Exposure to organophosphate esters, phthalates, and alternative plasticizers in association with uterine fibroids

Exposure to organophosphate esters, phthalates, and alternative plasticizers in association with uterine fibroids

Gowoon Lee[1], Sunmi Kim[1], Michiel Bastiaensen[2], Govindan Malarvannan[2], Giulia Poma[2], Noelia Caballero-Casero[2], Celine Gys[2], Adrian Covaci[2], Sunggyu Lee[3], Jae-Eun Lim[3], Sori Mok[3], Hyo-Bang Moon[3], Gyuyeon Choi[4], Kyungho Choi[1*]

[1] School of Public Health, Seoul National University, Seoul, Republic of Korea

[2] Toxicological Center, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

[3] Department of Marine Sciences and Convergent Technology, Hanyang University, Ansan, Republic of Korea

[4] College of Medicine, Soonchunhyang University, Seoul, Republic of Korea

* Corresponding author. School of Public Health, Seoul National University, Seoul, Republic of Korea

E-mail address: kyungho@snu.ac.kr (K. Choi).

Abstract

Exposure to endocrine disrupting chemicals is suggested to be responsible for the development or progression of uterine fibroids. However, little is known about risks related to emerging chemicals, such as organophosphate esters (OPEs) and alternative plasticizers (APs). A case-control study was conducted to investigate whether exposures to OPEs, APs, and phthalates, were associated with uterine fibroids in women of reproductive age. For this purpose, the cases (n = 32) and the matching controls (n = 79) were chosen based on the results of gynecologic ultrasonography among premenopausal adult women in Korea and measured for metabolites of several OPEs, APs, and major phthalates. Logistic regression models were employed to assess the associations between chemical exposure and disease status. Factor analysis was conducted for multiple chemical exposure assessments as a secondary analysis. Among OPE metabolites, diphenyl phosphate (DPHP), 2-ethylhexyl phenyl phosphate (EHPHP), and 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP) were detected in >80% of the subjects. Among APs, metabolites of di-isononyl phthalate (DINP) and di(2-propylheptyl) phthalate (DPrHpP) were detected in >75% of the urine samples. The odds ratios (ORs) of uterine fibroids were significantly higher among the women with higher exposures to tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) and tris(2-butoxyethyl) phosphate (TBOEP), di(2-ethylhexyl) terephthalate (DEHTP), DPrHpP, and di-(iso-nonyl)-cyclohexane-1,2-dicarboxylate (DINCH). In addition, urinary concentrations of mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), a sum of five di(2-ethylhexyl) phthalate metabolites (∑5DEHP), and mono(4-methyl-7-hydroxyoctyl) phthalate (OH-MINP) were significantly higher in the cases. In factor analysis, a factor heavily loaded with DPrHpP and DEHP was significantly associated with uterine fibroids, supporting the observation from the single chemical regression model. We found for the first time that several metabolites of OPEs and APs are associated with increased risks of uterine fibroids among pre-menopausal women. Further epidemiological and mechanistic studies are warranted to validate the associations observed in the present study.

Keywords: Organophosphate esters,alternative plasticizers, phthalates, fibroids, pre-menopausal women

1. Introduction

Uterine fibroids or leiomyomas are benign tumors common among women with prevalence up to 68.6% depending on the population and method of diagnosis (Stewart et al., 2017). While most patients with uterine fibroids have no symptoms, some may experience heavy menstrual bleeding or pain, pelvic pressure or pain, dysuria, and sterility or subfertility (Parker, 2007). The personal and societal economic burden is huge, with an additional annual cost estimated at $15,952 per patient worldwide, and at an estimated annual cost of up to $34.4 billion in the United States (Cardozo et al., 2012; Soliman et al., 2015). Uterine fibroids can also cause impairment of health-related quality of life, and productivity, according to a study on women living in Western Europe (Downes et al., 2010). The etiology of uterine fibroids is unknown, but several important factors have been suggested to be responsible for initiating or promoting this disease; these include hormones (estradiol and progesterone), growth factors, and regulatory changes of hormonal receptors, as well as demographic and physiological risk factors such as age, family history, weight, and parity (Flake et al., 2003; Parker, 2007).

Several chemicals have been reported to be associated with presence of uterine fibroids in epidemiological studies, which include di(2-ethylhexyl) phthalate (DEHP) metabolites (Fu et al., 2017; Huang et al., 2010; Kim et al., 2016; Sun et al., 2016) and environmental phenols, such as bisphenol A and nonylphenol (Shen et al., 2016). Exposure to persistent organic pollutants (POPs), such as *p,p′*-dichlorodiphenyl-dichloroethylene (*p,p'*-DDE) and polychlorinated biphenyls (PCBs), has also been suggested for potential association with uterine fibroids (Lambertino et al., 2011; Trabert et al., 2015). Endocrine-disruption which affects sex hormone balances is suspected as an underlying mechanism for these chemicals (Lan et al., 2017; Lee et al., 2019; Ptak et al., 2006).

Phthalates have been used in many applications, like food packaging, cosmetics, paintings, medical devices, and building materials. The use of phthalates, such as DEHP, benzyl butyl phthalate (BBzP), or dibutyl phthalate, has been regulated worldwide due to their potential adverse health effects (CPSIA, 2008; European Union, 2005; KATS, 2010). Subsequently, alternative chemicals have been introduced in increasing amounts into the market to replace the regulated phthalates (Bui et al., 2016; Stapleton et

al., 2012). Organophosphate esters (OPEs) which have been used as flame retardants or lubricants, are also one category of these alternative plasticizers (van der Veen and de Boer, 2012). In addition, phthalates have been replaced by several other alternative plasticizers (APs), such as 1,2-cyclohexane dicarboxylic acid, diisononyl ester (DINCH), and di-2-ethylhexyl terephthalate (DEHTP). Compared to conventional phthalates, much less information about the potential health consequences is currently available for these substituting chemicals. So far, there has been no study reporting the associations of these alternative plasticizers with uterine fibroids (Du et al., 2019; Engel et al., 2018; Kambia et al., 2019).

In this study, we investigated the association between several emerging chemicals that are used in growing amounts and uterine fibroids. For this purpose, we designed a case-control study among Korean women of reproductive age and assessed the associations of urinary metabolites of OPEs, APs, and phthalates with uterine fibroids. The results of this study will help identify the health hazards of new consumer chemicals emerging in the daily lives and facilitate further experimental and epidemiological studies on potential risk factors of uterine fibroids.

2. Materials and methods

2.1 Study population and sample collection

Adult Korean women before menopause (n = 516, 20–49 years of age) were recruited during 2015–2016 from medical institutes located in Seoul, Ansan, Incheon, and Jeju of Korea. Participants visited a public health center for a general health check, or the obstetrics and gynecology clinics of the university hospitals for routine gynecology checkup. In addition, a subset of the participants (n =70) was randomly chosen from Children's Health and Environmental Chemicals of Korea (CHECK) cohort. Among the women initially recruited, those with current pregnancy (n=38) were excluded. The participants were asked to fast for more than 8 hr, before they came for health examinations and urine sample collection. The urine samples were stored at -20°C immediately after the collection. The frozen

urine samples were subsequently, within several days following collection, transferred to Seoul National University and stored at -40°C until chemical analysis. The participants had undergone gynecologic ultrasonography for the diagnosis of gynecologic diseases including uterine fibroids and adenomyosis. Among the participating women, 95 cases of uterine fibroids were initially identified. In the present study, those of 'severe' degree of fibroids were defined as the case, in order to make a clearer contrast with the control. The severity of uterine fibroids is often determined with the number and size of the fibroids or symptoms (Ciavattini et al., 2015; Taran et al., 2010). Concurrent occurrence of adenomosis was also considered because adenomyosis contributes to symptomatology in women with leiomyomas (Brucker et al., 2014; Taran et al., 2010). Therefore, a total of 40 cases were chosen based on the following criteria: the size of uterine fibroids (>4 cm), the number of fibroids (>2), or concurrent diagnosis of adenomyosis. As the control, women of the same age without the disease were randomly chosen with 1:2 (case:control) ratio. Among the cases and controls initially chosen, those with no or insufficient amount of the samples for chemical analysis were excluded, and hence a total of 32 cases and 79 controls were finally included in this study. Demographic data were obtained using a questionnaire. Urinary cotinine was measured by Immulite 2000 Nicotine Metabolite kit (Siemens Healthcare Diagnostics, USA). The present study was approved by the Institutional Review Board of Seoul National University (IRB No. 1509/001-011).

