S. general population OCP exposure is primarily from the consumption of contaminated fatty foods, fish and dairy products (CDC 2009; Patterson et al. 2009). Additionally, continued global use and unintentional production of these chemicals may contribute to ongoing U.S. population exposure given that these chemicals have the capacity for long-range transport, appearing in locations far from where they are manufactured or used (Fisher 1999). Continuing OCP exposure is of potential human health concern, as these chemicals generally have demonstrated estrogenic properties in *in vitro* studies (Andersen et al. 2002; Shelby et al. 1996; Soto et al. 1995), and exhibited adverse reproductive system effects in laboratory animal studies, altering uterine and ovarian function and endogenous hormone production (Alvarez et al. 2000; Foster et al. 1995; Shelby et al. 1996; Van Velsen et al. 1986).

Despite evidence of endocrine disruptive properties of OCPs, the impact of these chemicals on endometriosis risk remains unclear, as prior epidemiologic studies have had conflicting results (Buck Louis et al. 2012; Cooney et al. 2010; Lebel et al. 1998; Niskar et al. 2009; Porpora et al. 2009; Quaranta et al. 2006; Tsukino et al. 2005). These studies investigating serum and adipose concentrations of OCPs in relation to endometriosis were primarily conducted among women undergoing surgical evaluation by laparoscopy, which may have produced biased results if the indication for such evaluation was associated with OCP body burden. Only one recently published study has evaluated endometriosis risk in relation to serum OCP concentrations using a population-based sampling framework, but that study cohort was limited by its small size (n=127) (Buck Louis et al. 2012). The purpose of the current analyses was to investigate, outside the specialized setting of women undergoing laparoscopy, the risk of

incident, surgically confirmed endometriosis in relation to environmental exposure to OCPs using data from a large, general population-based case-control study of endometriosis.

Materials and Methods

Study population

We conducted the current analyses using data from the "Women's Risk of Endometriosis" (WREN) study and the ancillary "Persistent Organic Pollutants and Endometriosis Risk" (POPs) study. WREN was a population-based case-control study of endometriosis conducted among 18-49 year old female enrollees of Group Health, a large integrated healthcare system in western Washington State. As previously described, cases in the WREN study were 340 women with incident first-time endometriosis diagnoses (International Classification of Disease 9th Revision (ICD-9) (WHO 1977) diagnostic codes 617.0-617.5, 617.8-617.9, excluding those with adenomyosis coded as 617.0, uterine endometriosis) between April 1, 1996 and March 31, 2001 (Marino et al. 2008; Trabert et al. 2010). The diagnoses were confirmed by record review indicating the presence of endometriosis through direct surgical visualization, with histologic confirmation when available. The reference date for cases was the date of first visit to Group Health for symptoms leading to endometriosis diagnosis. Population-based controls (n=741) in the WREN study were female Group Health enrollees without diagnosed endometriosis, randomly selected from computerized Group Health enrollment databases and frequency matched to cases on 5-year age groups. Controls were assigned reference dates to correspond with the distribution of reference dates among cases. Cases and controls without a uterus, at least one ovary, or six months minimum enrollment at reference date were excluded, as were menopausal or postmenopausal women. Because we were interested in

first diagnosis of endometriosis, we also excluded women with a past history of the disease. WREN subjects participated 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 before the reference date. The cases and controls who participated in WREN and completed the interview represented 73% of those invited to participate (Marino et al. 2008). In the POPs study, a subset of WREN study participants was invited to donate a blood sample to assess exposure to OCPs and polychlorinated biphenyls (PCBs); 89.7% of these cases (n=286) and 85.1% of these controls (n=592) agreed, and 283 cases and 585 controls donated samples.

Quantification of serum OCP concentrations was completed for 268 cases and 550 controls. Based on information collected during the WREN study interview, we discovered a past history of surgically confirmed endometriosis for 20 participants (8 cases and 12 controls), who we then excluded. We also excluded 11 cases not meeting the definition of definite or possible endometriotic disease (Holt and Weiss 2000), a definition that focuses on endometriosis with evidence of tissue invasiveness or interference with normal physiologic processes. One case whose endometriosis diagnosis was not confirmed surgically also was excluded. Thus, this analysis used data from 248 cases and 538 controls in the WREN study. The Fred Hutchinson Cancer Research Center Institutional Review Board approved this study and each participant provided written informed consent prior to enrollment and participation.

Serum organochlorine pesticide measurements

Study personnel collected non-fasting blood samples at the time of interview. The blood was processed by the Fred Hutchinson Cancer Research Center Specimen Processing Laboratory and serum was aliquoted into acid-washed glass vials and stored at -20°C prior to shipment to

the Toxicology Branch, Division of Laboratory Sciences, National Center for Environmental Health, Centers for Disease Control and Prevention (CDC) in Atlanta, GA, for measurement of serum OCP concentrations. OCP analytes were isolated from serum using solid phase extraction and quantified using isotope-dilution gas chromatography-high resolution mass spectrometry (Barr et al. 2006; Barr et al. 2003). The 11 OCPs or metabolites quantified were *beta*-hexachlorocyclohexane (β-HCH), *gamma*-hexachlorocyclohexane (γ-HCH), heptachlor epoxide, oxychlordane, *trans*-nonachlor, two isomers of dichlorodiphenyltrichloroethane (*p,p'*-DDT, *o,p'*-DDT) and dichlorodiphenyldichloroethylene (*p,p'*-DDE), dieldrin, hexachlorobenzene, and mirex. The limit of detection (LOD) for all OCPs was 10.0 pg/g serum with relative standard deviations of <15%. The laboratory staff was blinded to the case status of the laboratory specimens.

All analytes detected in ≥30% of samples were included in analyses (Table 1.1), based on the method used to impute values <LOD which has demonstrated minimal bias with 50-60% missing in a simulation study (Lubin et al. 2004). We used imputed values only in analyses requiring continuous OCP analyte concentrations. The OCP analyte *o,p'*-DDT was not included in statistical analyses as it was detected in only 8.8% of samples. Quality control (QC) procedures monitored the intrabatch variability in serum OCP measurements with 34 QC duplicate samples. The agreement between measurements for duplicate samples included in the same analytic run was high, with intraclass correlation coefficients ranging from 89% to 99% for individual OCP analytes.

Laboratory personnel quantified the individual lipid components total cholesterol, free cholesterol, triglycerides, and phospholipids in each serum sample using enzymatic methods (Roche Chemicals, Indianapolis, IN) (Phillips et al. 1989). We then determined serum total lipid

concentrations in milligrams per deciliter (mg/dL) by summing the individual lipid components using an established formula, total lipids = ((1.677*(total cholesterol – free cholesterol)) + free cholesterol + triglycerides + phospholipids)) (Akins et al. 1989). A shorter formula, total lipids = ((2.27*total cholesterol) + triglycerides + 62.3), was used if free cholesterol was not quantified (Akins et al. 1989; Bernert et al. 2007; Phillips et al. 1989).

Exposure coding

Serum OCP measurements that were not reportable due to interference with co-eluting chemicals were excluded (Table 1.1). We created categorical variables for each serum OCP analyte; values <LOD were always included in the lowest category. For analytes with ≤25% <LOD, we categorized the serum OCP concentration by quartiles of the control distribution. For two OCPs with >25% missing values due to non-detection (mirex and γ-HCH), the distribution was assessed using measurements ≥LOD, and we categorized the serum OCP concentration into three categories using the LOD and the median value (of those detected) among controls as the two cutpoints. By summing the molar concentrations of the individual analytes within each group, we also created three summary exposure variables among structurally-related or isomeric forms of OCPs: Σchlordane (sum of oxychlordane, heptachlor epoxide, and *trans*-nonachlor), ΣDDT (sum of *p,p'*-DDT and *p,p'*-DDE), and ΣHCH (sum of β-HCH and γ-HCH). The summary exposure variables were categorized by quartiles based on the distribution in controls.

We imputed serum measurements quantified as <LOD using a distribution-based multiple imputation procedure so as to be able to create the summary exposure variables and to conduct the test of trend analyses (Lubin et al. 2004). This procedure entailed creating a bootstrap sample of the data and estimating the parameters of the log-normal serum OCP

distribution among controls by maximum likelihood estimation, including covariates age, reference year, smoking, alcohol, education, race, natural log-transformed total lipids, breastfeeding, and body mass index (BMI). The log-normal distribution with the estimated parameters was then randomly sampled to impute values <LOD. The process was repeated to create five multiple imputed datasets.

Statistical analyses

The statistical analyses were conducted using STATA 12.0 (StataCorp, College Station, TX) and SAS version 9.3 (SAS Institute, Cary, NC). We used the significance level of α=0.05 in all analyses. We summarized the distribution of OCPs using the median and interquartile range. To evaluate the degree of collinearity among pairs of OCP analytes, we conducted pairwise Spearman correlation using the non-imputed continuous serum OCP concentration data restricted to values ≥LOD.

To test whether the risk of endometriosis was associated with serum concentrations of OCPs, unconditional logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (CI). The categories for each OCP analyte or summary metric were modeled as a set of indicator variables, with the lowest category serving as the reference category. We identified variables necessary for adjustment in the model using a directed acyclic graph (DAG), informed by previous studies of endometriosis risk factors and predictors of serum OCP concentrations (Greenland et al. 1999; Hernan et al. 2002). On this basis we adjusted for natural logarithm-transformed total serum lipids, education, race, smoking, alcohol intake and the frequency matching variables age and enrollment year. We did not adjust for parity as it may be in the causal pathway between exposure and development of endometriosis or downstream from

disease (Koepsell and Weiss 2003). Additionally, we did not adjust for other OCPs and PCBs due to the concern for collinearity, resulting unstable coefficient estimates or lack of model convergence when estimating multiple correlated exposure effects with maximum likelihood estimation. We instead conducted a conventional one-at-a-time analysis, considering each individual categorical OCP analyte or summary exposure variable in a separate logistic regression model. We also repeated the analyses restricting cases to those with ovarian endometriosis, which may be etiologically distinct from non-ovarian disease (Nisolle and Donnez 1997).

To test the trend across categories of an individual serum OCP analyte or summary exposure variable, we created a continuous variable assigning the median category values to participants in each category and included the variable in the adjusted logistic regression model. We interpreted the *P*-value accompanying the continuous variable for the test of trend.

In logistic regression analyses using imputed data, namely analyses of summary exposure variables and test of trend, we used the PROC MIANALYZE procedure in SAS to combine results from individual imputed datasets to account for uncertainty in the imputation and adjust the variance of the estimates.

We conducted two exploratory analyses. First, because we hypothesized that laparoscopic controls may have altered serum OCP concentrations due to the indication for surgical evaluation, we explored the distribution of serum OCPs concentrations among cases and controls who reported a history of a laparoscopic evaluation as well as among cases and controls with a history of infertility testing. Second, we considered an alternative conceptual framework with excretion factors of parity and breastfeeding as confounders and repeated the analyses adjusting for parity and breastfeeding using a composite variable (nulliparous women, parous women with

lifetime history of breastfeeding ≤six months, parous women with lifetime history of breastfeeding >6 months).

Results

We found that cases and controls were similar demographically, with the exception that cases were more likely to be of Hispanic ethnicity (Table 1.2). Additionally, cases were more likely than controls to be current alcohol consumers and nulliparous, less likely to have a history of breastfeeding for greater than one year, and had greater serum lipid concentrations. The distribution of characteristics in this subset of WREN subjects was similar to that found among WREN cases and controls in the parent study (data not shown). The demographic and lifestyle characteristics among participants of the WREN study closely mirror those of the general population in the surrounding region (Saunders et al. 2005).

The distribution of serum OCP concentrations was right-skewed (Table 1.1). Geometric mean lipid-adjusted serum concentrations of β-HCH, oxychlordane, *trans*-nonachlor, and *p,p'*-DDE were somewhat lower than those reported by the Centers for Disease Control and Prevention for the same years using data from the National Health and Nutrition Examination Survey (NHANES) on females ages 12 and older (CDC 2009) (data not shown). The pairwise Spearman correlations among serum OCP concentrations were ≥0.70 for β-HCH and oxyclordane (r=0.73), heptachlor epoxide and dieldrin (r=0.78), and oxychlordane and *trans*-nonachlor (r=0.90). Blood was collected 6 months to 5.8 years after diagnosis in cases (median 1.2 years).

Our data suggested increased risks of endometriosis in association with serum concentrations of β-HCH (third vs. lowest quartile: OR 1.7, 95% CI: 1.0-2.8, *P*-value=0.047;

highest vs. lowest quartile: OR 1.3, 95% CI: 0.8-2.4) and mirex (highest vs. lowest category: OR 1.5, 95% CI: 1.0-2.2, *P*-value=0.065), adjusted for natural logarithm-transformed total serum lipids, education, race, smoking, alcohol intake, age, and enrollment year (Table 1.3). We also found modest associations between endometriosis risk and various quartiles of serum concentrations of ∑HCH, heptachlor epoxide, *trans*-nonachlor, ∑chlordane, and hexachlorobenzene, although the confidence intervals for these associations included the null. None of the tests of trend across exposure categories was significant ($P>0.05$) (data not shown). The association between serum concentrations of β-HCH and endometriosis was stronger in analyses restricting cases to those with ovarian endometriosis (third vs. lowest quartile: OR 2.5, 95% CI: 1.2-5.2; highest vs. lowest quartile: OR 2.5, 95% CI: 1.1-5.3; $P=0.023$ for test of trend), a finding not replicated for mirex (Table 1.3).

In our exploratory analyses adjusting for the excretion factors parity and breastfeeding, we found that the association between β-HCH and ovarian endometriosis remained (third vs. lowest quartile: OR 1.9, 95% CI: 0.9-4.1; highest vs. lowest quartile: OR 1.7 95% CI: 0.7-3.8). Overall, the estimates of the associations between individual OCP analytes and endometriosis risk adjusted for parity and breastfeeding were closer to zero than the estimates not adjusted for these factors (Supplemental Materials, Table 1A). With regard to the distribution of serum OCP concentrations among controls who reported a history of laparoscopic evaluation or history infertility testing, we observed that these controls had greater median concentrations of several OCP analytes compared to all controls (Supplemental Materials, Table 1B).

Discussion

In this population-based study, our data suggested an increased risk of endometriosis in relation to serum concentrations of β-HCH. β-HCH is not intentionally produced but is a component of technical-grade HCH used as an agricultural insecticide in the U.S. until the mid 1970s as well as a biologically persistent unintentional by-product of γ-HCH (lindane) (ATSDR 2005; CDC 2009). Prior epidemiologic studies restricting the study population to women undergoing laparoscopy have had divergent findings for the association between serum or fat β-HCH concentrations and endometriosis, with odds ratio estimates ranging from 0.77 to 2.0 (Buck Louis et al. 2012; Cooney et al. 2010; Lebel et al. 1998; Tsukino et al. 2005). Because the indication for laparoscopy may be associated with β-HCH body burden, as illustrated in our exploratory analyses, women in these studies without endometriosis but undergoing laparoscopy may have had atypical β-HCH concentrations. Therefore, the studies' results may be subject to unpredictable bias. Our results are consistent with recently published findings on a population cohort of 127 women sampled from a California telephone directory and Utah population database (Buck Louis et al. 2012). The investigators reported an aOR of 1.72 (95% CI: 1.09, 2.72) for MRI-detected endometriosis (n=14 women) in relation to a one-standard deviation change in the log-transformed serum concentration of β-HCH (Buck Louis et al. 2012). Similar to the population cohort in that study, WREN controls likely represented the frequency of exposure in the underlying population, as controls were randomly sampled directly from the source population from which the incident endometriosis cases emerged. Thus, the use of a population-based sampling framework in both studies may have avoided selection bias present in analyses restricted to women undergoing laparoscopy and allowed for more accurate risk estimates.

We also found the suggestion of increased endometriosis risk associated with serum concentrations of mirex. Mirex was used in the 1960s and 1970s as part of an insect control program against fire ants, with aerial application on millions of acres of southeastern U.S. states, and the chemical was also used as a fire-retardant additive. With a half-life of up to 10 years, mirex is considered one of the most stable and persistent pesticides (ATSDR 1995b; Fisher 1999). In our study of U.S. Pacific Northwest healthplan enrollees, 57.6% of mirex concentration measurements were below the limit of detection, indicating that this risk factor is not likely a major contributor to endometriosis incidence in our population. However, the investigation of mirex in relation to endometriosis remains relevant as some populations may be exposed to greater concentrations of mirex, such as those who consume fish from mirex-contaminated lakes and waterways (ATSDR 1995b). The association we found between serum concentrations of mirex and endometriosis risk contrasts with the results of three small prior studies with null results (Cooney et al. 2010; Lebel et al. 1998; Tsukino et al. 2005). All three of these case-control studies were conducted among laparoscopic patients; and two of the studies did not provide numeric data for the association. Although our finding may have been due to chance given the multiple comparisons carried out across individual OCP analytes, for the reason previously mentioned it is possible that bias from using a sampling framework of laparoscopic patients obscured the association between serum concentrations of mirex and endometriosis risk in the earlier studies. Additionally, given the small sample sizes, these studies may have been underpowered to detect an association.

In our study, the possibility existed for controls to have undiagnosed disease, as the absence of endometriosis was not confirmed surgically in controls in the parent WREN study. However, the prevalence of undiagnosed endometriosis that meets the case definition of

endometriotic disease is likely to be small, possibly <2%, which would minimally bias our results (Holt and Weiss 2000). Additionally, the use of the endometriotic disease definition, which excludes ectopic endometrial implants without evidence of tissue damage or symptoms, may have strengthened our study by increasing the sensitivity of our study to detect an association between OCPs and endometriosis risk. Furthermore, the population-based sampling framework may have minimized selection bias, allowing us to more accurately estimate the association between serum concentrations of OCP analytes and endometriosis risk and yield a finding consistent with a small cohort population.

