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Phthalates and alternative plasticizers exposure characterization and associated reproductive health outcomes in women of reproductive age

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Abstract

Phthalates and alternative plasticizers exposure characterization and associated reproductive health outcomes in women of reproductive age

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Exposure to endocrine disrupting chemicals (EDCs) is important in women of reproductive age in terms of related adverse health effects. Personal care products (PCPs) is one of the common sources of EDCs. Considering the frequent and excessive use of PCPs by women, EDCs exposer through the use of PCPs is of great concern to the female population. The prenatal period could be the most sensitive period since exposure to EDCs could affect fetal development and the health of later life stage. It was important to evaluate and manage EDCs exposure during pregnancy.

Phthalates are known as EDCs displaying anti-androgenic and weak estrogenic properties. Phthalates are often used as plasticizers. Due to increasing health concerns of phthalates, their uses in many applications were regulated recently worldwide. Subsequently, alternative plasticizers have been used in increasing amounts. The alternative plasticizers with a similar

chemical structure of phthalates might have endocrine disruption function. Biomonitoring studies for the alternative plasticizers, however, were mostly limited to several European countries. A few studies have reported on the related health effects of alternative plasticizers.

The present study was conducted to assess related reproductive health effects and to characterize exposure of plasticizers in women of reproductive age. Uterine fibroids were selected as reproductive health effects of exposure to phthalates and their alternatives. For the purpose, we recruited premenopausal women in Korea, and the severe cases (n = 32) and controls (n = 79) were subsequently selected based on the results of gynecologic ultrasonography for the diagnosis of uterine fibroids, and measured for metabolites of organophosphate ester (OPEs), alternative plasticizers, and phthalates in urine. To evaluate the characteristics of plasticizer exposure in the most vulnerable populations, pregnant women were recruited from Korea (n = 81) and Thailand (n = 102). Twenty- four metabolites of 15 phthalates were measured in urine samples collected once each trimester, i.e., three times during pregnancy. To understand the changes in urine characteristics during pregnancy, concentrations of urinary correction factors, such as creatinine, specific gravity (SG), and osmolality, were measured in urine samples of the participants.

The first study (Chapter 2) suggested the importance of exposure to alternative plasticizers including phthalates in female reproductive health. Among alternative plasticizers, metabolites of di-isononyl phthalate (DINP)

and di(2-propylheptyl) phthalate (DPrHpP) were detected in >75% of the urine samples. Among OPEs 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. The concentrations of mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), the sum of five di(2-ethylhexyl) phthalate metabolites (∑5DEHP), and mono(4-methyl-7-hydroxyoctyl) phthalate (OH-MINP) were significantly higher in patients with uterine fibroids than in control participants. We found for the first time that several alternative plasticizers, such as di(2-ethylhexyl) terephthalate (DEHTP), DPrHpP, DINCH, tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) and tris(2-butoxyethyl) phosphate (TBOEP) were associated with increased risks of uterine fibroids among Korean women. While the mechanisms of those plasticizers leading to uterine fibroids are not well understood, sex endocrine disruption by these compounds may provide one potential explanation.

In the second study (Chapter 3), we described the exposure profile of gestational phthalates and alternative plasticizers and identified important plasticizers. While the levels of several phthalate metabolites were significantly different by trimester among Korean pregnant women, they were relatively stable across the three trimesters of pregnancy among Thai pregnant women. Urinary metabolites of diethyl phthalate (DEP) and dioctyl phthalate (DnOP) were two to three times higher in Thai pregnant women than those in Korean pregnant women. This observation may be linked to the more frequent

use of cosmetics which contain DEP and DnOP in Thai than in Korean women. The detection frequencies of a metabolite of DINCH were 67.4% and 44.9% among the Korean and Thai pregnant women, respectively. According to risk assessment, Korean and Thai pregnant women were considered at high risk of 11.9% and 5.3%, respectively, due to phthalate exposure, particularly DEHP, DnBP and DiBP. These observations indicated that the plasticizer exposure profile was clearly different by country and trimester. In addition, DINCH has been used by pregnant women in Korean and Thai.

The third study (Chapter 4) described the variations of urine characteristics in pregnancy. Three urinary correction factors were measured in urine samples collected in a pregnant female population of the second study. Among Korean pregnant women, urinary concentrations of creatinine and osmolality were significantly lowered at T3 than T1 suggesting more diluted urine as later stages of pregnancy. Those factors, however, were stable across the trimester of gestation in Thai pregnant women. Nationality or lifestyle may affect the changes in urinary dilution status during pregnancy. The correlations between unadjusted and adjusted concentrations during pregnancy were less than those of each adjusted phthalate concentrations. These results suggest that the variations of urinary correction factors may make the uncertainty propagation in exposure assessment of chemicals for pregnant women with urine samples. It is necessary to understand the changes in urinary correction factors for dilution by trimesters and nationality for a reduction in exposure misclassification of pregnant women. In addition,

it needs to be careful at understanding the exposure biomarker during pregnancy in terms of the changes in urine volume or a series of characteristics related to the absorption, distribution, metabolism, and excretion (ADME) of chemicals in the body.

The series of studies demonstrated important health effects of plasticizers and exposure characterization and in women of reproductive age. We determined the important emerging plasticizers associated with uterine fibroids. In addition, exposure profiles of plasticizers varied across the trimester of pregnancy. Variations of urine dilution during pregnancy due to physiological changes varied by country. These findings suggest that variations of exposure and related factors should be considered during study design and interpretation of results in biomonitoring studies of pregnant women. Considering the increasing levels of occurrences of alternative plasticizers in both environment and biospecimen, continuous exposure and risk assessment for other alternative plasticizers are warranted. Since the sample size of the present study is small, our observations need to be confirmed in larger cohorts or through experimental study. The present study is expected to contribute to improving the exposure assessment and management of EDCs in the vulnerable population.

Keywords: Phthalate; alternative plasticizer; gestation; urine correction; leiomyoma; uterine fibroids

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Contents

Chapter 1 Background

1.1 Women of reproductive age as a susceptible population to chemicals

Endocrine disrupting chemicals (EDCs) are man-made substances that interfere with hormone biosynthesis, metabolism, or action resulting in a deviation from normal homeostatic control or reproduction (Diamanti-Kandarakis et al., 2009). Results from epidemiological studies and experimental toxicity studies identify implications EDCs as a significant concern to public health. Scientists have been more interested in understanding of exposures and related health effects in industrials, over the past years. Recent research into the assess exposure to EDCs, however, included not only workers, but also general population and even susceptible populations such as infants, children, and pregnant women (Kim et al., 2015; Kim et al., 2018; Lee et al., 2019b).

Women of reproductive age including pregnant women were one of the vulnerable populations to both exposure and health risk to EDCs. Urinary levels of phthalates and bisphenol a (BPA) among females were higher than those of males in the Korean National Environmental Health Survey (KoNEHS) (Park et al., 2019). One of the reasons may be exposure to personal care products (PCPs) contained various EDCs. Considering the use

of PCPs more often and in greater quantities in women (Park et al., 2015), EDCs exposed through the use of PCPs is a concern, especially, in the female population.

Pregnant women, especially, were sensitive populations to EDCs exposures. Prenatal exposure to EDCs could affect the early development of the fetus and the health of later childhood (Barkoski et al., 2019; Hoepner, 2019; Kim et al., 2018; Zarean et al., 2019). Prenatal chemical exposures with endocrine disrupting properties were also associated with adverse birth outcomes (Govarts et al., 2018). Those adverse health effects due to prenatal chemical exposure were differed by the windows of exposure during pregnancy (Ferguson et al., 2014; Jiang et al., 2019; Qian et al., 2019). Thus, it is important to consider the specific characteristics of prenatal chemical exposure and related health effects.

1.2 Phthalates and alternative plasticizers exposure

Phthalates are plasticizers that have been used in numerous consumer applications, including food packaging, cosmetics, paintings, medical devices, and building materials. Because phthalates are not chemically bound, these chemicals can easily leak out of the products and cause eventual exposure to people. Major exposure routes to humans are via consumption of foods and drinking water, inadvertent dust ingestion, dermal absorption from cosmetics, and, to a lesser extent, inhalation of indoor or outdoor air (Fromme et al., 2013a; Koniecki et al., 2011; Serrano et al., 2014). Consequently, phthalates have been frequently detected worldwide in human urine samples. (Koch et al., 2017; Park et al., 2017; CDC, 2018).

Phthalates are endocrine-disrupting chemicals that are well known for anti-androgenic and weak estrogenic effects. The endocrine-disrupting effects of phthalates and their metabolites have been frequently demonstrated in experimental studies (Lee et al., 2019a; Sohn et al., 2016). Moreover, endocrine disruption due to phthalate exposure has been suggested particularly during pregnancy in human populations (Zarean et al., 2019). Recently, transgenerational effects through epigenetic modulation have also been reported in rats, suggesting the potential adverse health consequences of phthalate exposure during the gestation period in humans (Martinez-Arguelles and Papadopoulos, 2016).

Due to increasing concerns on the use of phthalates, regulations against the

use in many applications were implemented in the EU, the USA, and Korea, etc. (Directive 2005/84/EC; CPSIA, 2008; KATS, 2010). Subsequently, the use of alternative plasticizers, including di-(iso-nonyl)-cyclohexane-1,2-dicarboxylate (DINCH) and Di(2-ethylhexyl) terephthalate (DEHTP) has increased (Bui et al., 2016; Schütze et al., 2014). Organophosphate esters (OPEs) were also a group of chemicals that have been usually used to replace conventional flame retardants, but also used as plasticizers. Humans are expected to be exposed to these alternative chemicals in greater amounts, but biomonitoring studies for these alternative plasticizers are mostly limited to several European countries and a few other countries, such as USA, Australia and Israel (Bastiaensenet al., 2018; Castorina et al., 2017; Correia-Sá et al., 2017; Fromme et al., 2016; Giovanoulis et al., 2016; Gomez Ramos et al., 2016; Hoffman et al., 2017; Machtinger et al., 2018; Schütze et al., 2014) (Table 1-1). Such exposure profiles are largely unknown to many Asian countries (Sun et al., 2018).

Table 1-1. Biomonitoring of alternative plasticizers in the urine sample.

Sampling year	Nation	Population	n	Target compound	Target metabolite	Detection frequency (%)	Concentrations (median)	Unit	adjustment	Reference
2000-2012	U.S.	adults	23-121	1,2-Cyclohexanedicarboxylicacid, 1,2-diisononyl ester (DINCH)	OH-MINCH	0-19	<LOD	ng/ml	not adjusted	Silva et al., 2013
					cxMINCH	0-21	<LOD			
					oxo-MINCH	0-16	<LOD			
2010-2017	Germany	adult (20-29 y)	300	DINCH	OH-MINCH	95.7	0.42	ng/ml	not adjusted	Kasper-Sonnenberg et al., 2019
					cx-MINCH	66.3	0.14			
						85.3	0.22			
2011-2012	Germany	children (3-6 y)	208	DINCH	MINCH	22.6	< 0.1	ng/ml	not adjusted	Fromme et al., 2016
					OH-MINCH	100	1.66			
					cx-MINCH	100	1.14			
					oxo-MINCH	99	1.54			
2012 (1999-2012)	Germany	adult (20-30 y)	60	DINCH	MINCH	5	<LOQ	ng/ml	not adjusted	Schütze et al., 2014
					OH-MINCH	98.3	0.39			
					cx-MINCH	88.3	0.17			
					oxo-MINCH	85	0.25			

Table 1-1. (Continued)

Sampling year	Nation	Population	n	Target compound	Target metabolite	Detection frequency (%)	Concentrations (median)	Unit	adjustment	Reference
2012 (1999-2012)	Germany	adult (20-30 y)	60	DEHTP	cx-MPHxP	0	<LOQ	ng/ml	not adjusted	Schütze et al., 2015
					OH-MPHP	3.3	<LOQ			
					oxo-MPHP	21.7	<LOQ			
2012-2013	Australia	0->60	100	DINCH	OH-MINCH	100	3.9[a]	ng/ml	not adjusted	Gomez Ramos et al., 2016
2014-2015	Portugal	children (4-18y)	112	DINCH	OH-MINCH	100	2.14	ng/ml	not adjusted	Correia-Sá et al., 2017
					cx-MINCH	100	1.08			
					oxo-MINCH	99	1..10			
2014-2015	Portugal	children (4-17 y)	107	Di(2-ethylhexyl) terephthalate (DEHTP)	5OH-MEHTP	67	0.45	ng/ml	not adjusted	Lessmann et al., 2017
					5oxo-MEHTP	58	0.27			
					5cx-MEPTP	96	4.19			
					2cx-MMHTP	7	<LOQ			

Table 1-1. (Continued)

Sampling year	Nation	Population	n	Target compound	Target metabolite	Detection frequency (%)	Concentrations (median)	Unit	adjustment	Reference
2014-2015	U.S.	undergoing fertility treatment; female (18-40)	49	DINCH	OH-MINCH	42.9	<LOD		not adjusted	Wu et al., 2017
		male (18-55)	50			44	<LOD			
		female	49		cx-MINCH	14.3	<LOD			
		male	50			18	<LOD			
2014-2016	Israel	women undergoing a fresh IVF cycle	136	DINCH	OH-MINCH	92.6	1.2 (1.1)	ng/ml	not adjusted (SG adjusted)	Machtinger et al., 2018
					cx-MINCH	68.4	0.6 (0.6)			
				DEHTP	5OH-MEHTP	90.4	2.3(2.4)			
					5cx-MEPTP	100	7.7(8.2)			
2015	Sweden	children (3-4 y)	113	DINCH	oxo-MINCH	100	1.8	ng/ml	not adjusted	Larsson et al., 2017
2017 (1999-2017)	Germany	adult (20-29 y)	60	DEHTP	5OH-MEHTP	47	<LOQ			Larsson et al., 2019
					5oxo-MEHTP	40	<LOQ			
					5cx-MEPTP	100	3.35			
					2cx-MMHTP	2	<LOQ			

[a] arithmetic mean, LOD: limit of detection; LOQ: limit of quantification

1.3 Reproductive health effects of plasticizers in women of reproductive age

Disorders of the female reproductive system are common with a high prevalence of infertility. To manage to control the female reproductive health is very important because disorders of the female reproductive system can make adverse effects not only reproduction but also a health and quality of life. Endocrine disrupting chemicals (EDCs) are an one of the important factor which produces adverse developmental, reproductive effects in humans (Schug et al., 2011). Recently published paper suggested 10 key characteristics of chemicals that cause female reproductive toxicity: 1) alters hormone receptor signaling; alters reproductive hormone production, secretion, or metabolism; 2) chemical or metabolite is genotoxic; 3) induces epigenetic alterations; 4) causes mitochondrial dysfunction; 5) induces oxidative stress; 6) alters immune function; 7) alters cell signal transduction; 8) alters direct cell-cell interactions; 9) alters survival, proliferation, cell death, or metabolic pathways; and 10) alters microtubules and associated structures (Luderer et al., 2019). Throughout those mechanisms, EDCs could effect to the female reproductive system.

Several population studies and animal experiments indicate that EDCs can affect the structure and function of the uterus and lead to disorders such as endometriosis and fibroids (Scsukavo et al., 2016). Uterine fibroids or leiomyomas are one of the most common benign tumors with a prevalence ranging from 4.5 – 68.6% depending on population and diagnosis method

(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). Although the etiology of uterine fibroids is unknown, 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).

Previous studies have reported associations of chemical exposure with uterine fibroids (Table 1-1). In epidemiological studies, uterine fibroids have been associated with higher levels of urinary DEHP metabolites (Fu et al., 2017; Huang et al., 2010; Kim et al., 2016; Sun et al., 2016), and environmental phenols like bisphenol A and nonylphenol(Shen et al., 2016). Moreover, such associations have also been reported for persistent organic pollutants (POPs) such as p,p′-dichlorodiphenyldichloroethylene (p,p’-DDE) and polychlorinated biphenyls (PCBs) (Lambertino et al., 2011; Trabert et al., 2015). The research has been reported for having endocrine-disrupting effects while much less information is currently available for the endocrine-related adverse effects of most of these substituting chemicals, including uterine fibroids (Engel et al., 2018; Kambia et al., 2019; Kojima et al., 2013; Liu et al., 2012).

Table 1-2. The association between chemical exposure and uterine fibroids in literature.

N	Chemicals	Results (↑: positive association, ↓: negative association)	Location	Reference
Environmental phenols and phthalates				
Case: 15 Control: 20	Serum DEHP, MEHP	DEHP↓, MEHP↓	Italy	Luisi et al. 2009
Case: 36 Control: 29	Urine MMP, MEP, MBzP, MEHP, 5oxo-MEHP, 5OH-MEHP	MMP↑, MEHP↑, ∑MEHP↑	Taiwan	Huang et al.2010
Urine/Blood Case: 156/214 Control: 106/126	Urine and Plasma Bisphenol A (BPA), Octylphenol (OP),Nonylphenol (NP)	OP↑, NP↑, BPA↑	China	Shen et al. 2013
Case: 36 Control: 69	Urine MMP, MEP, MnBP, MBzP, MEHP, 5oxo-MEHP, 5OH-MEHP	∑MEHP↑, MnBP↑, MEP↑	Taiwan	Huang et al.2014
Case: 99 Control: 374 (ENDO study)	Urine (phthalates, benzophenones) MMP, MEP, MCPP, MnBP, MiBP, MECPP, MCMHP, MEHHP, MEOHP, MCHP, MBzP, MEHP, MOP, MNP, 2,4OH-BP, 4OH-BP, 2OH-4MeO-BP	Urine BPA↑, 2,4OH-BP↑, 2O-4MeO-BP↑, Urine MMP↓	USA	Pollack et al.2015

Table 1-2. (*Continued*)

N	Chemicals	Results (↑: positive association, ↓: negative association)	Location	Reference
Environmental phenols and phthalates (*Continued*)				
Case: 30 Control: 27	Urine MEHP, MEOHP, MEHHP, 2cx-MMHP, 5cx-MEPP, MnBP, MBzP, MiBP, MEP, MMP, MCHP, MOP, MCPP, MnPP, MiDP, MNP	MEHP↑, MEHHP↑, 2cx-MMHP↑, 3DEHP↑, ∑4DEHP↑, ∑5DEHP↑	Korea	Kim et al. 2016
Case: 300 Control: 300	Urine and Plasma BPA, OP, NP	OP↑, BPA↑, NP↑	China	Shen et al. 2016
Case: 61 Control: 61	Urine MMP, MEP, MiBP, MnBP, MEHP, MEOHP, MEHHP, MECPP, MCMHP	MiBP↑, MEHP↑, MEHHP↑, MECPP↑, ∑DEHP↑, ∑DBP↑, MnBP↑, MMP↓	China	Sun et al. 2016
Metals and persistent organic pollutants (POPs)				
Case: 99 Control: 374 (ENDO study)	Blood metals: Cd, Hg, Pb Urine trace elements: As, Ba, Be, Cd, Co, Cr, Cs, Cu, Hg, Mn, Mo, Ni, Pb, Sb, Sn, Te, Tl, U, W, Zn	Blood Cd↑, Hg↑, Pb↑ Urine Cd↑, Co↑, Cs↑, Mn↑, Pb↑, Tl↑ Urine W↓	USA	Johnstone et al. 2014
Case: 122 Control: 455	Serum ∑PCB congeners	∑PCB↑	USA	Lambertino et al. 2011

Table 1-2. (*Continued*)

N	Chemicals	Results (↑: positive association, ↓: negative association)	Location	Reference
Metals and persistent organic pollutants (POPs) (*Continued*)				
Case: 24 Control: 20	Subcutaneous and Visceral fat Organochlorine pesticides (OCPs), Polychlorinated biphenyls (PCBs), Polycyclic aromatic hydrocarbons (PAHs),Polybrominated diphenyl ethers (PBDEs), Metals	Subcutaneous fat DDTs↑, HCHs↑, PCBs↑, PAHs↑, PBDEs↑, As↑, Cd↑, Pb↑, Hg↑ Visceral fat As↑, Cd↑, Pb↑, Hg↑	China	Qin et al. 2010
Case: 99 Control: 374 (ENDO study)	Omental fat and Serum (n) HCB (5), Chlordanes (5), PBDEs (7), PCB (34),Per- and Polyfluoroalkyl Substances (PFAS, 9)	Omental fat HCB (2)↑, chlordanes (4)↑, PCB (27)↑ Serum HCB (1)↑, PBDE 209↑, PCB (16)↑, PFAS (PFUnDA)↑ Serum PBDE 153↓, 154↓, 209↓, PCB (7) ↓, PFAS (PFOA, PFHxS) ↓ Omental fat PCB 99 ↑, 138↑, 146↑, 153↑, 196↑, 206↑	USA	Trabert et al. 2015

1.4 Challenges of exposure assessment to pregnant women

Even though pregnant women are a vulnerable populations to chemical exposure and related health effects, exposure profile across trimesters of gestations and their detailed information were largely unknown. Previous studies have been suggested that temporal variability of phthalate exposure during pregnancy can be huge depending on the timing of sampling, sample size, and study population (Braun et al., 2012; Fisher et al., 2015; Huang et al., 2017; Li et al., 2019; Martino-Andrade et al., 2016; Warembourg et al., 2019; Watkins et al., 2017). The temporal variability of exposure makes difficulties for exposure assessment and risk management of EDCs.