2.2 Measurement of urinary OPEs and plasticizers

Metabolites of twelve OPEs, thirteen APs, and fifteen phthalates were analyzed in the urine of the participants. The OPE metabolites included: 4-hydroxyphenyl phenyl phosphate (4-HO-DPHP), diphenyl phosphate (DPHP), 2-ethyl-5-hydroxyhexyl diphenyl phosphate (5-HO-MEHTP), 2-ethylhexyl phenyl phosphate (EHPHP), 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP), bis(1-chloro-2-propyl) phosphate (BCIPP), tris(chloroethyl) phosphate (TCEP), bis(2-butoxyethyl) phosphate (BBOEP), 2-hydroxyethyl bis(2-butoxyethyl) phosphate (BBOEHEP), bis(2-butoxyethyl) 3′-hydroxy-2-butoxyethyl phosphate (3-HO-TBOEP), bis(1,3-dichloro-2-propyl)

phosphate (BDCIPP), and di-n-butyl phosphate (DNBP);the AP metabolites included: mono(4-methyl-7-hydroxyoctyl) phthalate (OH-MINP), mono(4-methyl-7-carboxyheptyl) phthalate (cxMINP), mono(2-ethyl-5-hydroxyhexyl) terephthalate (OH-MEHTP), mono(2-ethylhexyl) terephthalate (MEHTP), mono(2-ethyl-5-hydroxyhexyl) adipate (OH-MEHA), mono(2-ethyl-5-oxohexyl) adipate(oxoMEHA), mono(2-ethylhexyl) adipate (MEHA), mono(2-propyl-6-carboxyhexyl) phthalate (cxMPrHpP), mono(2-propyl-6-hydroxyheptyl) phthalate (OH-MPrHpP), mono(2-propyl-6-oxoheptyl) phthalate (oxoMPrHpP), cyclohexane-1,2-dicarboxylic mono carboxyisooctyl ester (cxMINCH), cyclohexane-1,2-dicarboxylic mono hydroxyisononyl ester (OH-MINCH), and cyclohexane-1,2-dicarboxylic mono isononyl ester (MINCH); and the phthalate metabolites included: monomethyl phthalate (MMP), monoethyl phthalate (MEP), mono-isopropyl phthalate (MiPP), mono-2-isobutyl phthalate (MiBP), mono-n-butyl phthalate (MBP), mono-n-pentyl phthalate (MPeP), monobenzyl phthalate (MBzP), monocyclohexyl phthalate (MCHP), monohexyl phthalate (MHxP), mono(2-ethy l-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-[(2-carboxymethyl)hexyl] phthalate (MCMHP), mono(2-ethylhexyl) phthalate (MEHP), and mono(3-carboxypropyl) phthalate (MCPP) (Table S1).

Urinary metabolites of OPEs were measured following the method described by Bastiaensen et al. (Bastiaensen et al., 2018). OPE metabolites were extracted using solid-phase extraction (SPE) after enzymatic deconjugation with ß-glucuronidase. Instrumental analysis was conducted on an Agilent 1290 Infinity liquid chromatography system coupled with an Agilent 6460 Triple Quadrupole mass spectrometer (MS) (Santa Clara, CA, USA) (Table S2).

AP metabolites were measured following Been et al. (2019) with a slight modification (Been et al., 2019). Briefly, 1.0 mL of urine samples were spiked with a mixture of internal standards and then extracted by Oasis MAX SPE after enzymatic deconjugation with ß-glucuronidase. Target analytes were separated by reversed-phase HPLC and quantified by electrospray ionization tandem MS (Agilent 1290 Infinity coupled to 6460 electrosprays Triple Quadrupole, Agilent Technologies) (Table S2).

For phthalate metabolites, the sample was prepared following a method described elsewhere (Guo et al., 2011). In brief, 0.5 mL of urine was spiked with a mixture of internal standards and buffered with ammonium acetate including β-glucuronidase. Phthalate metabolites were extracted using an SPE setup. For the chromatographic separation and quantification of target analytes, an Agilent 1260 Series high-performance liquid chromatography (HPLC) system (Agilent Technologies) and an API 4000 electrospray triple quadrupole MS (ESI–MS/MS; AB Sciex, Framingham, MA, USA) were used (Table S2). Additional procedural parameters and quality control information are shown in Table S3.

Urinary chemical concentrations were adjusted by specific gravity (SG) to correct for urine dilutions, using the following equation. The SG was determined on the URISYS 2400 Cassette (Roche).

SG-adjusted concentration = (chemical concentration) × $[(SG_{median} - 1)/(SG - 1)]$ where the median value of the specific gravity (SG_{median}) of all samples was 1.016.

2.3 Statistical analysis

For chemicals with a detection frequency of 75% or more, the non-detected was substituted with a limit of quantification (LOQ) divided by the square root of 2 before statistical analysis (Hornungm, 1990; Kim et al., 2015). For the comparison of demographic factors between the case and the control, the Wilcoxon signed-rank test was performed for continuous variables such as age and body mass index (BMI), considering their non-parametric distribution. For categorical variables such as income and alcohol consumption, the chi-squared test or Fisher's exact test (for categorical variables with an expected frequency of less than 5 such as parity, and urinary cotinine) were conducted (Table 1). Correlations among the metabolite concentrations (SG-adjusted) were determined by Spearman's correlation coefficients (Figure S1). For comparison of the metabolite concentrations between the case and the control groups, general linear regression models were constructed (analysis of covariance, ANCOVA) with covariates of age, BMI (continuously), income (categorically: low-middle for <4 million KRW a month; middle-high, for ≥4 million and <8 million KRW; and very high for ≥8 million

KRW), parity (categorically: 0, 1, ≥2), urinary cotinine (categorically: <10 and ≥10 ng/ml), and alcohol consumption (categorically: yes and no), based on previous reports (Kobrosly et al., 2012; Pavone et al., 2018). Due to the skewness of distribution, measured chemical concentrations were log-transformed.

For logistic regression analysis for association between chemical exposure and uterine fibroids, some independent variables were transformed into categorical variables depending on the frequency of detection. The chemicals which were detected in ≥75% of the samples were transformed into quartile variables. The chemicals which were detected in ≥50% and < 75% were transformed into tertiles, in which 0 was assigned for the samples not detected or below the LOQ, 1 was assigned for the samples with concentrations up to the median, and 2 for those with concentrations above the median. The chemicals which were detected in ≥25% and < 50% of the samples, were transformed into dichotomous variables, in which 0 was assigned for the samples < LOQ, and 1 for the samples detected. Odds ratios (ORs) of uterine fibroids were derived using logistic regression after adjusting for the same covariates as mentioned above. Covariates were chosen based on the previous studies that reported for association with uterine fibroids (Flake et al., 2003; Parker, 2007). Statistical significance was determined at p=0.05.

To further address concerns related to multiple chemical exposures, factor analysis with varimax rotation was conducted without constraints on the total number of the factors. Urinary chemicals with a detection frequency of 50% or more were included in the factor analysis. Before the analysis, the non-detected was substituted with an LOQ divided by the square root of 2 (Hornungm and Reed, 1990). The optimum number of the factors was determined by the eigenvalue, Scree plots, and proportion criterion. The loading on the factor with a factor loading >0.5 was considered significant. The factor scores derived from the factor analysis were categorized into tertiles, with the lowest tertile considered as the reference group. The associations between the factors and uterine fibroids were assessed by logistic regression analysis after adjusting covariates. All factors that were determined by the factor analysis were simultaneously included in a multiple chemical exposure model. Statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1 Study population

The average age of participants was 38 years (mean 38.2 ± 5.3 years for the cases, mean 37.8 ± 5.1 years for the controls). The average BMIs of the case and the control groups were 22.7±2.7 and 22.8±4.0 kg/m^2, respectively (Table 1). Age, income, urinary cotinine, alcohol consumption, and BMI were not different between the case and the control groups.The only exception was parity which was lower in the case group (Table 1). Several characteristics of the participants, such as age, income, parity, urinary cotinine, alcohol consumption, and BMI, were included for case-control comparisons and logistic regression analyses as covariates.