Given the number of statistical tests carried out in this study, it is possible that some of our findings were due to chance. It is also possible that our results were affected by misclassification of exposure. Similar to previous studies of serum and fat concentrations of OCPs and endometriosis risk, serum samples in our study were obtained near the time of diagnosis, and serum OCP concentrations may not reflect OCP body burden at the time of disease development and progression. The timing of sample collection may have resulted in an underestimate of exposure in women who subsequently gave birth or breastfed, excretion factors that may reduce OCP body burden (Sarcinelli et al. 2003; Soliman et al. 2003). Because parity and breastfeeding may be in the causal pathway between exposure and disease, or downstream from endometriosis onset, we decided *a priori* not to adjust for these factors in the main analyses. In our exploratory analyses considering an alternative conceptual framework with excretion factors of parity and breastfeeding as confounders, we found that the estimates were closer to zero than the estimates in our main analyses not adjusted for these factors. However, in both sets of analyses, we observed an association between increasing β-HCH and ovarian endometriosis risk.

Conclusion

In this study we found that among enrollees of a large healthcare system in the U.S. Pacific Northwest, serum concentrations of select pesticides were associated with a possible increased risk of endometriosis. Our study demonstrates the environmental persistence of OCPs and suggests that exposure from extensive past use of OCPs in the United States, or present use in other countries may impact the health of the current generation of reproductive-age women with regard to a hormonally-mediated disease.

Table 1.1. Laboratory measurement of serum organochlorine pesticides and distribution by case status, Group Health, 1996-2001 (n=786).

Pesticide (pg/g serum)	Measured ≥LOD n (%)	Measured <LOD n (%)	Interference n (%)	Cases (n=248) Median (IQR)	Controls (n=538) Median (IQR)
β-HCH	709 (90.2)	30 (3.8)	47 (6.0)	51.91 (29.19, 80.79)	43.06 (26.99, 74.03)
γ-HCH[a]	339 (43.1)	424 (53.9)	23 (2.9)	<10.00 (<10.00, 13.97)	<10.00 (<10.00, 13.34)
Heptachlor epoxide	542 (69.0)	102 (13.0)	142 (18.1)	27.52 (18.88, 42.89)	26.19 (16.93, 42.91)
Oxychlordane	613 (78.0)	114 (14.5)	59 (7.5)	60.59 (30.93, 94.78)	51.96 (28.31, 79.55)
trans-nonachlor	784 (99.7)	2 (0.3)	0 (0.0)	81.22 (53.76, 127.63)	75.15 (51.37, 107.65)
p,p'-DDE	784 (99.7)	0 (0.0)	2 (0.3)	1569.74 (947.52, 2799.01)	1575.51 (905.86, 2816.55)
p,p'-DDT	670 (85.2)	80 (10.2)	36 (4.6)	29.11 (19.03, 45.10)	28.36 (18.79, 44.38)
Dieldrin	513 (65.3)	210 (26.7)	63 (8.0)	48.79 (<10.00, 71.40)	48.18 (<10.00, 73.74)
Hexachlorobenzene	786 (100)	0 (0.0)	0 (0.0)	287.87 (177.03, 529.99)	279.00 (159.74, 589.30)
Mirex[a]	300 (38.2)	453 (57.6)	33 (4.2)	<10.00 (<10.00, 15.61)	<10.00 (<10.00, 13.11)

Abbreviations: LOD=limit of detection; IQR=Interquartile range; HCH=hexachlorocyclohexane; DDE=dichlorodiphenyldichloroethylene; DDT=dichlorodiphenyltrichloroethane.

[a]For categorizing exposures in the statistical analyses, median and IQR calculated among controls using values ≥LOD. For γ-HCH, median (IQR) was 13.89 (12.21, 17.65). For Mirex, median (IQR) was 15.47 (12.12, 23.32).

Table 1.2. Characteristics of WREN participants with serum organochlorine pesticide measurements, Group Health, 1996-2001.

Characteristic	Cases (n=248) n (%)	Controls (n=538) n (%)
Age (years)		
17-24	19 (7.7)	44 (8.2)
25-34	52 (21.0)	93 (17.3)
35-44	120 (48.4)	277 (51.5)
45-49	57 (23.0)	124 (23.1)
Race		
White	213 (85.9)	457 (85.1)
Black	8 (3.2)	22 (4.1)
Asian/Pacific Islander	20 (8.1)	38 (7.1)
American Indian	2 (0.8)	4 (0.7)
More than one race	5 (2.0)	16 (3.0)
Ethnicity		
Hispanic	16 (6.5)	18 (3.4)
Non-Hispanic	230 (93.5)	518 (96.6)
Income		
<$35,000	73 (30.5)	146 (28.0)
$35,000-$69,999	105 (43.9)	223 (42.7)
≥70,000	61 (25.5)	153 (29.3)
Education		
<HS	8 (3.2)	17 (3.2)
HS graduate	42 (16.9)	96 (17.8)
Some college	80 (32.3)	202 (37.6)
College graduate	79 (31.9)	120 (22.3)
Post graduate	39 (15.7)	103 (19.1)
Cigarette smoking		
Never	143 (57.7)	325 (60.4)
Former	56 (22.6)	124 (23.1)
Current	49 (19.8)	89 (16.5)
Alcohol use		
Never	68 (27.5)	186 (34.6)
Former	45 (18.2)	113 (21.0)
Current	134 (54.3)	239 (44.4)

Table 1.2. Continued.

Characteristic	Cases (n=248) n (%)	Controls (n=538) n (%)
BMI (kg/m^2)		
<18.5	8 (3.2)	11 (2.1)
18.5-<25.0	126 (50.8)	278 (52.1)
25.0-<30.0	60 (24.2)	140 (26.2)
≥30.0	54 (21.8)	105 (19.7)
Parity		
Nulliparous	121 (48.8)	157 (29.2)
Parous	127 (51.2)	380 (70.8)
Lifetime lactation history (weeks)		
Did not breastfeed – nulliparous	121 (48.8)	157 (29.2)
Did not breastfeed - parous	27 (10.9)	71 (13.2)
1-24 weeks	42 (16.9)	89 (16.6)
25-52 weeks	28 (11.3)	77 (14.3)
>52 weeks	30 (12.1)	143 (26.6)
Total lipids (mg/dL), median (IQR)	679.7 (593.4, 816.1)	661.4 (572.6, 777.1)

Abbreviations: WREN=Women's Risk of Endometriosis Study; HS=High School; BMI=body mass index; IQR=interquartile range.

Table 1.3. Odds ratios and 95% confidence intervals for the relationship between individual organochlorine pesticides and risk of endometriosis, Group Health, 1996-2001.

Pesticide	Overall endometriosis		Ovarian endometriosis	
Quartiles (pg/g serum)	Case/control (n)	aOR[a] (95% CI)	Case/Control (n)	aOR[a] (95% CI)
β-HCH[b]				
≤26.99	49/126	1.0	17/126	1.0
>26.99-43.06	41/127	0.8 (0.5,1.4)	21/127	1.2 (0.5, 2.4)
>43.06-74.01	78/127	1.7 (1.0, 2.8)	42/127	2.5 (1.2, 5.2)
>74.01	64/127	1.3 (0.8, 2.4)	42/127	2.5 (1.1, 5.3)
γ-HCH[c]				
≤10.0 (LOD)	129/295	1.0	69/295	1.0
>10.0-13.89	54/112	1.1 (0.8, 1.7)	29/112	1.1 (0.7, 1.9)
>13.89	61/112	1.3 (0.9, 1.9)	32/112	1.2 (0.7, 2.0)
∑HCH (mol/g serum)[d]				
≤0.12	54/134	1.0	24/134	1.0
>0.12-0.18	48/135	0.8 (0.5, 1.3)	21/135	0.8 (0.4, 1.7)
>0.18-0.29	79/134	1.4 (0.9, 2.2)	45/134	1.8 (1.0, 3.3)
>0.29	67/135	1.1 (0.6, 1.8)	42/135	1.6 (0.8, 3.0)
Heptachlor epoxide[e]				
≤17.10	40/110	1.0	21/110	1.0
>17.10-26.19	55/110	1.4 (0.8, 2.4)	22/110	1.0 (0.5, 2.0)
>26.19-42.91	59/110	1.4 (0.8, 2.3)	34/110	1.3 (0.7, 2.6)
>42.91	51/109	1.2 (0.7, 2.1)	28/109	1.1 (0.5, 2.2)
Oxychlordane				
≤28.31	52/124	1.0	24/124	1.0
>28.31-51.96	46/125	0.8 (0.5, 1.3)	23/125	0.8 (0.4, 1.6)
>51.96-79.55	57/126	1.0 (0.6, 1.6)	34/126	1.2 (0.6, 2.4)
>79.55	73/124	1.2 (0.7, 2.1)	45/124	1.5 (0.8, 3.0)
trans-nonachlor				
≤51.37	55/134	1.0	21/134	1.0
>51.37-75.15	55/135	1.0 (0.6, 1.6)	31/135	1.4 (0.7, 2.7)
>75.15-107.65	56/134	1.0 (0.6, 1.6)	33/134	1.3 (0.7, 2.6)
>107.65	82/135	1.4 (0.8, 2.4)	47/135	1.8 (0.9, 3.7)
∑Chlordane (mol/g serum)[d]				
≤0.21	53/134	1.0	23/134	1.0
>0.21-0.33	56/135	1.0 (0.6, 1.7)	24/135	1.0 (0.5, 1.9)
>0.33-0.52	57/134	1.1 (0.6, 1.8)	36/134	1.5 (0.8, 2.9)
>0.52	82/135	1.4 (0.8, 2.5)	49/135	1.8 (0.9, 3.7)

Table 1.3. Continued.

Pesticide	Overall endometriosis		Ovarian endometriosis	
Quartiles (pg/g serum)	Case/control (n)	aOR[a] (95% CI)	Case/Control (n)	aOR[a] (95% CI)
p,p'-DDE				
≤905.86	55/134	1.0	25/134	1.0
>905.86-1575.51	69/134	1.2 (0.8, 2.0)	38/134	1.4 (0.8, 2.6)
>1575.51-2816.55	62/134	1.1 (0.7, 1.7)	31/134	1.1 (0.6, 2.1)
>2816.55	61/135	1.0 (0.6, 1.7)	37/135	1.2 (0.6, 2.4)
p,p'-DDT				
≤18.79	58/128	1.0	21/128	1.0
>18.79-28.36	58/128	0.9 (0.6, 1.5)	33/128	1.4 (0.7, 2.6)
>28.36-44.38	55/128	0.8 (0.5, 1.4)	32/128	1.2 (0.6, 2.3)
>44.38	66/129	0.9 (0.6, 1.5)	38/129	1.3 (0.7, 2.6)
∑DDT (mol/g serum)[d]				
≤2.88	55/135	1.0	25/135	1.0
>2.88-5.03	71/134	1.3 (0.8, 2.0)	39/134	1.5 (0.8, 2.7)
>5.03-8.95	59/135	1.0 (0.6, 1.7)	29/135	1.1 (0.6, 2.1)
>8.95	63/134	1.1 (0.6, 1.8)	39/134	1.4 (0.7, 2.7)
Dieldrin[f]				
≤10.0 (LOD)	59/151	1.0	32/151	1.0
>10.0-48.18	52/97	1.3 (0.8, 2.1)	17/97	0.7 (0.4, 1.5)
>48.18-73.74	65/123	1.1 (0.7, 1.8)	39/123	1.2 (0.7, 2.1)
>73.74	52/124	0.8 (0.5, 1.3)	30/124	0.8 (0.4, 1.4)
Hexachlorobenzene				
≤159.74	49/135	1.0	23/135	1.0
>159.74-278.998	71/134	1.4 (0.9, 2.1)	40/134	1.6 (0.9, 3.0)
>278.998-589.30	72/134	1.4 (0.9, 2.1)	35/134	1.4 (0.7, 2.6)
>589.30	56/135	0.9 (0.6, 1.5)	34/135	1.1 (0.6, 2.1)
Mirex[c]				
≤10.0 (LOD)	133/320	1.0	68/320	1.0
>10.0-15.47	45/97	1.1 (0.7, 1.8)	28/97	1.3 (0.8, 2.2)
>15.47	60/98	1.5 (1.0, 2.2)	29/98	1.2 (0.7, 2.1)

Abbreviations: OR=Odds ratio; 95% CI=95% confidence interval; LOD=limit of detection; HCH=hexachlorocyclohexane; DDE=dichlorodiphenyldichloroethylene; DDT=dichlorodiphenyltrichloroethane.

[a]Odds ratio adjusted for age, reference year, smoking, alcohol, education, natural logarithm transformed total lipids, and race.

[b]Cutpoint between third and fourth quartile modified to provide equal number across control groups (74.01 versus 74.03).

[c]Lowest category consists of values <LOD. Middle and top categories, ≤median or >median, based on observations with values among controls.

[d]Summed chemicals used multiple imputation data (five datasets); Quartile cutpoints and frequencies from first imputed dataset.

[e]Cutpoint between first and second quartile modified to provide equal number across control groups (17.10 versus 16.93).

[†]All samples with values below the level of detection were categorized in the lowest category. Subsequent categories were created using the 50th and 75th percentile among the distribution in controls.

Supplemental Materials, Table 1A. Odds ratios and 95% confidence intervals for the relationship between individual organochlorine pesticides and risk of endometriosis additionally adjusting for parity and breastfeeding, Group Health, 1996-2001.

Pesticide			Ovarian endometriosis	
Quartiles (pg/g serum)	Case/control (n)	aOR[a] (95% CI)	Case/Control (n)	aOR[a] (95%CI)
β-HCH[b]				
≤26.99	49/126	1.0	17/126	1.0
>26.99-43.06	41/127	0.7 (0.4, 1.3)	21/127	1.0 (0.5, 2.2)
>43.06-74.01	78/127	1.3 (0.8, 2.3)	42/127	1.9 (0.9, 4.1)
>74.01	64/127	1.0 (0.5, 1.8)	42/127	1.7 (0.7, 3.8)
γ-HCH[c]				
≤10.0 (LOD)	129/295	1.0	69/295	1.0
>10.0-13.89	54/112	1.1 (0.8, 1.7)	29/112	1.1 (0.6, 1.9)
>13.89	61/112	1.3 (0.9, 1.9)	32/112	1.2 (0.7, 2.0)
∑HCCH (mol/g serum)[d]				
≤0.12	54/134	1.0	24/134	1.0
>0.12-0.18	48/135	0.8 (0.5, 1.2)	21/135	0.8 (0.4, 1.7)
>0.18-0.29	79/134	1.2 (0.7, 1.9)	45/134	1.5 (0.8, 2.9)
>0.29	67/135	0.8 (0.5, 1.4)	42/135	1.1 (0.6-2.3)
Heptachlor epoxide[e]				
≤17.10	40/110	1.0	21/110	1.0
>17.10-26.19	55/110	1.5 (0.9, 2.5)	22/110	1.0 (0.5, 2.1)
>26.19-42.91	59/110	1.3 (0.7, 2.2)	34/110	1.2 (0.6, 2.3)
>42.91	51/109	1.0 (0.5, 1.8)	28/109	0.8 (0.4, 1.8)
Oxychlordane				
≤28.31	52/124	1.0	24/124	1.0
>28.31-51.96	46/125	0.7 (0.4, 1.2)	23/125	0.7 (0.4, 1.5)
>51.96-79.55	57/126	0.8 (0.5, 1.4)	34/126	1.0 (0.5, 1.9)
>79.55	73/124	0.8 (0.5, 1.5)	45/124	0.9 (0.5, 2.0)
trans-nonachlor				
≤51.37	55/134	1.0	21/134	1.0
>51.37-75.15	55/135	0.9 (0.6, 1.5)	31/135	1.2 (0.6, 2.4)
>75.15-107.65	56/134	0.9 (0.5, 1.4)	33/134	1.1 (0.5, 2.2)
>107.65	82/135	1.1 (0.6, 1.9)	47/135	1.3 (0.6, 2.8)
∑Chlordane (mol/g serum)[d]				
≤0.21	53/134	1.0	23/134	1.0
>0.21-0.33	56/135	1.0 (0.6, 1.7)	24/135	1.0 (0.5, 1.9)
>0.33-0.52	57/134	1.0 (0.6, 1.6)	36/134	1.3 (0.6, 2.5)
>0.52	82/135	1.1 (0.7, 2.0)	49/135	1.3 (0.6, 2.8)

Supplemental Materials, Table 1A. continued.

Pesticide			Ovarian endometriosis	
Quartiles (pg/g serum)	Case/control (n)	aOR[a] (95% CI)	Case/Control (n)	aOR[a] (95% CI)
p,p'-DDE				
≤905.86	55/134	1.0	25/134	1.0
>905.86-1575.51	69/134	1.1 (0.7, 1.8)	38/134	1.2 (0.7, 2.3)
>1575.51-2816.55	62/134	0.9 (0.5, 1.4)	31/134	0.8 (0.4, 1.6)
>2816.55	61/135	0.8 (1.5, 1.3)	37/135	0.9 (0.4, 1.8)
p,p'-DDT				
≤18.79	58/128	1.0	21/128	1.0
>18.79-28.36	58/128	0.8 (0.5, 1.4)	33/128	1.2 (0.6, 2.3)
>28.36-44.38	55/128	0.7 (0.4, 1.1)	32/128	1.0 (0.5, 1.9)
>44.38	66/129	0.8 (0.4, 1.3)	38/129	1.0 (0.5, 2.0)
∑DDT (mol/g serum)[d]				
≤2.88	55/135	1.0	25/135	1.0
>2.88-5.03	71/134	1.2 (0.8, 1.9)	39/134	1.3 (0.7, 2.4)
>5.03-8.95	59/135	0.8 (0.5, 1.4)	29/135	0.8 (0.4, 1.6)
>8.95	63/134	0.9 (0.5, 1.5)	39/134	1.0 (0.5, 2.1)
Dieldrin[f]				
≤10.0 (LOD)	59/151	1.0	32/151	1.0
>10.0-48.18	52/97	1.2 (0.8, 2.0)	17/97	0.7 (0.3, 1.4)
>48.18-73.74	65/123	1.1 (0.7, 1.7)	39/123	1.0 (0.6, 1.9)
>73.74	52/124	0.7 (0.4, 1.2)	30/124	0.6 (0.3, 1.1)
HCB				
≤159.74	49/135	1.0	23/135	1.0
>159.74-278.998	71/134	1.3 (0.8, 2.1)	40/134	1.5 (0.8, 2.8)
>278.998-589.30	72/134	1.3 (0.8, 2.0)	35/134	1.3 (0.7, 2.4)
>589.30	56/135	0.9 (0.5, 1.5)	34/135	1.0 (0.5, 1.8)
Mirex[c]				
≤10.0 (LOD)	133/320	1.0	68/320	1.0
>10.0-15.47	45/97	1.1 (0.7, 1.7)	28/97	1.3 (0.7, 2.2)
>15.47	60/98	1.3 (0.9, 2.0)	29/98	1.0 (0.6, 1.8)

Abbreviations: OR=Odds ratio; 95% CI=95% confidence interval; LOD=limit of detection.