The underlying reasons of temporal variability of exposure are largely unknown but physiological and behavior changes are considerable during pregnancy such as maternal weight, individual organ volumes, and blood flows glomerular filtration rate (GFR) which is a renal function marker, and some drug-metabolizing enzyme activities (Abduljalil et al., 2012; Hsieh et al., 2019; Zhao et al., 2018). The increased renal blood flow leads to an increase in renal size (Soma-Pillay et al., 2016) and an activity of metabolic enzymes such as cytochrome P450 (CYP) is variable during pregnancy (Tracy et al., 2005). Human CYP2C9 and CYP2C19, for example, are the major CYP isoforms producing DEHP metabolites (Choi et al., 2012), but the activity of CYP2C9 and CYP2C19 are changed during pregnancy (Anderson, 2005;

Jeong, 2010). Although the underlying mechanism of all physiological changes is not clear, pregnancy-associated hormones, such as estradiol and progesterone, during pregnancy may influence these changes (Jeong, 2010; Katharine et al., 2013; Soma-Pillay et al., 2016). Change of using patterns of PCPs may be also another reason for variability of exposure profile of EDCs during pregnancy. The use of several PCPs was associated with urinary phthalates in pregnant women (Buckley et al., 2012). It, therefore, is important to understand the characteristics of exposure profiles during pregnancy, for accurate exposure assessment.

1.5 Study design and objectives

The aim of this study is to identify both female reproductive health risk of plasticizers in women of reproductive age and exposure characteristics of plasticizers during pregnancy. We identified the plasticizers including alternative substances that are associated with a reproductive health outcome in adult women. In addition, we characterized the exposure profile of major phthalates and their alternatives in pregnant women by considering the importance of vulnerable windows of chemical exposure. Furthermore, we described a variety of urine characteristics during pregnancy by providing new insights into urinary correction factors. The knowledge from the present study will be useful to manage and accurate exposure and risk assessment of plasticizers in premenopausal women including pregnant women.

The present study consists of three parts (Fig. 1-1). In the first part (Chapter 2), the association between exposure to plasticizers and reproductive health effect were investigated with uterine fibroids case (n = 32) and control (n = 79) participant. We measured for metabolites of several phthalates, alternative plasticizers, and organophosphate esters (OPEs), in the urine. We identified several plasticizers associated with increased risks of uterine fibroids among Korean women.

In the second part (Chapter 3), the exposure profile of urinary plasticizer metabolites and their general risk based on a reference dose were investigated in pregnant women. We measured the urinary concentrations of phthalate and alternative plasticizer metabolites throughout pregnancy among Korean (n =

81) and Thai women (n = 102). Metabolites (n = 24) from phthalates (n = 15) were measured in the urine samples collected three times during pregnancy. Important exposure sources of plasticizers in each country were also suggested.

In the third part (Chapter 4), the variable exposure profile of plasticizers across three trimesters of gestation which was found in Chapter 3ies have a different ethnicity, climate, and lifestyle can show the various profiles were described with their variation of urinary correction factors such as creatinine, specific gravity, and osmolality. The study with participants from the two countries of urine correction factors depending on populations.

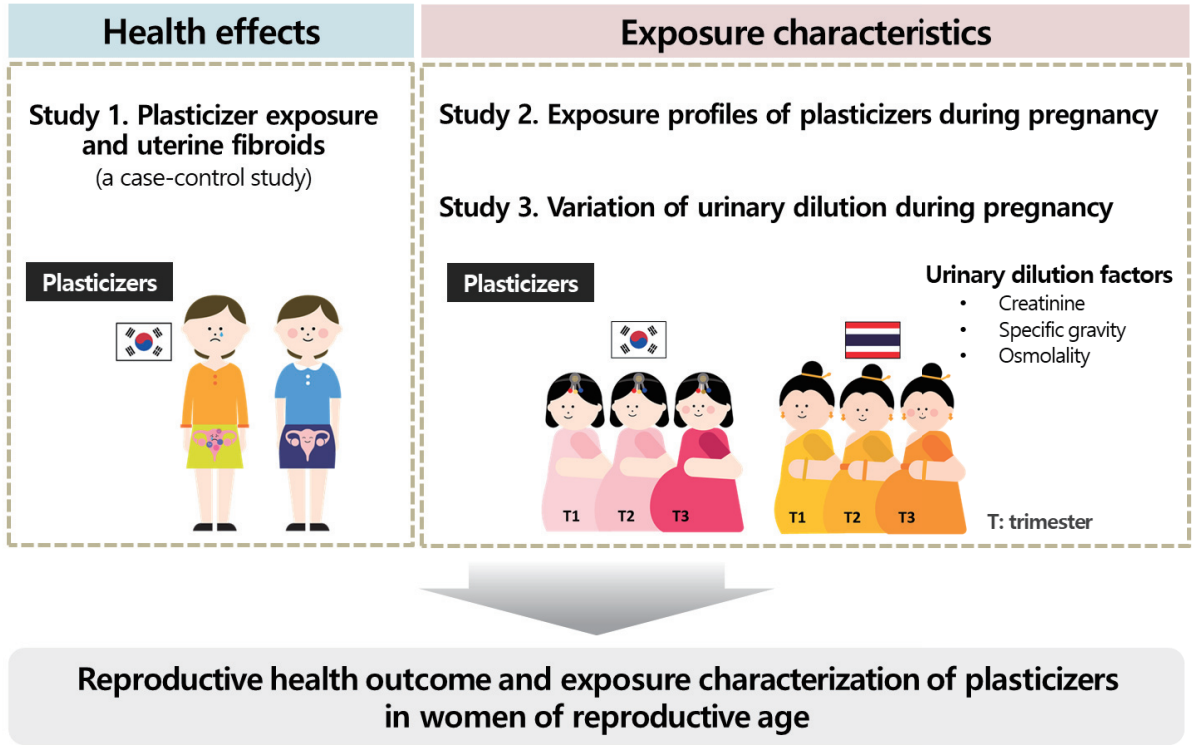


Fig.1-1. Study design to investigate plasticizer exposure characterization and associated reproductive health outcomes in women of reproductive age.

Chapter 2 Exposure to organophosphate esters, alternative plasticizers, and phthalates in association with uterine fibroids: a case-control study

2.1 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 costs estimated at $15,952 per patient worldwide, and at an estimated annual cost of $34.4 billion in the United States (Cardozo et al., 2012; Soliman et al., 2015). 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 associated with uterine fibroids in a number of epidemiological studies, and these chemicals included DEHP metabolites (Fu et al., 2017; Huang et al., 2010; Kim et al., 2016; Sun et al., 2016) and

environmental phenols like bisphenol A and nonylphenol (Shen et al., 2016). Exposure to persistent organic pollutants (POPs) such as *p,p′*-dichlorodiphenyldichloroethylene (*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.

Recently, the use of plasticizers has been regulated worldwide due to their potential adverse health effects. Subsequently, new alternative chemicals have been introduced in increasing amounts into the market to replace the regulated chemicals (Bui et al., 2016; Stapleton et al., 2012). For example, organophosphate esters (OPEs) have been extensively used as plasticizers or lubricants, and as flame retardants (van der Veen and de Boer, 2012). Phthalates that have been used in many applications like food packaging, cosmetics, paintings, medical devices, and building materials, etc. have been replaced by several alternative plasticizers (APs), such as 1,2-cyclohexane dicarboxylic acid, diisononyl ester (DINCH) and di-2-ethylhexyl terephthalate (DEHTP). Compared to the chemicals being replaced, much less information is currently available for these substituting chemicals, in terms of their potential adverse health effects, such as uterine fibroids. (Engel et al., 2018; Kambia et al., 2019; Kojima et al., 2013; Liu et al., 2012).

In this study, we hypothesized that several alternative plasticizers that are used in growing amounts would be associated with uterine fibroids, a typical endocrine-related benign tumor of women. In the present study, we designed a

case-control study design with 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 chemical determinants of uterine fibroids.

2.2 Materials and methods

2.2.1 Study population and sample collection

Adult Korean women before menopause (*n* = 516, 20–49 years of age) were recruited from 2015–2016 at 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 & gynecology clinics of the university hospitals for routine gynecology checkup. In addition, a subset of the participating women (*n* =70) was randomly chosen from an existing cohort, i.e., Children's Health and Environmental Chemicals of Korea (CHECK) cohort. Among the women initially recruited, women with current pregnancy and past potential occupational exposure were excluded. The participating women were asked to fast for > 8 hr before they came for health examinations and urine sample collection. Once collected, the urine samples were stored at -20°C immediately until chemical analysis. The participating women 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 identified. Among these asymptomatic women, a total of 32 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 matching ages without the disease (*n* = 79) were selected. Therefore, the final study population consisted of 111 participants, i.e., 32 cases and 79 controls. Demographic data were obtained using a questionnaire. The present study was

approved by the Institutional Review Board of Seoul National University (IRB No. 1509/001-011).

2.2.2 Measurement of urinary OPEs and plasticizers

Metabolites of twelve OPEs, thirteen APs, and fifteen phthalates were analyzed in the urine of the participating women. 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 2-1).

Urinary metabolites of OPEs were measured following a previously reported method (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 to an Agilent 6460 Triple Quadrupole mass spectrometer (MS) (Santa Clara, CA, USA). AP metabolites were measured following Been et al. (2019) with a slight modification. 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). For phthalate metabolites, the sample was prepared

following a method described in detail 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 (Tables 2-2 – 2-3).

Table 2-1. Target parent compounds and their urinary metabolites

Parent compound	Target metabolite
Organophosphate esters	
Triphenyl phosphate (TPHP)	4-Hydroxyphenyl phenyl phosphate (4-HO-DPHP)
	Diphenyl phosphate (DPHP)
2-Ethylhexyldiphenyl phosphate (EHDPHP)	2-Ethyl-5-hydroxyhexyl diphenyl phosphate (5-HO-EHDPHP)
	2-Ethylhexyl phenyl phosphate (EHPHP)
Tris(2-chloroisopropyl) phosphate (TCIPP)	1-Hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP)
	Bis(1-chloro-2-propyl) phosphate (BCIPP)
Tris(chloroethyl) phosphate (TCEP)	Tris(chloroethyl) phosphate (TCEP)*
Tris(2-butoxyethyl) phosphate (TBOEP)	Bis(2-butoxyethyl) phosphate (BBOEP)
	2-Hydroxyethyl bis(2-butoxyethyl) phosphate (BBOEHEP)
	Bis(2-butoxyethyl) 3′-hydroxy-2-butoxyethyl phosphate (3-HO-TBOEP)
Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP)	Bis(1,3-dichloro-2-propyl) phosphate (BDCIPP)
Tri-n-butyl phosphate (TNBP)	Di-n-butyl phosphate (DNBP)
Alternative plasticizers	
Di-isononyl phthalate (DINP)	Mono(4-methyl-7-hydroxyoctyl) phthalate (OH-MINP)
	Mono(4-methyl-7-carboxyheptyl) phthalate (cxMINP)
Di(2-ethylhexyl) terephthalate (DEHTP)	Mono(2-ethyl-5-hydroxyhexyl) terephthalate (OH-MEHTP)
	Mono(2-ethylhexyl) terephthalate (MEHTP)
Bis(2-ethylhexyl) adipate (DEHA)	Mono(2-ethyl-5-hydroxyhexyl) adipate (OH-MEHA)
	Mono(2-ethyl-5-oxohexyl) adipate (oxoMEHA)
	Mono(2-ethylhexyl) adipate (MEHA)
Di(2-propylheptyl) phthalate (DPrHpP)	Mono(2-propyl-6-carboxyhexyl) phthalate (cxMPrHpP)
	Mono(2-propyl-6-hydroxyheptyl) phthalate (OH-MPrHpP)
	Mono(2-propyl-6-oxoheptyl) phthalate (oxoMPrHpP)
Di-(iso-nonyl)-cyclohexane-1,2-dicarboxylate (DINCH)	Cyclohexane-1,2-dicarboxylic mono carboxyisooctyl ester (cxMINCH)
	Cyclohexane-1,2-dicarboxylic mono hydroxyisononyl ester (OH-MINCH)
	Cyclohexane-1,2-dicarboxylic mono isononyl ester (MINCH)

Table 2-1. (*Continued*)

Parent compound	Target metabolite
Phthalates	
Dimethyl phthalate (DMP)	Monomethyl phthalate (MMP)
Diethyl phthalate (DEP)	Monoethyl phthalate (MEP)
Di-isopropyl phthalate (DiPP)	Mono-isopropyl phthalate (MiPP)
Di-2-isobutyl phthalate (DiBP)	Mono-2-isobutyl phthalate (MiBP)
Di-n-butyl phthalate (DBP)	Mono-n-butyl phthalate (MBP)
Di-n-pentyl phthalate (DPeP)	Mono-n-pentyl phthalate (MPeP)
Benzyl butyl phthalate (BBzP)	Monobenzyl phthalate (MBzP)
Dicyclohexyl phthalate (DCHP)	Monocyclohexyl phthalate (MCHP)
Dihexyl phthalate (DHxP)	Monohexyl phthalate (MHxP)
Di(2-ethylhexyl) phthalate (DEHP)	Mono(2-ethyl-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)
Dioctyl phthalate (DnOP)	Mono(3-carboxypropyl) phthalate (MCPP)

* Parent compound

Table2-2. Detailed summary of sample preparation and instrumental analysis.

	OPE metabolites in urine (Bastiaensen et al., 2018)	**AP** metabolites in urine (modified from Been et al., 2019)	**Phthalate metabolites** in urine (Guo et al., 2011)
Sample volume	2 mL	1 mL	0.5 mL
Internal standard addition	5 ng before extraction (DPHP-d_{10}, TBOEP-d_6, TCEP-d_{12}, BBOEP-d_4, BBOEHEP-d_4, BDCIPP-d_{10})	5 ng before extraction (MINCH-d_2, OH-MINCH-d_4, cxMINCH-d_2, OH-MINP-d_4, cxMINP-d_4, OH-MPrHpP-d_4, oxoMPrHpP-d_4, cx-MPrHpP-d_4)	20 ng before extraction (^{13}C-MMP, ^{13}C-MEP, ^{13}C-MBP, ^{13}C-MBzP, ^{13}C-MCHP, ^{13}C-MCPP, ^{13}C-MEHP, ^{13}C-MEOHP, ^{13}C-MEHHP, ^{13}C-MECPP, and ^{13}C-MCMHP)
Deconjugation	50 µL of β-glucuronidase for 2 h (from *E. coli* powder, 2 mg/mL in phosphate buffer) (Sigma-Aldrich, Bornem, Belgium)	25 µL of β-glucuronidase for 2 h (from *E. coli* powder, 2 mg/mL in phosphate buffer) (Sigma-Aldrich, Bornem, Belgium)	40 µL of β-glucuronidase for 12 h (from *Helix pomatia,* 2 µL/mL in ammonium acetate buffer (Sigma-Aldrich, St. Louis, MO).
pH adjustment	1.5 mL of phosphate buffer (1 M, pH 6)	1 mL of phosphate buffer (1 M, pH 6)	1.2 mL of phosphate buffer (pH 2)
Extraction	Bond-Elut C18 (200 mg, 3 mL, Agilent)	Oasis Max (30 mg, 3 mL, Waters)	ABS ELUT-Nexus (60 mg, 3 mL, Varian, Walnut Creek, CA, USA)
Elution	3 mL of methanol	5 mL of methanol with 2% formic acid	1.2 mL of acetonitrile and 1.1 mL of ethyl acetate
Column: stationary phase, dimensions	Phenomenex Kinetex Biphenyl reversed-phase column (2.1 × 100 mm, 2.6 µm; Torrance, CA, USA)	Phenomenex Kinetex Biphenyl reversed-phase column (2.1 × 100 mm, 2.6 µm; Torrance, CA, USA)	Betasil C18 column (Thermo Electron, Bellefonte, PA; 2.1 x 100 mm, 5 µm)
Mobile phase A	Water:Methanol (98:2)	Water	Water
Mobile phase B	Methanol:Water (98:2)	Acetonitrile	Acetonitrile
Mobile Phase Buffer	5 mM ammonium acetate	0.1% acetic acid	0.1% acetic acid
Instrument	Agilent 1290 Infinity LC - 6460 Triple Quadrupole MS	Agilent 1290 Infinity LC - 6460 Triple Quadrupole MS	Agilent 1260 HPLC – AB Sicex API 4000 Triple Quadrupole MS
Calibration	Solvent	Solvent	Solvent

Table 2-3. Average quality control accuracies and method recoveries.