3.2 Chemical exposure

Among twelve target OPE metabolites, DPHP, EHPHP, and BCIPHIPP were detected in over 80% of the urine samples (Table 2). Other metabolites, such as BBOEHEP, BDCIPP, DNBP, and BBOEP, were detected in < 70%. EHPHP was detected at the highest concentrations (median 1.01 ng/ml) in the control group (Table 2). Among APs, metabolites of DINP and DPrHpP were detected in > 90% and > 75% of the urine samples, respectively. Median concentrations of cxMINP and cxMPrHpP in the control group were 2.57 ng/ml, and 0.66 ng/ml, respectively. Metabolites of DINCH, DEHA, and DEHTP were detected in fewer urine samples, < 68%, < 53%, and < 40%, respectively. Among measured phthalates, metabolites of DMP, DEP, DiBP, DBP, BBzP, and DEHP were detected in > 90% of urine samples. Among the phthalate metabolites, MECPP showed the highest median concentrations of 11.61 ng/ml. Significant positive correlations were observed between cxMINP and cxMPrHpP, and MBP and MEHHP, based on Spearman's correlation analysis (Figure S1).

3.3 Association of urinary metabolite concentrations with uterine fibroids

General linear regression after the adjustment of covariates showed that urinary concentrations of MEOHP, the sum of five DEHP metabolites (∑5DEHP, nmol/L), and OH-MINP were significantly higher in the urine of the cases than in the controls (Table 2). Other chemicals did not show any statistical differences between the two groups.

When the samples were dichotomously grouped based on the detection status (non-detected versus detected), BDCIPP, BBOEP, and BBOEHEP showed positive associations with uterine fibroids (adjusted OR 5.51 with 95% CI: 1.93, 15.76 for BDCIPP; adjusted OR 2.81 with 95% CI: 1.01, 7.83 for BBOEP; and adjusted OR 2.94 with 95% CI: 1.05, 8.24 for BBOEHEP) (Figure 1a, Table S5). Among those who were positive for BDCIPP, 21 out of 48, or 43.8% women were identified to have uterine fibroids, while only 11 out of 63 women had uterine fibroids among those who were not detected for BDCIPP. Among AP metabolites, those positive for OH-MEHTP, OH-MPrHpP, and OH-MINCH, showed significantly higher ORs of the uterine fibroids (Figure 1b, Table S5). For urinary OH-MPrHpP, 15 out of 35 (42.9 % of women) showed uterine fibroids among those in the highest tertile, while only 8 cases were identified out of 41 women, among those who were not detected (below LOQ). For OH-MINCH, uterine fibroids were shown in 46.9% of women (15 out of 32) among those detected, while only 21.5% of the case were identified among those below LOQ. Among the phthalate metabolites, women with the highest quartiles of DEHP metabolites, (e.g., MEOHP, MEHHP, MECPP, and ∑5DEHP metabolites), showed significantly elevated ORs than the women in the lowest quartile (Figure 1c). In addition, urinary concentrations of MBzP were significantly associated with increased ORs of uterine fibroids (Q2 vs. Q1: 4.82 with 95% CI, 1.09–21.27) (Figure 1c, Table S4).

According to the factor analysis, four significant factors were identified which accounted for 61.8%, 26.3%, 7.7%, and 5.1% of the total variance, respectively (Table S6). Factor 1 was characterized by the high loading of the metabolites of DEHP, DBP, and BBzP. Factor 2 was heavily loaded with metabolites of DPrHpP and DEHP.Factor 3 was loaded with the metabolites of DINP. Factor 4 had a high loading of metabolites of TPHP and DMP. In the multiple chemical exposure model, factor 2 was significantly associated with increased OR of uterine fibroids (tertile 1 vs tertile 3: 4.60 with 95% CI, 1.10 – 19.20)

(Table 3).

4. Discussion

In the present study, metabolites of major OPEs, phthalates, and APs were detected in the urine of Korean women of reproductive age. Among them, exposure to TDCIPP, TBOEP, BBzP, DEHP, DEHTP, and DPrHpP were significantly associated with uterine fibroids. While the association of uterine fibroids with DEHP metabolites has been reported previously, the associations of OPEs and APs are first reported in a population study. Underlying mechanisms of these observations warrant further investigation, even though estrogenic mode of action may partly explain the observed association of these chemicals.

Frequent detection of DPHP, EHPHP, and BCIPHIPP in the urine shows that premenopausal women of Korea were widely exposed to OPEs such as TPHP, EHDPHP, and TCIPP. The SG-adjusted median concentration of DPHP measured in the present female population (0.28 ng/ml) was lower than those of the US adults participating in the National Health and Nutrition Examination Survey (NHANES 2013-2014) (n = 1672; median 0.72 ng/ml, unadjusted) (Boyle et al., 2019), but higher than that reported in Chinese adults in Shanghai (n = 132; median 0.07 ng/ml, SG-adjusted) (Sun et al., 2018). Among the measured OPE metabolites in the present population, EHPHP showed the highest median concentration (1.01 ng/ml, SG-adjusted). The SG-adjusted median concentration of BCIPHIPP was 0.42 ng/ml in the control, which was lower than that of Belgian adults (n = 14, 3.93 ng/ml unadjusted) and the US (California) mothers (n = 28, 2.4 ng/ml SG-adjusted) (Bastiaensen et al., 2018; Butt et al., 2016). The detection frequency of BBOEHEP was <60% in the present Korean population, while BBOEHEP, BCIPHIPP, TCEP, and DPHP were most frequently detected in the urine samples of Japanese children (Bastiaensen et al., 2019) suggesting that usage pattern of these chemicals may vary by country and by population.

The exposure profiles of APs observed in the present population were different from those reported

in other countries even though population characteristics are different. The present Korean women are more frequently exposed to DINCH and DPrHpP than to DEHTP. Among the control group, cxMINCH was detected in 68.4% of the population with a median concentration of 0.45 ng/ml (SG-adjusted). DINCH appears to be introduced to Korean market since 2003, according to the registration number of DINCH in Korea (2003-3-2499). The detection level of cxMINCH was higher than that reported for Norwegian adults (age 20-66; 16 males and 45 females living in Oslo) with a geometric mean of 0.23 ng/ml (unadjusted) (Giovanoulis et al., 2016) and the general German young adult population (age range: 20-30; n = 60 recruited in 2012) with an unadjusted median of 0.17 ng/ml (Schütze et al., 2014). However, among the US general population participating in different cycles of NHANES (n = 5171), the detection frequency of OH-MINCH was low at 27.4% (Chen et al., 2019).

DPrHpP metabolites were detected in over 70% of the Korean women's urine samples. Among the present population, oxoMPrHpP was detected in 71.9% (case) and 62% (control) of the urine samples, while in the general German population (age range: 20-30; n = 60 recruited in 2012), the detection frequency of oxoMPrHpP was 21.7% (LOQ of 0.25 ng/ml) (Schütze et al., 2015). In contrast, OH-MEHTP, a metabolite of DEHTP, was detected only in 29.1% of the control urine samples of the present population, but among the US general population participating in NHANES 2015-2015 (n = 2970), this DEHTP metabolite was detected in 96% of the samples (Silva et al., 2019). Considering the different exposure profile by country, and the increasing use and exposure to APs such as DINCH, DPHP, and DEHTP worldwide (Schütze et al., 2015; Silva et al., 2013; Silva et al., 2017), continuous surveillance of APs in human urine samples is warranted.