[a]Odds ratio adjusted for age, reference year, smoking, alcohol, education, natural logarithm transformed total lipids, race, and a composite variable for parity and breastfeeding.

[b]Cutpoint between third and fourth quartile modified to provide equal number across control groups (74.01 versus 74.03).

[c]Lowest category consists of values <LOD. Middle and top categories, ≤median or >median, based on observations with values among controls.

[d]Summed chemicals used multiple imputation data (five datasets); Quartile cutpoints and frequencies from first imputed dataset.

[e]Cutpoint between first and second quartile modified to provide equal number across control groups (17.10 versus 16.93).

[f]All samples with values below the level of detection were categorized in the lowest category. Subsequent categories were created using the 50th and 75th percentile among the distribution in controls.

Supplemental Materials, Table 1B: Distribution of serum organochlorine pesticides by history of laparoscopy and infertility testing, Group Health, 1996-2001.

	History of laparoscopy[a]		History of infertility testing[b]	
Pesticide (pg/g serum)	Cases (n=109) Median (IQR)	Controls (n=47) Median (IQR)	Cases (n=40) Median (IQR)	Controls (n=39) Median (IQR)
β-HCH	40.68 (25.57, 59.63)	47.58 (38.27,98.72)	44.77 (29.19, 60.52)	49.33 (34.51, 83.61)
γ-HCH[a]	<10.0 (<10.00, 13.14)	<10.00 (<10.00, 15.13)	10.29 (<10.00, 13.14)	10.74 (<10.00, 14.20)
Heptachlor epoxide	22.17 (14.84, 31.63)	25.69 (19.59, 41.46)	23.47 (<10.00, 32.22)	23.99 (16.15, 41.37)
Oxychlordane	46.39 (27.37, 66.07)	62.40 (43.23, 99.66)	41.36 (21.24, 65.91)	54.25 (38.85, 81.49)
trans-nonachlor	65.45 (44.62, 95.99)	81.14 (64.14, 131.97)	63.49 (51.63, 108.46)	81.24 (69.09, 116.09)
p,p'-DDE	1363.09 (815.01, 2220.50)	1751.52 (825.69, 3798.72)	1143.79 (803.93, 2066.65)	1732.02 (940.30, 2901.02)
p,p'-DDT	23.05 (16.57, 42.58)	27.43 (17.49, 54.86)	23.13 (17.99, 34.50)	28.86 (17.51, 51.19)
Dieldrin	41.15 (<10.00, 60.02)	55.88 (<10.00, 77.96)	42.05 (<10.00, 54.51)	50.59 (<10.00, 75.05)
Hexachlorobenzene	222.94 (148.97, 425.54)	387.85 (237.84, 742.02)	232.64 (140.45, 433.64)	350.13 (179.59, 685.24)
Mirex[a]	<10.00 (<10.00, 15.07)	10.96 (<10.00, 16.71)	<10.00 (<10.00, 19.63)	<10.00 (<10.00, 17.44)

Abbreviations: LOD=limit of detection; IQR=Interquartile range; HCH=hexachlorocyclohexane; DDE=dichlorodiphenyldichloroethylene; DDT=dichlorodiphenyltrichloroethane.

[a]History of laparoscopy was based on self-report of laparoscopy before the reference date for cases and controls and laparoscopic diagnosis of endometriosis among cases.

[b]History of infertility was based on self-report of having tests done for infertility and if reason for first visit leading to endometriosis diagnosis among cases included infertility.

Chapter 2: Phthalates and risk of endometriosis

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.

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 1995a, 2001, 2002; 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 care and cosmetic products (ECB 2007; IPCS 2003; Kavlock et al. 2002a, b; 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, b; 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 (Buck Louis et al. 2013; 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.

Methods

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, 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, 2009). Cases (n=340) were female GH enrollees diagnosed for the first time with endometriosis (International Classification of Disease 9th 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; 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.

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 30mL 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 2.1). 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 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, 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.

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 2.2). 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.

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/√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, Figure 2A) (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.

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 2.3). 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 (data not shown).

The majority of women in our study had detectable concentrations of phthalate metabolites (Table 2.2). The distribution of individual urinary phthalate concentrations was right-skewed (Table 2.2) and metabolites of DEHP were highly correlated ($r>0.80$) (Supplemental Material, Table 2A). 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 2B and 2C). 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 2.4). 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 2.4).

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 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, histopathologic ovarian changes, and altered ovarian steroidogenesis such as decreased estradiol and progesterone production (Davis et al. 1994; Li et al. 2012; Takai 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 three studies restricting the study population to women undergoing laparoscopy. Those studies reported a positive association between MEHP and endometriosis, with adjusted ORs ranging from 1.20-1.57 (Buck Louis et al. 2013; Huang et al. 2010; Itoh et al. 2009). Our results also differ from findings on a population cohort of 127 women in which endometriosis, primarily ovarian endometrioma, was detected by MRI screening (Buck Louis et al. 2013). In that study, the investigators reported an adjusted OR of 2.59 (95% CI: 1.17-5.75) for endometriosis in relation to one standard deviation change in log-transformed urinary MEHP concentrations (Buck Louis et al. 2013). 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). Even though only our study and that of Weuve et al (2010) reported inverse associations, our findings are consistent with the limited animal studies indicating that DEHP is an ovarian toxicant and suppresses ovarian production of

hormones. 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. As for the positive association found for the population cohort in the study by Buck Louis et al (2013), the possibility exists that DEHP may act differentially by entities of endometriosis which may be etiologically distinct. That is to say, DEHP may increase the risk of ovarian endometrioma but decrease the risk of peritoneal endometriosis. Due to the small number of cases and controls in our study, we were not able to further investigate urinary MEHP concentrations in relation to distinct ovarian or non-ovarian disease entities.

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).

Three prior studies that restricted the study population to laparoscopic patients reported divergent adjusted odds ratios for the associations between MBzP and MEP and endometriosis risk (Buck Louis et al. 2013; Huang et al. 2010; Itoh et al. 2009). 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, Buck Louis et al (2013) reported adjusted ORs indicating no association with MEP and suggesting a positive association with MBzP for the population cohort. Given that primarily ovarian endometrioma was diagnosed in that population cohort, differing results by disease entity are possible as described in our discussion of DEHP. Also conflicting with 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 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.

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.

Table 2.1. 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.

Table 2.2. 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.

Table 2.3. 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)
≥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)

Table 2.3. Continued.

Characteristic	Cases (n=92) n (%)	Controls (n=195) n (%)
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.

Table 2.4. Odds ratios and 95% confidence intervals for the relationship between individual urinary phthalate metabolites and risk of endometriosis, Group Health, 1996-2001.

Phthalate metabolite	Cases (n=92)	Controls (n=195)		
Quartiles (ng/ml urine)	n (%)	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

Table 2.4. Continued.

Phthalate metabolite Quartiles (ng/ml urine)	Cases (n=92) n (%)	Controls (n=195) n (%)	aOR[a] (95% CI)	aOR[b] (95% CI)
∑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)
>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.

Supplemental Material, Table 2A. Pairwise Spearman correlation among phthalate metabolites using concentrations above the limit of quantitation for cases and controls in the WREN study with measured urinary phthalate metabolite concentrations, Group Health 1996-2001.

	MEHP	MEHHP	MEOHHP	MECPP	MBzP	MEP	MiBP	MnBP
MEHP	1.0000							
MEHHP	0.8751	1.0000						
MEOHP	0.8713	0.9897	1.0000					
MECPP	0.8153	0.9670	0.9694	1.0000				
MBzP	0.4227	0.5989	0.6137	0.5807	1.0000			
MEP	0.2501	0.3784	0.3875	0.3855	0.3137	1.0000		
MiBP	0.4227	0.5735	0.6066	0.5723	0.6435	0.3770	1.0000	
MnBP	0.5295	0.6454	0.6760	0.6488	0.6748	0.4357	0.6698	1.0000

Abbreviations: WREN=Women's Risk of Endometriosis; 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.

Supplemental Material, Table 2B. Comparison of geometric means and confidence intervals of urinary phthalate metabolite concentrations (µg/L) among WREN controls and female NHANES sample.

	WREN Controls[a]		NHANES - females (≥6 years of age)					
Phthalate metabolite	2001-2002		1999-2000		2001-2002		2003-2004	
	n	GM (95% CI)	n	GM (95% CI)	n	GM (95% CI)	n	GM (95% CI)
MEHP	195	3.49 (2.76-4.43)	1326	3.21 (2.91-3.54)	1411	4.23 (3.67-4.86)	1355	2.15 (1.92-2.42)
MEHHP	195	20.2 (16.2-25.1)			1411	18.3 (15.7-21.4)	1355	19.7 (17.4-22.2)
MEOHP	195	11.2 (9.1-13.9)			1411	12.5 (10.8-14.6)	1355	13.4 (11.9-15.1)
MECPP	195	18.9 (15.3-23.3)					1355	31.9 (28.1-36.2)
MBzP	195	4.7 (3.9-5.6)	1326	10.5 (9.17-12.0)[b]	1411	10.5 (9.40-11.7)[b]	1355	9.33 (8.53-10.2)[b]
MEP	195	53.2 (42.2-67.1)	1322	118 (102-136)[c]	1411	115 (101-131)[c]	1355	125 (106-148)[c]
MiBP	195	1.54 (1.32-1.80)			1411	2.68 (2.44-2.96)	1355	3.56 (3.19-3.97)
MnBP	195	10.5 (8.9-12.5)			1411	20.2 (18.2-22.4)	1355	22.2 (21.2-23.3)

Abbreviations: GM=Geometric mean; CI=confidence interval; WREN=Women's Risk of Endometriosis Study; NHANES=National Health and Nutrition Evaluation Survey; LOQ=limit of quantitation; 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.

[a]WREN data handled in same way as NHANES data described in the CDC's Fourth National Report on human exposure to environmental chemicals. Observations below the LOQ were substituted with the value LOQ/√2.
[b]NHANES MBzP concentrations corrected for inadequate purity in the analytical standards (adjusted by a factor of 0.72).
[c]NHANES MEP concentrations corrected for inadequate purity in the analytical standards (adjusted by a factor of 0.66).

Supplemental Material, Table 2C. Comparison of geometric means and confidence intervals of creatinine-corrected urinary phthalate metabolite concentrations (µg/g creatinine) among WREN controls and female NHANES sample.

	WREN Controls[a]		NHANES - females (≥6 years of age)					
Phthalate metabolite	2001-2002 n	GM (95% CI)	1999-2000 n	GM (95% CI)	2001-2002 n	GM (95% CI)	2003-2004 n	GM (95% CI)
MEHP	188	6.34 (5.23-7.69)	1326	3.36 (3.11-3.63)	1411	4.54 (4.02-5.13)	1355	2.40 (2.15-2.69)
MEHHP	188	37.0 (31.4-43.6)			1411	19.7 (17.3-22.4)	1355	21.9 (19.7-24.5)
MEOHP	188	20.6 (17.7-24.0)			1411	13.5 (11.9-15.2)	1355	14.9 (13.4-16.7)
MECPP	188	34.4 (29.5-40.1)					1355	35.5 (31.6-40.0)
MBzP	188	8.5 (7.6-9.6)	1326	11.0 (9.96-1.1)[b]	1411	11.3 (10.2-12.4)[b]	1355	10.4 (9.65-11.2)[b]
MEP	188	93.3 (76.5-113.8)	1322	123 (109-139)[c]	1411	123 (110-139)[c]	1355	139 (119-163)[c]
MiBP	188	2.80 (2.53-3.10)			1411	2.88 (2.61-3.18)	1355	3.96 (3.56-4.42)
MnBP	188	19.4 (17.3-21.8)			1411	21.7 (19.6-23.9)	1355	24.8 (22.9-26.8)

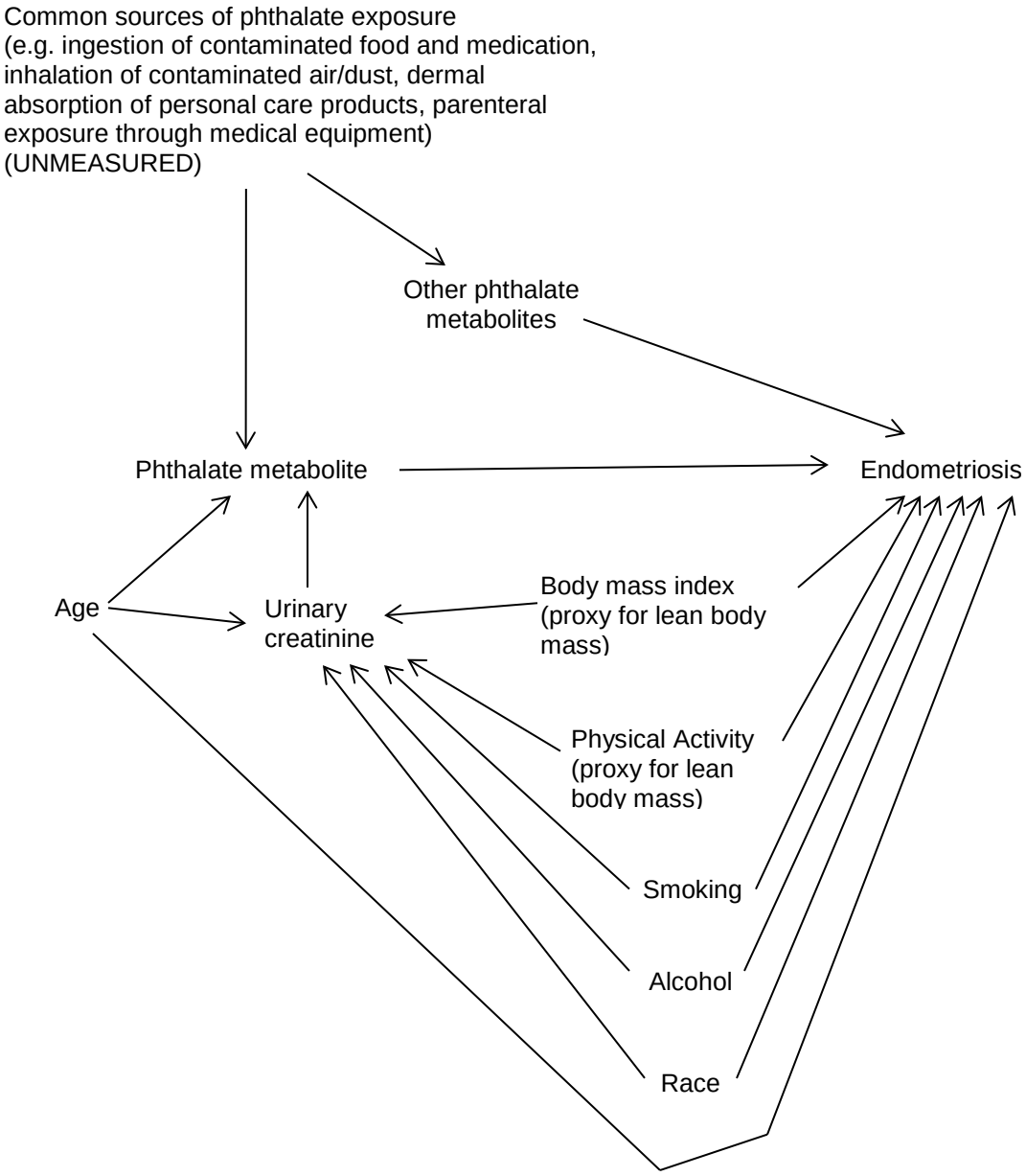
Abbreviations: GM=Geometric mean; CI=confidence interval; WREN=Women's Risk of Endometriosis Study; NHANES=National Health and Nutrition Evaluation Survey; LOQ=limit of quantitation; MEHP=mono-(2-ethyl-5-hexyl) phthalate; MEHHP=mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP=mono-(2-ethyl-5-oxohexyl) 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.

[a]WREN data handled in same way as NHANES data described in the CDC's Fourth National Report on human exposure to environmental chemicals. Observations below the LOQ were substituted with the value LOQ/√2. We excluded seven WREN controls due to creatinine values being below the limit of quantitation.

[b]NHANES MBzP concentrations corrected for inadequate purity in the analytical standards (adjusted by a factor of 0.72).

[c]NHANES MEP concentrations corrected for inadequate purity in the analytical standards (adjusted by a factor of 0.66).

Supplemental Material, Figure 2A. Proposed directed acyclic graph for the relationship between urinary phthalate metabolites and endometriosis risk



Chapter 3: Demographic, temporal, and anthropometric characteristics and phthalate metabolite concentrations among premenopausal women

Abstract

Despite U.S. biomonitoring studies reporting that women have greater exposure to select phthalates than men and children, there is a paucity of basic information on demographic, temporal, and anthropometric characteristics associated with increased phthalate body burden among premenopausal women. We investigated these characteristics in relation to urinary phthalate metabolite concentrations using data on 195 premenopausal women who served as controls in a case-control study of endometriosis and for whom eight urinary phthalate metabolites were measured on single, spot urine samples. Ratios of median phthalate metabolite concentrations and 95% confidence intervals (CI) across characteristics were estimated using multivariable linear regression, adjusting for urinary creatinine and potential confounding factors. We observed trends across hours of the day (summed metabolites of di(2-ethylhexyl) phthalate, P=0.028), days of the week (mono-ethyl phthalate, P=0.009), and seasons of the year (mono-isobutyl phthalate (MiBP), P=0.041). MiBP concentrations were inversely associated with waist circumference (fourth vs. first quartile: ratio of median concentrations 0.67, 95% CI: 0.49-0.91) and waist-to-hip ratio (fourth vs. first quartile: ratio 0.66, 95% CI: 0.49-0.90). Our findings suggest that subgroups of women have increased phthalate body burden. Further research is warranted to confirm our findings and to elucidate specific behaviors associated with phthalate exposure among women.