Compound	LOQ (ng/mL)	QC low (%)	Q C mid (%)	Blank concentration (ng/mL)	Recovery (%)	ICI/ EQUAS*
OPEs metabolites						
4HO-DPHP	0.50	146	144	ND	107	No
DPHP	0.10	93	78	0.076	101	Yes
5-HO-EHDPHP	0.01	109	110	0.015	107	No
EHPHP	0.05	123	128	ND	104	No
BCIPHIPP	0.04	93	105	ND	101	No
BCIPP	1.00	263	286	ND	85	Yes
TCEP	0.04	98	98	0.039	112	No
BBOEP	0.05	131	99	ND	109	No
BBOEHEP	0.01	94	94	0.029	105	No
3-HO-TBOEP	0.01	123	124	ND	104	No
BDCIPP	0.05	111	101	ND	99	Yes
DNBP	0.15	273	205	0.283	102	No
Alternative plasticizers metabolites						
OH-MINP	0.2	-	92	0.01	90	Yes
cxMINP	0.2	-	109	0.02	101	Yes
OH-MEHTP	0.2	-	98	0.01	95	No
MEHTP	0.2	-	109	0.10	102	No
OH-MEHA	0.2	-	87	0.01	90	No
oxoMEHA	0.2	-	88	0.01	92	No
MEHA	0.2	-	101	0.14	96	No
cxMPrHpP	0.2	-	108	0.01	101	Yes
OH-MPrHpP	0.2	-	97	0.01	93	Yes
oxoMPrHpP	0.2	-	95	0.01	92	No
cxMINCH	0.2	-	105	0.02	95	Yes
OH-MINCH	0.2	-	87	0.01	92	Yes
MINCH	0.2	-	95	0.01	90	No
Phthalates metabolites					IS	
MMP	0.05	-	88	ND	67	-
MEP	0.05	-	93	ND	68	-
MiPP	0.05	-	100	ND	-	-
MiBP	0.05	-	104	0.49	-	-
MBP	0.01	-	91	0.76	78	-
MPeP	0.01	-	89	ND	-	-
MBzP	0.01	-	95	ND	76	-
MCHP	0.01	-	95	ND	79	-
MHxP	0.05	-	127	ND	-	-
MEOHP	0.05	-	92	ND	68	-
MEHHP	0.05	-	116	ND	68	-
MECPP	0.01	-	138	0.12	69	-
MCMHP	0.05	-	122	ND	67	-
MEHP	0.10	-	66	1.04	71	-
MCPP	0.25	-	136	ND	86	-

* External quality control is assured by successful participation in inter-laboratory comparison exercises, such as Human Biomonitoring for the Europe External Quality Assurance Scheme (HBM4EU ICI/EQUAS, 2018) and the External Quality Assessment Scheme for Organic Substances in urine (OSEQAS, 2018).

2.2.3 Statistical analysis

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

$= (\text{chemical concentration}) \times [(SG_{median} - 1)/(SG - 1)]$

where the median value of the specific gravity (SG_{median}) of all samples was 1.016.

For those chemicals with the 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. For the comparison of demographic factors between the case and the control, Wilcoxon signed-rank test (for continuous variables such as age, and body mass index (BMI)), the chi-squared test (for categorical variables such as income, and alcohol consumption), or Fisher's exact test (for categorical variables with expected frequency less than 5 such as parity, and urinary cotinine) were conducted (Table 1). Correlations among the metabolite concentrations were determined by Spearman's correlation coefficients. For comparison of chemical concentrations between the case and the control groups, general linear regression models were constructed with covariates of age, BMI (continuously), income (categorically: low-middle, middle-high, and very high), parity (categorically: 0, 1, > 2), urinary cotinine (categorically: < 10 and ≥ 10 ng/ml), and alcohol consumption (categorically: yes and no). Due to

the skewness of distribution, measured chemical concentrations were log-transformed.

For logistic regression analysis, 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. Statistical significance was determined at $p = 0.05$. Statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

2.3 Results

2.3.1 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/m2, respectively (Table 2-4). There were no differences in age, income, urinary cotinine, alcohol consumption, and BMI between the case and the control groups. The only exception was parity which was lower in the case group (Table 2-4). A number of characteristics of participating women were included for case-control comparisons and logistic regression analyses as covariates (i.e., age, income, parity, urinary cotinine, alcohol consumption, and BMI). There were no significantly different demographic factors between selected and non-selected case women in the parent population (Table 2-5).

Table 2-4. Characteristics of the participating women.

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[a]				0.106
Low-Middle	10 (31.3)	25 (31.6)	35 (31.5)	
Middle-High	8 (25.0)	34 (43.0)	42 (37.8)	
> Very high	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]Low-Middle income: < 4 million KRW/ Middle-High income: < 8 million KRW/ Very high income: ≥ 8 million KRW (KRW: Korean Won). Values are the number of women and those in parentheses are percent unless otherwise noted. Statistical analysis was conducted by the Wilcoxon rank-sum 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, $p < 0.05$).

Table 2-5. Characteristics of the selected and non-selected case women in the parent population.

Characteristics	Selected case	Non-selected case	Total case	*P* value
Total	32 (28. 8)	63 (71.2)	95 (100.0)	
Age (year) (mean±SD)	38.3±5.3	37.9±5.0	38.1±5.1	0.587
20-29	3 (9.4)	6 (9.5)	9 (9.5)	
30-39	14(43.8)	30 (47.6)	44 (46.3)	
40-49	15 (46.9)	27 (42.9)	42 (44.2)	
Income[a]				0.894
Low-Middle	10 (31.3)	17 (28.8)	27 (29.7)	
Middle-High	8 (25.0)	16 (27.1)	24 (26.4)	
> Very high	14 (43.8)	26 (44.1)	40 (44.0)	
No response	0	4	4	
Parity				0.719
0	11 (35.5)	23 (36.5)	34 (36.2)	
1	4 (12.9)	7 (11.1)	11 (11.7)	
≥ 2	16 (51.6)	33 (52.4)	49 (52.1)	
No response	1	0	1	
Urinary cotinine (ng/mL)				0.897
< 10	29 (90.6)	57 (90.5)	86 (91.5)	
> 10	3 (9.4)	6 (9.5)	9 (9.6)	
Alcohol consumption				0.707
Yes	21 (65.6)	41 (69.5)	62 (68.1)	
No	11 (34.4)	18 (30.5)	29 (31.9)	
No response	0	4		
Body mass index (kg/m^2) (mean±SD)	22.7±2.7	22.7±3.6	22.7±3.3	0.460
< 18.5 (underweight)	0 (0.0)	1 (1.6)	1 (1.1)	
18.5-24.9 (normal weight)	23 (71.9)	38 (61.3)	61 (64.9)	
25-29.9 (pre-obesity)	5 (15.6)	10 (16.1)	15 (16.0)	
≥ 30 (obesity)	4 (12.5)	13 (21.0)	17 (18.1)	
No response	0	1		

[a]Low-Middle income: < 4 million KRW/ Middle-High income: < 8 million KRW/ Very high income: ≥ 8 million KRW (KRW: Korean Won). Values are the number of women and those in parentheses are percent unless otherwise noted. Statistical analysis was conducted by the Wilcoxon rank-sum 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.

2.3.2 Chemical exposure

Among twelve target OPE metabolites, DPHP, EHPHP, and BCIPHIPP were detected in over 80% of the urine samples (Table 2-6). 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 2-6). 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, i.e., < 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. Several chemicals showed significant positive correlations with each other based on Spearman's correlation analysis and strong correlations were generally observed between high molecular weight phthalates (Figure 2-1).

Table 2-6. 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 (25th, 75th)**	**DF (%)**	**Median (25th, 75th)**	
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	-

Table 2-6. (Continued)

Parent compound	Target compound	Total (*n* = 111)	Case (*n* = 32)		Control (*n* = 79)		*P* value (case-control)
		Median	DF (%)	Median (25th, 75th)	DF (%)	Median (25th, 75th)	
APs (continued)							
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	-

Table 2-6. (Continued)

Parent compound	Target compound	Total (*n* = 111)	Case (*n* = 32)		Control (*n* = 79)		*P* value (case-control)
		Median	DF (%)	Median (25th, 75th)	DF (%)	Median (25th, 75th)	
Phthalates (continued)							
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%. * indicates statistical significance 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: organophosphate esters, SG: specific gravity, DF: detection frequency, BMI: body mass index

OPEs		Phthalates									Alternative plasicizers				
BCIPHIPP	**EHPHP**	**MMP**	**MEP**	**MiBP**	**MBP**	**MBzP**	**MEOHP**	**MEHHP**	**MECPP**	**MCMHP**	**OH-MINP**	**cxMINP**	**cxMPrHpP**		
0.12	**0.34**	**0.37**	0.13	**0.21**	0.18	0.06	0.16	**0.32**	0.18	0.12	**0.21**	0.18	**0.23**	**DPHP**	**OPEs**
	0.00	0.10	**0.23**	**0.22**	**0.27**	**0.20**	0.10	**0.19**	0.03	0.14	**-0.37**	**-0.23**	-0.17	**BCIPHIPP**	
		0.17	0.07	0.16	0.17	0.05	**0.30**	**0.31**	**0.23**	**0.26**	0.14	**0.28**	**0.37**	**EHPHP**	
			0.13	0.08	0.13	-0.08	0.06	0.18	0.13	0.08	0.16	0.08	0.12	**MMP**	**Phthalates**
				0.15	0.11	0.01	0.01	-0.01	0.06	0.15	0.16	0.09	0.01	**MEP**	
					0.61	**0.36**	**0.30**	**0.41**	**0.40**	**0.41**	0.14	**0.31**	0.16	**MiBP**	
						0.42	**0.47**	**0.52**	**0.34**	**0.48**	0.01	**0.21**	0.07	**MBP**	
							0.28	**0.33**	**0.32**	**0.41**	-0.05	0.09	-0.03	**MBzP**	
								0.84	**0.59**	**0.60**	0.14	**0.25**	**0.20**	**MEOHP**	
									0.56	**0.59**	0.07	**0.27**	**0.22**	**MEHHP**	
										0.57	**0.24**	**0.33**	**0.34**	**MECPP**	
											0.14	**0.31**	**0.21**	**MCMHP**	
												0.60	**0.41**	**OH-MINP**	**Alternative plasicizers**
													0.55	**cxMINP**	

Spearman's correlation

0  **1**

Fig. 2-1. Spearman's correlation coefficients observed among the urinary metabolites which were detected in ≥ 75% of samples. Values bold represent significant results ($p < 0.05$).

2.3.3 Association of urinary metabolite concentrations with uterine fibroids

Urinary concentrations of MEOHP, the sum of 5 DEHP metabolites ($\sum$ 5DEHP, nmol/L), and OH-MINP were significantly higher in the urine of the case than in the control (Table 2-6). Other chemicals showed no differences between the two groups.

When the samples were dichotomously grouped based on the detection status (i.e., non-detected or detected), BDCIPP, BBOEP, and BBOEHEP showed positive associations with uterine fibroids (OR 5.51 with 95% CI: 1.93, 15.76; OR 2.81 with 95% CI: 1.01, 7.83; and OR 2.94 with 95% CI: 1.05, 8.24, respectively) (Fig. 2-2, Table 2-7). Among those who were detected for BDCIPP, 43.8%, or 21 out of 48 women, were identified to have uterine fibroids, while only 11 out of 63 women had uterine fibroids among those not detected for BDCIPP. Among AP metabolites, those detected for OH-MEHTP, OH-MPrHpP, and OH-MINCH, showed significantly higher ORs of the uterine fibroids (Fig. 2-2, Table 2-8). 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 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) (Fig. 2-2, Table 2-7).

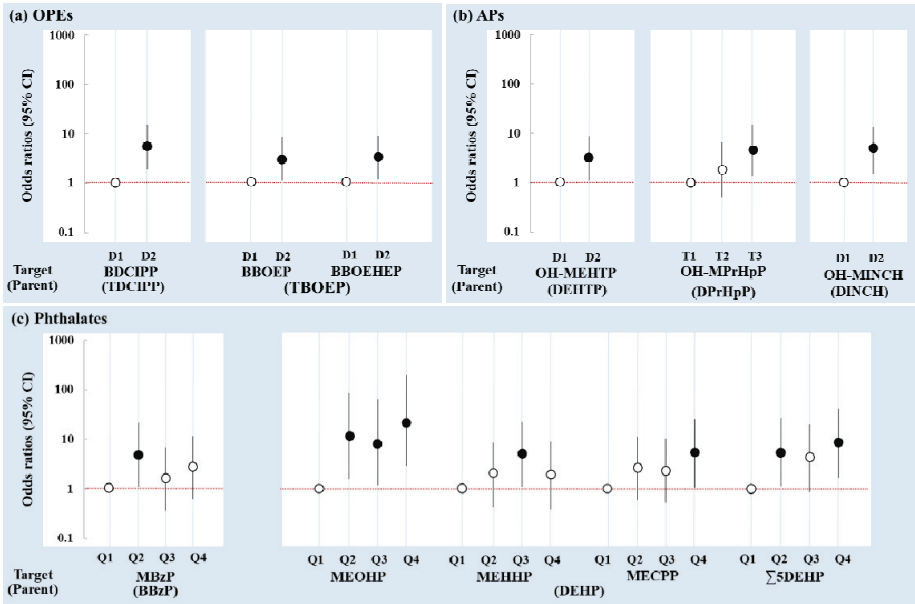


Fig. 2-2. Odds ratios (ORs) between uterine fibroids and (a) urinary OPEs, (b) APs, and (c) phthalates concentrations (DF ≥75%: quartile group comparison (Q), 50%≤DF<75%: tertile group comparison (T), two detected groups and not detected, 25%≤DF<50%: dichotomy group comparison (D), detected or not). The ORs were estimated for the 1st quartile, 1st tertile, 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

Table 2-7. Odds ratios (ORs) of uterine fibroids by grouping into four categories based on the range of detection frequencies (DF) ≥ 75%.

Parent compound	Target compound	Q	ORs	95% CI		Parent compound	Target compound	Q	ORs	95% CI	
OPEs						**Phthalates continued**					
TPHP	DPHP	Q1	reference			DiBP	MiBP	Q1	reference		
		Q2	1.67	0.40	6.95			Q2	0.92	0.23	3.67
		Q3	1.58	0.35	7.07			Q3	0.80	0.20	3.19
		Q4	2.24	0.50	9.95			Q4	1.78	0.47	6.77
EHDPHP	EHPHP	Q1	reference			DBP	MBP	Q1	reference		
		Q2	1.13	0.30	4.32			Q2	1.39	0.37	5.27
		Q3	0.57	0.14	2.36			Q3	0.77	0.19	3.11
		Q4	1.33	0.33	5.32			Q4	2.33	0.61	8.85
TCIPP	BCIPHIPP	Q1	reference			**BBzP**	**MBzP**	Q1	reference		
		Q2	0.61	0.17	2.25			**Q2**	**5.60**	**1.16**	**27.05**
		Q3	0.31	0.08	1.26			Q3	1.57	0.36	6.93
		Q4	0.41	0.11	1.57			Q4	2.66	0.61	11.73
APs						**DEHP**	**MEOHP**	Q1	reference		
DINP	OH-MINP	Q1	reference					**Q2**	**11.82**	**1.61**	**86.80**
		Q2	0.60	0.13	2.83			**Q3**	**8.91**	**1.21**	**65.66**
		Q3	2.93	0.75	11.48			**Q4**	**24.22**	**3.02**	**194.43**
		Q4	2.33	0.60	9.13						

Table 2-7. (Continued)

Parent compound	Target compound	Q	ORs	95% CI	
APs continued					
DINP (continued)	cxMINP	Q1	reference		
		Q2	0.95	0.26	3.45
		Q3	0.46	0.12	1.86
		Q4	1.22	0.33	4.50
DPrHpP	cxMPrHpP	Q1	reference		
		Q2	1.57	0.43	5.76
		Q3	0.53	0.12	2.37
		Q4	1.10	0.27	4.55
Phthalates					
DMP	MMP	Q1	reference		
		Q2	0.39	0.09	1.78
		Q3	1.28	0.32	5.06
		Q4	2.47	0.65	9.34
DEP	MEP	Q1	reference		
		Q2	0.80	0.21	3.11
		Q3	0.27	0.06	1.16
		Q4	0.71	0.19	2.61

Parent compound	Target compound	Q	ORs	95% CI	
Phthalates continued					
	MEHHP	Q1	reference		
		Q2	1.99	0.45	8.77
		Q3	**5.05**	**1.14**	**22.28**
		Q4	1.90	0.40	9.10
	MECPP	Q1	reference		
		Q2	2.62	0.60	11.44
		Q3	2.38	0.54	10.54
		Q4	**5.25**	**1.07**	**25.76**
	MCMHP	Q1	reference		
		Q2	1.51	0.36	6.38
		Q3	1.41	0.35	5.71
		Q4	2.60	0.62	10.90
∑5 DEHP		Q1	reference		
		Q2	**5.60**	**1.16**	**27.05**
		Q3	4.18	0.87	20.22
		Q4	**8.49**	**1.71**	**42.10**

Table 2-7 (Continued)

Boldface values indicate statistically significant differences with respect to the reference category ($p < 0.05$). Odds ratios between uterine fibroids and urinary OPEs and plasticizer concentrations (DF ≥75%) were estimated for the 1st quartile as a reference. The target population number was 111 (case = 32). Adjusted for age, income, parity, urinary cotinine, alcohol consumption, and BMI.

OPEs: organophosphate esters, APs: alternative plasticizers, DF: detection frequency, BMI: body mass index

Table 2-8. Odds ratios (ORs) of uterine fibroids by grouping into two or three categories based on the DF range (25% ≤ DF < 75%).

Parent compound	Target compound	DF (%)		OR	95% CI	
OPEs						
TNBP	DNBP	30.7	D1	reference		
			D2	0.39	0.12	1.21
TDCIPP	**BDCIPP**	43.9	D1	reference		
			D2	**5.51**	**1.93**	**15.76**
TBOEP	**BBOEP**	29.8	D1	reference		
			D2	**2.81**	**1.01**	**7.83**
	BBOEHEP	45.6	D1	reference		
			D2	**2.94**	**1.05**	**8.24**
APs						
DEHA	OH-MEHA	36.0	D1	reference		
			D2	2.70	0.93	7.82
	oxoMEHA	46.5	D1	reference		
			D2	1.08	0.42	2.77
DEHTP	**OH-MEHTP**	31.6	D1	reference		
			D2	**3.13**	**1.11**	**8.84**
DPrHpP	**OH-MPrHpP**	62.3	T1	reference		
			T2	1.75	0.49	6.29
			T3	**4.40**	**1.31**	**14.80**
	oxoMPrHpP	64.0	T1	reference		
			T2	0.85	0.26	2.82
			T3	1.89	0.57	6.28
DINCH	cxMINCH	63.2	T1	reference		
			T2	0.53	0.16	1.68
			T3	0.32	0.09	1.10
	OH-MINCH	28.9	D1	reference		
			D2	**4.74**	**1.63**	**13.80**
Phthalates						
DHxP	MHxP	27.0	D1	reference		
			D2	0.30	0.09	1.06
DEHP	MEHP	45.9	D1	reference		
			D2	1.87	0.71	4.94
DnOP	MCPP	45.0	D1	reference		
			D2	1.52	0.55	4.18

Boldface values indicate a statistically significant difference with respect to the reference category ($p < 0.05$). For metabolites with detection frequencies between 50 and 75%, samples were grouped into three categories: T1 = the non-detected, T2 = below the median, and T3 = above the median. For metabolites with detection frequencies between 25% and 50%, samples were grouped into two categories: D1 = the non-detected, D2 = the detected. ORs were estimated against the first tertile (T1) or the non-detected (D1). The target population number was 111 (case = 32). Adjusted for age, income, parity, urinary cotinine, alcohol consumption, and BMI.

OPEs: organophosphate esters, APs: alternative plasticizers, DF: detection frequency, BMI: body mass index

2.4 Discussion

Frequently detection of DPHP, EHPHP, and BCIPHIPP in the urine shows that premenopausal women of Korea were widely exposed to OPEs like 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 pregnant women in the US (median 1.3 and 0.93 ng/ml) (Hoffman et al., 2017; Castorina et al., 2017), but higher than that reported in Chinese adults (median 0.06 ng/ml) (Sun et al., 2018). Among the measured OPE metabolites, EHPHP showed the highest median concentration (1.01 ng/ml) in the present population. The SG adjusted median concentration of BCIPHIPP was 0.42 ng/ml in the control, which was similar to that of the US pregnant women (Hoffman et al., 2017), but lower than that of Belgian adults (n = 14, 3.93 ng/ml unadjusted) (Bastiaensen et al., 2018). The detection frequency of BBOEHEP was <60% in the present Korean population (Table 2-5), while BBOEHEP, BCIPHIPP, TCEP, and DPHP were most frequently detected in the urine samples of Japanese children (Bastiaensen et al., 2019), showing that usage patterns vary by country and by population.