Our observation suggests that several OPEs are associated with uterine fibroids. While the low detection frequency of most OPEs did not allow logistic regression analysis, the analytical results based on detection categories for BDCIPP, BBOEP, and BBOEHEP show clear and significant associations of these chemicals with higher ORs for uterine fibroids. In human populations, an association between OPE exposure and uterine fibroids has never been reported. The modes of action of OPEs in the development of uterine fibroids are not well understood, however, one possible explanation can be

297 found from the endocrine disruption potential of OPEs. Uterine fibroids are considered to be estrogen-
298 sensitive and it is well documented that sex regulating hormones, such as estrogen and progesterone,
299 promote the growth of uterine fibroids (Flake et al., 2003; Parker, 2007). Estrogenicity of several OPEs
300 has been documented in both *in vitro* and *in vivo* studies. TDCIPP and TBOEP could influence the
301 synthesis and metabolism of estradiol and TDCIPP could behave as an estrogen receptor agonist (Liu
302 et al., 2012; Zhang et al., 2014). In humans, its association with sex hormone regulation or other
303 reproductive health outcomes has been suggested. TDCIPP concentrations in house dust were
304 associated with a prolactin increase in male adults (Meeker and Stapleton, 2010). Prolactin is a
305 reproductive hormone that was suggested as an adjuvant biomarker in uterine fibroids (Baban, 2009;
306 Levy et al., 2013).

307 Significantly high ORs observed for OH-MEHTP and OH-MINCH could be partially supported by
308 the estrogenic effects of DINCH and DEHTP reported *in vitro*. DINCH showed a stimulatory effect on
309 steroid production *in vitro* (Boisvert et al., 2016). DINCH metabolites activate nuclear receptors,
310 estrogen receptors (α and β), androgen receptors, and peroxisome proliferator-activated receptors (α
311 and γ), suggesting weak estrogenic effects related with lipid and glucose metabolisms in reporter gene
312 assays (Engel et al., 2018). OH-MEHTP, a DEHTP metabolite, has an agonistic effect on estrogen
313 receptors and also increases steroid hormone synthesis *in vitro* (Kambia et al., 2019). While further
314 confirmation in other populations and the experimental studies are warranted, these observations
315 suggest these APs may not be safer substitutes for DEHP, at least in terms of the risk of uterine fibroids.
316 For DPrHpP, neither experimental nor epidemiological reports supporting our observation are available.
317 Considering that the exposure to DPrHpP appears to increase over time in the general population
318 (Schütze et al., 2015), studies are warranted to investigate an effect of this compound on development
319 of uterine fibroids.

320 The significant associations of DEHP metabolites with uterine fibroids have been previously reported
321 from several populations (Fu et al., 2017; Huang et al., 2010; Kim et al., 2016; Sun et al., 2016). In
322 contrast, null associations of urinary DEHP metabolites with fibroids are also available, in women who

underwent laparoscopy recruited from clinical centers of the US and with self-reported history of the disease (Pollack et al., 2015; Weuve et al., 2010). Several *in vitro* studies show that DEHP could enhance proliferative activity in myometrial and leiomyoma cells (Kim, 2018; Kim et al., 2017). In addition, exposure to DEHP was reported to be positively associated with increased uterine volume in premenopausal women (Zota et al., 2019). However, the mechanisms of action of DEHP underlying pathogenesis of uterine fibroids are not fully understood, therefore, further mechanistic studies are necessary to explain the associations.

Among measured phthalates, urinary MBzP concentrations showed a significant association with uterine fibroids, but without a dose dependent pattern (Figure 1, Table S4). This observation is discrepant with several previous studies that have reported null associations (Huang et al., 2010; Kim et al., 2016; Pollack et al., 2015). One possible explanation for the significant association of MBzP may be found from shared exposure sources of BBzP and DEHP, which is supported by moderate correlations between urinary metabolites of DEHP and BBzP in the present population (Figure S1). Both phthalates were widely used in flooring or wall coverings in houses (Jeon et al., 2016; Just et al., 2015; Shinohara and Uchino, 2020; Shu et al., 2019). Whether the observed association of MBzP represents a real cause of fibroids or simply reflects the effects of DEHP coexposed is subject to further investigation in other populations.

In the multiple chemical model following the factor analysis, factor 2 which is heavily loaded with metabolites of DPrHpP and DEHP showed a significant association with fibroids (OR of 4.60 with 95% CI of 1.10, 19.20; Table 3). This observation is comparable to those observed in the single exposure model, which showed significant positive associations (Figure 1). In summary, the positive associations of DPrHpP and DEHP with uterine fibroids in the single and multiple chemical models suggest the potential involvement of phthalates in the etiology of uterine fibroids. Due to less frequent detection of the target chemicals, however, most of the alternative plasticizers and OPEs could not be included in the factor analysis, and therefore could not be tested.

Considering several suggested theories of the development ofuterine fibroids, a few hypotheses can

be proposed as possible underlying mechanisms. One hypothesis is the regulation of ovarian hormones such as estrogen and progesterone, both of which have been recognized as promoters of uterine fibroids. The effects of endocrine-disrupting chemicals such as phthalates on sex hormonal change are well understood (Lee et al., 2019; Sohn et al., 2016). Another hypothesis is the mediation of nuclear receptors such as estrogen receptors,which is an important signaling process in differentiation and regulating cell proliferation in uterine myometrium cells (Bakas et al., 2008; Luo et al., 2014). In fact, chemicals like TDCIPP, BBzP, and DINCH have been suggested as weak agonists of the estrogen receptor (Engel et al., 2018; Mankidy et al., 2013; Zhang et al., 2014). Finally, the exposure to endocrine-disrupting chemicals during developmental stages may induce genetic and epigenetic regulatory changes in stem cells which lead to uterine fibroids (Katz et al., 2016).

There are several limitations to the present study. First, the limited sample size may lead to insufficient statistical power. Second, biological half-lives of most OPEs, phthalates, and APs are less than 24 hours (Koch et al., 2004; 2013; Wang et al., 2020). Therefore, spot urine measurement may not represent longer term exposure profile of the target chemicals. Third, observations from the cross-sectional case and control design cannot explain causality. Because the timing of the diagnosis of uterine fibroids and measurement of urinary chemicals were similar, reasonable inference of causation and contribution of chemical exposure to uterine fibroids cannot be made. Finally, the participating women had fasted for > 8 hr before the urine collection. Because of the short half-lives of target chemicals, the concentrations of metabolites measured in the urine of the participating women may be lower than those expected in normal situations. Therefore, direct comparison of the measured levels with those reported in other population may underestimate the exposure among the present population. Considering these limitations, interpretation of the association observed in the present study warrants cautions because chance findings cannot be ruled out. Despite these shortcomings, we found that, for the first time, several new consumer chemicals including OPEs (TDCIPP and TBOEP) and APs (DEHTP, DPrHpP, and DINCH) are associated with uterine fibroids among women of reproductive age. Our observations could be supported partly by several previous experimental and epidemiological studies that reported sex hormones related effects of the target chemicals. Further experimental mechanistic studies and

epidemiological studies focusing on uterine fibroids are warranted to confirm the present observation.

Acknowledgments

This research was supported by the National Research Foundation of Korea (NRF-2017K2A9A1A06037826) and Research Foundation – Flanders (FWO- VS00118N).

References

Baban, R. S., 2009. Serum protein and prolactin as diagnostic markers. Saudi Medical Journal. 30, 4.

Bakas, P., Liapis, A., Vlahopoulos, S., Giner, M., Logotheti, S., Creatsas, G., Meligova, A. K., Alexis, M. N., Zoumpourlis, V., 2008. Estrogen receptor alpha and beta in uterine fibroids: a basis for altered estrogen responsiveness. Fertil Steril. 90, 1878-1885.

Bastiaensen, M., Ait Bamai, Y., Araki, A., Van den Eede, N., Kawai, T., Tsuboi, T., Kishi, R., Covaci, A., 2019. Biomonitoring of organophosphate flame retardants and plasticizers in children: Associations with house dust and housing characteristics in Japan. Environ Res. 172, 543-551.