Introduction

Phthalates (dialkyl or alkyl aryl esters of *o*-phthalic acid) are a family of synthetic chemicals used as plasticizers to impart flexibility to plastics or as solvents or fixatives and are found in a wide range of industrial and consumer products. (ATSDR 1995a, 2001, 2002; CERHR 2003). Biomonitoring studies have detected urinary metabolites of di(2-ethylhexyl) phthalate (DEHP), benzylbutyl phthalate (BzBP), diethyl phthalate (DEP) and dibutyl phthalate (DBP, sum of isomers di-n-butyl phthalate (DiBP) and di-n-butyl phthalate (DnBP)) in ≥78% of the U.S. general population with greater concentrations of DEP and DBP metabolites detected in women than in men and children. (ATSDR 1995a, 2001, 2002; Blount et al. 2000; CERHR 2003; Silva et al. 2004).

DEP and to some extent DnBP are found in products specifically marketed to women. DEP is used as an emollient, fragrance-fixative, or solvent in the manufacturing of personal care products, such as hair products and cosmetics, and is used in high concentrations in deodorants and perfumes (Houlihan et al. 2002; Hubinger and Havery 2006; Koniecki et al. 2011; Koo and Lee 2004). Women are exposed to DEP through the dermal application of these products (Wormuth et al. 2006). DnBP is added to nailpolish to prevent brittleness and cracking and to personal care products as a fragrance-fixative but it is also used to produce other products including pharmaceuticals, adhesives, paints, printing inks for paper and packaging, and floor carpets (ATSDR 2001; Koo and Lee 2004). Women are believed to be mainly exposed to DEHP, DiBP, DnBP, and BzBP from the oral ingestion of food contaminated by the leaching of these phthalates from processing equipment and packaging materials used in the food industry (Wormuth et al. 2006), although additional exposure is possible through general contamination in the environment such as by inhalation of indoor air (ATSDR 1995a, 2001, 2002; CERHR

2003; Schettler 2006; Wormuth et al. 2006). DEHP is commonly used as a plasticizer to soften or provide flexibility to polyvinyl chloride (PVC) and is found in numerous products including building materials (such as flooring, wall covering, upholstery, and cables), home furnishings, and food contact materials (ECB 2008). DiBP is used in similar products as its isomer DnBP (CPSC 2011) and in combination with high molecular weight phthalates such as DEHP as a gelling aid to produce PVC (NICNAS 2006). 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).

Exposure to phthalates has been a source of public and scientific concern as these phthalates have demonstrated endocrine disruptive effects *in vitro* and *in vivo* on several organ systems including the female reproductive system. Ovarian toxicity has been observed in limited female animal studies of adult exposure to DEHP (Davis et al. 1994; Li et al. 2012; Takai et al. 2009) and some *in vitro* studies have demonstrated estrogenic effects of DEP, DnBP, DiBP, and BzBP (Coldham et al. 1997; Harris et al. 1997; Jobling et al. 1995; Soto et al. 1995; Zacharewski et al. 1998). Additionally, subsequent investigations into the health effects of phthalates among women have suggested that exposure to DEP, DnBP, DiBP, BzBP, and DEHP may be associated with endocrine-related conditions, including uterine fibroids, endometriosis, breast cancer, diabetes, altered glucose metabolism and increased insulin resistance (Huang et al. 2010; James-Todd et al. 2012; Lopez-Carrillo et al. 2010; Svensson et al. 2011; Weuve et al. 2010). Hence, the premenopausal years may be an etiologically relevant time period for disease development from exposure to these chemicals.

Identifying demographic and anthropometric characteristics of premenopausal women with increased exposure to phthalates may help inform efforts to limit phthalate exposure,

particularly given that not all sources and pathways of exposure have been identified (Schettler 2006) and that exposure may be influenced by behavior. These characteristics as well as temporal characteristics may serve as proxies for behaviors such as diet and personal care product use or suggest other exposure sources. Only three studies to date have investigated demographic, temporal, and anthropometric characteristics in relation to phthalate body burden specifically among premenopausal women (Hatch et al. 2008; Kobrosly et al. 2012; Peck et al. 2010). Two of these studies were conducted using data from the National Health and Nutrition Examination Survey (NHANES) (Hatch et al. 2008; Kobrosly et al. 2012). Although NHANES collects extensive data on socioeconomic factors, it offers only limited anthropometric and temporal data. The third study to report on urinary concentrations of phthalates in relation to select demographic, temporal, anthropometric characteristics was conducted among a population of reproductive-age Hmong women and may not be generalizable to other populations (Peck et al. 2010). The purpose of the current analyses was to further investigate demographic, temporal, and anthropometric characteristics in relation to urinary phthalate metabolite concentrations among premenopausal women using data from a case-control study conducted among female enrollees of large healthplan in the U.S. Pacific Northwest.

Materials and methods

Study population

For the current cross-sectional analyses, we used data available on controls from a case-control study of phthalate metabolites and endometriosis risk that was nested in the Women's Risk of Endometriosis study (WREN) (Marino et al. 2008, 2009). WREN was a five-year population-based case-control study investigating reproductive and contraceptive risk factors in relation to

endometriosis among 18-49 year old female enrollees of Group Health, a large integrated healthcare delivery system in western Washington State. WREN controls were women without endometriosis who were randomly selected from Group Health enrollment databases between April 1, 1996 and March 31, 2001 and matched to cases in 5-year age bands. Eligibility criteria for the WREN study included premenopausal status, an intact uterus and at least one ovary, no past history of surgically confirmed endometriosis, and enrollment in Group Health for at least six months prior to the reference date. The reference date for WREN cases was the date of the first Group Heath visit for symptoms leading to endometriosis diagnosis; controls were assigned reference dates to correspond with the case distribution of dates. WREN study activities entailed participation in a structured, in-person interview covering a range of reproductive health topics that included demographic and lifestyle information. A subset of WREN study participants also took part in a two-year ancillary study, 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). The case-control study of phthalate metabolites and endometriosis risk consisted of 92 WREN cases and 195 WREN controls with available archived urine samples from the POPs study that had not undergone a thaw-refreeze cycle, had creatinine concentrations ≤300 mg/dl, and were measured for urinary phthalate metabolites. Dehydration, indicated by creatinine concentrations >300mg/dl, may alter renal elimination of phthalate metabolites (Barr et al. 2005). The current cross-sectional analyses used data on the 195 controls from that case-control study to explore demographic, temporal, and anthropometric characteristics in relation to urinary concentrations of phthalate metabolites. We used only data for controls to prevent confounding from the inclusion of cases as endometriosis is associated with demographic and anthropometric characteristics and select

phthalate metabolites. We received institutional review board approval from the Fred Hutchinson Cancer Research Center and each participant provided written informed consent prior to enrollment and participation in the WREN study.

Demographic characteristics

Data on demographic characteristics ascertained as part of the in person structured interview and explored in the current analyses included age at interview in years (19-24, 25-34, 35-44, 45-53), race (White, Black, and Asian/Pacific Islander), the country in which the participant had lived the longest during childhood (United States, other country), educational attainment (≤high school, some college, college graduate/post graduate education), and total household income in US dollars (<35,000, 35,000-69,000, ≥70,000).

Temporal characteristics

The date and time of urine sample collection were recorded by the interviewer during the study visit. Using this information, we created variables for the hour of day, day of week, and season (summer, autumn, winter, spring) of urine sample collection.

Anthropometric characteristics

In this investigation, we used body mass index (BMI) as the measure of overall adiposity and waist circumference and waist-to-hip ratio as indicators of abdominal adiposity. We additionally explored ratios of waist-to-thigh and waist-to-height as additional indicators of abdominal adiposity. During the in person structured interview, each participant was asked to report her current height without shoes and her reference date weight. If she was pregnant or

nursing on the reference date, she was asked to recall her weight just before that pregnancy. We used the self-reported weight and height to calculate BMI in kg/m^2. We categorized BMI according to pre-defined cutpoints (<25.0, ≥25.0-<30.0, ≥30.0). During the study visit, the interviewer took measurements in duplicate of the waist, hip, and thigh circumference, according to the study protocol and recording measurements to the nearest ¼-inch. The interviewer took a third measurement if there was more than a 0.5 inch difference between the first two measurements. We averaged the measurements of waist, hip, and thigh circumference in centimeters and calculated the waist-to-hip, waist-to-thigh, and waist-to-height ratio. We categorized waist circumference, and the ratios of waist-to-hip, waist-to-thigh, and waist-to-height into quartiles.

Urinary phthalate metabolite measurements

In the POPs study, non-fasting spot urine samples were collected in person from WREN participants in 2001 and 2002 and immediately refrigerated. A polypropylene container with a screw-top lid was used for urine collection. Urine specimens were processed by the Fred Hutchinson Cancer Research Center Specimen Processing Laboratory, aliquoted into 30mL flint glass vials with Teflon screw caps, and archived in -20ºC freezer storage. The urine samples were analyzed by the Environmental Health Laboratory at the University of Washington (UW) 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). We measured phthalate metabolites of BzBP (mono-benzyl phthalate (MBzP)), DEP (mono-ethyl phthalate (MEP)), DiBP (mono-isobutyl phthalate (MiBP)), DnBP (mono-n-butyl phthalate (MnBP)), and DEHP (mono-(2-ethyl-5-hexyl) phthalate

(MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP)). Internal laboratory control procedures included process blanks and instrumental duplicates on 10% of samples included in each analytic run. We monitored the interbatch and intrabatch reliability by including a pooled sample and a duplicate sample in each batch and blinding the laboratory staff with regard to the inclusion of these samples. We observed good interbatch reliability, with a coefficient of variation (CV%) for phthalate metabolites of <16% for MBzP and MiBP and <5% for all other phthalate metabolites, and strong intrabatch reliability with intraclass correlation coefficient (ICC) >99% for all phthalate metabolites except MBzP (63%) and MEHP (82%). The UW Department of Laboratory Medicine, Research Testing Services, measured urinary creatinine concentrations using the spectrophotometric Jaffe reaction rate method and the Beckman Coulter Synchron System (Beckman Coulter, Brea, CA, USA). The limit of quantitation (LOQ) for urinary creatinine was 10.0 mg/dL with <4% observations measured below the LOQ.

Statistical analyses

For urinary phthalate metabolite and creatinine concentrations measured below the LOQ, we replaced missing observations with the value of LOQ divided by two (Hornung and Reed 1990). Creatinine-corrected phthalate metabolite concentrations (μg/g creatinine) were used only for summary statistics and were estimated by dividing the absolute phthalate metabolite concentrations (μg/L) by creatinine concentrations (mg/dL) and then multiplying by 100. We summarized the distribution of uncorrected and creatinine-corrected phthalate metabolite concentrations using the median and interquartile range.

We conducted multivariable linear regression analyses to evaluate the associations between demographic, temporal, and anthropometric characteristics and urinary phthalate metabolite concentrations. Using the natural log of the dependent variable, an individual urinary phthalate concentration, and exponentiating the regression beta coefficients and 95% confidence intervals (CI), we interpreted the results as the ratio of the median phthalate metabolite concentrations across predictor categories or per one unit change in the characteristic of interest (Rao et al. 2008). We modeled each characteristic separately and in most analyses adjusted for age, race, hour of sample collection, and natural log-transformed urinary creatinine concentration (Barr et al. 2005). When evaluating the association between season and phthalate metabolite concentrations, we additionally adjusted for year of sample collection. The covariates for adjustment were selected *a priori*, informed by prior studies of predictors of urinary phthalate metabolite concentrations. Test of trend was generally conducted by including the categorical characteristic as a grouped linear variable in the adjusted model. To evaluate the trend across categories of hours, we created a continuous variable by assigning values equal to the median time to participants in each category and including the variable in the adjusted linear regression model. Since MEHP, MEHHP, MEOHP, and MECPP are metabolites of DEHP, we created a summary dependent variable, ∑DEHP, for use in the main analyses, by summing the molar concentrations of the four DEHP metabolites (nmol/ml). We decided to use ∑DEHP in the main analyses given our interest in understanding characteristics associated with DEHP exposure. Results for the individual metabolites, MEHP, MEHHP, MEOHP, and MECPP, are presented in Supplemental Materials, Tables 3A-C. Statistical significance was defined to be two-sided $P<0.05$ in all analyses and statistical analyses were conducted using STATA 12.0 (StataCorp, College Station, TX). We report results for ratio estimates ≥ 1.50 (or ≤ 0.67) with a confidence

limit ratio <3.0 (ratio of the upper and lower confidence limits as the measure of precision). We carried out regression diagnostics with a particular focus on linearity for continuous covariates and on the identification of outliers and influential measurements. We conducted robust regression to evaluate the impact of outlier and influential observations on the estimates of association. We also graphed boxplots of select creatinine-corrected phthalate metabolite concentrations across categories of temporal and anthropometric characteristics.

Results

The 195 women in the current investigation predominantly were of white race and had at least some college education. The characteristics of these women were generally similar to all WREN controls, whose distribution of characteristics mirrored that of the general population of western Washington State (Saunders et al. 2005).

Urinary phthalate metabolites were detected in the majority of women, with a small percent of missing data below the LOQ, <4% for all urinary phthalate metabolites except MBzP (7%) and MEHP (13%) (Table 3.1). Of all the metabolites, MEP had the greatest creatinine-corrected median concentration. The distributions of uncorrected and creatinine-corrected phthalate metabolite concentrations were right-skewed, with some women having urinary concentrations of MEP, MnBP, MEHHP, MECPP metabolites several orders of magnitude greater than the median concentration for the study population. With the exception of lower creatinine-corrected MEP concentration and higher creatinine-corrected concentrations of DEHP metabolites, the median phthalate metabolite concentrations among the women in the current analyses were generally comparable to the geometric means reported by the Centers for Disease

Control and Prevention (CDC) for females ≥ 6 years of age using the nationally representative NHANES data (Chapter 2, Supplemental Material, Table 2C) (CDC 2012).

With regard to demographic characteristics in relation to urinary phthalate metabolite concentrations, we observed lower concentrations of MBzP and MnBP among women ages 24-34 years compared to women ages 35-44 years adjusted for race, hour of sample collection, and urinary creatinine concentrations (Table 3.2). However, these estimates were driven by a few influential observations and attenuated with robust regression. We did not observe a clear pattern between race, country of childhood, and socioeconomic factors of education and income and urinary phthalate metabolite concentrations.

In our explorations of hour of day of urine sample collection in relation to phthalate metabolite concentration, we found that women who donated a urine sample during the evening hours of 6-10 pm had ∑DEHP metabolites concentrations 1.6 times (95% CI: 0.94-0.73) higher than women who donated a urine sample during the morning hours of 7-11 am (Table 3.3). The test of trend across daytime hours was significant for ∑DEHP (P=0.028) (Figure 3.1) and all of the other DEHP metabolites except MECPP (P<0.05 (Supplemental Materials, Table 3B)). Considering day of week of urine sample collection, we observed a significant trend (P=0.009) of increasing urinary concentrations of MEP across days of the week of urine sample collection, from Monday to Saturday (Figure 3.2). We also observed decreased concentrations of ∑DEHP among samples collected on Tuesday compared to Monday, but we did not observe a dose-response pattern in ∑DEHP concentrations across days of the week. As for seasons, the test of trend was significant for MiBP concentrations (P=0.041) across seasons, from summer to spring, although the ratios of the median concentrations were similar for winter and spring (Figure 3.3).

We found an inverse association between indicators of central adiposity, waist circumference and waist-to-hip ratio, and urinary concentration of MiBP, particularly in comparisons of the fourth and first quartiles (waist circumference: ratio of median concentrations 0.67, 95% CI: 0.49-0.91; waist-to-hip ratio: ratio 0.66, 95% CI: 0.49-0.90) (Table 3.4, Figures 3.4 and 3.5). An inverse association also was found between concentrations of MiBP and another indicator of central adiposity, waist-to-height ratio (data not shown). We also observed decreased concentrations of ∑DEHP comparing the second and first quartile of waist-to-hip ratio, however, we did not observe a pattern across categories of waist-to-hip ratio.

Discussion

In this exploratory cross-sectional analysis, we found several associations between temporal and anthropometric characteristics and urinary phthalate metabolite concentrations. We did not see a clear pattern among demographic factors in relation to phthalate metabolite concentrations. Our findings contrast with the one prior study of reproductive age women, which found associations between select phthalate metabolites and socioeconomic status, education and race. That study was conducted using NHANES data and had additional measurements of socioeconomic factors (Kobrosly et al. 2012).

We considered temporal characteristics of time of day, day of week, and season as proxies of temporal variation in behaviors, such as diet and personal care product use. Prior studies of various populations investigating time of day and DEHP metabolite concentrations have consistently reported greater concentrations of these metabolites in the evening compared to other times of the day (Aylward et al. 2011; Meeker et al. 2012; Preau Jr et al. 2010; Saravanabhavan et al. 2013; Silva et al. 2004). In accord with those studies, we observed

increased concentrations of ∑DEHP and individual DEHP metabolites across samples collected at later daytime hours, with the greatest concentrations observed among women who donated a urine sample between 6 and 10 pm, compared to samples collected between 7 and 11 am. Our findings are also in alignment with the measurement of DEHP concentrations in diverse foods (Wormuth et al. 2006). Additionally, two dosing studies found that DEHP is rapidly metabolized into its monoester MEHP metabolite with peak urinary concentrations at 2 hours and an elimination half-life of 5 hours after dosing and that the majority of MEHP was further metabolized into oxidative metabolites, including MEHHP, MEOHP, MECPP, with peak urinary concentrations at 4 hours and an elimination half-life of 10-15 hours (Koch et al. 2004; Koch et al. 2005; Koch et al. 2006). Given these elimination characteristics after exposure, DEHP metabolite concentrations in the evening reflect exposure from ingestion of meals throughout the day and may explain the lowest concentrations mid-morning.