The exposure profiles of APs observed in the present population were different from those reported in other countries. Based on our observations, 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 (Table 2-5). The

detection level of cxMINCH was higher than that found in Norwegian adults (detection frequency 85%, geometric mean (GM 0.23 ng/ml) (Giovanoulis et al., 2016), and in the general German population (detection frequency 88.3%, median 0.17 ng/ml) (Schütze et al., 2014). However, the median concentration reported for German children (n= 208, 1.14 ng/ml) was higher than that found in the present Korean female population (0.33 ng/ml) (Fromme et al., 2016). For DPrHpP metabolites, over 70% of the Korean women's urine samples were detected (Table 2-5). Among the present population, oxoMPrHpP was detected in 71.9% (case) and 62% (control) of the urines, while in the general German population, 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 in only 29.1% of the control urine samples of the present population, while being detected in 96% of the US general population (Silva et al., 2019). Considering the different exposure profiles by country, and the increasing use and exposure to APs such as DINCH, DPHP, and DEHTP (Schütze et al., 2015; Silva et al., 2013; 2016), continuous surveillance of APs in human urine samples is warranted.

Our observation suggests that several OPEs are associated with increased 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 (Fig. 2-2). While the modes of action of OPEs in the development of uterine fibroids are

not well understood, one possible explanation can be found from the endocrine disruption potential of OPEs. Uterine fibroids are considered to be estrogen-dependent and it is well documented that sex regulating hormones, such as estrogen and progesterone, promote the growth of uterine fibroids (Flake et al., 2003; Parker et al., 2007). Estrogenicity of several OPEs has been documented in both in vitro and in vivo studies. TDCIPP and TBOEP could influence the synthesis and metabolism of estradiol and TDCIPP could behave as an estrogen receptor agonist (Liu et al., 2012; Zhang et al., 2014). In humans, an association between OPE exposure and uterine fibroids has never been reported. However, its association with sex hormone regulation or other reproductive health outcomes has been suggested. TDCIPP concentrations in house dust were associated with a prolactin increase in men (Meeker and Stapleton, 2010). Prolactin is a reproductive hormone that was suggested as an adjuvant biomarker in uterine fibroids (Baban, 2009; Levy et al., 2013). Among men and women, urinary concentrations of TDCIPP metabolites and ∑OPEs were negatively associated with successful in vitro fertilization (Carignan et al., 2017). These epidemiological observations on adverse sex hormone and reproductive effects support the observed association of OPE exposure with uterine fibroids.

Significantly high ORs observed for OH-MEHTP and OH-MINCH (Fig. 2-2, Table 2-8) can be also supported by the estrogenic effects of DINCH and DEHTP reported in vitro experimental studies. While further confirmation in other populations and the experimental studies are warranted, these

observations suggest these APs may not be safer substitutes for DEHP, at least in terms of the risk of uterine fibroids. DINCH showed a stimulatory effect on steroid production in vitro (Boisvert et al., 2016). DINCH metabolites activate nuclear receptors, estrogen receptors (α and β), androgen receptors, and peroxisome proliferator-activated receptors (α and γ), suggesting weak estrogenic effects related with lipid and glucose metabolisms in reporter gene assays (Engel et al., 2018). OH-MEHTP, a DEHTP metabolite, has an agonistic effect on estrogen receptors and also increases steroid hormone synthesis in vitro (Kambia et al., 2019). For OH-MPrHpP, while exposure to DPrHpP appears to increase over time among the general population (Schütze et al., 2014), neither experimental nor epidemiological reports supporting our observation are not available.

The significant associations of DEHP metabolites (MEOHP, MEHHP, MECPP, and ∑5DEHP) with uterine fibroids have been previously reported from several populations (Fu et al., 2017; Huang et al., 2010; Kim et al., 2016; Sun et al., 2016). The mechanisms of action of DEHP underlying pathogenesis of uterine fibroids are not fully understood, but several in vitro studies show that DEHP could enhance proliferative activity in myometrial and leiomyoma cells (Kim et al., 2017; Kim, 2018). In addition, exposure to DEHP was reported to be positively associated with increased uterine volume (Zota et al., 2019). While reports of a null association between urinary DEHP metabolites and fibroids are available, these results are either based on women who underwent laparoscopy recruited from clinical centers of the US or

depended on self-reported history of the disease. (Pollack et al., 2015; Weuve et al., 2010). Among measured phthalates, urinary MBzP concentrations showed a significant association with uterine fibroids, but without a dose-response relationship (Fig. 2-2, Table 2-7). Because several previous studies have reported null associations between MBzP and uterine fibroids, the association between MBzP and uterine fibroids should be tested in other populations (Huang et al., 2010; Pollack et al., 2015; Kim et al., 2016).

Considering several suggested theories of initiation uterine fibroids, a few hypotheses can be proposed as possible underlying mechanisms. One hypothesis is that chemical exposure may promote the uterine fibroids through an increase of estrogen or progesterone levels. Two hormones, estrogen, and progesterone have been recognized as a promoter of uterine fibroids, and the effects of endocrine-disrupting chemicals such as phthalates on sex hormonal change have been well investigated (Lee et al., 2019a; Sohn et al., 2016). Another hypothesis is the mediation of the nuclear receptor 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). The target chemicals or their metabolites in this present study such as TDCPP, BBzP, and DINCH may act as weak agonists of the estrogen receptor (Engel et al., 2018; Mankidy et al., 2013; Zhang et al., 2014). Finally, the developmental exposure to endocrine-disrupting chemicals may induce genetic and epigenetic regulation of stem cells which lead to uterine fibroids development (Katz et al., 2016). Even

though several studies have reported the epigenetic changes in uterine fibroids (Navarro et al., 2012; Yang et al., 2016), the specific mechanism is largely unclear. Identification of the role of epigenetics in the tumorigenesis of uterine leiomyoma warrants further investigations.

There are limitations to the present study. First, 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 may be lower than those expected in normal situations. Second, observations from the cross-sectional case and control design cannot explain causality. Third, the limited sample size may have insufficient statistical power to find significant associations and therefore 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. While the results of this study should be validated by further experimental and epidemiological studies, our observations could be supported by previous experimental and epidemiological studies that reported sex hormones related to disruptive effects. Our observations suggest that several OPEs and APs may not be safer alternatives to more conventional flame retardants and plasticizers.

Chapter 3 Urinary levels of phthalates and DINCH metabolites in Korean and Thai pregnant women across three trimesters

3.1 Introduction

Due to a wide range of physico-chemical properties, phthalates have been used in numerous consumer applications, including food packaging, cosmetics, paintings, medical devices, and building materials. Because phthalates are not chemically bound with the products, these chemicals can easily leach out of the products and eventually enter the body. Major exposure routes to humans include consumption of foods and drinking water, inadvertent dust ingestion, dermal absorption from cosmetic use, and, to a lesser extent, inhalation of indoor or outdoor air (Fromme et al., 2013a; Koniecki et al., 2011; Serrano et al., 2014). Consequently, phthalates have been frequently detected in human urine samples worldwide (Koch et al., 2017; Park et al., 2017; Centers for Disease Control and Prevention, 2019).

Phthalates and their metabolites have been frequently demonstrated for endocrine-disrupting effects in experimental studies (Lee et al., 2019a; Sohn et al., 2016). For example, following prenatal exposure to di(2-ethylhexyl) phthalate (DEHP), disruption in sex hormone balances were reported in adult rats (Martinez-Arguelles and Papadopoulos, 2016). Due to increasing health concerns on phthalates, their uses in many applications were regulated in recent decades worldwide (Directive 2005/84/EC; CPSIA, 2008; KATS,

2010). Subsequently, alternative plasticizers, e.g., di-(iso-nonyl)-cyclohexane-1,2-dicarboxylate (DINCH) and di(2-ethylhexyl) terephthalate (DEHTP) have been used in increasing amount (Bui et al., 2016; Schütze et al., 2014). However, biomonitoring studies for these alternative plasticizers are mostly limited to several European countries, and a few other countries, such as Australia and Israel (Correia-Sá et al., 2017; Fromme et al., 2016; Giovanoulis et al., 2016; Gomez Ramos et al., 2016; Machtinger et al., 2018; Schütze et al., 2014). For many Asian countries, information related to exposure to alternative plasticizers is largely unknown.

Available experimental and human observational studies suggest that pregnancy is one of the most vulnerable windows for phthalate exposure (Ferguson et al., 2014; Martino-Andrade et al., 2016; Zarean et al., 2019). Because prenatal exposure to phthalates could affect the fetal development and the health of later life stages (Kim et al., 2018; Zarean et al., 2019), it is important to evaluate and manage phthalate exposure during pregnancy. Exposure profiles of major phthalates during the gestation period have been reported worldwide, but most studies used limited number of spot urine samples (generally 1 or 2) to characterize phthalate exposure (Bornehag et al., 2015; Kobrosly et al., 2014; Zhao et al., 2015). However, because of short biological half-life of phthalates in the body, the use of spot urine has limited value (Koch et al., 2005). In addition, due to substantial changes in behavior and physiology of pregnant women, phthalate exposure is expected to vary significantly over the course of pregnancy (Hsieh et al., 2019; Serrano et al., 2014; Zhao et al., 2018). Recent studies suggest that the temporal variability

of phthalate exposure during pregnancy can be huge depending on the timing of sampling, sample size, and study population (Braun et al. 2012; Huang et al., 2017; Li et al., 2019; Martino-Andrade et al., 2016; Warembourg et al., 2019; Watkins et al., 2017).

In the present study, we recruited women in early pregnancy from Korea and Thailand, and investigated the exposure profile of major phthalates and an alternative plasticizer using urine samples collected in each trimester. Because of different climate and lifestyle, the exposure pattern was also expected to be different by country. While a convenience sampling design was employed and therefore the participating women may not be representative of each country, the information gleaned from the present study will provide a novel insight into the exposure profiles, temporal variations, and associated risks of phthalates and their alternative over the course of pregnancy in each country.

3.2 Materials and methods

3.2.1 Study population and sample collection

Healthy women in early pregnancy (<14weeks of gestation) were recruited from obstetrics and gynecology hospitals in Seoul, Korea and an antenatal care clinic of a health promoting hospital in Bangkok, Thailand, between May and December 2016. Among the women recruited, those with endocrine diseases or pregnancy-related medical histories were excluded from the further assessment. The final number of participants was 81 in Korea and 102 in Thailand. The participating women were sampled for spot urine for three times during pregnancy, generally one sample in each trimester. The first trimester (T1) was defined as before 14 gestational weeks, the second trimester (T2) was defined as between weeks 15 and 28, and the third trimester (T3) was defined as from week 29 until term delivery. Urine samples were collected in polypropylene tubes and stored at −40°C until analysis. In the cases where more than one urine sample were collected in a given trimester, an average of the measurements for the samples was used to represent the level of exposure for the trimester. The cases where more than one sample were collected in a given trimester were 19 (11 and 8 for the second and third trimester, respectively) in Korea, and 28 (22 and 6 for the second and third trimester, respectively) in Thailand. A questionnaire survey was carried out, and demographic and physiological characteristics, such as age, alcohol consumption, smoking status, body mass index (BMI) before pregnancy and the gestational period, were obtained. The Institutional Review

Boards of the School of Public Health, Seoul National University (SNU), and Mahidol University (MU) approved the study (No. 1604/ 001-012 for SNU and No. 2016-018-01 for MU), and informed consent was obtained from the participating women. All samples and data were processed blindly.

3.2.2 Measurement of urinary plasticizers

Metabolites of major phthalates and DINCH were analyzed in the urine (Table 3-1). Monohydroxyisononyl phthalate (OH-MiNP), mono-(7-carboxy-2,7-dimethylheptyl) phthalate (cx-MiDP), cyclohexane-1,2-dicarboxylate-mono-4-methyloctyl ester (MINCH), cyclohexane-1,2-dicarboxylate-mono-(7-hydroxy-4-methyl)octyl ester (OH-MINCH), mono(2-ethylhexyl) terephthalate (MEHTP), and OH-MINCH-d8 were purchased from Toronto Research Chemical (North York, ON, CA), and the rest were purchased from Cambridge Isotope Laboratory (Andover, MA, USA). After enzymatic hydrolysis, the samples were pretreated using the Strata X SEP Cartridge (30 mg/cc, Phenomenex, USA), following Servaes et al. (2013) with a minor modification (Fig. 3-1). For measurement, ultra-high performance liquid chromatography (UHPLC-MS/MS) was used (UHPLC Nexera X2, Shimadzu Corporation, Kyoto, Japan, and API 4500, AB SCIEX, Ontario, Canada). The UHPLC-MS/MS conditions, multiple reaction monitoring parameters, and information regarding quality assurance and quality control, including the limit of detection (LOD), are shown in Tables 3-2 – 3-4.

Table 3-1. Target plasticizers and their urinary metabolites.

Parent compound	**Chemical formula**	**Molecular weight (g/mole)**	**Metabolite**	**Chemical formula**	**Molecular weight (g/mol)**
Dimethyl phthalate (DMP)	$C_{10}H_{10}O_4$	194.1	Monomethyl phthalate (MMP)	$C_9H_8O_4$	180.2
Diethyl phthalate (DEP)	$C_{12}H_{14}O_4$	222.2	Monoethyl phthalate (MEP)	$C_{10}H_{10}O_4$	194.2
Di-isopropyl phthalate (DiPP)	$C_{14}H_{18}O_4$	250.3	Mono-isopropyl phthalate (MiPP)	$C_{11}H_{12}O_4$	208.2
Di-2-isobutyl phthalate (DiBP)	$C_{16}H_{22}O_4$	278.3	Mono-2-isobutyl phthalate (MiBP)	$C_{12}H_{14}O_4$	222.2
Di-n-butyl phthalate (DnBP)	$C_{16}H_{22}O_4$	278.3	Mono-n-butyl phthalate (MnBP)	$C_{12}H_{14}O_4$	222.2
Di-n-pentyl phthalate (DPeP)	$C_{18}H_{26}O_4$	306.4	Mono-n-pentyl phthalate (MPeP)	$C_{13}H_{16}O_4$	236.3
Benzyl butyl phthalate (BBzP)	$C_{19}H_{20}O_4$	312.4	Monobenzyl phthalate (MBzP)	$C_{15}H_{12}O_4$	256.3
Dicyclohexyl phthalate (DCHP)	$C_{20}H_{26}O_4$	334.4	Monocyclohexyl phthalate (MCHP)	$C_{14}H_{16}O_4$	248.3
Dihexyl phthalate (DHxP)	$C_{20}H_{30}O_4$	334.5	Monohexyl phthalate (MHxP)	$C_{14}H_{18}O_4$	250.3
Di(2-ethylhexyl) phthalate (DEHP)	$C_{24}H_{38}O_4$	390.6	Mono(2-ethylhexyl) phthalate (MEHP)	$C_{16}H_{22}O_4$	278.3
			Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)	$C_{16}H_{22}O_5$	292.3
			Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	$C_{16}H_{22}O_5$	294.3

Table 3-1. (*Continued*)

Parent compound	Chemical formula	Molecular weight (g/mole)	Metabolite	Chemical formula	Molecular weight (g/mol)
			Mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)	$C_{16}H_{20}O_6$	308.3
			Mono-[(2-carboxymethyl)hexyl] phthalate (MCMHP)	$C_{16}H_{20}O_6$	308.3
Dioctyl phthalate (DnOP)	$C_{24}H_{38}O_4$	390.6	Mono(3-carboxypropyl) phthalate (MCPP)	$C_{12}H_{12}O_6$	252.2
			Monooctyl phthalate (MnOP)	$C_{16}H_{22}O_4$	278.3
Di-isononyl phthalate (DiNP)	$C_{26}H_{42}O_4$	422.6	Mono-isononyl phthalate (MiNP)	$C_{17}H_{24}O_4$	292.4
			Monohydroxyisononyl Phthalate (OH-MiNP)	$C_{17}H_{24}O_5$	308.4
Diisodecyl phthalate (DiDP)	$C_{28}H_{46}O_4$	446.67	Mono-isodecyl phthalate (MiDP)	$C_{18}H_{26}O_4$	306.4
			Mono-(7-carboxy-2,7-dimethylheptyl) Phthalate (cx-MiDP)	$C_{18}H_{24}O_6$	336.4
Di(2-ethylhexyl) terephthalate (DEHTP)	$C_{24}H_{38}O_4$	390.6	Mono(2-ethylhexyl) terephthalate (MEHTP)	$C_{16}H_{22}O_4$	278.3
Di-(iso-nonyl)-cyclohexane-1,2-dicarboxylate (DINCH)	$C_{26}H_{48}O_4$	424.7	Cyclohexane-1,2-dicarboxylate-mono-4-methyloctyl ester (MINCH)	$C_{17}H_{30}O_4$	298.4
			Cyclohexane-1,2-dicarboxylate-mono-(7-hydroxy-4-methyl)octyl ester (OH-MINCH)	$C_{17}H_{30}O_5$	314.4

Table 3-2. UHPLC-MS/MS conditions.

Parameter		Condition						
UHPLC	Column	ACQUITY UPLC BEH phenyl (100 mm × 2.1 mm, 1.7 μm)						
	Mobile phase	A: 0.1% Acetic acid in water						
		B: 0.1% Acetic acid in acetonitrile						
	Gradient	Time (min)	0	1	9	9.5	10	12
		B (%)	5	25	85	85	5	5
	Flow rate	0.3 mL/min						
	Injection volume	5 μL						
MS/MS	Ionization mode	ESI negative						
	Curtain gas (CUR)	25 psi						
	Collision gas (CAD)	9						
	Ion spray voltage (V)	-4500						
	Temperature	500						
	Ion source gas 1	50						
	Ion source gas 2	60						

Table 3-3. Multiple reaction monitoring (MRM) parameters of the UHPLC–MS/MS method.