Bastiaensen, M., Xu, F., Been, F., Van den Eede, N., Covaci, A., 2018. Simultaneous determination of 14 urinary biomarkers of exposure to organophosphate flame retardants and plasticizers by LC-MS/MS. Anal Bioanal Chem. 410, 7871-7880.

Been, F., Malarvannan, G., Bastiaensen, M., Yin, S., van Nuijs, A. L. N., Covaci, A., 2019. Development and validation of a bioanalytical assay based on liquid chromatography-tandem mass spectrometry for measuring biomarkers of exposure of alternative plasticizers in human urine and serum. Talanta. 198, 230-236.

Boisvert, A., Jones, S., Issop, L., Erythropel, H. C., Papadopoulos, V., Culty, M., 2016. In vitro functional screening as a means to identify new plasticizers devoid of reproductive toxicity. Environ Res. 150, 496-512.

Boyle, M., Buckley, J. P., Quiros-Alcala, L., 2019. Associations between urinary organophosphate ester metabolites and measures of adiposity among U.S. children and adults: NHANES 2013-2014. Environ Int. 127, 754-763.

Brucker, S. Y., Huebner, M., Wallwiener, M., Stewart, E. A., Ebersoll, S., Schoenfisch, B., Taran, F. A., 2014. Clinical characteristics indicating adenomyosis coexisting with leiomyomas: a retrospective, questionnaire-based study. Fertil Steril. 101, 237-241 e231.

Bui, T. T., Giovanoulis, G., Cousins, A. P., Magner, J., Cousins, I. T., de Wit, C. A., 2016. Human exposure, hazard and risk of alternative plasticizers to phthalate esters. Sci Total Environ. 541, 451-467.

Butt, C. M., Hoffman, K., Chen, A., Lorenzo, A., Congleton, J., Stapleton, H. M., 2016. Regional comparison of organophosphate flame retardant (PFR) urinary metabolites and tetrabromobenzoic acid (TBBA) in mother-toddler pairs from California and New Jersey.

412 Environ Int. 94**,** 627-634.
413 Cardozo, E. R., Clark, A. D., Banks, N. K., Henne, M. B., Stegmann, B. J., Segars, J. H., 2012. The
414 estimated annual cost of uterine leiomyomata in the United States. Am J Obstet Gynecol.
415 206**,** 211 e211-219.
416 Chen, L., Luo, K., Etzel, R., Zhang, X., Tian, Y., Zhang, J., 2019. Co-exposure to environmental endocrine
417 disruptors in the US population. Environ Sci Pollut Res Int. 26**,** 7665-7676.
418 Ciavattini, A., Clemente, N., Delli Carpini, G., Di Giuseppe, J., Giannubilo, S. R., Tranquilli, A. L., 2015.
419 Number and size of uterine fibroids and obstetric outcomes. J Matern Fetal Neonatal Med.
420 28**,** 484-488.
421 CPSIA, 2008. Consumer Product Safety Improvement Act of 2008, Public Law 110–314, 110th
422 Congress (Available online at https://www.cpsc.gov/s3fs-public/pdfs/blk_pdf_cpsia.pdf
423 (accessed Jun 11, 2020))
424 Downes, E., Sikirica, V., Gilabert-Estelles, J., Bolge, S. C., Dodd, S. L., Maroulis, C., Subramanian, D.,
425 2010. The Burden of Uterine Fibroids in Five European Countries. Eur J Obstet Gynecol
426 Reprod Biol. 152(1):96-102.
427 Du, J., Li, H., Xu, S., Zhou, Q., Jin, M., Tang, J., 2019. A review of organophosphorus flame retardants
428 (OPFRs): occurrence, bioaccumulation, toxicity, and organism exposure. Environ Sci Pollut
429 Res Int. 26**,** 22126-22136.
430 Engel, A., Buhrke, T., Kasper, S., Behr, A. C., Braeuning, A., Jessel, S., Seidel, A., Volkel, W., Lampen, A.,
431 2018. The urinary metabolites of DINCH((R)) have an impact on the activities of the human
432 nuclear receptors ERalpha, ERbeta, AR, PPARalpha and PPARgamma. Toxicol Lett. 287**,** 83-
433 91.
434 European Union, 2005. Directive 2005/84/EC of the European Parliament and of the Council (2005)
435 Vol 344, Official Journal of the European Union (Available online at http://eur-
436 ex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:344:0040:0043:EN:PDF (accessed Jun
437 11, 2020)).
438 Flake, G. P., Andersen, J., Dixon, D., 2003. Etiology and pathogenesis of uterine leiomyomas: a review.
439 Environ Health Perspect. 111**,** 1037-1054.
440 Fromme, H., Schütze, A., Lahrz, T., Kraft, M., Fembacher, L., Siewering, S., Burkardt, R., Dietrich, S.,
441 Koch, H. M., Volkel, W., 2016. Non-phthalate plasticizers in German daycare centers and
442 human biomonitoring of DINCH metabolites in children attending the centers (LUPE 3). Int
443 J Hyg Environ Health. 219**,** 33-39.
444 Fu, Z., Zhao, F., Chen, K., Xu, J., Li, P., Xia, D., Wu, Y., 2017. Association between urinary phthalate
445 metabolites and risk of breast cancer and uterine leiomyoma. Reprod Toxicol. 74**,** 134-142.
446 Giovanoulis, G., Alves, A., Papadopoulou, E., Cousins, A. P., Schütze, A., Koch, H. M., Haug, L. S., Covaci,
447 A., Magner, J., Voorspoels, S., 2016. Evaluation of exposure to phthalate esters and DINCH
448 in urine and nails from a Norwegian study population. Environ Res. 151**,** 80-90.
449 Guo, Y., Alomirah, H., Cho, H. S., Minh, T. B., Mohd, M. A., Nakata, H., Kannan, K., 2011. Occurrence
450 of phthalate metabolites in human urine from several Asian countries. Environ Sci Technol.

45, 3138-3144.
Hornungm, R. W., Reed, L.D., 1990. Estimation of Average concentration in the presence of Nondetectable Values. Appl. Occup. Environ. Hyg. 5, 6.
Huang, P. C., Tsai, E. M., Li, W. F., Liao, P. C., Chung, M. C., Wang, Y. H., Wang, S. L., 2010. Association between phthalate exposure and glutathione S-transferase M1 polymorphism in adenomyosis, leiomyoma and endometriosis. Hum Reprod. 25, 986-994.
Jeon, S., Kim, K. T., Choi, K., 2016. Migration of DEHP and DINP into dust from PVC flooring products at different surface temperature. Sci Total Environ. 547, 441-446.
Just, A. C., Miller, R. L., Perzanowski, M. S., Rundle, A. G., Chen, Q., Jung, K. H., Hoepner, L., Camann, D. E., Calafat, A. M., Perera, F. P., Whyatt, R. M., 2015. Vinyl flooring in the home is associated with children's airborne butylbenzyl phthalate and urinary metabolite concentrations. J Expo Sci Environ Epidemiol. 25, 574-579.
Kambia, N. K., Severin, I., Farce, A., Moreau, E., Dahbi, L., Duval, C., Dine, T., Sautou, V., Chagnon, M. C., 2019. In vitro and in silico hormonal activity studies of di-(2-ethylhexyl)terephthalate, a di-(2-ethylhexyl)phthalate substitute used in medical devices, and its metabolites. J Appl Toxicol. 39, 1043-1056.
KATS, 2010. Safety standards for hazardous substances common to children's products (Notification No. 2010-676), Korean Agency for Technology and Standards.
Katz, T. A., Yang, Q., Trevino, L. S., Walker, C. L., Al-Hendy, A., 2016. Endocrine-disrupting chemicals and uterine fibroids. Fertil Steril. 106, 967-977.
Kim, J. H., 2018. Analysis of the in vitro effects of di-(2-ethylhexyl) phthalate exposure on human uterine leiomyoma cells. Exp Ther Med. 15, 4972-4978.
Kim, J. H., Kim, S. H., Oh, Y. S., Ihm, H. J., Chae, H. D., Kim, C. H., Kang, B. M., 2017. In vitro effects of phthalate esters in human myometrial and leiomyoma cells and increased urinary level of phthalate metabolite in women with uterine leiomyoma. Fertil Steril. 107, 1061-1069 e1061.
Kim, S., Park, J., Kim, H. J., Lee, J. J., Choi, G., Choi, S., Kim, S., Kim, S. Y., Lee, D. H., Moon, H. B., Kim, S., Choi, K., 2015. Association between Several Persistent Organic Pollutants in Serum and Adipokine Levels in Breast Milk among Lactating Women of Korea. Environ Sci Technol. 49, 8033-8040.
Kim, Y. A., Kho, Y., Chun, K. C., Koh, J. W., Park, J. W., Bunderson-Schelvan, M., Cho, Y. H., 2016. Increased Urinary Phthalate Levels in Women with Uterine Leiomyoma: A Case-Control Study. Int J Environ Res Public Health. 13.
Kobrosly, R. W., Parlett, L. E., Stahlhut, R. W., Barrett, E. S., Swan, S. H., 2012. Socioeconomic factors and phthalate metabolite concentrations among United States women of reproductive age. Environ Res. 115, 11-17.
Lambertino, A., Turyk, M., Anderson, H., Freels, S., Persky, V., 2011. Uterine leiomyomata in a cohort of Great Lakes sport fish consumers. Environ Res. 111, 565-572.
Lan, H. C., Wu, K. Y., Lin, I. W., Yang, Z. J., Chang, A. A., Hu, M. C., 2017. Bisphenol A disrupts steroidogenesis and induces a sex hormone imbalance through c-Jun phosphorylation in