We observed increased MEP concentrations among urine samples collected later in the week, compared to urine samples collected on Mondays. One study of pregnant women that measured MEP concentrations using samples from the first morning void observed no association with day of sampling (Mortamais et al. 2012). We did not collect serial samples, thus the trend we observed may be explained by differences among women who provided samples on different days. However, it is also possible that greater MEP concentrations on Thursday, Friday, and Saturday may be due to a greater number of products used on those days of the week given the excretion of DEP within eight hours after use (Janjua et al. 2008). Prior studies investigating the relationship between personal care product use and phthalates metabolite concentrations have observed a dose-response relationship of increasing urinary MEP

concentrations with greater number of personal care products used (Berman et al. 2009; Duty et al. 2005; Parlett et al. 2012; Romero-Franco et al. 2011; Sathyanarayana et al. 2008).

We also observed a significant test of trend for MiBP concentrations across seasons from summer to spring. However, a significant trend test does not validate a linear relationship and we observed similar ratios of the median concentrations for winter and spring seasons. Two prior studies of MiBP concentrations and seasons reported no association (Mortamais et al. 2012; Peck et al. 2010). Given that women are believed to be exposed to DiBP through oral ingestion of contaminated food, we speculate that our findings may be related to seasonal changes in diet (Wormuth et al. 2006). However, the rationale for our finding is unclear and may be due to seasonal changes in other behaviors or exposure to general environmental contamination.

We found an inverse association between indicators of central adiposity, waist circumference and waist-to-hip ratio, and urinary concentrations of MiBP. Similar to our study, Hatch et al (2008) found the suggestion of an inverse association between BMI, waist circumference and urinary concentrations of monobutyl phthalate (MBP), the sum of both MiBP and MnBP, among women ages 20-59 years using data from NHANES. However, that study also reported a positive relationship between MEP and BMI and waist circumference and an inverse association between MEHP and BMI and waist circumference, associations not observed in our study (Hatch et al. 2008). *In vitro* and *in vivo* studies have suggested that phthalates may disrupt metabolism and adipogenesis (Feige et al. 2010; Hurst and Waxman 2003). However, in interpreting the results from our cross-sectional analyses, we are not able to speculate as to the temporal sequence between phthalate exposure to DiBP and waist circumference and waist-to-hip ratio. Additionally, only one study to date has described human metabolism of DiBP (Koch et al. 2012). While that oral dosing study of one normal weight adult male showed that 90% of

the DiBP dose was excreted in the first 24 hours after dosing, it is possible that metabolism and excretion of this lipophilic chemical may vary by adiposity. The inverse associations observed with MiBP in our study and with MBP in the study of Hatch et al (2008) warrant further investigation.

In this cross-sectional study we carried out numerous statistical tests to explore associations between several characteristics in relation to eight phthalate metabolites, with some comparisons involving a small number of women. Therefore, some of the associations we observed may be due to chance, and findings from this exploratory analysis should be considered as hypothesis generating.

Another limitation was the use of a single, spot urine sample to characterize exposure to phthalates. Multiple samples would have allowed us to better characterize phthalate exposure given the relatively short elimination half-life of these chemicals, which for some phthalates is a few hours (Koch et al. 2012). Despite generally poorer temporal reliability for urinary DEHP metabolites, creatinine-corrected urinary concentrations of other metabolites including MEP and MBzP have demonstrated moderate (ICC>0.48) and high reproducibility (ICC>0.53), respectively, across sampling intervals of two days to four weeks among studies of reproductive age women (Baird et al. 2010; Hoppin et al. 2002; Peck et al. 2010). To address this limitation in our analyses, we adjusted for hour of sample collection in the majority of our analyses when investigating demographic, temporal, and anthropometric characteristics in relation to urinary phthalate metabolites. On the other hand, the use of an untimed sample allowed us to explore the association between hour of day and phthalate metabolite concentrations and capture fluctuations in urinary phthalate metabolite concentrations from ingestion of meals and dermal application of personal care products. Information on hour of day as well as day of week and season of year, to

our knowledge, is not available in the public-use NHANES dataset that is frequently used for studies of urinary phthalate metabolites

Lastly, in this study we did not have information on behaviors associated with phthalate exposure, such as use of personal care products and diet in the previous 24 hours. However, we selected demographic, temporal, and anthropometric characteristics that may be related to or proxies for these behaviors to understand patterns associated with increased phthalate exposure.

Conclusions

In this study of premenopausal women, we found phthalate metabolite concentrations to vary by time of day, day of week, season of year, waist circumference, and waist-to-hip ratio. Assuming that these characteristics are proxies for behaviors, our findings suggest that subgroups of women have increased phthalate exposure and body burden. Given the possible health effects associated with these endocrine disruptive chemicals, further research is warranted to confirm our findings and to elucidate specific behaviors associated with phthalate exposure.

Table 3.1. Laboratory measurement and distribution of uncorrected (µg/L) and creatinine-corrected (µg/g) urinary phthalate metabolite concentrations for 195 premenopausal women, Group Health, 1996-2001[a].

Phthalate metabolite	LOQ (µg/L)	<LOQ n (%)	Uncorrected (µg/L)		Creatinine-corrected (µg/g)	
			Median (IQR)	min, max	Median (IQR)	min, max
MBzP	0.5	13 (6.7)	5.0 (2.0-11.5)	<0.5, 91.6	8.4 (5.1-12.8)	0.5, 163.6
MEP	0.8	3 (1.5)	43.9 (16.8-144.4)	<0.8, 12168	79.9 (32.7-258.6)	2.7, 17506
MiBP	0.2	8 (4.1)	1.5 (0.7-3.1)	<0.2, 48.8	2.6 (1.7-4.2)	0.6, 31.1
MnBP	0.3	0 (0.0)	10.0 (4.9-23.5)	0.4, 793.7	17.8 (11.5-30.1)	3.6, 1946.9
MEHP	0.4	26 (13.3)	3.4 (1.0-11.1)	<0.4, 197.6	5.6 (2.3-14.0)	0.1, 262.8
MEHHP	0.2	1 (0.5)	18.8 (6.3-56.5)	<0.2, 1513.2	30.1 (16.5-66.2)	0.9, 1269.2
MEOHP	0.2	0 (0.0)	10.8 (3.5-29.1)	0.2, 780.3	16.7 (10.0-35.5)	2.7, 534.4
MECPP	0.3	0 (0.0)	18.0 (5.8-51.9)	0.4, 747.8	27.3 (17.9-58.3)	3.6, 1121.9

Abbreviations: MBzP=mono-benzyl phthalate; MEP=mono-ethyl phthalate; MiBP=mono-isobutyl phthalate; MnBP=mono-n-butyl 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. LOQ=limit of quantitation; IQR=interquartile range.

[a]Urinary phthalate metabolite and creatinine measurements below the LOQ were substituted with the value of LOQ divided by two.

[b]MnBP is also a minor metabolite of BzBP.

Table 3.2. Estimated ratio of median urinary phthalate metabolite concentrations and 95% confidence intervals across categories of demographic characteristics among 195 premenopausal women, Group Health, 1996-2001.

Characteristic	n (%)	MBzP Ratio (95% CI)	MEP Ratio (95% CI)	MiBP Ratio (95% CI)	MnBP Ratio (95% CI)	∑DEHP Ratio (95% CI)
Age at interview (years)[a]						
19-24	11 (5.6)	0.98 (0.56-1.70)	0.67 (0.26-1.73)	0.89 (0.56-1.42)	0.81 (0.48-1.37)	1.93 (0.97-3.86)
25-34	23 (11.8)	0.63 (0.42-0.93)	0.62 (0.31-1.22)	0.95 (0.68-1.33)	0.67 (0.46-0.99)	1.11 (0.67-1.31)
35-44	84 (43.1)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
45-53	77 (39.5)	0.74 (0.56-0.97)	1.04 (0.65-1.65)	0.88 (0.69-1.10)	0.88 (0.68-1.14)	1.12 (0.80-1.57)
Race[b]						
White	165 (88.0)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Black	11 (5.8)	0.86 (0.50-1.47)	2.10 (0.85-5.16)	1.01 (0.65-1.57)	1.17 (0.70-1.95)	0.74 (0.38-1.42)
Asian/ Pacific Islander	12 (6.3)	1.14 (0.66-1.95)	0.67 (0.27-1.66)	1.49 (0.96-2.33)	0.95 (0.57-1.59)	0.93 (0.48-1.81)
Childhood country[c]						
United States	183 (93.9)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Other country	12 (6.2)	0.92 (0.52-1.62)	0.94 (0.36-2.45)	1.45 (0.91-2.32)	0.94 (0.55-1.61)	0.63 (0.31-1.26)
Education[c]						
≤High school	43 (22.1)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Some college	80 (41.0)	1.17 (0.84-1.62)	1.16 (0.67-2.01)	1.07 (0.82-1.41)	0.94 (0.69-1.28)	0.93 (0.62-1.39)
College/post graduate	72 (36.9)	1.09 (0.78-1.52)	0.79 (0.45-1.38)	1.24 (0.94-1.63)	0.98 (0.72-1.35)	1.05 (0.70-1.59)
Income[c]						
<$35,000	50 (26.5)	0.99 (0.71-1.37)	1.39 (0.81-2.40)	1.29 (0.99-1.68)	1.23 (0.91-1.67)	1.17 (0.79-1.73)
$35,000-$69,999	82 (43.4)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
≥70,000	57 (30.2)	0.85 (0.63-1.16)	0.88 (0.53-1.46)	1.14 (0.89-1.46)	1.16 (0.88-1.54)	1.08 (0.74-1.56)

Abbreviations: MBzP=mono-benzyl phthalate; MEP=mono-ethyl phthalate; MiBP=mono-isobutyl phthalate; MnBP=mono-n-butyl phthalate; ΣDEHP=summed metabolites of di(2-ethylhexyl) phthalate.

[a]Adjusted for race, hour of urine sample collection, and natural log-transformed urinary creatinine concentrations.

[b]Excluded four women of other race. Adjusted for age, hour of urine sample collection, and natural log-transformed urinary creatinine concentrations.

[c]Adjusted for age, race, hour of urine sample collection, and natural log-transformed urinary creatinine concentrations.

Table 3.3. Estimated ratio of median urinary phthalate metabolite concentrations and 95% confidence intervals across categories of temporal characteristics among 195 premenopausal women, Group Health, 1996-2001.

Timing of urine sample collection	n (%)	MBzP Ratio (95% CI)	MEP Ratio (95% CI)	MiBP Ratio (95% CI)	MnBP Ratio (95% CI)	∑DEHP Ratio (95% CI)
Time (hour)[a]						
7:00AM-10:59AM	36 (18.7)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
11:00AM-12:59PM	49 (25.4)	0.95 (0.65-1.39)	1.43 (0.76-2.71)	0.88 (0.64-1.20)	0.90 (0.63-1.28)	0.77 (0.48-1.22)
1:00PM-3:59PM	50 (25.9)	0.92 (0.63-1.34)	1.03 (0.55-1.94)	0.96 (0.70-1.31)	0.87 (0.61-1.24)	0.91 (0.57-1.44)
4:00PM-5:59PM	29 (15.0)	1.24 (0.81-1.91)	1.43 (0.69-2.94)	1.41 (0.99-2.02)	0.98 (0.66-1.48)	1.15 (0.68-1.96)
6:00PM-9:59PM	29 (15.0)	1.15 (0.75-1.77)	1.15 (0.55-2.37)	1.09 (0.76-1.56)	0.91 (0.60-1.37)	1.61 (0.94-2.73)
P_{trend}		*P*=0.292	*P*=0.799	*P*=0.122	*P*=0.801	*P*=0.028
Day of week[b]						
Monday	32 (16.4)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Tuesday	48 (24.6)	0.99 (0.66-1.48)	1.06 (0.54-2.05)	0.88 (0.63-1.23)	0.99 (0.68-1.44)	0.66 (0.40-1.07)
Wednesday	32 (16.4)	0.91 (0.59-1.42)	1.13 (0.55-2.34)	0.76 (0.53-1.09)	1.21 (0.80-1.83)	0.74 (0.43-1.28)
Thursday	44 (22.6)	0.78 (0.52-1.17)	1.80 (0.91-3.56)	0.69 (0.49-0.97)	0.90 (0.61-1.33)	0.75 (0.46-1.24)
Friday	27 (13.9)	0.70 (0.44-1.12)	2.43 (1.11-5.31)	0.72 (0.49-1.07)	0.91 (0.58-1.42)	1.04 (0.58-1.85)
Saturday	12 (6.2)	0.81 (0.45-1.48)	1.80 (0.66-4.86)	0.96 (0.59-1.57)	0.88 (0.50-1.55)	0.71 (0.34-1.49)
P_{trend}		*P*=0.075	*P*=0.009	*P*=0.107	*P*=0.509	*P*=0.979
Season[c]						
Jun-Aug (Summer)	50 (25.6)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Sep-Nov (Autumn)	50 (25.6)	0.76 (0.53-1.07)	0.78 (0.43-1.40)	1.09 (0.82-1.46)	0.98 (0.71-1.37)	0.81 (0.53-1.25)
Dec-Feb (Winter)	43 (22.1)	0.80 (0.55-1.17)	1.03 (0.55-1.95)	0.75 (0.55-1.03)	0.79 (0.55-1.14)	0.92 (0.58-1.48)
Mar-May (Spring)	52 (26.7)	0.83 (0.56-1.21)	1.35 (0.71-2.60)	0.76 (0.55-1.04)	0.92 (0.64-1.33)	0.93 (0.58-1.51)
P_{trend}		*P*=0.335	*P*=0.338	*P*=0.041	*P*=0.476	*P*=0.831

Abbreviations: MBzP=mono-benzyl phthalate; MEP=mono-ethyl phthalate; MiBP=mono-isobutyl phthalate; MnBP=mono-n-butyl phthalate; ΣDEHP=summed metabolites of di(2-ethylhexyl) phthalate.

[a]Adjusted for age, race, and natural log-transformed urinary creatinine concentrations.

[b]Adjusted for age, race, natural log-transformed urinary creatinine concentrations, and hour of sample collection.

[c]Adjusted for age, race, natural log-transformed urinary creatinine concentrations, and hour and year of sample collection.

Table 3.4. Estimated ratio of median urinary phthalate metabolite concentrations and 95% confidence intervals across categories of anthropometric characteristics among 195 premenopausal women, Group Health, 1996-2001.[a]

Body size characteristics	n (%)	MBzP Ratio (95% CI)	MEP Ratio (95% CI)	MiBP Ratio (95% CI)	MnBP Ratio (95% CI)	∑DEHP Ratio (95% CI)
BMI (kg/m^2) per 5 unit	192	1.02(0.93-1.12)	1.08 (0.93-1.25)	0.94 (0.87-1.01)	1.04 (0.95-1.13)	1.04 (0.93-1.16)
<25.0	107 (55.7)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
25.0-<30.0	42 (21.9)	1.38 (1.01-1.89)	1.34 (0.79-2.31)	0.91 (0.70-1.18)	1.03 (0.76-1.39)	1.03 (0.69-1.52)
≥30.0	43 (22.4)	1.14 (0.82-1.58)	1.18 (0.67-2.06)	0.77 (0.59-1.02)	1.14 (0.83-1.56)	1.08 (0.72-1.63)
Waist circumference (cm) per 5 cm	185	0.99 (0.95-1.03)	1.01 (0.94-1.08)	0.95 (0.92-0.98)	1.00 (0.96-1.04)	1.02 (0.97-1.08)
≤74	49 (26.5)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
>74-82	46 (24.9)	1.20 (0.83-1.73)	1.01 (0.55-1.85)	0.93 (0.69-1.25)	1.13 (0.80-1.59)	0.87 (0.56-1.36)
>82-96	44 (23.8)	1.20 (0.82-1.74)	1.04 (0.56-1.93)	0.82 (0.61-1.11)	0.94 (0.66-1.32)	0.92 (0.59-1.45)
>96	46 (24.9)	1.00 (0.68-1.47)	1.28 (068-2.42)	0.67 (0.49-0.91)	1.07 (0.74-1.53)	1.01 (0.63-1.61)
Waist to hip ratio per 0.1 unit	183	0.95 (0.78-1.16)	1.00 (0.72-1.39)	0.83 (0.71-0.98)	0.97 (0.81-1.17)	1.03 (0.81-1.31)
≤0.74	46 (25.1)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
>0.74-0.78	46 (25.1)	1.09 (0.74-1.56)	1.53 (0.82-2.82)	0.89 (0.66-1.20)	1.19 (0.85-1.69)	0.66 (0.42-1.03)
>0.78-0.83	46 (25.1)	0.95 (0.65-1.37)	1.23 (0.66-2.28)	0.86 (0.63-1.16)	0.94 (0.66-1.32)	0.80 (0.51-1.26)
>0.83	45 (24.6)	0.79 (0.54-1.16)	0.94 (0.50-1.77)	0.66 (0.49-0.90)	1.00 (0.70-1.41)	0.91 (0.58-1.43)

Abbreviations: MBzP=mono-benzyl phthalate; MEP=mono-ethyl phthalate; MiBP=mono-isobutyl phthalate; MnBP=mono-n-butyl phthalate; ΣDEHP=summed metabolites of di(2-ethylhexyl) phthalate; BMI=body mass index; CI=confidence interval.

[a]Adjusted for age, race, hour of urine sample collection, and natural log-transformed urinary creatinine concentrations.

Figure 3.1. Boxplot of creatinine-corrected ∑DEHP concentrations, by hour of day.