Compound	RT(min)	Parent ion (Q1)	Daughter ion (Q3)	DP(volts)	CE(volts)	CXP(volts)
MMP	3.61	178.8	77.0 (107.0)	-40	-24 (-16)	-7 (-9)
$^{13}C_2$-MMP	3.61	182.9	79.0	-10	-24	-7
MEP	4.1	192.8	77.1 (120.9)	-5	-26 (-14)	-5
$^{13}C_2$-MEP	4.1	197.2	78.9	-30	-22	-5
MiPP	4.58	206.9	77.1 (57.0)	-40	-26 (-18)	-5
MiBP	5.3	220.9	77.0 (133.8)	-40	-22 (-20)	-13 (-9)
MnBP	5.4	220.9	77.1 (71.1)	-50	-24 (-18)	-1 (-19)
$^{13}C_2$-MnBP	5.4	225.0	79.0	-55	-24	-7
MPeP	6.03	234.9	85.0 (76.9)	-50	-20 (-26)	-5
MBzP	5.71	255.0	76.9 (106.9)	-5	-32 (-20)	-7 (-9)
$^{13}C_2$-MBzP	5.71	258.9	107.1	-15	-18	-9
MCHP	5.91	246.9	77.0 (97.0)	-60	-26 (-22)	-11 (-7)
$^{13}C_2$-MCHP	5.91	250.9	79.0	-45	-28	-7
MHxP	6.63	249.0	77.0 (99.0)	-55	-32 (-20)	-7 (-3)
MEHP	7.47	277.1	134.1 (77.0)	-65	-20 (-34)	-5 (-9)
$^{13}C_2$-MEHP	7.47	281.0	137.0	-65	-20	-7
MEOHP	5.41	290.9	121.0 (143.0)	-65	-24 (-18)	-3 (-5)
$^{13}C_4$-MEOHP	5.41	295.0	124.0	-55	-24	-3
MEHHP	5.21	293.0	121.0 (120.6)	-35	-28	-5
$^{13}C_4$-MEHHP	5.21	297.0	124.0	-85	-26	-7
MECPP	5.12	306.9	159.0 (77.0)	-20	-18 (-50)	-5
$^{13}C_4$-MECPP	5.12	311.0	159.0	-20	-16	-5
MCMHP	5.50	306.9	159.1 (141.0)	-25	-22 (-32)	-1
$^{13}C_4$-MCMHP	5.50	311.0	158.9	-30	-16	-5
MCPP	3.64	250.9	102.9 (120.9)	-10	-14 (-28)	-9 (-11)

Table 3-3. (Continued)

Compound	RT(min)	Parent ion (Q1)	Daughter ion (Q3)	DP(volts)	CE(volts)	CXP(volts)
$^{13}C_2$-MCPP	3.64	255.0	103.0	-30	-17	-3
MnOP	7.67	276.9	77.0 (121.0)	-45	-36 (-20)	-1
$^{13}C_2$-MnOP	7.67	281.0	79.1	-55	-34	-7
MiNP	7.69	291.0	141.0 (76.9)	-70	-26 (-32)	-11 (-9)
$^{13}C_2$-MiNP	7.69	295.0	141.1	-70	-26	-7
OH-MiNP	5.51	307.0	121.0 (77.0)	-75	-24 (-44)	-3 (-7)
MiDP	8.35	305.0	155.0 (77.0)	-60	-22 (-40)	-1 (-5)
cx-MiDP	5.82	335.0	187.1 (120.8)	-30	-20 (-32)	-5 (-9)
MEHTP	8	277.0	233.0 (121.0)	-80	-24	-7 (-11)
MINCH	8.49	297.1	153.0 (109.1)	-70	-20 (-36)	-9 (-11)
OH-MINCH	5.97	313.1	153.0 (109.0)	-75	-24 (-40)	-5 (-3)
OH-MINCH-d8	5.97	321.3	160.9	-60	-24	-13

*DP : declustering potential (V), CE : collision energy (V), CXP : collision cell exit potential (V)

Table 3-4. Limit of detection, accuracy and precision of the analytical method.

Metabolite	LOD (ng/mL)	Intra – day						Inter - day					
		Accuracy (%)			Precision (%)			Accuracy (%)			Precision (%)		
		L	M	H	L	M	H	L	M	H	L	M	H
MMP	0.18	86.33	112.47	114.23	6.20	5.95	2.11	99.60	107.43	102.84	4.50	5.08	6.10
MEP	0.31	88.83	105.07	109.93	2.84	7.32	0.69	88.36	104.38	97.53	3.28	5.55	2.76
MiPP	0.13	118.72	118.44	118.92	3.54	8.74	0.35	100.11	109.09	106.66	3.58	4.76	3.31
MiBP	0.34	106.33	98.67	95.53	3.32	5.20	5.06	108.58	102.68	101.16	2.71	3.45	3.46
MnBP	0.11	80.67	107.33	103.67	2.22	4.88	5.33	100.00	102.70	98.48	2.23	3.14	3.39
MPeP	0.47	113.20	119.00	116.30	4.08	8.06	0.61	117.03	116.73	115.76	7.77	6.62	3.13
MBzP	0.22	94.87	110.53	116.85	7.73	7.65	2.99	83.66	102.88	106.06	7.89	6.17	4.53
MCHP	0.12	98.50	103.30	110.40	4.57	10.66	4.36	103.97	104.74	105.97	8.68	6.80	4.57
MHxP	0.08	96.20	101.18	99.50	10.48	9.51	4.97	95.67	95.27	94.25	9.17	6.83	3.23
MEHP	0.26	80.03	106.47	104.73	9.73	10.78	1.17	82.82	100.78	103.20	5.42	7.69	2.09
MEOHP	0.30	100.33	84.07	103.43	7.58	4.39	4.35	97.00	95.47	103.11	4.80	3.88	2.52
MEHHP	0.38	105.67	88.73	82.77	2.83	1.79	2.83	101.83	86.18	82.91	3.72	1.98	4.09
MECPP	0.12	95.00	118.80	111.00	2.05	2.60	4.24	101.83	106.44	103.39	2.95	3.12	5.03
MCMHP	0.13	101.00	115.60	115.10	5.14	2.50	1.54	89.21	104.19	107.87	2.45	2.60	1.40
MCPP	0.12	97.27	100.73	95.73	10.74	9.59	0.69	92.88	98.56	101.38	7.39	6.49	4.41
MnOP	0.31	110.00	110.80	113.20	5.72	12.23	3.75	98.70	103.77	103.55	6.41	7.48	6.14
MiNP	0.40	106.40	112.00	107.20	8.16	8.82	8.18	108.97	108.23	104.45	9.19	5.15	5.70
OH-MiNP	0.09	88.47	99.73	91.07	6.96	7.70	2.33	94.93	94.13	92.55	4.45	4.38	3.44
MiDP	0.19	114.00	115.20	106.40	9.72	9.34	3.99	114.65	111.21	112.20	9.97	5.02	3.79

Table 3-4. (Continued)

Metabolite	LOD (ng/mL)	Intra – day						Inter - day					
		Accuracy (%)			Precision (%)			Accuracy (%)			Precision (%)		
		L	M	H	L	M	H	L	M	H	L	M	H
cx-MiDP	0.04	95.05	100.25	92.23	8.48	11.22	0.15	102.94	99.06	94.72	6.72	6.84	3.38
MEHTP	0.20	106.63	117.09	119.88	10.42	13.99	0.82	101.54	109.50	110.05	4.45	7.48	2.61
MINCH	0.09	111.33	111.73	110.69	6.24	12.72	1.20	116.89	115.36	110.16	6.34	6.05	3.23
OH-MINCH	0.13	109.93	112.89	113.32	4.25	12.73	1.86	109.18	103.65	104.14	4.37	5.96	3.33

- L: low concentration, M: middle concentration, H: high concentration

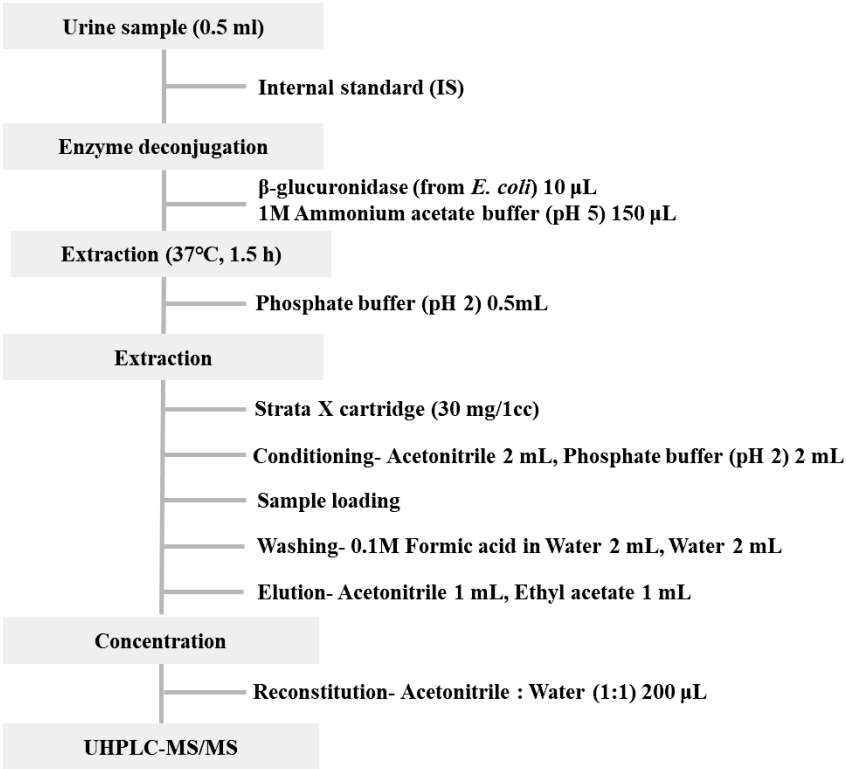


Fig. 3-1. Procedures of sample pretreatment.

3.2.3 Estimation of daily intake and associated risk

The concentrations of target metabolites are shown following specific gravity (SG) adjustment using the following equation to correct for urine dilution. The reasons for using SG as correction factors for urine dilution were physiological characteristics, such as age, pregnancy, physical activity, and disease, could affect to creatinine levels (Boeniger et al., 1993). Especially, physiologic increase in glomerular filtration rate during pregnancy results in changes in concentration of serum creatinine (Maynard and Thadhani, 2009). In addition to, creatinine adjusted phthalates concentration might not be appropriate for phthalates because organic compounds that are glucuronidated in the liver, like phthalates, are eliminated by active tubular secretion (Boeniger et al., 1993; Hauser et al., 2004). Creatinine, however, is a low molecular weight, its passage is accomplished through glomerular filtration, not re-absorbed by renal tubules (Boeniger et al., 1993).

SG-adjusted concentration

$= (\text{chemical concentration}) \times [(SG_{median} - 1)/(SG - 1)]$

SG of urine was measured using a handheld digital refractometer (Master-SUR/Na; Atago Co, Tokyo Japan). The median specific gravity of the urine samples was determined at 1.012 and 1.008 in Korea and Thailand, respectively. Daily intake amount of a given plasticizer was estimated using the following equation (Fromme et al., 2013b):

Daily intake (DI) (μg/kg bw-day)

$$= \text{UC } (\mu g/L) \text{ x } UV \ (L/day) \text{ x } \frac{MWp}{MWm} \text{ x } \frac{1}{fue} \text{ x } \frac{1}{kgbw}$$

where UC was the SG adjusted urinary concentration of a metabolite (μg/L), UV was the total urine volume per day (2 L/d from a Norwegian

mother and child cohort study) (Ye et al., 2009), MWp and MWm are the molecular weights of the parent plasticizer and the given metabolite (g/mol), respectively, and kgbw was the body weight. The urinary excretion fraction (f_{ue}) for monomethyl phthalate (MMP), monoethyl phthalate (MEP), mono-isobutyl phthalate (MiBP), and mono-n-butyl phthalate (MnBP) was 0.69 (Anderson et al., 2001; Itoh et al., 2007), and that for monobenzyl phthalate (MBzP) was 0.73 (Anderson et al., 2001). For mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-[(2-carboxymethyl)hexyl] phthalate (MCMHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), fue values of 0.059, 0.18, 0.042, 0.23 and 0.15 were used, respectively (Koch et al., 2005). In addition, fue values for OH-MiNP and OH-MINCH, were 0.202 (Koch and Angerer, 2007) and 0.107 (Koch et al., 2013a), respectively. The HQ was calculated by dividing the estimated daily intake of a given compound by the dose at which no adverse health effects are expected, e.g., reference dose (RfD) or tolerable daily intake (TDI), whichever was more conservative. HQ greater than unity was considered to have potential risk. The RfD values for benzyl butyl phthalate (BBzP) and DEHP were 200 and 20μg/kg_{bw}/day, respectively (US EPA, 1987; US EPA, 1988). The TDI values for diethyl phthalate (DEP), di-n-butyl phthalate (DnBP), and di-isononyl phthalate (DiNP) were 500, 10, and 150μg/kg_{bw}/day, respectively (WHO, 2003; EFSA, 2005a,b). The TDI for di-2-isobutyl phthalate (DiBP) was not available; therefore, the TDI for DnBP were used instead. RfDs for dimethyl phthalate (DMP) and DINCH were 375 and 700μg/kg_{bw}/day, respectively

(Giovanoulis et al., 2016; Bhat et al., 2014). DI for DEHP was derived from the fue weighted average of each metabolite, that is, MEHP, MEOHP, MEHHP, and MECPP (Christensen et al., 2014). The hazard index (HI) was estimated as the sum of HQs calculated for the measured phthalates (DMP, DEP, DiBP, DnBP, BBzP, DEHP, DiNP and DINCH). Contribution of each phthalate to the HI was derived by dividing the HQ calculated for a given phthalate by the HI summed up for all phthalates for each trimester, and averaging ratios for three trimesters. Relative proportions of each DEHP metabolite (f_i), i.e., the ratio of a DEHP metabolite (i) to the sum of all five of its metabolites, were calculated using the following equation: $f_i=C_i/\Sigma C_i$ * 100% where Ci is the molar concentration (mol/ L) of each DEHP metabolite, i.e., MEHP; MEHHP; MEOHP, MECPP or MCMHP (Hauser et al., 2006; Zhao et al., 2018).

3.2.4 Statistical analysis

For statistical analyses, the metabolites detected in 70% and more of the samples were used. For these chemicals, the non-detected values were substituted with the LOD divided by the square root of 2. Those that were detected in <70%, while statistical comparison was not conducted, the non-detected were regarded as zero for other presentation. To determine the differences in urinary chemical concentrations between the two countries, analysis of covariance (ANCOVA) was conducted using PROC GLM of SAS 9.4 (SAS Institute Inc., Cary, NC, US). For regression analyses, urinary

phthalate metabolite concentrations were log-transformed because of their right-skewed distributions. In full statistical models, covariates such as age, pre-pregnancy BMI (continuous variables), education (categorical: less than or equal to high school graduate and greater than or equal to undergraduate school), and income level (categorical: low, low-middle, middle, upper middle, and high) were included, based on previous reports (Kobrosly et al., 2012; Rodríguez-Carmona et al., 2019). To determine the differences among the trimesters, Wilcoxon signed-rank test was used because of the abnormally distributed data. For this analysis, SAS 9.4 was employed. Statistical significance was defined as $p < 0.05$. For multiple comparison, the Bonferroni adjusted p value was used. Intraclass correlation coefficients (ICCs) for the three trimesters were calculated as the ratio of the between-person variance to the total (between+within) variance employing the Statistical Package for Social Sciences for Windows version 25 (SPSS, IBM, Armonk, NY).

3.3 Results

3.3.1 Characteristics of the study population

Average age (± SD) in Korean and Thai pregnant women were 31.6 (± 3.3) and 27.4 (± 5.2) years, respectively. The pre-pregnancy BMI ranged between 17.2 kg/m^2 and 38.8 kg/m^2 for Korean pregnant women, and between 15.4 kg/m^2 and 30.9 kg/m^2 for Thai women (Table 3-5). For income, we used categorical variables after adjustment of the relative income levels for each country because the absolute income level is different between the two countries. Between two populations, several characteristics such as age, alcohol consumption, education, and income levels of participants, were significantly different (Table 3-5).

Table 3-5. Characteristics of the participating women by country.

Country	**Korea**	**Thailand**	***P***
Characteristic	**N (%)**	**N (%)**	**value**
Total	81	102	
Age (years) mean±SD (range)	**31.6±3.3 (26, 42)**	**27.4±5.2 (17, 39)**	**<0.000***
<30	15 (23.4)	69 (67.6)	
≥30	49 (76.6)	33(32.4)	
No response	20	0	
Stages of pregnancy, N (mean±SD)			-
1st trimester (T1)	63 (10.5±2.0)	76 (9.8 ± 2.7)	
2nd trimester (T2)	57 (23.8 ± 3.7)	96 (25.2 ± 2.7)	
3rd trimester (T3)	59 (35.3 ± 2.8)	102 (32.8 ± 1.8)	
Pre-pregnancy BMI (kg/m^2), mean±SD (range)	21.6±3.5 (17.2, 38.8)	21.8±3.1 (15.4, 30.9)	0.285
Underweight (<18.5)	8 (12.5)	12 (12.2)	
Normal weight(18.5 to <23)	41 (64.1)	56 (57.1)	
Overweight (23 to <25)	7 (10.9)	17 (17.4)	
Obese (>25)	8 (12.5)	13 (13.2)	
No response	17	4	
Smoking status during pregnancy			0.4179
Yes	1 (1.6)	0 (0.0)	
No	63 (98.4)	89 (100)	
No response	17	13	
Alcohol consumption during pregnancy			**0.030***
Yes	2 (3.1)	14 (14.1)	
No	62 (96.9)	85 (85.9)	
No response	17	3	
Education			**<0.000***
Less than high school	0 (0.0)	31 (30.4)	
High school or equivalent	5 (7.7)	33 (32.4)	
Undergraduate degree (including college)	48 (73.9)	36 (35.3)	
More than a graduate school	12 (18.5)	2 (2.0)	
No response	16	0	

Table 3-5. (*Continued*)

Country	**Korea**	**Thailand**	***P***
Characteristic	**N (%)**	**N (%)**	**value**
Total	81	102	
Income level[a]			**<0.000***
Low income	2 (3.2)	10 (10.3)	
Lower middle income	11 (17.5)	45 (46.4)	
Middle income	18 (28.6)	25 (25.8)	
Upper middle income	14 (22.2)	11 (11.3)	
High income	18 (28.6)	6 (6.2)	
No response	18	5	

[a] Low income: < 2 million KRW/ < 10,000 THB, Lower middle income: < 4 million KRW/ < 20,000 THB, Middle income: < 6 million KRW/ < 30,000 THB, Upper middle income: < 10 million KRW/ < 50,000 THB, High income: > 10 million KRW/ > 50,000 THB (KRW: Korea Won, THB: Thailand Bah). For comparing two countries, statistical analysis was conducted by the Wilcoxon rank-sum test for continuous variables, age, and pre-pregnancy BMI, and the Fisher's exact test for categorical variables with expected frequency less than 5, i.e., smoking status, alcohol consumption, education, income.

3.3.2 Urinary concentrations of target metabolites and potential exposure sources

In both countries, MMP, MEP, MiBP, MnBP, MBzP, metabolites of DEHP (MEHP, MEOHP, MEPHHP, MECPP, and MCMHP), and OH-MiNP were detected in ≥ 70% of the urine samples (SG-adjusted), regardless of trimester (Table 3-7). OH-MINCH, a secondary metabolite of DINCH, was detected in 44 - 73% of the urine samples. However, mono-isopropyl phthalate (MiPP), mono-n-pentyl phthalate (MPeP), monocyclohexyl phthalate (MCHP), monohexyl phthalate (MHxP), monooctyl phthalate (MnOP), mono-isononyl phthalate (MiNP), mono-isodecyl phthalate (MiDP), MEHTP, and MINCH were detected in < 40% of the urine (Table 3-6). Korean women showed higher concentrations of MMP, MiBP, MEHP, MEOHP, and MEHHP in the urine samples, compared to the Thai women (Table 3-7). The detection frequency and median level of cx-MiDP were higher in Korea. On the other hand, MEP and mono(3-carboxypropyl) phthalate (MCPP) concentrations were detected at significantly higher concentrations in the urine of Thai women (Table 3-7). The ratio of DINCH to DEHP metabolites (molar concentrations) was significantly higher in the urine of Thai women than that of Korean women (Fig. 3-2).

Several factors were identified to be associated with higher or lesser levels of phthalate metabolites in urine samples (Tables 3-8 and 3-9). Among the behavior factors, moping the floor and hand washing were identified to be negatively correlated with urinary MMP and MEP in Korean women.

Frequencies of electronic mosquito repellent incense use were positively correlated with urinary metabolites of DEHP in Korean women. The use of optical media such as CD and DVD was positively correlated with most of metabolites of phthalate in Thai women.