Leydig cells. Chemosphere. 185, 237-246.
Lee, H., Lee, J., Choi, K., Kim, K. T., 2019. Comparative analysis of endocrine disrupting effects of major phthalates in employed two cell lines (MVLN and H295R) and embryonic zebrafish assay. Environ Res. 172, 319-325.
Lee, J., Jeong, Y., Mok, S., Choi, K., Park, J., Moon, H. B., Choi, G., Kim, H. J., Kim, S. Y., Choi, S. R., Kim, S., 2020. Associations of exposure to phthalates and environmental phenols with gynecological disorders. Reprod Toxicol. 95, 19-28.
Levy, G., Hill, M. J., Plowden, T. C., Catherino, W. H., Armstrong, A. Y., 2013. Biomarkers in uterine leiomyoma. Fertil Steril. 99, 1146-1152.
Liu, X., Ji, K., Choi, K., 2012. Endocrine disruption potentials of organophosphate flame retardants and related mechanisms in H295R and MVLN cell lines and in zebrafish. Aquat Toxicol. 114-115, 173-181.
Luo, N., Guan, Q., Zheng, L., Qu, X., Dai, H., Cheng, Z., 2014. Estrogen-mediated activation of fibroblasts and its effects on the fibroid cell proliferation. Transl Res. 163, 232-241.
Mankidy, R., Wiseman, S., Ma, H., Giesy, J. P., 2013. Biological impact of phthalates. Toxicol Lett. 217, 50-58.
Meeker, J. D., Stapleton, H. M., 2010. House dust concentrations of organophosphate flame retardants in relation to hormone levels and semen quality parameters. Environ Health Perspect. 118, 318-323.
Parker, W. H., 2007. Etiology, symptomatology, and diagnosis of uterine myomas. Fertil Steril. 87, 725-736.
Pavone, D., Clemenza, S., Sorbi, F., Fambrini, M., Petraglia, F., 2018. Epidemiology and Risk Factors of Uterine Fibroids. Best Pract Res Clin Obstet Gynaecol. 46, 3-11.
Pollack, A. Z., Buck Louis, G. M., Chen, Z., Sun, L., Trabert, B., Guo, Y., Kannan, K., 2015. Bisphenol A, benzophenone-type ultraviolet filters, and phthalates in relation to uterine leiomyoma. Environ Res. 137, 101-107.
Ptak, A., Ludewig, G., Robertson, L., Lehmler, H. J., Gregoraszczuk, E. L., 2006. In vitro exposure of porcine prepubertal follicles to 4-chlorobiphenyl (PCB3) and its hydroxylated metabolites: effects on sex hormone levels and aromatase activity. Toxicol Lett. 164, 113-122.
Schütze, A., Gries, W., Kolossa-Gehring, M., Apel, P., Schroter-Kermani, C., Fiddicke, U., Leng, G., Bruning, T., Koch, H. M., 2015. Bis-(2-propylheptyl)phthalate (DPHP) metabolites emerging in 24h urine samples from the German Environmental Specimen Bank (1999-2012). Int J Hyg Environ Health. 218, 559-563.
Schütze, A., Kolossa-Gehring, M., Apel, P., Bruning, T., Koch, H. M., 2014. Entering markets and bodies: increasing levels of the novel plasticizer Hexamoll(R) DINCH(R) in 24 h urine samples from the German Environmental Specimen Bank. Int J Hyg Environ Health. 217, 421-426.
Shen, Y., Dong, Y. M., Lu, Q., Xu, J., Wu, Y. T., Yun, S. S., Ren, M. L., 2016. Phenolic environmental estrogens in urine and blood plasma from women with uterine leiomyoma: Epidemiological survey. J Obstet Gynaecol Res. 42, 440-445.

529 Shinohara, N., Uchino, K., 2020. Diethylhexyl phthalate (DEHP) emission to indoor air and transfer
530 to house dust from a PVC sheet. Sci Total Environ. 711, 134573.
531 Shu, H., Jonsson, B. A. G., Gennings, C., Lindh, C. H., Nanberg, E., Bornehag, C. G., 2019. PVC flooring
532 at home and uptake of phthalates in pregnant women. Indoor Air. 29, 43-54.
533 Silva, M. J., Jia, T., Samandar, E., Preau, J. L., Jr., Calafat, A. M., 2013. Environmental exposure to the
534 plasticizer 1,2-cyclohexane dicarboxylic acid, diisononyl ester (DINCH) in U.S. adults (2000-
535 2012). Environ Res. 126, 159-163.
536 Silva, M. J., Wong, L. Y., Samandar, E., Preau, J. L., Calafat, A. M., Ye, X., 2017. Exposure to di-2-
537 ethylhexyl terephthalate in a convenience sample of U.S. adults from 2000 to 2016. Arch
538 Toxicol. 91, 3287-3291.
539 Silva, M. J., Wong, L. Y., Samandar, E., Preau, J. L., Jr., Jia, L. T., Calafat, A. M., 2019. Exposure to di-2-
540 ethylhexyl terephthalate in the U.S. general population from the 2015-2016 National Health
541 and Nutrition Examination Survey. Environ Int. 123, 141-147.
542 Sohn, J., Kim, S., Koschorreck, J., Kho, Y., Choi, K., 2016. Alteration of sex hormone levels and
543 steroidogenic pathway by several low molecular weight phthalates and their metabolites in
544 male zebrafish (Danio rerio) and/or human adrenal cell (H295R) line. J Hazard Mater. 320,
545 45-54.
546 Soliman, A. M., Yang, H., Du, E. X., Kelkar, S. S., Winkel, C., 2015. The direct and indirect costs of
547 uterine fibroid tumors: a systematic review of the literature between 2000 and 2013. Am J
548 Obstet Gynecol. 213, 141-160.
549 Stapleton, H. M., Sharma, S., Getzinger, G., Ferguson, P. L., Gabriel, M., Webster, T. F., Blum, A., 2012.
550 Novel and high volume use flame retardants in US couches reflective of the 2005 PentaBDE
551 phase out. Environ Sci Technol. 46, 13432-13439.
552 Stewart, E. A., Cookson, C. L., Gandolfo, R. A., Schulze-Rath, R., 2017. Epidemiology of uterine fibroids:
553 a systematic review. BJOG. 124, 1501-1512.
554 Sun, J., Zhang, M. R., Zhang, L. Q., Zhao, D., Li, S. G., Chen, B., 2016. Phthalate monoesters in
555 association with uterine leiomyomata in Shanghai. Int J Environ Health Res. 26, 306-316.
556 Sun, Y., Gong, X., Lin, W., Liu, Y., Wang, Y., Wu, M., Kannan, K., Ma, J., 2018. Metabolites of
557 organophosphate ester flame retardants in urine from Shanghai, China. Environ Res. 164,
558 507-515.
559 Taran, F. A., Weaver, A. L., Coddington, C. C., Stewart, E. A., 2010. Characteristics indicating
560 adenomyosis coexisting with leiomyomas: a case-control study. Hum Reprod. 25, 1177-1182.
561 Trabert, B., Chen, Z., Kannan, K., Peterson, C. M., Pollack, A. Z., Sun, L., Buck Louis, G. M., 2015.
562 Persistent organic pollutants (POPs) and fibroids: results from the ENDO study. J Expo Sci
563 Environ Epidemiol. 25, 278-285.
564 van der Veen, I., de Boer, J., 2012. Phosphorus flame retardants: properties, production,
565 environmental occurrence, toxicity and analysis. Chemosphere. 88, 1119-1153.
566 Weuve, J., Hauser, R., Calafat, A. M., Missmer, S. A., Wise, L. A., 2010. Association of exposure to
567 phthalates with endometriosis and uterine leiomyomata: findings from NHANES, 1999-2004.