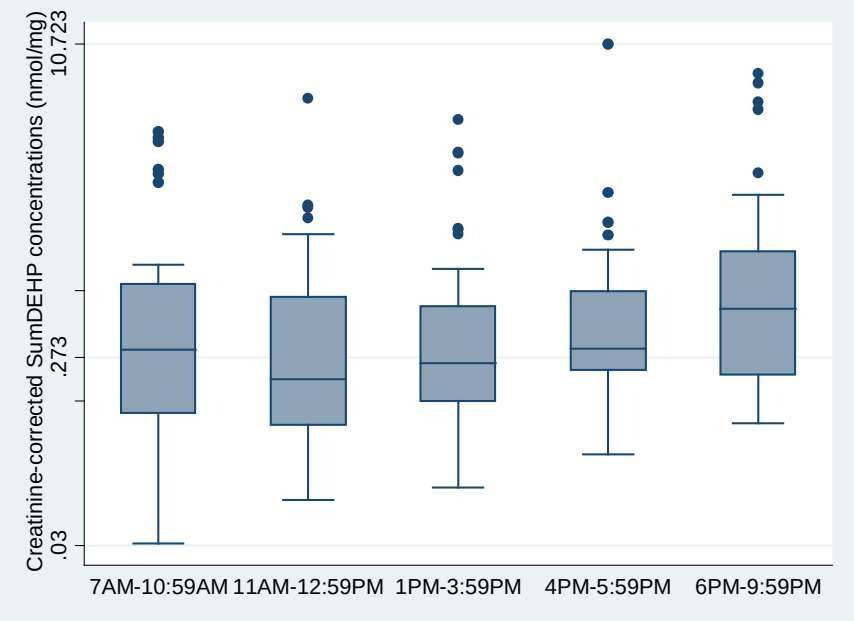


Figure 3.2. Boxplot of creatinine-corrected MEP concentrations, by days of week.

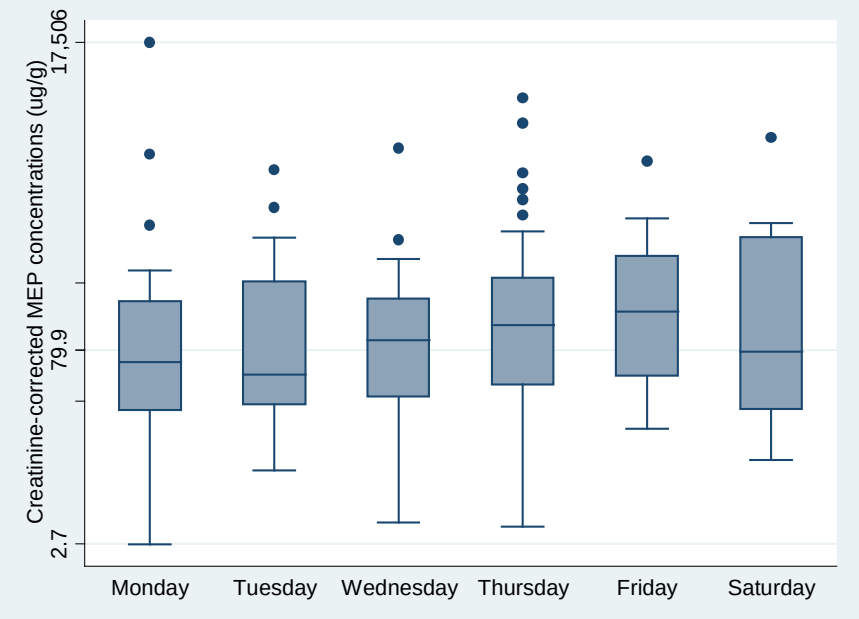


Figure 3.3. Boxplot of creatinine-corrected MiBP concentrations, by seasons of year.

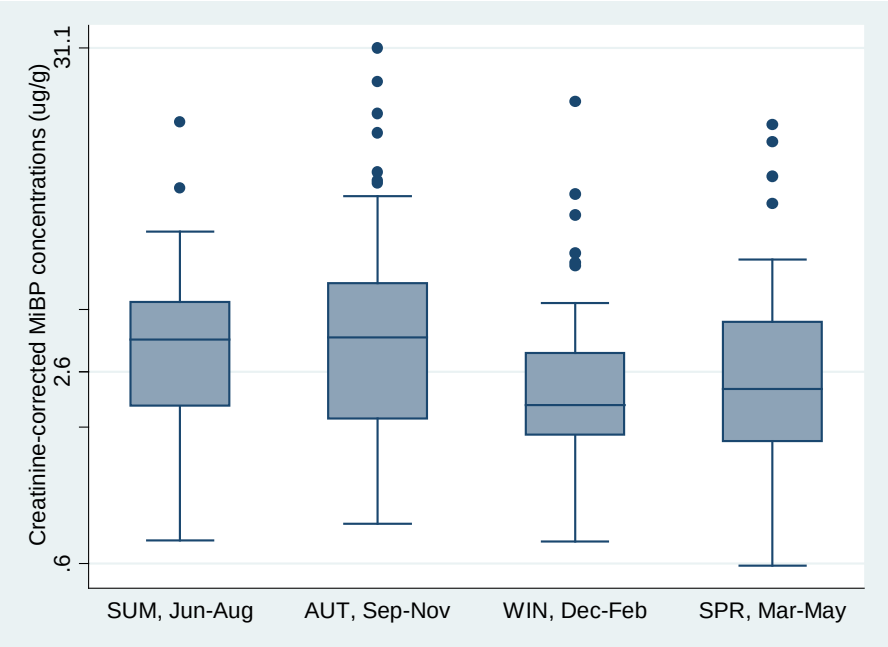


Figure 3.4. Boxplot of creatinine-corrected MiBP concentrations, by categories of waist circumference (cm).

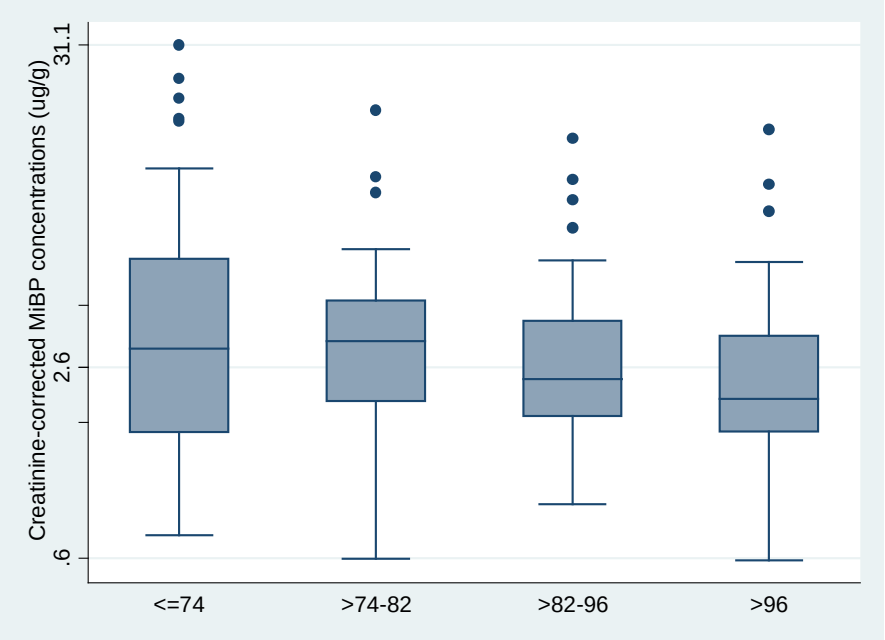
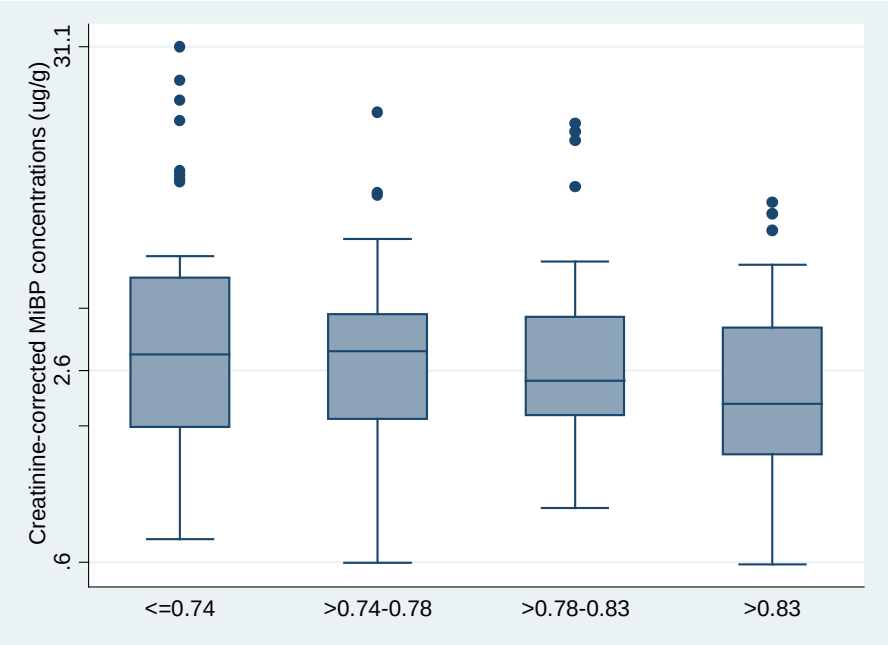


Figure 3.5. Boxplot of creatinine-corrected MiBP concentrations, by categories of waist-to-hip ratio.



Supplemental Materials, Table 3A. Estimated ratio of median urinary phthalate metabolite concentrations and 95% confidence intervals across categories of demographic characteristics among 195 premenopausal women, Group Health, 1996-2001.

Characteristic	n (%)	MEHP Ratio (95% CI)	MEHHP Ratio (95% CI)	MEOHP Ratio (95% CI)	MECPP Ratio (95% CI)
Age at interview (years)[a]					
19-24	11 (5.6)	2.74 (1.17-6.42)	1.74 (0.84-3.60)	1.98 (1.01-3.89)	1.81 (0.92-3.59)
25-34	23 (11.8)	0.98 (0.53-1.82)	1.08 (0.64-1.83)	1.07 (0.66-1.74)	1.21 (0.74-1.99)
35-44	84 (43.1)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
45-53	77 (39.5)	1.09 (0.72-1.66)	1.09 (0.76-1.56)	1.05 (0.75-1.47)	1.24 (0.88-1.73)
Race[b]					
White	165 (88.0)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Black	11 (5.8)	0.71 (0.32-1.62)	0.78 (0.39-1.55)	0.83 (0.43-1.58)	0.64 (0.33-1.23)
Asian/ Pacific Islander	12 (6.3)	1.15 (0.50-2.61)	0.90 (0.45-1.80)	0.86 (0.45-1.65)	1.01 (0.53-1.95)
Childhood country[c]					
United States	183 (93.9)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Other country	12 (6.2)	0.61 (0.26-1.45)	0.62 (0.30-1.29)	0.62 (0.31-1.22)	0.68 (0.34-1.36)
Education[c]					
≤High school	43 (22.1)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Some college	80 (41.0)	0.97 (0.59-1.59)	0.94 (0.62-1.44)	0.93 (0.62-1.37)	0.96 (0.64-1.44)
College/post graduate	72 (36.9)	1.14 (0.69-1.91)	1.07 (0.69-1.65)	1.12 (0.75-1.68)	1.02 (0.67-1.53)
Income[c]					
<$35,000	50 (26.5)	1.18 (0.72-1.93)	1.13 (0.74-1.72)	1.14 (0.78-1.69)	1.25 (0.85-1.85)
$35,000-$69,999	82 (43.4)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
≥70,000	57 (30.2)	1.15 (0.73-1.82)	1.05 (0.71-1.56)	1.03 (0.72-1.48)	1.12 (0.78-1.60)

Abbreviations: 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.

[a]Adjusted for race, hour of urine sample collection, and natural log-transformed urinary creatinine concentrations.

[b]Excluded four women of other race. Adjusted for age, hour of urine sample collection, and natural log-transformed urinary creatinine concentrations.

[c]Adjusted for age, race, hour of urine sample collection, and natural log-transformed urinary creatinine concentrations.

Supplemental Materials, Table 3B. Estimated ratio of median urinary phthalate metabolite concentrations and 95% confidence intervals across categories of temporal characteristics among 195 premenopausal women, Group Health, 1996-2001.

Timing of urine sample collection	n (%)	MEHP Ratio (95% CI)	MEHHP Ratio (95% CI)	MEOHP Ratio (95% CI)	MECPP Ratio (95% CI)
Time (hour)[a]					
7:00AM-10:59AM	36 (18.7)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
11:00AM-12:59PM	49 (25.4)	0.62 (0.35-1.11)	0.81 (0.50-1.32)	0.79 (0.50-1.24)	0.76 (0.48-1.21)
1:00PM-3:59PM	50 (25.9)	0.82 (0.46-1.46)	0.97 (0.60-1.58)	0.93 (0.59-1.46)	0.84 (0.53-1.33)
4:00PM-5:59PM	29 (15.0)	1.42 (0.74-2.73)	1.24 (0.71-2.17)	1.20 (0.71-2.01)	1.01 (0.60-1.70)
6:00PM-9:59PM	29 (15.0)	2.09 (1.08-4.04)	1.78 (1.01-3.12)	1.61 (0.96-2.71)	1.35 (0.80-2.29)
P_{trend}		P=0.003	P=0.014	P=0.022	P=0.153
Day of week[b]					
Monday	32 (16.4)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Tuesday	48 (24.6)	0.51 (0.28-0.94)	0.64 (0.38-1.08)	0.65 (0.40-1.05)	0.71 (0.44-1.16)
Wednesday	32 (16.4)	0.81 (0.42-1.58)	0.76 (0.43-1.34)	0.75 (0.44-1.27)	0.69 (0.41-1.18)
Thursday	44 (22.6)	0.77 (0.42-1.44)	0.69 (0.41-1.17)	0.75 (0.46-1.22)	0.82 (0.50-1.35)
Friday	27 (13.9)	0.81 (0.40-1.65)	1.01 (0.55-1.86)	1.08 (0.61-1.89)	1.13 (0.64-2.01)
Saturday	12 (6.2)	0.80 (0.32-1.98)	0.71 (0.33-1.53)	0.73 (0.36-1.50)	0.71 (0.34-1.48)
P_{trend}		P=0.792	P=0.898	P=0.877	P=0.864
Season[c]					
Jun-Aug (Summer)	50 (25.6)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Sep-Nov (Autumn)	50 (25.6)	0.90 (0.53-1.55)	0.76 (0.48-1.20)	0.84 (0.55-1.29)	0.84 (0.55-1.30)
Dec-Feb (Winter)	43 (22.1)	1.16 (0.65-2.07)	0.92 (0.56-1.50)	0.95 (0.60-1.51)	0.90 (0.57-1.43)
Mar-May (Spring)	52 (26.7)	0.99 (0.55-1.80)	0.91 (0.55-1.51)	0.91 (0.57-1.46)	0.94 (0.59-1.52)
P_{trend}		P=0.885	P=0.790	P=0.761	P=0.821

Abbreviations: 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.

[a]Adjusted for age, race, and natural log-transformed urinary creatinine concentrations.

[b]Adjusted for age, race, natural log-transformed urinary creatinine concentrations, and hour of sample collection.

[c]Adjusted for age, race, natural log-transformed urinary creatinine concentrations, and hour and year of sample collection.

Supplemental Material, Table 3C. Ratio of median urinary phthalate metabolite concentrations and 95% confidence intervals across categories of anthropometric characteristics among 195 premenopausal women, Group Health, 1996-2001.[a]

Body size characteristics	n (%)	MEHP Ratio (95% CI)	MEHHP Ratio (95% CI)	MEOHP Ratio (95% CI)	MECPP Ratio (95% CI)
BMI (kg/m^2) per 5 unit	192	0.98 (0.86-1.13)	1.04 (0.92-1.16)	1.03 (0.92-1.14)	1.06 (0.95-1.19)
<25.0	107 (55.7)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
25.0-<30.0	42 (21.9)	1.07 (0.66-1.73)	1.06 (0.70-1.60)	1.01 (0.68-1.48)	1.02 (0.69-1.51)
≥30.0	43 (22.4)	0.90 (0.54-1.49)	1.09 (0.71-1.68)	1.04 (0.69-1.55)	1.17 (0.78-1.76)
Waist circumference (cm) per 5 cm	185	1.00 (0.93-1.06)	1.03 (0.97-1.08)	1.02 (0.97-1.07)	1.03 (0.98-1.08)
≤74	49 (26.5)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
>74-82	46 (24.9)	0.71 (0.42-1.22)	0.87 (0.55-1.39)	0.93 (0.60-1.43)	0.92 (0.59-1.42)
>82-96	44 (23.8)	0.83 (0.47-1.45)	0.89 (0.55-1.42)	0.92 (0.60-1.44)	0.97 (0.62-1.53)
>96	46 (24.9)	0.78 (0.44-1.39)	1.02 (0.62-1.66)	0.98 (0.62-1.54)	1.08 (0.68-1.71)
Waist to hip ratio per 0.1 unit	183	0.97 (0.72-1.31)	1.03 (0.80-1.33)	1.02 (0.81-1.29)	1.06 (0.84-1.35)
≤0.74	46 (25.1)	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
>0.74-0.78	46 (25.1)	0.69 (0.40-1.22)	0.66 (0.41-1.06)	0.63 (0.41-0.98)	0.69 (0.44-1.07)
>0.78-0.83	46 (25.1)	0.78 (0.45-1.37)	0.76 (0.47-1.22)	0.78 (0.51-1.21)	0.87 (0.56-1.36)
>0.83	45 (24.6)	0.80 (0.45-1.41)	0.91 (0.56-1.48)	0.88 (0.56-1.37)	0.97 (0.62-1.53)

Abbreviations: 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; BMI=body mass index; CI=confidence interval.

[a]Adjusted for age, race, hour of urine sample collection, and natural log-transformed urinary creatinine concentrations.