Table 3-6. Detection frequency (DF) of the test metabolites which exhibited low detection frequency (< 40%) in the urine samples in Korean (T1: n = 63, T2: n = 57, T3: n = 59) and Thai (T1: n = 76, T2: n = 96, T3: n = 102) pregnant women.

Parent compound	Metabolite	Stages	Korea	Thailand
			DF (%)	DF (%)
DiPP	MiPP	T1	15.9	1.3
		T2	10.5	2.1
		T3	10.2	4.9
DPeP	MPeP	T1	3.2	25.0
		T2	7.0	38.5
		T3	0.0	20.6
DCHP	MCHP	T1	3.2	0.0
		T2	6.5	0.0
		T3	0.0	1.0
DHxP	MHxP	T1	27.0	19.7
		T2	14.0	10.4
		T3	10.2	5.9
DnOP	MnOP	T1	0.0	0.0
		T2	0.0	0.0
		T3	0.0	0.0
DiNP	MiNP	T1	0.0	0.0
		T2	0.0	1.0
		T3	0.0	0.0
DiDP	MiDP	T1	3.2	1.3
		T2	5.3	0.0
		T3	22.0	0.0
DEHTP	MEHTP	T1	6.3	9.2
		T2	24.6	4.2
		T3	33.9	5.9
DINCH	MINCH	T1	14.3	7.9
		T2	22.8	13.5
		T3	16.9	12.7

Table 3-7. Concentrations of plasticizer metabolites in urine samples of pregnant women by trimester (SG-adjusted, ng/ml), and comparison between Korea (T1: n = 63, T2: n = 57, T3: n = 59) and Thailand (T1: n = 76, T2: n = 96, T3: n = 102).

Parent compound	Metabolite	Stages	Korea					Thailand					*P*
			DF(%)	GM	25th	50th	75th	DF (%)	GM	25th	50th	75th	
DMP	MMP	A11	98.4	4.3	2.5	5.0	7.7	98.2	2.5	1.6	2.6	4.0	-
		T1	**97.1**	**5.0**	**3.3**	**5.5**	**8.6**	97.4	2.8	1.7	3.0	4.4	**0.007**
		T2	98.2	3.2	2.0	4.1	6.2	99.0	2.4	1.5	2.4	4.0	0.184
		T3	**100**	**4.8**	**2.5**	**4.8**	**7.7**	98.0	2.5	1.5	2.4	3.9	**0.009**
DEP	MEP	A11	99.5	10.2	3.4	7.0	17.8	100	25.2	7.8	23.9	62.4	-
		T1	98.5	9.5	3.4	6.6	11.8	**100**	**33.0**	**10.9**	**24.5**	109.6	**0.000**
		T2	100	8.6	3.1	7.1	17.8	**100**	**23.1**	**7.5**	**19.7**	65.6	**0.017**
		T3	100	13.0	3.6	7.1	25.2	100	22.3	6.8	24.4	58.8	0.073
DiBP	MiBP	A11	100	10.4	4.4	8.7	18.9	96.0	4.1	2.3	4.4	8.7	-
		T1	**100**	**9.7**	**4.8**	**8.7**	**18.9**	94.7	4.1	2.0	4.1	8.2	**0.001**
		T2	**100**	**9.1**	**3.6**	**6.8**	**18.5**	95.8	3.7	1.8	3.5	6.8	**<.000**
		T3	**100**	**12.4**	**4.6**	**9.8**	**25.2**	97.1	4.4	2.4	4.7	7.2	**<.000**
DnBP	MnBP	A11	100	14.3	11.0	14.9	21.6	100	12.2	6.0	11.3	21.1	-
		T1	100	16.2	12.2	16.5	24.3	100	12.2	7.0	12.2	19.7	0.454
		T2	100	13.5	10.0	14.3	21.3	100	10.9	5.8	10.0	22.4	0.052
		T3	100	13.3	9.6	13.3	16.9	100	13.5	8.3	12.8	25.2	0.490
BBzP	MBzP	A11	97.8	2.0	1.0	1.8	3.9	94.9	3.3	1.1	3.1	7.7	-
		T1	100	2.7	1.3	2.1	5.1	94.7	3.0	1.0	2.7	7.1	0.3553
		T2	96.5	1.9	0.9	1.7	3.5	97.9	2.9	0.7	2.7	9.2	0.4111
		T3	96.6	1.7	0.9	1.6	3.1	**92.2**	**4.0**	**1.4**	**3.8**	**10.8**	0.0343
DEHP	MEHP	A11	96.7	2.7	1.4	2.8	5.0	81.8	1.3	0.7	1.5	3.0	-
		T1	**94.1**	**3.3**	**1.7**	**3.8**	**5.6**	78.9	1.2	0.7	1.3	2.0	**0.000**
		T2	**98.2**	**2.7**	**1.9**	**2.8**	**4.3**	83.3	1.4	0.7	1.6	2.6	**<.000**
		T3	**98.3**	**2.1**	**1.1**	**2.0**	**4.6**	82.4	1.3	0.6	1.3	2.5	**0.000**
	MEOHP	A11	99.5	7.0	4.3	6.6	10.9	97.8	3.7	2.0	3.8	6.6	-
		T1	**98.5**	**7.7**	**4.9**	**7.6**	**11.5**	94.7	2.9	1.6	3.2	5.0	**<.000**
		T2	**100**	**6.3**	**4.0**	**6.1**	**9.0**	100	3.8	2.2	4.2	6.4	**0.000**
		T3	**100**	**7.0**	**4.3**	**6.5**	**11.4**	98.0	4.2	2.5	4.2	7.9	**0.003**

Table 3-7. (*Continued*)

Parent compound	Metabolite	Stages	Korea					Thailand					*P*
			DF(%)	GM	25th	50th	75th	DF (%)	GM	25th	50th	75th	
DEHP (*continued*)	MEHHP	A11	100	11.8	7.1	11.4	18.8	98.9	5.6	2.8	5.4	9.1	-
		T1	**100**	**14.5**	**10.6**	**14.3**	**23.4**	97.4	5.3	3.1	5.5	8.9	**<.000**
		T2	**100**	**10.4**	**6.7**	**9.6**	**17.1**	100	5.5	3.1	6.0	9.0	**<.000**
		T3	**100**	**10.5**	**6.0**	**10.1**	**16.9**	99.0	5.9	3.2	6.6	10.8	**0.004**
	MECPP	A11	100	16.0	10.3	15.4	23.0	100	10.0	5.5	8.8	14.9	-
		T1	**100**	**18.3**	**11.7**	**19.2**	**27.4**	100	9.5	6.0	9.1	13.9	**<.000**
		T2	**100**	**14.2**	**9.6**	**14.5**	**19.7**	100	9.7	6.0	9.8	14.9	**0.001**
		T3	**100**	**15.2**	**9.7**	**14.1**	**20.7**	100	10.7	6.4	11.0	16.4	**0.016**
	MCMHP	A11	99.5	9.6	6.5	10.1	14.0	98.9	8.6	4.8	8.2	13.2	-
		T1	**100**	**11.4**	**8.0**	**11.6**	**17.6**	100	7.8	5.7	8.1	11.7	**0.009**
		T2	100	8.8	6.5	9.3	13.3	96.9	8.1	5.5	8.7	13.4	0.772
		T3	98.3	8.5	5.8	8.9	13.1	100	9.6	5.9	9.7	14.5	0.114
DnOP	MCPP	A11	84.2	1.4	0.5	1.9	5.4	93.4	3.6	2.0	3.6	6.9	-
		T1	94.1	2.2	1.3	2.3	5.3	**93.4**	**5.4**	**2.7**	**5.3**	12.5	**<.000**
		T2	87.7	1.1	0.3	1.1	3.4	**92.7**	**3.1**	**1.7**	**3.4**	6.8	**0.010**
		T3	69.5	1.2	0.1	1.9	6.3	**94.1**	**3.2**	**1.9**	**3.7**	6.1	**0.006**
DiNP	OH-MiNP	A11	97.8	0.7	0.4	0.7	1.2	96.4	0.8	0.4	0.8	1.4	-
		T1	97.1	0.8	0.5	0.8	1.2	90.8	0.9	0.5	0.7	1.7	0.344
		T2	98.2	0.7	0.4	0.7	1.3	97.9	0.8	0.4	0.9	1.3	0.795
		T3	98.3	0.6	0.4	0.6	0.9	**99.0**	**0.8**	**0.5**	**0.8**	1.4	**0.016**
DiDP	cx-MiDP	A11	94.6	0.7	0.3	1.0	1.5	61.3	-	<LOD	0.1	0.1	-
		T1	95.6	0.5	0.2	0.8	1.7	61.8	-	<LOD	0.1	0.1	-
		T2	93.0	1.0	0.6	1.2	1.6	56.3	-	<LOD	0.1	0.1	-
		T3	94.9	0.5	0.1	0.8	1.4	65.7	-	<LOD	0.1	0.1	-
DINCH	OH-MINCH	A11	67.4	-	<LOD	0.2	0.4	44.9	-	<LOD	<LOD	0.5	-
		T1	73.5	-	<LOD	0.2	0.6	47.4	-	<LOD	<LOD	0.5	-
		T2	54.4	-	<LOD	0.2	0.3	43.8	-	<LOD	<LOD	0.5	-
		T3	64.4	-	<LOD	0.2	0.4	44.1	-	<LOD	<LOD	0.5	-

Table 3-7. (*Continued*)

Boldface p-value indicates statistical significance ($p < 0.05$), and boldface values represents significantly higher concentration compared with the other country. Plasticizer metabolites concentrations were log-transformed for statistical comparison. Adjusted for age, pre-pregnancy BMI, education and income. Stages of pregnancy were classified as 1st trimester (T1), 2nd trimester (T2), and 3rd trimester (T3).

Table 3-8. Spearman correlations between SG-corrected concentrations of phthalate metabolites in urine samples and potential exposure-related sources in Korean women.

	Behavior			personal care products									Foods	
ρ	Moping the floor	Aircleaner	Hand washing	Electronic mosquito coils	Lotion /Skin	Body lotion /hand cream	Perfume	Nail polish	Bath products	Anti-bacterial soap	Liquid soap	Optical media (CD and DVD)	plastic container for food	Eat out
N	64	61	64	63	64	63	63	63	64	64	64	63	64	64
MMP	0.15	0.02	0.05	0.10	-0.03	-0.08	-0.24	-0.16	0.14	**0.26**	-0.09	0.11	0.07	-0.23
MEP	**-0.32**	-0.02	**-0.26**	0.17	-0.09	0.00	0.11	-0.01	-0.09	0.00	-0.09	-0.04	-0.06	-0.04
MiBP	0.09	0.15	-0.20	0.16	-0.19	-0.13	**-0.28**	0.06	-0.05	-0.01	-0.23	0.24	-0.18	-0.05
MnBP	-0.05	0.02	-0.12	0.13	**-0.28**	-0.20	-0.23	0.08	-0.11	-0.08	-0.11	0.01	-0.08	-0.12
MBzP	-0.13	-0.01	-0.18	0.13	-0.15	-0.15	-0.23	-0.02	**-0.25**	-0.17	-0.15	-0.04	-0.18	-0.23
MEHP	-0.01	0.04	0.13	0.22	**-0.28**	-0.24	-0.23	-0.11	-0.16	-0.22	-0.01	0.11	-0.06	0.00
MEOHP	0.00	0.08	0.04	**0.32**	**-0.25**	-0.19	**-0.26**	0.03	-0.20	-0.13	0.01	0.05	0.01	0.00
MEHHP	-0.01	0.07	0.13	**0.38**	-0.21	-0.12	-0.20	0.04	-0.18	-0.11	0.05	0.08	0.04	0.03
MECPP	-0.08	0.08	0.01	**0.36**	**-0.29**	-0.18	-0.20	0.08	-0.22	-0.22	-0.01	-0.01	0.02	0.12
MCMHP	-0.02	0.07	-0.06	**0.32**	-0.22	-0.07	-0.23	0.00	-0.17	-0.18	-0.10	0.02	0.02	0.02
MCPP	-0.18	-0.01	-0.05	0.21	**-0.39**	**-0.29**	-0.18	0.04	-0.20	**-0.32**	-0.13	-0.22	-0.10	**-0.26**
OHMiNP	-0.14	0.17	0.00	0.19	-0.11	-0.02	-0.02	0.00	-0.09	-0.08	-0.21	-0.15	-0.20	-0.03
cxMiDP	0.09	0.20	0.16	0.04	-0.12	0.12	0.02	-0.14	-0.01	0.10	-0.06	0.12	-0.20	-0.06

Boldface values indicate statistical significance ($p < 0.05$).

Table 3-9. Spearman correlations between SG-corrected concentrations of phthalate metabolites in urine samples and potential exposure-related sources in Thai women.

	Behavior			personal care products									Foods	
ρ	Moping the floor	Aircleaner	Hand washing	Electronic mosquito coils	Lotion /Skin	Body lotion /hand cream	Perfume	Nail polish	Bath products	Anti-bacterial soap	Liquid soap	Optical media (CD and DVD)	plastic container for food	Eat out
N	101	89	100	92	102	101	100	102	102	93	97	100	102	101
MMP	**-0.21**	-0.04	-0.07	0.09	-0.02	0.05	-0.10	0.05	0.17	0.05	-0.01	**0.25**	-0.05	0.05
MEP	0.07	-0.04	0.09	0.03	0.14	**0.20**	0.17	0.15	0.03	0.01	0.02	0.05	0.10	0.17
MiBP	0.09	-0.01	-0.05	0.03	0.10	0.13	0.08	**0.21**	**0.24**	-0.06	**0.23**	**0.24**	0.08	**0.37**
MnBP	-0.03	-0.07	-0.09	-0.01	-0.08	-0.01	0.06	0.18	0.11	0.00	-0.01	**0.21**	0.14	**0.24**
MBzP	-0.19	**-0.22**	-0.17	0.06	-0.02	-0.01	-0.13	0.12	-0.01	-0.05	-0.09	0.10	**0.27**	0.13
MEHP	-0.03	-0.12	-0.17	0.01	-0.05	-0.03	-0.08	0.11	0.07	0.02	-0.09	0.15	-0.05	0.07
MEOHP	0.01	-0.08	-0.15	-0.02	-0.05	0.05	-0.07	0.15	0.07	-0.02	-0.09	**0.20**	0.05	0.14
MEHHP	0.02	-0.06	-0.16	0.01	-0.01	0.09	-0.04	0.16	0.10	-0.04	-0.06	**0.21**	0.04	0.16
MECPP	0.01	-0.03	-0.17	-0.04	-0.10	0.02	-0.07	0.14	0.06	0.07	-0.15	**0.20**	0.09	**0.23**
MCMHP	-0.01	-0.04	-0.12	0.06	0.03	0.05	-0.16	0.10	0.08	-0.14	-0.04	**0.23**	0.17	**0.27**
MCPP	0.02	-0.13	0.06	-0.13	-0.14	-0.11	-0.04	0.10	0.07	-0.06	-0.09	0.05	0.08	0.07
OHMiNP	-0.03	-0.04	-0.08	0.06	-0.08	0.02	-0.04	0.18	0.06	-0.12	-0.05	**0.24**	0.20	**0.25**
cxMiDP	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Boldface values indicate statistical significance ($p < 0.05$).

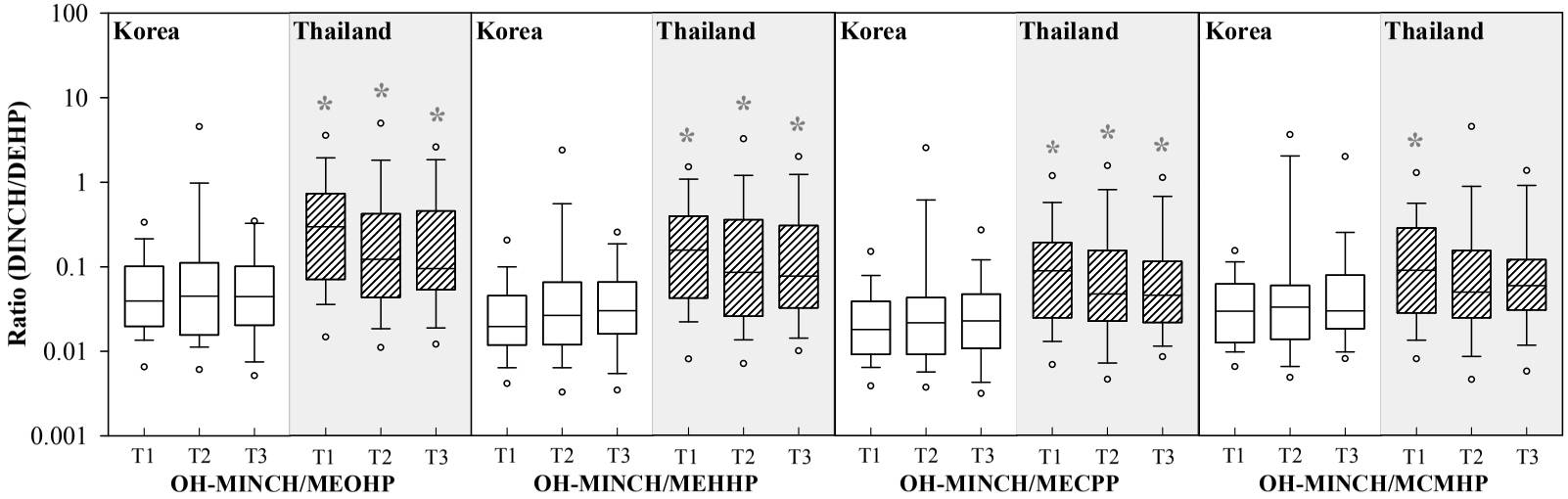


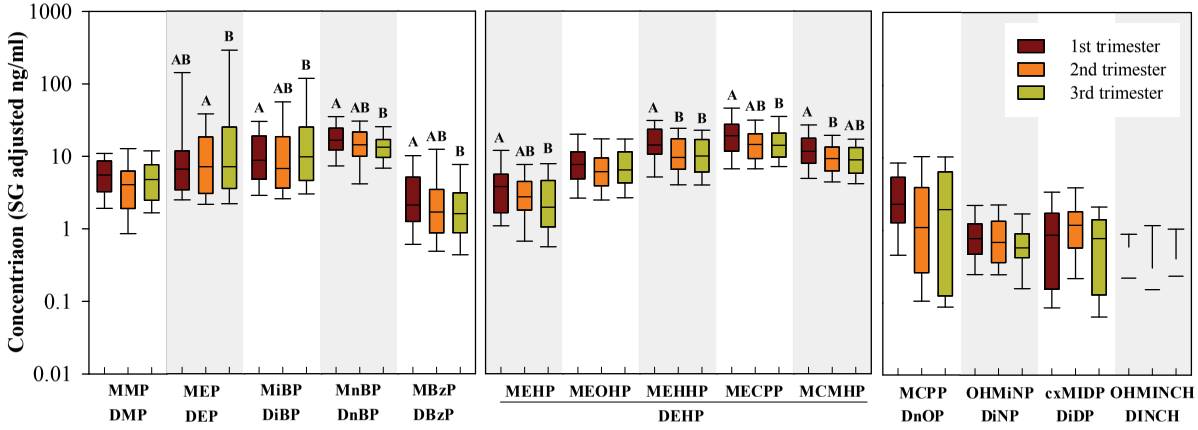
Fig. 3-2. Comparison of the ratio of DINCH to DEHP metabolites (concentration unit: mol/L) between Korean (T1: n = 46, T2: n = 32, T3: n = 40) and Thai (T1: n = 36, T2: n = 51, T3: n = 48) pregnant women (except < LOD values). * indicates a significant higher ratio between Korea and Thailand for a given trimester. Statistical analysis was conducted by the Wilcoxon signed-rank test ($p<0.05$).