Environ Health Perspect. 118**,** 825-832.

Zhang, Q., Lu, M., Dong, X., Wang, C., Zhang, C., Liu, W., Zhao, M., 2014. Potential estrogenic effects of phosphorus-containing flame retardants. Environ Sci Technol. 48**,** 6995-7001.

Zota, A. R., Geller, R. J., Calafat, A. M., Marfori, C. Q., Baccarelli, A. A., Moawad, G. N., 2019. Phthalates exposure and uterine fibroid burden among women undergoing surgical treatment for fibroids: a preliminary study. Fertil Steril. 111**,** 112-121.

575 Table 1. Characteristics of the participating women
576

Characteristics	Case	Control	Total	*P* value
Total	32 (28. 8)	79 (71.2)	111 (100.0)	
Age (year) (mean±SD)	38.3±5.3	37.8±5.1	37.9±5.1	0.516
20-29	3 (9.4)	7 (8.9)	10 (9.0)	
30-39	14(43.8)	36 (45.6)	50 (45.0)	
40-49	15 (46.9)	36 (45.6)	51 (45.9)	
Income (monthly)				0.106
Low-Middle (< 4 million KRW[a])	10 (31.3)	25 (31.6)	35 (31.5)	
Middle-High (≥4 and < 8 million KRW)	8 (25.0)	34 (43.0)	42 (37.8)	
> Very high (≥8 million KRW)	14 (43.8)	20 (25.3)	34 (30.6)	
Parity				**0.036***
0	11 (34.4)	10 (12.7)	21 (18.9)	
1	4 (12.5)	14 (17.7)	18 (16.2)	
≥ 2	16 (50.0)	54 (68.4)	70 (63.1)	
No response	1 (3.1)	1 (1.3)	2 (1.8)	
Urinary cotinine (ng/mL)				0.754
< 10	29 (90.6)	69 (87.3)	98 (88.3)	
> 10	3 (9.4)	10 (12.7)	13 (11.7)	
Alcohol consumption				0.817
Yes	21 (65.6)	50 (63.3)	71(64.0)	
No	11 (34.4)	29 (36.7)	40 (36.0)	
Body mass index (kg/m^2) (mean±SD)	**22.7±2.7**	**22.8±4.0**	**22.8±3.7**	0.445
< 18.5 (underweight)	0 (0.0)	5 (6.3)	5 (4.5)	
18.5-24.9 (normal weight)	23 (71.9)	46 (58.2)	69 (62.2)	
25-29.9 (pre-obesity)	5 (15.6)	10 (12.7)	15 (13.5)	
≥ 30 (obesity)	4 (12.5)	18 (22.8)	22 (19.8)	

[a)]KRW: Korean Won
Values are number of women and those in parentheses are percent, unless otherwise noted.
Statistical analysis was conducted by the Wilcoxon signed-rank test for continuous variables, age, and BMI; chi-squared tests for categorical variables, income, and alcohol consumption; Fisher's exact test for categorical variables with expected frequency < 5, parity, and urinary cotinine (* indicates statistical significance, $p < 0.05$).

577

Table 2. Concentrations of urinary OPEs and plasticizer metabolites (SG-adjusted, ng/ml) in the case and control groups

Parent compound	Target compound	Total (*n*=111)		Case (*n* = 32)		Control (*n* = 79)	*P* value (case-control)
		Median	DF (%)	Median (25^{th}, 75^{th})	DF (%)	Median (25^{th}, 75^{th})	
OPEs							
TPHP	4-HO-DPHP	<LOQ	21.9	<LOQ	24.1	<LOQ	-
	DPHP	0.28	81.3	0.30 (0.20, 0.46)	82.3	0.27 (0.17, 0.41)	0.400
EHDPHP	5-HO-EHDPHP	<LOQ	25.0	<LOQ (<LOQ, 0.05)	13.9	<LOQ	-
	EHPHP	1.01	93.8	1.02 (0.69, 1.56)	97.5	1.01 (0.54, 1.40)	0.624
TCIPP	BCIPHIPP	0.41	84.4	0.35 (0.19, 1.02)	87.3	0.42 (0.23, 0.75)	0.239
	BCIPP	<LOQ	6.3	<LOQ	2.5	<LOQ	-
TCEP	TCEP	<LOQ	9.4	<LOQ	3.8	<LOQ	-
TBOEP	BBOEP	<LOQ	43.8	<LOQ (<LOQ, 0.56)	22.8	<LOQ	-
	BBOEHEP	<LOQ	59.4	0.12 (<LOQ, 0.30)	40.5	<LOQ (<LOQ, 0.21)	-
	3-HO-TBOEP	<LOQ	9.4	<LOQ	26.6	<LOQ	-
TDCIPP	BDCIPP	<LOQ	65.6	0.42 (<LOQ, 0.79)	34.2	<LOQ (<LOQ, 0.16)	-
TNBP	DNBP	<LOQ	18.8	<LOQ	36.7	<LOQ (<LOQ, 0.17)	-
APs							
DINP	**OH-MINP**	1.68	**93.8**	**2.05 (1.12, 3.80)**	**91.1**	**1.37 (0.83, 2.39)**	**0.042**
	cxMINP	2.48	78.1	2.34 (1.51, 4.65)	78.5	2.57 (1.52, 3.50)	0.727
DEHTP	OH-MEHTP	<LOQ	40.6	<LOQ (<LOQ, 0.33)	29.1	<LOQ (<LOQ, 0.21)	-
	MEHTP	<LOQ	0.0	<LOQ	0.0	<LOQ	-
DEHA	OH-MEHA	<LOQ	53.1	0.17 (<LOQ, 0.55)	29.1	<LOQ (<LOQ, 0.19)	-
	oxoMEHA	<LOQ	46.9	<LOQ (<LOQ, 0.55)	48.1	<LOQ (<LOQ, 0.60)	-
	MEHA	<LOQ	0.0	<LOQ	0.0	<LOQ	-
DPrHpP	cxMPrHpP	0.62	75.0	0.57 (0.43, 0.86)	79.7	0.66 (0.37, 0.98)	0.977
	OH-MPrHpP	0.31	75.0	0.46 (0.41, 0.75)	58.2	0.26 (<LOQ, 0.50)	-
	oxoMPrHpP	0.28	71.9	0.30 (<LOQ, 0.77)	62.0	0.26 (<LOQ, 0.50)	-
DINCH	cxMINCH	0.33	50.0	0.09 (<LOQ, 0.44)	68.4	0.45 (<LOQ, 0.98)	-
	OH-MINCH	<LOQ	46.9	<LOQ (<LOQ, 0.54)	21.5	<LOQ	-
	MINCH	<LOQ	3.1	<LOQ	1.3	<LOQ	-
Phthalates							
DMP	MMP	1.85	96.9	2.43 (1.18, 3.96)	91.1	1.78 (0.99, 2.53)	0.101
DEP	MEP	5.11	100	3.67 (2.40, 8.49)	98.7	5.36 (3.10, 12.72)	0.436
DiPP	MiPP	<LOQ	21.9	<LOQ	13.9	<LOQ	-
DiBP	MiBP	2.68	100	2.81 (1.18, 5.16)	93.7	2.54 (1.22, 4.15)	0.426
DBP	MBP	5.88	100	6.73 (4.46, 12.61)	100	5.60 (3.66, 8.29)	0.161
DPeP	MPeP	<LOQ	9.4	<LOQ	10.1	<LOQ	-
BBzP	MBzP	0.65	100	0.66 (0.44, 1.15)	100	0.65 (0.41, 1.26)	0.934
DCHP	MCHP	<LOQ	3.1	<LOQ	3.8	<LOQ	-
DHxP	MHxP	<LOQ	12.5	<LOQ	32.9	<LOQ	-
DEHP	**MEOHP**	1.45	**100**	**1.73 (1.09, 2.57)**	**87.3**	**1.23 (0.72, 2.26)**	**0.032**
	MEHHP	2.85	100	3.21 (2.21, 4.02)	98.7	2.59 (1.55, 4.25)	0.155
	MECPP	11.61	100	14.33 (9.99, 23.50)	100	11.67 (7.42, 18.06)	0.050
	MCMHP	4.13	100	4.95 (3.36, 7.27)	100	4.04 (2.38, 6.07)	0.245
	MEHP	<LOQ	50.0	0.09 (<LOQ, 3.55)	44.3	<LOQ (<LOQ, 1.18)	-
	∑5DEHP[a)]	76.40	**100**	**87.11 (66.74, 133.73)**	**100**	**71.36 (45.48, 97.18)**	**0.023**
DnOP	MCPP	<LOQ	46.9	<LOQ (<LOQ, 0.75)	44.3	<LOQ (<LOQ, 0.50)	-