Conclusion

In this dissertation, I investigated the risk of endometriosis in relation to organochlorine pesticides (OCPs) and phthalate metabolites using data from a U.S. case-control study of endometriosis, the Women's Risk of Endometriosis (WREN), that employed a population-based sampling frame and surgically confirmed cases. I additionally explored demographic, temporal, and anthropometric characteristics in relation to urinary concentrations to phthalate metabolites.

The most notable associations that I observed were the increased endometriosis risk with serum concentrations of two OCPs, β-hexachlorocyclohexane (HCH) and mirex, and the inverse associations between endometriosis risk and urinary concentrations of di(2-ethylhexyl) phthalate (DEHP) metabolites, particularly mono-(2-ethyl-5-hexyl) phthalate (MEHP). I also found phthalate metabolite concentrations to vary by time of day, day of week, season of year, waist circumference, and waist-to-hip ratio. Assuming that these characteristics are proxies for behaviors, my findings suggest subgroups of women with increased phthalate exposure and body burden.

Two key themes emerge from this dissertation research. First, I found that two classes of environmental endocrine disrupting chemicals, OCP and phthalates, are associated with altered risk of endometriosis among reproductive age women. These findings provide support for my hypothesis that organochlorine pesticides and phthalates may affect the normal dynamic structural changes of hormonally-responsive endometrial tissue during the menstrual cycle, promoting the establishment and persistence of refluxed endometrial tissue, and may interfere with endogenous hormonal activity to alter endometriosis disease risk. In addition to the menstrual cycle during the reproductive years, it is possible that other windows for disease susceptibility exist given that uterine gland development begins in utero and continues until the

onset of puberty (Crain et al. 2008). However, it has been hypothesized that even with endocrine disruptive exposure in utero, subsequent adult endocrine disruption and/or other hormonal and immune irregularities are necessary for endometriosis onset (Crain et al. 2008).

Second, when considering the notable associations in my dissertation research in the context of results from prior studies using population-based or laparoscopy-based sampling frames, I observed an interesting pattern (Table 4A). The results of my dissertation research were consistent with other studies using a population-based sampling frame investigating similar entities of endometriosis. This is exemplified by the study of two different chemicals, β-HCH and MEHP. We observed similar results for β-HCH in relation to ovarian endometriosis in our study and the population cohort in the study by Buck Louis et al (2012). We also observed similar findings of an inverse association between MEHP and all endometriosis entities in our study and the population-based cross-sectional study by Weuve et al (2010), consistent with animal studies demonstrating the ovarian toxicity of DEHP (Kavlock et al. 2002b). Conversely, studies using a laparoscopy-based sampling frame had divergent results that were not consistent with established biologic plausibility.

This second key theme has the potential to move the field of endometriosis research forward in understanding environmental determinants of disease. The majority of prior studies of OCPs and phthalates have been case-control studies conducted among a convenience sample of laparoscopic patients as surgical evaluation is required for definitive disease diagnosis. However, the use of laparoscopic controls may result in unpredictable bias and may hinder the understanding of the relationship between these chemicals and endometriosis. As discussed in Chapters 1 and 2 of this dissertation research, laparoscopic controls may not represent a random sample of the source population that gave rise to the endometriosis cases. Additionally, the

indication for surgical evaluation among controls may be associated with altered concentrations of OCPs and phthalates (Holt and Weiss 2000; Zondervan et al. 2002). Conversely, it has been hypothesized that bias from undiagnosed endometriotic disease among population-based controls is likely to be minimal (Holt and Weiss 2000; Zondervan et al. 2002). In addition to selection bias from the use of laparoscopic controls, endometriosis research may also be hindered by the collection of biospecimens near the time of surgical diagnosis of disease and not during the etiologically relevant time period. The consistent results across population-based studies investigating similar entities of endometriosis and alignment with biological plausibility from animal studies help to provide evidence for the relationship between endocrine-disrupting chemicals and endometriosis in the face of these research challenges.

Findings from Chapter 3 of this dissertation research investigating demographic, temporal, and anthropometric characteristics in relation to urinary concentrations of phthalate metabolites also may move the field of endometriosis research forward by informing the design of future studies. Basic information on characteristics of premenopausal women associated with altered phthalate metabolite concentrations may help to identify potential confounding factors for adjustment to minimize bias when assessing the association between phthalates and endometriosis. The finding of a trend in urinary phthalate concentrations across hours of the day suggests that samples should be collected at various times of the day to capture exposure and to increase the sensitivity of a study to detect associations that could be missed if only samples of first morning voids are used.

In conclusion, this dissertation research suggests that exposure to environmental endocrine disruptive chemicals, OCPs and phthalates, during the childbearing years alters the risk of endometriosis. This dissertation research also suggests that population-based sampling

may reduce unpredictable selection bias and provide evidence about relationships between environmental determinants and endometriosis risk despite the research challenges. Additionally, this dissertation research provides basic information on characteristics associated phthalate exposure that may inform the design of future endometriosis research studies to minimize bias from confounding and to optimize the timing of sample collection to increase the sensitivity of a study to detect an association.

Table 4A. Odds ratios and 95% confidence intervals for mono-(2-ethyl-5-hexyl) phthalate (MEHP) and β-hexachlorocyclohexane (β-HCH) across epidemiologic studies using population-based and laparoscopic -based sampling.

Chemical	Sampling	Study	Exposure unit	Exposure	OR (95%CI)
MEHP					
	Population-based	Upson et al. (2013)	ng/ml urine	Quartile 1: ≤1.0	1.0
				Quartile 2: >1.0-3.4	0.6 (0.3-1.3)
				Quartile 3: >3.4-11.1	0.7 (0.3-1.5)
				Quartile 4: >11.1	0.3 (0.1-0.7)
	Population-based	Buck Louis et al. (2013)	ng/mL urine	Per one-standard deviation increase	2.59 (1.17-5.75)
	Population-based	Weuve et al. (2010)	ng/mg creatinine	Quartile 1: 0.2-1.4	1.00
				Quartile 2: 1.5-3.2	0.78 (0.38-1.58
				Quartile 3: 3.3-6.3	0.89 (0.42-1.90)
				Quartile 4: 6.4-538	0.39 (0.16-0.95)
	Laparoscopy-based	Buck Louis et al. (2013)	ng/mL urine	Per one-standard deviation increase	1.20 (0.97-1.20)
	Laparoscopy-based	Huang et al. (2010)	Not specified	≤Median	1.00
				>Median	1.42 (0.45-4.50)
	Laparoscopy-based	Itoh et al. (2009)	μg/g creatinine	≤Median	1.00
				>Median 4.2	1.57 (0.74-3.30)
β-HCH					
	Population-based	Upson et al. (2013)	pg/g serum	Quartile 1: ≤26.99	1.0
				Quartile 2: >26.99-43.06	0.8 (0.5-4.1)
				Quartile 3: >43.06-74.01	1.7 (1.0-2.8)
				Quartile 4: >74.01	1.3 (0.8-2.4)
	Population-based	Buck Louis et al. (2012)	ng/g serum	Per one-standard deviation increase	1.72 (1.09-2.72)
	Laparoscopy-based	Buck Louis et al. (2012)	ng/g serum	Per one-standard deviation increase	0.77 (0.54-1.14)
	Laparoscopy-based	Cooney et al. (2010)	ng/g serum	≤LOD	1.0
				>LOD	2.0 (0.3-15.8)

Table 4A. Continued.

Chemical	Sampling	Study	Exposure unit	Exposure	OR (95%CI)
	Laparoscopy-based	Tsukino et al. (2005)	Serum lipid adjusted; Unit not specified	Not specified	No association
	Laparoscopy-based	Lebel et al. (1998)	Serum lipid adjusted; Unit not specified	Not specified	No association

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Appendix 1: Application of hierarchical regression when investigating correlated phthalate metabolites in relation to endometriosis risk

In Chapter 2 of this dissertation, I investigated the association between urinary phthalate metabolite concentrations and the risk of endometriosis. One methodologic challenge in conducting analyses of phthalate metabolites is the issue of multiple correlated exposures. Exposure to a single phthalate, such as di(2-ethylhexyl) phthalate (DEHP), may be metabolized and excreted by the body as multiple metabolites in urine that are highly correlated. This was observed in Chapter 2 (Supplemental Material, Table 2A) for several of the phthalate metabolites, including the four metabolites of DEHP that had large pairwise Spearman correlation coefficients ranging from 0.82 to 0.91. To address the multiple correlated exposures in Chapter 2, I considered each individual phthalate metabolite in a separate logistic regression model. This analysis approach was selected due to the concern for unstable coefficient estimates or lack of model convergence with maximum likelihood estimation if all of the phthalate metabolites were included in the same regression model. However, this approach did not allow for simultaneous evaluation or control for multiple phthalate metabolites.

A technique that permits simultaneous evaluation of multiple correlated exposures is hierarchical regression. In hierarchical regression, information about the relationship between multiple exposure effects is used to adjust effect estimates towards a prior distribution. In doing so, hierarchical regression yields estimates with greater accuracy and precision, on average, than with conventional maximum likelihood estimation. In this appendix, I report on exploratory analyses in which I simultaneously investigated the eight phthalate metabolites in relation to endometriosis risk using semi-Bayes hierarchical regression. I compared the results obtained from hierarchical regression to those obtained from a conventional full model analysis by

maximum likelihood estimation and those reported in Chapter 2 using separate models for each phthalate metabolite.

Hierarchical regression was conducted using established software, SAS macro GLIMMIX, that was modified for epidemiologic analyses (Witte et al. 1998) and further modified for epidemiologic analyses of environmental exposures (De Roos et al. 2003). The conventional full model using unconditional logistic regression with maximum likelihood estimation took the form of $\text{logit}(p|\mathbf{X},\mathbf{W})=\alpha+\mathbf{X}\underline{\beta}+\mathbf{W}\underline{\gamma}$ where p is the risk of endometriosis, α is the intercept term, X is the n-row matrix of quartiles of phthalate metabolites, W is the m-row matrix of potential confounders and frequency matching variables (imputed natural-logarithm transformed urinary creatinine, age, and reference year), and $\underline{\beta}$ and $\underline{\gamma}$ are vectors of logistic regression coefficients corresponding to the variables for the eight phthalate metabolites and three covariates. In terms of hierarchical regression modeling, the conventional full model may be considered the first-stage model.

The second-stage model considered additional information about the relationships between multiple exposures effects to adjust odds ratios and 95% confidence intervals obtained in the first-stage model towards a prior distribution. The second-stage model was a linear regression model in which β coefficients for the association between endometriosis and phthalate metabolites from the first-stage model were regressed on prior covariates. The second-stage model took the form of $\underline{\beta} = \mathbf{Z}\underline{\pi} + \underline{\delta} = \mu + \underline{\delta}$ where $\mathbf{Z}$ is the design matrix for the second-stage covariates, $\underline{\pi}$ is the vector of second-stage coefficients, and $\underline{\delta}$ is the independent random normal variable with mean zero and variance τ^2. The second-stage coefficients obtained through an iterative procedure using Monte Carlo Markov Chain sampling were used to estimate the prior

mean. This prior mean was used in turn to estimate the posterior coefficient for each phthalate metabolite exposure, or the adjusted semi-Bayes estimate.

Before seeing the data, I specified the second-stage design z-matrix (Appendix 1, Table 1). I assumed that phthalate metabolites derived from the same parent phthalate diester would draw from a common prior distribution. In Z, there is an i^{th} row for each phthalate metabolite dummy variable and j^{th} column for the intercept and parent phthalate grouping of phthalate metabolites for DBP and DEHP, coded as 0 or 1. I also assumed that phthalate metabolites MBzP and MEP shared a common underlying distribution. I also explored the use of two additional z-matrices; one matrix that additionally grouped first-stage exposures for BzBP and DEP (Appendix 1, Table 2) and one matrix that grouped first-stage exposure by quartiles within parent phthalates (Appendix 1, Table 3).

I took the semi-Bayes approach by specifying a range of τ^2 values for which I believed that 95% of the coefficient estimates of the association between individual phthalate metabolites and endometriosis risk would lie (De Roos et al. 2001; MacLehose et al. 2007). Previous studies of the association between individual urinary phthalate metabolite concentrations and risk of endometriosis have reported a maximum confidence limit ratio of 10 (ratio of upper confidence limit to that of the lower confidence limit) (Huang et al. 2010; Itoh et al. 2009; Weuve et al. 2010). This is in agreement with the assertion that relative risk values for binary risk factors do not often exceed 25-fold and are usually within 10-fold (Greenland 1994). Therefore, the range of τ^2 values considered were τ^2=0.35 (10-fold risk), τ^2=0.50 (16-fold risk, a prior variance with greater uncertainty) and τ^2=0.17 (5-fold risk, a prior variance with less uncertainty).

I found that the odds ratios and 95% confidence intervals for the full model conventional analysis by maximum likelihood estimation were unstable with wide confidence intervals and

confidence limit ratios ranging from 5 to 537 (MEP, third vs. first quartile, 95% CI: 0.7-3.5; MEHHP, fourth vs. first quartile, 95% CI: 0.03-10.7) (Appendix 1, Table 4). In comparison, the adjusted odds ratios and 95% confidence intervals in the hierarchical regression models were more precise and reasonable, particularly for the highly correlated DEHP metabolites. In this application of hierarchical regression, the choice of τ^2 did not substantially alter the results. When I repeated the analyses using the z-matrices depicted in Appendix 1, Table 2 (Model 2) and Table 3 (Model 3), I obtained similar estimates across hierarchical regression models, although the confidence intervals widened slightly for MBzP and MEP estimates in Model 2 and Model 3 (Appendix 1, Table 5).

In this exploratory analysis, hierarchical regression appeared to improve the precision and stability of estimates over maximum likelihood estimation when simultaneously evaluating multiple correlated exposures. The estimates across hierarchical regression models suggested an inverse association between MEHP concentration and endometriosis risk and increased endometriosis risk with greater concentrations of MBzP and MEP. The results across hierarchical models provided a similar impression as that found in Chapter 2 using logistic regression models that included one phthalate metabolite. However, unlike the results reported in Chapter 2, the estimates across hierarchical regression for the three other DEHP metabolites, MEHHP, MEOHP, and MECPP appeared to be closer to the null. I did not report the results of the hierarchical regression in Chapter 2 given that the appropriateness of our prior distribution was unknown.

Appendix 1, Table 1. The Z-matrix for second-stage covariates in the hierarchical regression (Model 1).

Phthalate metabolite	Intercept	DBP	DEHP
MBzP Quartile 2	1	0	0
MBzP Quartile 3	1	0	0
MBzP Quartile 4	1	0	0
MEP Quartile 2	1	0	0
MEP Quartile 3	1	0	0
MEP Quartile 4	1	0	0
MiBP Quartile 2	1	1	0
MiBP Quartile 3	1	1	0
MiBP Quartile 4	1	1	0
MnBP Quartile 2	1	1	0
MnBP Quartile 3	1	1	0
MnBP Quartile 4	1	1	0
MEHP Quartile 2	1	0	1
MEHP Quartile 3	1	0	1
MEHP Quartile 4	1	0	1
MEHHP Quartile 2	1	0	1
MEHHP Quartile 3	1	0	1
MEHHP Quartile 4	1	0	1
MEOHP Quartile 2	1	0	1
MEOHP Quartile 3	1	0	1
MEOHP Quartile 4	1	0	1
MEHHP Quartile 2	1	0	1
MEHHP Quartile 3	1	0	1
MEHHP Quartile 4	1	0	1
MECPP Quartile 2	1	0	1
MECPP Quartile 3	1	0	1
MECPP Quartile 4	1	0	1

Appendix 1, Table 2. The Z-matrix for second-stage covariates in the hierarchical regression with a prior covariate for each parent phthalate (Model 2).

Phthalate metabolite	Intercept	BzBP	DEP	DBP	DEHP
MBzP Quartile 2	1	1	0	0	0
MBzP Quartile 3	1	1	0	0	0
MBzP Quartile 4	1	1	0	0	0
MEP Quartile 2	1	0	1	0	0
MEP Quartile 3	1	0	1	0	0
MEP Quartile 4	1	0	1	0	0
MiBP Quartile 2	1	0	0	1	0
MiBP Quartile 3	1	0	0	1	0
MiBP Quartile 4	1	0	0	1	0
MnBP Quartile 2	1	0	0	1	0
MnBP Quartile 3	1	0	0	1	0
MnBP Quartile 4	1	0	0	1	0
MEHP Quartile 2	1	0	0	0	1
MEHP Quartile 3	1	0	0	0	1
MEHP Quartile 4	1	0	0	0	1
MEHHP Quartile 2	1	0	0	0	1
MEHHP Quartile 3	1	0	0	0	1
MEHHP Quartile 4	1	0	0	0	1
MEOHP Quartile 2	1	0	0	0	1
MEOHP Quartile 3	1	0	0	0	1
MEOHP Quartile 4	1	0	0	0	1
MEHHP Quartile 2	1	0	0	0	1
MEHHP Quartile 3	1	0	0	0	1
MEHHP Quartile 4	1	0	0	0	1
MECPP Quartile 2	1	0	0	0	1
MECPP Quartile 3	1	0	0	0	1
MECPP Quartile 4	1	0	0	0	1

Appendix 1, Table 3. The Z-matrix for second-stage covariates in the hierarchical regression with a prior covariate for each quartile of the parent phthalate (Model 3).