3.3.3 Comparison of urinary metabolite concentrations by trimester

The concentrations of urinary metabolites (SG-adjusted) measured in each trimester are shown in Fig. 3-3. Over the course of pregnancy, the levels of most metabolites varied (lower ICC) among Korean women (Table 3-10). Among Thai women, moderate ICC, e.g., > 0.5, was observed for most metabolites such as MMP, MEP, MiBP, MBzP, MEHP, MEOHP, and MEHHP (Table 3-10).

Among Korean women, the MEP and MiBP concentrations were significantly higher in the T3 urine compared to those collected in T1 and T2 (Fig. 3-3). In contrast, MnBP, MBzP, and most DEHP metabolites were significantly lower in the urine of T2 or T3 compared to those of T1. In Thai women, the MEP concentration was significantly lower in the urine of T3, and the MEOHP concentration was significantly higher in the urine of T3. The ratio of MEHP to the sum of DEHP metabolites was significantly lower but that of MEOHP was significantly higher in T3 urine samples in both countries (Fig. 3-3).

(A) Korean pregnant women



(B) Thai pregnant women

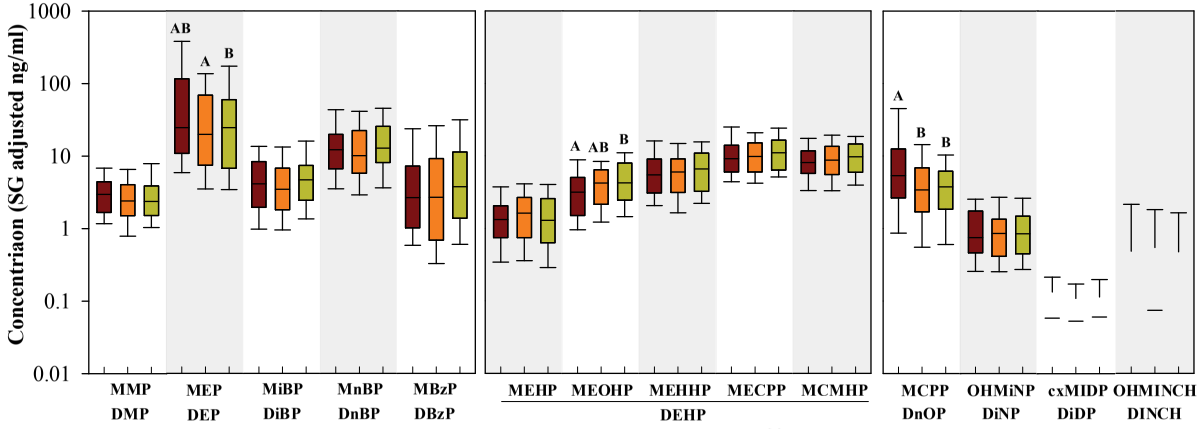


Fig. 3-3. Comparison of urinary plasticizer metabolites concentration by trimesters: (A) Korean pregnant women (T1: n = 63, T2: n = 57, T3: n = 59), and (B) Thai pregnant women (T1: n = 76, T2: n = 96, T3: n = 102). To determine the statistical difference among trimesters, the Wilcoxon signed-rank test was conducted. Statistical significance was defined at $p < 0.0167$, after adjustment by the Bonferroni correction (0.05/n, n = 3; number of hypothesis). The median is depicted as a line splitting the box and the edge of the box indicated the interquartile range (IQR, Q1 and Q3). The ends of the whiskers represented the maximum and minimum of the data (Q1-1.5*IQR, Q3+1.5*IQR). Metabolites with low detection frequency were not shown.

Table 3-10. Intraclass correlation coefficients (ICC) of urinary metabolite concentrations measured across trimesters in Korean and Thai pregnant women.

Parent compound	**Metabolite**	**Korean Pregnant woman (n = 29)**	**Thai Pregnant woman (n = 70)**
DMP	MMP	-0.074	**0.709****
DEP	MEP	0.201	**0.501****
DiBP	MiBP	0.247	**0.780****
DnBP	MnBP	-0.172	**0.316***
BBzP	MBzP	0.210	**0.505****
DEHP	MEHP	0.332	**0.540****
	MEOHP	0.230	**0.541****
	MEHHP	0.155	**0.509****
	MECPP	0.281	**0.561****
	MCMHP	0.350	0.115
DnOP	MCPP	-0.018	0.030
DiNP	OH-MiNP	0.362	**0.314***
DiDP	cx-MiDP	0.146	-
DINCH	OH-MINCH	-	-

* $P < 0.05$, ** $p < 0.01$

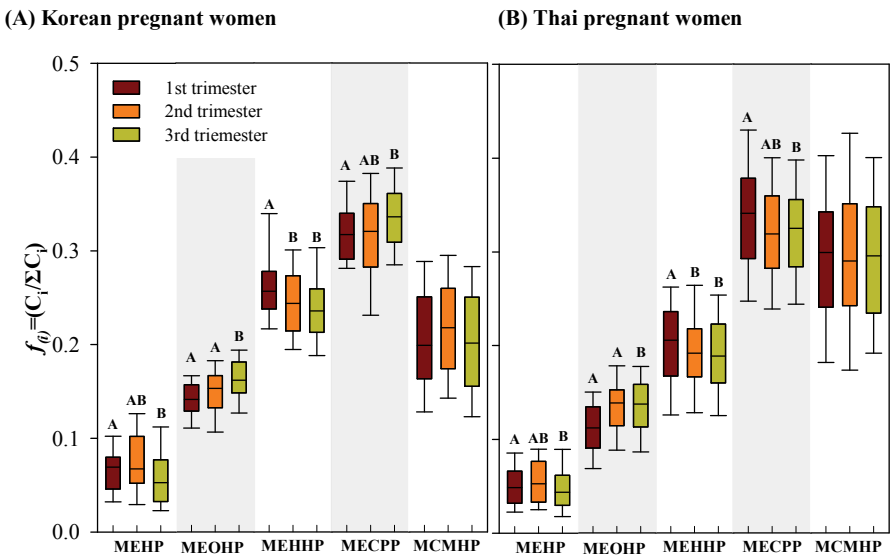


Fig. 3-4. Comparison of the percentage of each DEHP metabolite in different trimester of pregnant women in Korea (T1: n = 63, T2: n = 57, T3: n = 59) and Thailand (T1: n = 76, T2: n = 96, T3: n = 102). $f_{(i)} = C_i/\Sigma C_i \times 100$ % (C_i: molar concentration (mol/L) of the individual monoesters). To determine the statistical difference among trimesters, the Wilcoxon rank-sum test was conducted. Statistical significance was defined at $p < 0.0167$ after adjustment by the Bonferroni correction (0.05/n, n = 3; number of hypothesis).

3.3.4 Risk assessment

Based on the calculated HIs, up to 11.9% of the women were determined to be at potential risk due to exposure to phthalates. By trimester, 7.9% (T1), 10.5% (T2), and 11.9% (T3) of Korean women were found to be at risk, i.e., HI>1. In Thai women, 5.3% (T1), 4.2% (T2), and 3.9% (T3) of the women were identified at risk (Fig. 3-5). Among the measured phthalates, DiBP, DEHP, and DnBP were identified as the most important contributors of the risk, explaining 41.8%, 35.3%, and 20.4% of the HI in Korean pregnant women. In Thai pregnant women, DnBP, DEHP, and DiBP contribute 41.4%, 37.8%, and 14.8% of the HI, respectively (Fig. 3-5).

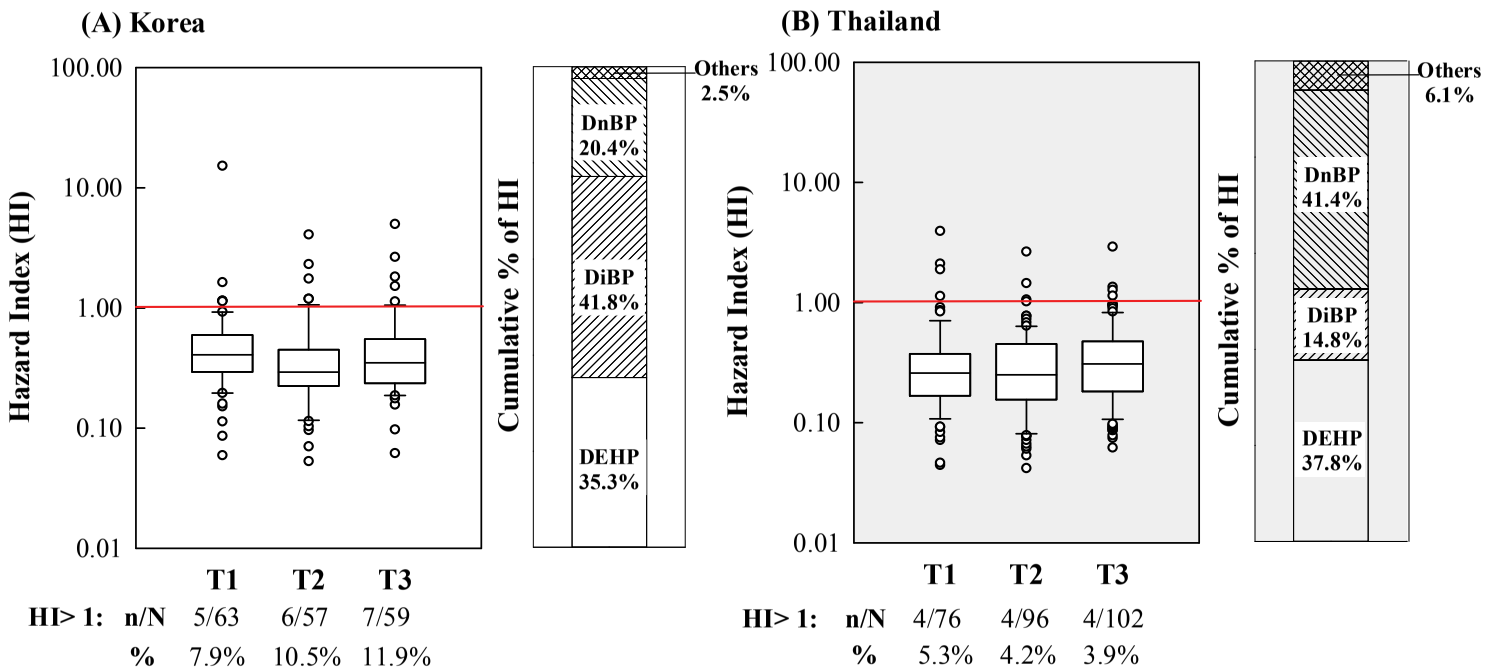


Fig. 3-5. Hazard Index (HI) calculated for measured phthalates, i.e., DMP, DEP, DiBP, DnBP, BBzP, DEHP, DiNP and DINCH, in (A) Korean and (B) Thai women. Contribution of major phthalates to HI is shown in cumulative bar graphs (right). others: contribution from exposure to DMP, DEP, BBzP, DiNP, and DINCH.

3.4 Discussion

The present study reports the exposure profiles of major phthalates and alternative plasticizers across three trimesters in pregnant women of Korea and Thailand. Our results show that variations in urinary metabolite levels by trimester are different by country. In addition, the levels of urinary phthalate metabolites were clearly different by country. Our observations highlight the importance of source identification and exposure mitigation measures specific to each country.

3.4.1. Difference in urinary metabolite levels by trimester

Several metabolites showed significantly different levels by trimester, while some difference was observed by country (Fig. 3-3). In particular, among Korean women, urinary MEHP, MEHHP, MECPP, and MCMHP concentrations were significantly lower at T2 or T3 compared to T1 urine, while the opposite pattern was observed for MEOHP among Thai women. This observation suggests that the amount of DEHP exposure decreases over the course of gestation among Korean women, while such change was not notable among Thai women. Similar temporal patterns of DEHP exposure during gestation have been reported in other countries. The urinary levels of MEHHP, MEOHP, and MECPP were lower in T2 compared to T1, and recovered slightly in T3 urine in China (n = 847) and the USA (n =482) (Ferguson et al., 2014; 2016, Zhao et al., 2018). Another study also reported a similar U-shaped trend across the three trimesters during pregnancy (Li et al.,

2019). Relatively higher level of DEHP metabolites in T1 urine of Korean pregnant women warrants caution, because early stage of pregnancy is considered as vulnerable window of phthalate exposure. Exposure to anti-androgenic chemicals in the first trimester may be associated with permanent adverse outcomes such as hypospadias and cryptorchidism (Martino-Andrade et al., 2016; Swan et al., 2015).

A recent study that reported different DEHP metabolite levels during gestation suggested that physiological changes that occur during pregnancy might lead to different metabolic activities and finally explain different levels of DEHP metabolites in the urine during the gestation period (Zhao et al., 2018). The $f_{(MEHP)}$ and $f_{(MEHHP)}$ were significantly decreased at T3, but the $f_{(MEOHP)}$ was significantly increased at T3 in both countries, indicating that the metabolism of DEHP was more effective in T3 than in T1 or T2 (Fig. 3-4). Our observation, which showed a similar pattern of change in the relative composition of each DEHP metabolite by trimester, may in part support the hypothesis of changing metabolic activities during pregnancy. The fact that the levels of DEHP metabolites in Thai women, however, showed virtually negligible differences by trimester also implies that the concentrations of DEHP metabolites in different trimesters may simply reflect the different patterns of exposure over time.

The higher ICC values generally observed for Thai women suggest that exposure to phthalates is relatively consistent over the course of gestation in Thai women. In contrast, in Korean women, greater variation of phthalate exposure during pregnancy was observed. Studies have shown that ICCs

derived for phthalate metabolites over the course of gestation varied by population. In the USA, the urinary levels of MnBP (ICC = 0.45) and MEP (ICC = 0.50) in pregnant women were relatively stable, while those of MBzP (ICC = 0.25) and ΣDEHP (ICC = 0.08) were not (Braun et al., 2012). Additional studies are needed to further identify the main causes of this variation in Korean pregnant women population.

3.4.2. Difference in urinary metabolite levels by country

Most frequently detected phthalate metabolites in the present study, i.e., DnBP, BBzP, and DEHP, were generally comparable to those frequently reported in other populations (Lin et al., 2011; Shapiro et al., 2015). However, the levels of major phthalate metabolites were clearly different between Korean and Thai women. The levels of DMP, DiBP, and DEHP metabolites were significantly higher in Korean women than in Thai pregnant women, while those of MEP and MCPP were two- to three-fold higher in Thai pregnant women, after adjustment for relevant covariates by ANCOVA (Table 3-7). The different level of exposure between countries may be partially explained by different exposure sources for each population. Food or non-food consumption has been suggested as the major source of exposure to DEHP and DiBP, respectively (Koch et al., 2013b), while indoor air has been associated with exposure to DMP (Wormuth et al., 2006). Dermal exposure by the use of personal care products is known to be an important exposure

source of DEP (Koniecki et al., 2011), and the use of sun cream has also been associated with DnOP exposure (Lee et al., 2019b).

Urinary DEHP metabolite levels among Korean pregnant women are similar or even higher than those of the USA, Puerto Rico, and China (Li et al., 2019; Martino-Andrade et al., 2016; Sun et al., 2018), but much lower than those of Mexican and Spanish pregnant women (Valvi et al., 2015; Watkins et al., 2017) (Table 3-11). The urinary MEP concentrations of Thai pregnant women are not higher than those of the USA, Canada, Mexico, and Spain (Fisher et al., 2015; Martino-Andrade et al., 2016; Valvi et al., 2015; Watkins et al., 2017), but are two to three times higher than those of Chinese pregnant women (Li et al., 2019; Sun et al., 2018) (Table 3-11). Further studies are warranted to identify major sources and pathways of exposure to these phthalates among pregnant women of both countries.

OH-MINCH, one of the metabolites of DINCH, was detected in more than two-thirds (67.4%) of Korean women and approximately a half (44.9%) of Thai women (Table 3-7). The level of DINCH metabolite (median 0.2 ng/ml in Korean pregnant women) are generally comparable to those reported in other populations, including Israeli pregnant women (median of 0.6ng/mL), the German general population (median of 0.39ng/mL), and the Norwegian general population (median of 0.25ng/mL) (Giovanoulis et al., 2016; Machtinger et al., 2018; Schütze et al., 2014). Our observations suggest that DINCH has been used by our pregnant women in both countries. The ratio of DINCH to DEHP metabolites, which was significantly higher among Thai women (Fig. 3-2), suggests that even though the absolute level of OH-

MINCH was lower among Thai women, the extent of replacement of DEHP with DINCH might be greater in Thailand. DINCH was introduced into the world market in 2002, but the timing of introduction and amounts of use may vary by country. Because the use of other alternative plasticizers, including adipates and terephthalates, should be different by country, further effort is warranted to profile patterns of exposure to major alternative plasticizers.

3.4.3. Risk assessment of phthalate exposure during pregnancy

Population at risk who showed HI>1 in the present study, i.e., 11.9% (Korean women) and 5.3% (Thai women), was generally lower than other populations, including French pregnant women (approximately 30%), Belgian children (25%), and Chinese young adults (39.8%), but similar to Belgian adults (6.2%) (Dewalque et al., 2014; Gao et al., 2016; Zeman, et al., 2013). In Korean pregnant women, the contributions of DiBP, DEHP, and DnBP were major, i.e., explaining 41.8%, 35.3% and 20.4% of HI, respectively (Fig. 3-5). Among Thai women, DnBP was the most important risk contributor (41.4%), followed by DEHP (37.8%). Similarly, DEHP, DnBP and DiBP have been reported as major risk contributors in other populations, such as in Belgium, China, Denmark, the USA, and France (Christensen et al., 2014; Dewalque et al., 2014; Gao et al., 2016; Søeborg et al., 2012; Zeman et al., 2013). The risk estimated for DiNP and DINCH was negligible. However, considering the increasing levels of occurrences of alternative plasticizers in both the environment and urine (Bui et al., 2016; Weiss et al., 2018),

continuous monitoring and risk assessment of these alternative plasticizers are warranted.

Risk estimates derived for pregnant women should be interpreted with caution because exposure to phthalates during pregnancy may influence the not only health of exposed women but also their fetuses (Bornehag et al., 2015; Kobrosly et al., 2014; Swan et al., 2005; Valvi et al., 2015). The long-term adverse health consequences of phthalate exposure during pregnancy are not well understood and hence not reflected in their RfDs established for phthalates. Therefore, efforts to identify sources and mitigate exposure during pregnancy are crucial.

Table 3-11. Plasticizer metabolites concentrations in urine samples of pregnant women (SG-adjusted, median, ng/mL).