[a)] unit: nM(nmol/L)

Statistical analyses were conducted when detection frequencies were ≥75%. Boldface p-values indicate statistically significant differences between cases and controls ($p < 0.05$). Urinary metabolites concentrations were log-transformed for statistical analysis. Adjusted for age, income, parity, urinary cotinine, alcohol consumption, and BMI.

OPEs: phosphorus flame retardants, APs: alternative plasticizers, SG: specific gravity, DF: detection frequency, BMI: body mass index

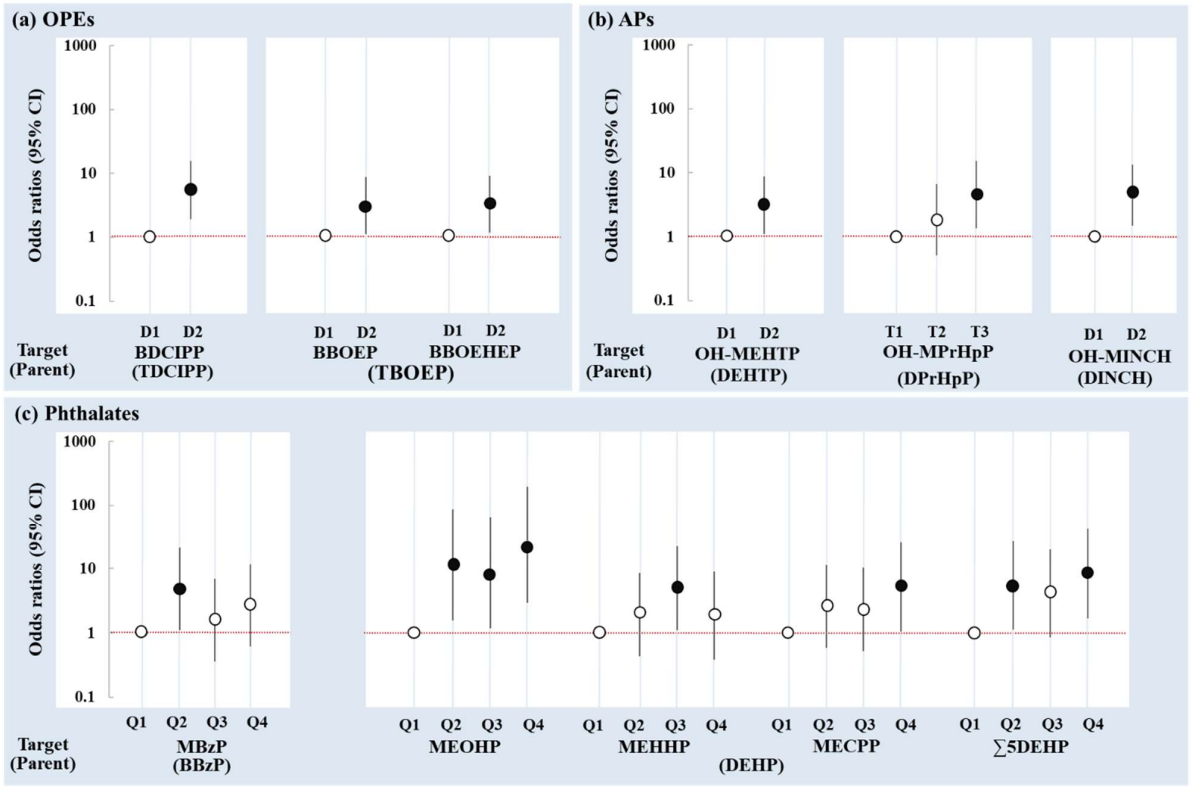

Figure 1. Associations ofchemical exposure and uterine fibroids. The figure shows adjustedOdds ratios (ORs) (a) urinary OPEs, (b) APs, and (c) phthalates concentrations (DF ≥75%: quartile group comparison (Q), 50%≤DF<75%: tertile group comparison (T), two detected groups and not detected, 25%≤DF<50%: dichotomy group comparison (D), detected or not). The ORs were estimated for the 1st quartile, 1sttertile, or 1st dichotomized (less than LOQ) as a reference. The target population number was 111 (case = 32). Adjusted for age, income, parity, urinary cotinine, alcohol consumption, and BMI.A solid circle indicates statistical significance ($p < 0.05$), while an open circle indicates reference or no significance.

OPEs: organophosphate esters, APs: alternative plasticizers, DF: detection frequency, LOQ: limit of quantification, BMI: body mass index

601 Table 3. Odds ratios (ORs) for the association between uterine fibroids and tertiles of factor scores (n
602 = 111) in the multiple chemical exposure model.

	ORs (95% CI)
Factor1- DEHP, DBP and BBzP	
Tertile 1	reference
Tertile 2	2.03 (0.54, 7.65)
Tertile 3	2.71 (0.73, 10.12)
Factor2- DPrHpP and DEHP	
Tertile 1	reference
Tertile 2	3.62 (0.87, 15.10)
Tertile 3	**4.60 (1.10, 19.20)**
Factor3- DINP	
Tertile 1	reference
Tertile 2	1.83 (0.53, 6.38)
Tertile 3	1.07 (0.31, 3.65)
Factor4- TPHP and DMP	
Tertile 1	reference
Tertile 2	1.56 (0.40, 6.00)
Tertile 3	2.39 (0.70, 8.20)

603 Factor analysis was conducted with 18 metabolites in the urine which were detected in ≥ 50% of samples.
604 Boldface numbers indicate statistically significant ORs ($p < 0.05$).
605 The factor scores were categorized into tertiles, with the lowest tertile considered as the reference
606 group.All factors were included in a logistic regression model.ORs were adjusted for age, income, parity,
607 urinary cotinine, alcohol consumption, and BMI.
608

Highlights

- Urinary BDCIPP, BBOEP, and BBOEHEP were associated with increased risk of fibroids.
- DEHTP, DPrHpP, and DINCH metabolites showed higher odds of uterine fibroids.
- Among phthalates, BBzP and DEHP metabolites were associated with fibroids.