Phthalate metabolite	Intercept	BzBP Q2	BzBP Q3	BzBP Q4	DEP Q2	DEP Q3	DEP Q4	DBP Q2	DBP Q3	DBP Q4	DEHP Q2	DEHP Q3	DEHP Q4
MBzP Quartile 2	1	1	0	0	0	0	0	0	0	0	0	0	0
MBzP Quartile 3	1	0	1	0	0	0	0	0	0	0	0	0	0
MBzP Quartile 4	1	0	0	1	0	0	0	0	0	0	0	0	0
MEP Quartile 2	1	0	0	0	1	0	0	0	0	0	0	0	0
MEP Quartile 3	1	0	0	0	0	1	0	0	0	0	0	0	0
MEP Quartile 4	1	0	0	0	0	0	1	0	0	0	0	0	0
MiBP Quartile 2	1	0	0	0	0	0	0	1	0	0	0	0	0
MiBP Quartile 3	1	0	0	0	0	0	0	0	1	0	0	0	0
MiBP Quartile 4	1	0	0	0	0	0	0	0	0	1	0	0	0
MnBP Quartile 2	1	0	0	0	0	0	0	1	0	0	0	0	0
MnBP Quartile 3	1	0	0	0	0	0	0	0	1	0	0	0	0
MnBP Quartile 4	1	0	0	0	0	0	0	0	0	1	0	0	0
MEHP Quartile 2	1	0	0	0	0	0	0	0	0	0	1	0	0
MEHP Quartile 3	1	0	0	0	0	0	0	0	0	0	0	1	0
MEHP Quartile 4	1	0	0	0	0	0	0	0	0	0	0	0	1
MEHHP Quartile 2	1	0	0	0	0	0	0	0	0	0	1	0	0
MEHHP Quartile 3	1	0	0	0	0	0	0	0	0	0	0	1	0
MEHHP Quartile 4	1	0	0	0	0	0	0	0	0	0	0	0	1
MEOHP Quartile 2	1	0	0	0	0	0	0	0	0	0	1	0	0
MEOHP Quartile 3	1	0	0	0	0	0	0	0	0	0	0	1	0
MEOHP Quartile 4	1	0	0	0	0	0	0	0	0	0	0	0	1
MEHHP Quartile 2	1	0	0	0	0	0	0	0	0	0	1	0	0
MEHHP Quartile 3	1	0	0	0	0	0	0	0	0	0	0	1	0
MEHHP Quartile 4	1	0	0	0	0	0	0	0	0	0	0	0	1
MECPP Quartile 2	1	0	0	0	0	0	0	0	0	0	1	0	0
MECPP Quartile 3	1	0	0	0	0	0	0	0	0	0	0	1	0
MECPP Quartile 4	1	0	0	0	0	0	0	0	0	0	0	0	1

Appendix 1, Table 4. Odds ratios and 95% confidence intervals for the relationship between urinary phthalate metabolites and risk of endometriosis using a one-at-a-time conventional model, full conventional model, and hierarchical regression models with range of prior variances specified.

			Conventional		Hierarchical regression[c]		
Phthalate metabolite (ng/ml urine)	Cases (n=92) n (%)	Controls (n=195) n (%)	One exposure aOR[a] (95% CI)	Full model aOR[b] (95% CI)	τ^2=0.17 aOR[b] (95% CI)	τ^2=0.35 aOR[b] (95% CI)	τ^2=0.50 aOR[b] (95% CI)
MEHP							
≤1.0	33 (35.9)	50 (25.6)	1.0	1.0	1.0	1.0	1.0
>1.0-3.4	21 (22.8)	47 (24.1)	0.6 (0.3-1.3)	0.4 (0.1-1.0)	0.8 (0.4-1.4)	0.7 (0.3-1.4)	0.7 (0.3-1.4)
>3.4-11.1	26 (28.3)	49 (25.1)	0.7 (0.3-1.5)	0.4 (0.1-1.3)	0.9 (0.5-1.7)	0.9 (0.4-1.8)	0.8 (0.4-1.8)
>11.1	12 (13.0)	49 (25.1)	0.3 (0.1-0.7)	0.2 (0.03-0.8)	0.6 (0.3-1.2)	0.5 (0.2-1.2)	0.5 (0.2-1.2)
MEHHP							
≤6.3	25 (27.2)	48 (24.6)	1.0	1.0	1.0	1.0	1.0
>6.3-18.8	31 (33.7)	51 (26.2)	1.1 (0.5-2.4)	1.0 (0.2-4.0)	1.0 (0.5-1.8)	1.0 (0.5-2.1)	1.0 (0.4-2.2)
>18.8-56.5	22 (23.9)	48 (24.6)	0.8 (0.3-2.0)	0.8 (0.1-5.5)	0.9 (0.5-1.8)	0.9 (0.4-2.1)	0.9 (0.4-2.3)
>56.5	14 (15.2)	48 (24.6)	0.5 (0.2-1.5)	0.6 (0.03-10.7)	0.8 (0.4-1.7)	0.8 (0.3-2.1)	0.8 (0.3-2.4)
MEOHP							
≤3.5	23 (25.0)	49 (25.1)	1.0	1.0	1.0	1.0	1.0
>3.5-10.8	33 (35.9)	47 (24.1)	1.4 (0.6-2.9)	1.5 (0.4-6.1)	1.1 (0.6-2.1)	1.2 (0.6-2.6)	1.3 (0.6-2.9)
>10.8-29.1	21 (22.8)	50 (25.6)	0.8 (0.3-2.1)	0.8 (0.1-5.8)	0.8 (0.4-1.6)	0.8 (0.3-1.9)	0.8 (0.3-2.0)
>29.1	15 (16.3)	49 (25.1)	0.6 (0.2-1.7)	0.9 (0.04-19.8)	0.8 (0.4-1.7)	0.8 (0.3-2.2)	0.8 (0.3-2.6)
MECPP							
≤5.8	22 (23.9)	48 (24.6)	1.0	1.0	1.0	1.0	1.0
>5.8-18.0	30 (32.6)	50 (25.6)	1.3 (0.6-2.9)	1.5 (0.5-4.6)	0.9 (0.5-1.7)	1.0 (0.5-2.0)	1.0 (0.4-2.1)
>18.0-51.9	23 (25.0)	48 (24.6)	1.2 (0.5-3.0)	3.4 (0.7-16.3)	1.0 (0.5-2.0)	1.1 (0.5-2.5)	1.2 (0.5-2.9)
>51.9	17 (18.5)	49 (25.1)	0.8 (0.3-2.3)	4.6 (0.6-35.2)	0.9 (0.5-1.9)	1.1 (0.4-2.6)	1.2 (0.4-3.2)

Appendix 1, Table 4. Continued.

			Conventional		Hierarchical regression[c]		
Phthalate metabolite (ng/ml urine)	Cases (n=92) n (%)	Controls (n=195) n (%)	One exposure aOR[a] (95% CI)	Full model aOR[b] (95% CI)	τ^2=0.17 aOR[b] (95% CI)	τ^2=0.35 aOR[b] (95% CI)	τ^2=0.50 aOR[b] (95% CI)
MBzP							
≤2.0	21 (22.8)	51 (26.2)	1.0	1.0	1.0	1.0	1.0
>2.0-5.0	29 (31.5)	47 (24.1)	1.7 (0.8-3.8)	1.6 (0.6-4.4)	1.5 (0.7-3.2)	1.5 (0.7-3.4)	1.5 (0.7-3.5)
>5.0-11.5	22 (23.9)	49 (25.1)	1.5 (0.6-4.0)	1.5 (0.5-5.0)	1.5 (0.7-3.2)	1.5 (0.6-3.6)	1.5 (0.6-3.7)
>11.5	20 (21.7)	48 (24.6)	1.3 (0.4-4.0)	1.6 (0.4-5.8)	1.4 (0.6-3.3)	1.4 (0.5-3.7)	1.4 (0.5-3.9)
MEP							
≤16.8	19 (20.7)	49 (25.1)	1.0	1.0	1.0	1.0	1.0
>16.8-43.9	20 (21.7)	48 (24.6)	1.1 (0.5-2.4)	0.9 (0.4-2.1)	1.2 (0.6-2.3)	1.1 (0.5-2.3)	1.1 (0.5-2.3)
>43.9-144.4	30 (32.6)	50 (25.6)	1.8 (0.8-3.8)	1.5 (0.7-3.5)	1.6 (0.8-3.2)	1.6 (0.8-3.4)	1.6 (0.8-3.5)
>144.4	23 (25.0)	48 (24.6)	1.7 (0.7-4.1)	1.5 (0.6-3.8)	1.6 (0.7-3.3)	1.6 (0.7-3.5)	1.6 (0.7-3.6)
MiBP							
≤0.7	26 (28.3)	50 (25.6)	1.0	1.0	1.0	1.0	1.0
>0.7-1.5	27 (29.4)	53 (27.2)	0.9 (0.4-2.0)	0.9 (0.4-2.2)	0.9 (0.5-1.8)	0.9 (0.4-1.9)	0.9 (0.4-2.0)
>1.5-3.1	20 (21.7)	44 (22.6)	0.8 (0.3-2.2)	0.9 (0.3-2.7)	0.9 (0.4-2.0)	0.9 (0.4-2.2)	0.9 (0.4-2.2)
>3.1	19 (20.7)	48 (24.6)	0.8 (0.3-2.6)	0.8 (0.2-2.8)	0.9 (0.4-2.2)	0.9 (0.3-2.4)	0.9 (0.3-2.5)
MnBP							
≤4.9	22 (23.9)	48 (24.6)	1.0	1.0	1.0	1.0	1.0
>4.9-10.0	25 (27.2)	50 (25.6)	1.2 (0.5-2.8)	0.9 (0.3-2.6)	0.9 (0.4-1.9)	0.9 (0.4-2.2)	0.9 (0.4-2.1)
>10.0-23.5	25 (27.2)	49 (25.1)	1.5 (0.6-3.9)	1.3 (0.4-4.0)	1.1 (0.5-2.3)	1.1 (0.5-2.6)	1.1 (0.5-2.7)
>23.5	20 (21.7)	48 (24.6)	1.3 (0.4-3.9)	1.4 (0.4-5.0)	1.0 (0.4-2.5)	1.1 (0.4-2.9)	1.1 (0.4-3.2)

Abbreviations: OR=odds ratio; CI=confidence interval; MBzP=mono-benzyl phthalate; MEP=mono-ethyl phthalate; MiBP=mono-isobutyl phthalate; MnBP=mono-n-butyl 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.
[a]Odds ratio adjusted for age, reference year, and natural logarithm transformed imputed urinary creatinine.
[b]Odds ratio adjusted for age, reference year, natural log-transformed imputed creatinine, and all other phthalate metabolites.

[c]Hierarchical regression used a z-matrix that included an intercept and prior covariates for DBP and DEHP (Model1).

Appendix 1, Table 5. Odds ratios and 95% confidence intervals for the relationship between urinary phthalate metabolites and risk of endometriosis using a one-at-a-time conventional model, full conventional model, and three hierarchical regression models that varied by specification of prior covariates (z-matrix) using a prior variance τ^2=0.35.

			Conventional		Hierarchical regression		
Phthalate metabolite (ng/ml urine)	Cases (n=92) n (%)	Controls (n=195) n (%)	One exposure aOR[a] (95% CI)	Full model aOR[b] (95% CI)	Model 1 aOR[b] (95% CI)	Model 2 aOR[b] (95% CI)	Model 3 aOR[b] (95% CI)
MEHP							
≤1.0	33 (35.9)	50 (25.6)	1.0	1.0	1.0	1.0	1.0
>1.0-3.4	21 (22.8)	47 (24.1)	0.6 (0.3-1.3)	0.4 (0.1-1.0)	0.7 (0.3-1.4)	0.7 (0.3-1.4)	0.7 (0.3-1.4)
>3.4-11.1	26 (28.3)	49 (25.1)	0.7 (0.3-1.5)	0.4 (0.1-1.3)	0.9 (0.4-1.8)	0.9 (0.4-1.8)	0.9 (0.4-1.8)
>11.1	12 (13.0)	49 (25.1)	0.3 (0.1-0.7)	0.2 (0.03-0.8)	0.5 (0.2-1.2)	0.5 (0.2-1.2)	0.5 (0.2-1.2)
MEHHP							
≤6.3	25 (27.2)	48 (24.6)	1.0	1.0	1.0	1.0	1.0
>6.3-18.8	31 (33.7)	51 (26.2)	1.1 (0.5-2.4)	1.0 (0.2-4.0)	1.0 (0.5-2.1)	1.0 (0.5-2.1)	1.0 (0.5-2.1)
>18.8-56.5	22 (23.9)	48 (24.6)	0.8 (0.3-2.0)	0.8 (0.1-5.5)	0.9 (0.4-2.1)	0.9 (0.4-2.1)	0.9 (0.4-2.1)
>56.5	14 (15.2)	48 (24.6)	0.5 (0.2-1.5)	0.6 (0.03-10.7)	0.8 (0.3-2.1)	0.8 (0.3-2.1)	0.8 (0.3-2.0)
MEOHP							
≤3.5	23 (25.0)	49 (25.1)	1.0	1.0	1.0	1.0	1.0
>3.5-10.8	33 (35.9)	47 (24.1)	1.4 (0.6-2.9)	1.5 (0.4-6.1)	1.2 (0.6-2.6)	1.2 (0.6-2.6)	1.2 (0.6-2.7)
>10.8-29.1	21 (22.8)	50 (25.6)	0.8 (0.3-2.1)	0.8 (0.1-5.8)	0.8 (0.3-1.9)	0.8 (0.3-1.8)	0.8 (0.3-1.9)
>29.1	15 (16.3)	49 (25.1)	0.6 (0.2-1.7)	0.9 (0.04-19.8)	0.8 (0.3-2.2)	0.8 (0.3-2.2)	0.8 (0.3-2.2)
MECPP							
≤5.8	22 (23.9)	48 (24.6)	1.0	1.0	1.0	1.0	1.0
>5.8-18.0	30 (32.6)	50 (25.6)	1.3 (0.6-2.9)	1.5 (0.5-4.6)	1.0 (0.5-2.0)	1.0 (0.5-2.0)	1.0 (0.5-2.0)
>18.0-51.9	23 (25.0)	48 (24.6)	1.2 (0.5-3.0)	3.4 (0.7-16.3)	1.1 (0.5-2.5)	1.1 (0.5-2.5)	1.1 (0.5-2.5)
>51.9	17 (18.5)	49 (25.1)	0.8 (0.3-2.3)	4.6 (0.6-35.2)	1.1 (0.4-2.6)	1.0 (0.4-2.6)	1.0 (0.4-2.5)

Appendix 1, Table 5. Continued.

Phthalate metabolite (ng/ml urine)	Cases (n=92) n (%)	Controls (n=195) n (%)	Conventional		Hierarchical regression		
			One exposure aOR[a] (95% CI)	Full model aOR[b] (95% CI)	Model 1[c] aOR[b] (95% CI)	Model 2[d] aOR[b] (95% CI)	Model 3[e] aOR[b] (95% CI)
MBzP							
≤2.0	21 (22.8)	51 (26.2)	1.0	1.0	1.0	1.0	1.0
>2.0-5.0	29 (31.5)	47 (24.1)	1.7 (0.8-3.8)	1.6 (0.6-4.4)	1.5 (0.7-3.4)	1.6 (0.6-4.4)	1.6 (0.6-4.4)
>5.0-11.5	22 (23.9)	49 (25.1)	1.5 (0.6-4.0)	1.5 (0.5-5.0)	1.5 (0.6-3.6)	1.6 (0.5-4.9)	1.5 (0.5-5.2)
>11.5	20 (21.7)	48 (24.6)	1.3 (0.4-4.0)	1.6 (0.4-5.8)	1.4 (0.5-3.7)	1.5 (0.4-5.1)	1.5 (0.4-5.4)
MEP							
≤16.8	19 (20.7)	49 (25.1)	1.0	1.0	1.0	1.0	1.0
>16.8-43.9	20 (21.7)	48 (24.6)	1.1 (0.5-2.4)	0.9 (0.4-2.1)	1.1 (0.5-2.3)	1.1 (0.5-2.4)	1.0 (0.4-2.4)
>43.9-144.4	30 (32.6)	50 (25.6)	1.8 (0.8-3.8)	1.5 (0.7-3.5)	1.6 (0.8-3.4)	1.6 (0.7-3.6)	1.7 (0.7-3.9)
>144.4	23 (25.0)	48 (24.6)	1.7 (0.7-4.1)	1.5 (0.6-3.8)	1.6 (0.7-3.5)	1.5 (0.6-3.8)	1.6 (0.6-4.1)
MiBP							
≤0.7	26 (28.3)	50 (25.6)	1.0	1.0	1.0	1.0	1.0
>0.7-1.5	27 (29.4)	53 (27.2)	0.9 (0.4-2.0)	0.9 (0.4-2.2)	0.9 (0.4-1.9)	0.9 (0.4-1.9)	0.9 (0.4-1.9)
>1.5-3.1	20 (21.7)	44 (22.6)	0.8 (0.3-2.2)	0.9 (0.3-2.7)	0.9 (0.4-2.2)	0.9 (0.4-2.2)	1.0 (0.4-2.3)
>3.1	19 (20.7)	48 (24.6)	0.8 (0.3-2.6)	0.8 (0.2-2.8)	0.9 (0.3-2.4)	0.9 (0.3-2.4)	0.9 (0.3-2.6)
MnBP							
≤4.9	22 (23.9)	48 (24.6)	1.0	1.0	1.0	1.0	1.0
>4.9-10.0	25 (27.2)	50 (25.6)	1.2 (0.5-2.8)	0.9 (0.3-2.6)	0.9 (0.4-2.2)	0.9 (0.4-2.0)	0.9 (0.4-2.0)
>10.0-23.5	25 (27.2)	49 (25.1)	1.5 (0.6-3.9)	1.3 (0.4-4.0)	1.1 (0.5-2.6)	1.1 (0.5-2.6)	1.1 (0.5-2.6)
>23.5	20 (21.7)	48 (24.6)	1.3 (0.4-3.9)	1.4 (0.4-5.0)	1.1 (0.4-2.9)	1.1 (0.4-2.9)	1.1 (0.4-3.2)

Abbreviations: OR=odds ratio; CI=confidence interval; MBzP=mono-benzyl phthalate; MEP=mono-ethyl phthalate; MiBP=mono-isobutyl phthalate; MnBP=mono-n-butyl 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.

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

[b]Odds ratio adjusted for age, reference year, natural log-transformed imputed creatinine, and all other phthalate metabolites.

[c]Using Z-matrix with intercept and grouping the first-stage covariates by DBP and DEHP.

[d]Using Z-matrix with intercept and grouping the first-stage covariates by BzBP, DEP, DBP, and DEHP.

[e]Using Z-matrix with intercept and grouping first-stage covariates by quartiles of BzBP, DEP, DBP, and DEHP.