Country	Sampling year	N	Stages	MEP	MiBP	MnBP	MBzP	MEHP	MEOHP	MEHHP	MECPP	MCPP	OH-MiNP	OH-MINCH	Reference
Korea	2016	63	T1	6.6	8.7	16.5	2.1	3.8	7.6	14.2	18.8	2.3	0.8	0.2	This study
	2016	57	T2	7.1	6.8	14.3	1.7	2.8	6.1	9.6	14.5	1.1	0.7	0.2	This study
	2016	59	T3	7.1	9.8	13.3	1.6	2.0	6.5	10.1	14.1	1.9	0.6	0.2	This study
Thailand	2016	76	T1	24.5	4.1	12.2	2.7	1.3	3.2	5.5	9.1	5.3	0.7	<LOD	This study
	2016	96	T2	19.7	3.5	10.0	2.7	1.6	4.2	6.0	9.8	3.4	0.9	<LOD	This study
	2016	102	T3	24.4	4.7	12.8	3.8	1.3	4.2	6.6	11.0	3.7	0.8	<LOD	This study
China	2014-2015	951	T1	10.6	18.3	52.8	0.1	3.03	5.22	6.74	10.2	-	-	-	Li et al., 2019
		951	T2	7.27	12.1	37.3	0.06	2	3.92	4.57	7.58	-	-	-	
		951	T3	8.05	14.9	54.7	0.07	2.12	4.62	5.69	9.09	-	-	-	
China	2014-2015	553	T1	10.5	16.7	45.4	0.1	3.1	5	6.7	10	-	-	-	Sun et al., 2018
		553	T2	7.4	11.8	35.2	0.1	2.1	3.9	4.6	7.7	-	-	-	
		553	T3	7.9	14.2	56.7	0.1	2	4.1	5.1	8	-	-	-	
China	2013	3294	T1[b,c]	9	48.7	-	0.08	2.5	6.9	5.1	-	-	-	-	Gao et al., 2017
		3186	T2[b,c]	6.8	64.2	-	0.07	6.3	11.2	9.8	-	-	-	-	
		3049	T3[b,c]	5.8	60.9	-	0.05	5	9.3	7.9	-	-	-	-	
China	-	847	T1[a]	-	-	-	-	0.0109	0.0174	0.0228	0.0325	-	-	-	Zhao et al., 2018
		847	T2[a]	-	-	-	-	0.0071	0.0132	0.0153	0.0241	-	-	-	
		847	T3[a]	-	-	-	-	0.0066	0.0146	0.0179	0.0273	-	-	-	
Taiwan	2010	112	T1[b]	20.23	28.36	37.8	2.34	30.37	11.54	16.55	20.63	-	-	-	Huang et al., 2017
		112	T2[b]	25.89	34.52	41.4	2.23	30.66	12.82	15.56	21.83	-	-	-	
		112	T3[b]	36.89	30.17	35.28	2.22	26.27	14.42	15.96	23.13	-	-	-	
USA	2010-2012	168	T1[c]	30.37	3.46	6.04	2.98	1.7	3.4	5.22	8.54	1.86	-	-	Martino-Andrade et al., 2016
		168	T2[c]	28.14	4.03	5.36	2.94	1.41	3.86	4.81	8.51	1.63	-	-	
		168	T3[c]	40.25	5.34	6.98	3.31	1.33	7.28	4.92	9.55	1.69	-	-	

Table 3-11. (*Continued*)

Country	Sampling year	N	Stages	MEP	MiBP	MnBP	MBzP	MEHP	MEOHP	MEHHP	MECPP	MCPP	OH-MiNP	OH-MINCH	Reference
USA	2006-2008	439	Visit1 (4.7-19.1 w)[c]	145	7.66	18.3	7.36	10.6	18.6	34.7	44.4	2.11	-	-	Johns et al., 2016
		439	Visit 2 (14.9-32.1 w)[c]	144	7.14	18.4	7.34	10.9	18.3	34.8	42.6	2.25	-	-	
		439	Visit 3 (22.9-36.3 w)[c]	141	7.45	17.3	7.05	9.46	15.6	27.2	36.8	1.94	-	-	
		439	Visit 4 (33.1-38.3)[c]	156	9.05	19.7	8.03	9.83	20.9	36.6	49.3	2.04	-	-	
USA	2004-2009	100	1T	64	9	26	6.5	12.5	-	-	-	-	-	-	Braun et al. 2012
		100	2T	82	9.6	27	5.8	6.2	-	-	-	-	-	-	
		100	3T	87	11	26	5.9	5.7	-	-	-	-	-	-	
USA	2005-2015	208	T1	32.0	5.8	10.5	2.9	-	-	-	-	3.8	-	-	James-Todd et al., 2018
		209	T2	48.0	6.2	12.0	2.8	-	-	-	-	2.9	-	-	
Puerto Rico	2010-2012	106	Visit 1 (18 ± 2 w)	97.7	10.3	19.3	3.73	3.14	8.33	10.5	20.8	2.08	-	-	Johns et al., 2015
		106	Visit 2 (26 ± 2 w)	83.6	10.9	18.6	3.22	3.08	9.86	11.1	20.8	1.86	-	-	
Canada	2009-2010	90	T2	32.1		20.64	8.75	1.85	5.59	9.15	-	1.45	-	-	Fisher et al., 2015
		90	T3	33		23.5	10	2.1	9.09	13	-	2.05	-	-	
Mexico	1997-2004	107	Visit 1 (9-24 w)[c]	147	1.09	63.6	2.65	4.95	9.43	17.5	31.4	1.12	-	-	Watkins et al., 2017
		109	Visit 2 (19-37 w)[c]	121	0.82	55.4	2.36	4.86	11.3	18.8	34.1	1.07	-	-	
		117	Visit 3 (28-43 w)[c]	115	2.03	55	4.11	5.06	11.7	19.2	31.2	1.06	-	-	

Table 3-11. (*Continued*)

Country	Sampling year	N	Stages	MEP	MiBP	MnBP	MBzP	MEHP	MEOHP	MEHHP	MECPP	MCPP	OH-MiNP	OH-MINCH	Reference
Spain	2014-2015	146-152	T2[b]	47.1	25.2	15.2	3.9	3.2	6	9.2	14.9	-	5.6	-	Warembourg et al., 2019
		146-152	T3[b]	49.1	26.3	15.8	4.1	2.7	5.7	8.3	14.1	-	5.1	-	
Spain	2004-2006	391	T1	202	25.2	26.2	9.5	8.4	17	24.6	32.4	-	1.1	-	Valvi et al. 2015
		391	T3	338	26.2	24.4	8.9	7.9	16.8	24.5	31.4	-	1.2		

[a] nmol/ml
[b] creatinine-adjusted, µg/g creatinine
[c] geometric mean

3.4.4. Limitations and implication

Limited sample size should be considered as a major limitation of the present study. Reduced statistical power resulting from small sample size may lead to difficulties in detecting variability among the trimesters and differences between two countries. In addition, lack of information on potential exposure sources and pathways for the study populations should be noted. Finally, the participants recruited from the hospitals in the capital cities were not representative of the pregnant women of each country, thus findings may lack generalizability. However, the present study provides a new information on temporal variation of phthalates exposure over the course of pregnancy, and identifies priority phthalates among the pregnant women in each country. The results of this study will facilitate follow up studies focusing on exposure assessment for phthalates and their alternatives among pregnant women, and potential health outcomes associated with phthalate exposure during this critical window of susceptibility.

Chapter 4 Variability of urine correction factors during pregnancy: phthalates as a case study

4.1 Introduction

Biomonitoring has been widely utilized for exposure assessment of many environmental chemicals worldwide. Urine is one of the most frequently and widely used biological specimen for biomonitoring of a wide range of many chemicals such as phthalates, phenolic compounds, polycyclic aromatic hydrocarbons, and metals among many. With relevant pharmacokinetic information, e.g., urinary excretion fractions or Fues, one can estimate daily intake amount of a given chemical, e.g., di(2-ethylhexyl) adipate (DEHA) or propylparaben, by measuring its metabolites in the urine sample (Nehring et al., 2019; Shin et al., 2019).

Urinary biomonitoring data are subject to variation due to dilution of urine driven by hydration status of a given individual (Middleton et al., 2019). Therefore, the 24 h urine collection is often employed for exposure assessment, in order to address diurnal variation in hydration status or urinary dilution within a subject. However, because of cost and difficulty of sampling, the 24 h urine collection is not feasible in most biomonitoring studies. For one time or spot urine sample, creatinine, and specific gravity are often used in order to correct urinary dilution. Creatinine is a breakdown product of muscle tissue, and is thought to be excreted in relatively constant excretion rate

through kidneys to urine, depending on age and sex. Studies indicate that urinary creatinine excretion could be influenced by several demographic factors such as age, sex, and BMI. Specific gravity has been also used to correct the urinary dilution, as this is thought to be less influenced by these factors (Sauvé et al., 2015). However, what is the best approach for correcting urinary dilution is still controversial (Boeniger et al., 1993; Heavner et al., 2006; O'Brien et al., 2016; 2017; Yeh et al., 2015).

Another important source of variation in urinary analyte concentrations is changes in toxicokinetics (Sauvé et al., 2015). Normally, intra-individual changes in toxicokinetics that may result in significant variations in urinary concentrations of analytes may not be as big as those of intra-individual. However, during pregnancy remarkable physiological changes in kidney function occur and may lead to significant fluctuations in urinary chemical concentrations. Over the course of pregnancy, the kidneys become larger because of fluid retention, and GFR rises to a peak of 40 – 50% that of pre-pregnancy (Katharine et al., 2013; Soma-Pillay et al., 2016). Moreover, urinary volume also tended to increase during pregnancy (Maikranzetal., 1989). These structural and functional changes of kidney may affect urine flow during pregnancy and factors that have been used to correct urinary dilution, such as the urinary creatinine, specific gravity (SG) and osmolality.

Dramatic changes in renal excretion during pregnancy could directly affect the levels of urinary analytes and may possibly bias the true associations between exposure and health outcomes. A recent study indeed showed that the

associations between chemical exposure and oxidative stress biomarkers in pregnant women were different by the method of urine dilution adjustment (Ferguson et al., 2019). Most of biomonitoring research for pregnant women used spot urinary concentration of analytes following adjusting with creatinine or SG (Huang et al., 2017; James-Todd et al., 2018; Li et al., 2019; Warembourg et al., 2019). Urine osmolality was also suggested as an alternative correction method for urinary dilution (Middleton et al., 2016; Ye et al., 2015). Indeed, previous studies suggested higher reproducibility of specific gravity than that of creatinine of repeat urine sample during pregnancy (Adibi et al., 2008; MacPherson et al., 2018), there is still lack of information on urinary dilution of pregnant women.

Considering the importance of chemical exposure during pregnancy and increasing number of epidemiological studies that involve biomonitoring of chemicals in urine, understanding the variation of the factors for correcting urinary dilution over the course of pregnancy is essential. However, to date, temporal variation in major adjusting factors such as urinary creatinine, specific gravity, and osmolality, has seldom been assessed among pregnant women, especially in Asia. In the present study, we aimed to investigate the variation of creatinine, SG, and osmolality of urine that were collected each trimester during pregnancy. For this purpose, Korean and Thai pregnant women were recruited in Seoul and Bangkok in order to see potential differences in these adjusting parameters due to different ethnicity, climate, and lifestyle. In addition, implication of the variation of these urinary

measurements was demonstrated by showing associated changes in concentrations of major phthalate metabolites in urine after the adjustment. Information obtained from the present study will provide new insight into the exposure assessment of chemicals in pregnant women.

4.2 Materials and methods

4.2.1 Study population and sample collection

Healthy women in their early pregnancy (<14 weeks of gestation) were recruited from two obstetrics and gynecology hospitals in Seoul, Korea and an antenatal care clinic of a health-promoting hospital in Bangkok, Thailand, between May and December 2016. Those with endocrine diseases or pregnancy-related medical histories were excluded from recruitment. In addition, those with <2 times of urine collection were excluded. The final number of pregnant women was 69 in Korea and 102 in Thailand. The participating women were sampled for spot urine for three times during pregnancy, generally one sample in each trimester. The first trimester (T1) was defined as before 14 gestational weeks; the second trimester (T2), between weeks 15 and 28; and the third trimester (T3), from week 29 until term delivery. Urine samples were collected in polypropylene tubes and stored at −40℃ until analysis. In the cases where more than one urine sample was collected in a given trimester, an average of the measurements for the samples was used to represent the level of exposure for the given trimester. The number of cases where more than one sample was collected in a given trimester was 18 (10 and 8 for the second and third trimester, respectively) in Korea and 28 (22 and 6 for the second and third trimester, respectively) in Thailand.

A questionnaire survey was carried out, and demographic and physiological characteristics, such as age, alcohol consumption, smoking status, and body mass index (BMI) before pregnancy and the gestational period, were obtained. The Institutional Review Boards of Seoul National University (SNU), and Mahidol University (MU) approved the study (No. 1604/001-012 for SNU and No. 2016-018-01 for MU), and informed consent was obtained from the participating women. All samples and data were processed blindly.

4.2.2 Measurement and adjustment of urinary correction factors

Three of urinary correction factors were used for adjustment for urine dilution. Urinary creatinine, SG and osmolality were measured by the Jaffe reaction (Modular P800, Roche), by a handheld digital refractometer (Atago Master-SUR/Na), and by freezing point depression method (Advanced ® Model 2020 Multi-sample Osmometer), respectively. Concentrations of urinary phthalate metabolites were adjusted by three urinary correction factors using the following equation, respectively.

Creatinine-adjusted concentrations (μg/g creatinine)

= chemical concentration (ng/ml) / creatinine concentrations (md/dL) × 100

SG-adjusted concentration (ng/ml)

$= \text{chemical concentration (ng/ml)} \times [(SG_{median} - 1)/(SG - 1)]$

Osmolality-adjusted concentrations (ng/ml)

$= \text{chemical concentration (ng/ml)} \times (Osmolality_{median}/Osmolality)$

The median SG values of the samples of each trimester were 1.014 for T1, 1.010 for T2, and 1.010 for T3 in Korea, and 1.007, 1.008, and 1.008 in Thailand. The median osmolality values of the urine samples for each trimester were 654 mOsm/kg for T1, 427 mOsm/kg for T2, and 463 mOsm/kg for T3 in Korea, and 339.5 mOsm/kg, 383.5 mOsm/kg and 315.5 mOsm/kg in Thailand, respectively.

4.2.3 Measurement of urinary phthalates metabolites and health related indicators

Metabolites of major phthalates were analyzed in the urine. These phthalate metabolites included monomethyl phthalate (MMP) of dimethyl phthalate (DMP), monoethyl phthalate (MEP) of diethyl phthalate (DEP), mono-2-isobutyl phthalate (MiBP) of di-2-isobutyl phthalate (DiBP), mono-n-butyl phthalate (MnBP) of di-n-butyl phthalate (DnBP), mono(2-ethylhexyl) phthalate (MEHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), and mono-[(2-carboxymethyl)hexyl] phthalate (MCMHP) of di(2-ethylhexyl) phthalate (DEHP), mono(3-carboxypropyl)

phthalate (MCPP) of dioctyl phthalate (DnOP), and mono-hydroxy-isononyl phthalate (OH-MiNP) of di-isononyl phthalate (DiNP) (Table 4-1). For measurement, ultra-high performance liquid chromatography (UHPLC-MS/MS) was used (UHPLC Nexera X2, Shimadzu Corporation, Kyoto, Japan, and API 4500, AB SCIEX, Ontario, Canada). The UHPLC-MS/MS conditions, multiple reaction monitoring parameters, and information regarding quality assurance and quality control, including the limit of detection (LOD) can be found elsewhere (Lee et al., in press)

Table 4-1. Target plasticizers and their urinary metabolites.

Parent compound	**Chemical formula**	**Molecular weight (g/mole)**	**Metabolite**	**Chemical formula**	**Molecular weight (g/mol)**
Dimethyl phthalate (DMP)	$C_{10}H_{10}O_4$	194.1	Monomethyl phthalate (MMP)	$C_9H_8O_4$	180.2
Diethyl phthalate (DEP)	$C_{12}H_{14}O_4$	222.2	Monoethyl phthalate (MEP)	$C_{10}H_{10}O_4$	194.2
Di-isobutyl phthalate (DiBP)	$C_{16}H_{22}O_4$	278.3	Mono-isobutyl phthalate (MiBP)	$C_{12}H_{14}O_4$	222.2
Di-n-butyl phthalate (DnBP)	$C_{16}H_{22}O_4$	278.3	Mono-n-butyl phthalate (MnBP)	$C_{12}H_{14}O_4$	222.2
Benzyl butyl phthalate (BBzP)	$C_{19}H_{20}O_4$	312.4	Monobenzyl phthalate (MBzP)	$C_{15}H_{12}O_4$	256.3
Di(2-ethylhexyl) phthalate (DEHP)	$C_{24}H_{38}O_4$	390.6	Mono(2-ethylhexyl) phthalate (MEHP)	$C_{16}H_{22}O_4$	278.3
			Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)	$C_{16}H_{22}O_5$	292.3
			Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	$C_{16}H_{22}O_5$	294.3
			Mono(2-ethyl-5-carboxypentyl) phthalate (MECPP)	$C_{16}H_{20}O_6$	308.3
			Mono-[(2-carboxymethyl)hexyl] phthalate (MCMHP)	$C_{16}H_{20}O_6$	308.3
Dioctyl phthalate (DnOP)	$C_{24}H_{38}O_4$	390.6	Mono(3-carboxypropyl) phthalate (MCPP)	$C_{12}H_{12}O_6$	252.2
Di-isononyl phthalate (DiNP)	$C_{26}H_{42}O_4$	422.6	Monohydroxyisononyl Phthalate (OH-MiNP)	$C_{17}H_{24}O_5$	308.4

4.2.4 The Korean National Environmental Health Survey (KoNEHS) 2015-2017

A dataset from the Korean National Environmental Health Survey (KoNEHS) 2015-2017 was used to obtain urinary concentrations of urinary creatinine concentration and SG of the non-pregnant women of matching age. KoNEHS is a nationwide population-based survey of Korea, which has been conducted since 2009, which was designed to determine the exposure level of environmental chemicals among the Korean population (Park et al., 2016). For the present study, only the data from KoNEHS 2015-2017 survey were used because this was the only cycle that measured both urinary creatinine and SG. A total of 249 non-pregnant women were chosen by matching the age and pre-pregnancy BMI. KoNEHS survey was approved by the Research Ethics Committee of the National Institute of Environmental Research (NIER), and all participants provided informed consent.

4.2.5 Statistical analysis

The non-detected values were substituted with the LOD divided by the square root of 2 (Hornung and Reed, 1990). Non-parametric statistical analyses were conducted because of the abnormally distributed data. Wilcoxon signed-rank test was used to determine the significant difference across the trimesters when the urine samples were collected more than two times per each person. The number of participants in Korea was 55 at T1, 53

at T2, 59 at T3, and those of Thailand were 76 at T1, 96 at T2, and 102 at T3. Mann-Whitney U test was used to determine the significant difference between pregnant women and non-pregnant women from KoNEHS.

Spearman's correlation coefficients were estimated between the arithmetic mean of adjusted urinary concentrations of the phthalate metabolites in three trimesters: unadjusted, creatinine adjusted, SG adjusted, or osmolality adjusted. The coefficients were scored on a scale of 1 to 4, 1 with $\rho \geq 0.6$, 2 with $\rho \geq 0.7$, 3 with $\rho \geq 0.8$, and 4 with $\rho \geq 0.9$. The rank-sum was the sum of all phthalate metabolites. Data analysis was performed using SAS 9.4 (SAS Institute Inc., Cary, NC, US). Statistical significance was defined as $p < 0.05$. For multiple comparisons, the Bonferroni adjusted p-value was used. Intraclass correlation coefficients (ICCs) for the three trimesters were calculated as the ratio of the between-person variance to the total (between + within) variance, employing the Statistical Package for Social Sciences for Windows version 25 (SPSS, IBM, Armonk, NY). ICC was calculated only among the participants who had three urine measurements (n = 29 in Korean and n = 70 in Thai pregnant women).

4.3 Results

4.3.1 Characteristics of the study population

The mean ± SD of age in Korean and Thai pregnant women was 31.8 ± 3.3 and 27.4 ± 5.2 years, respectively, which are significantly higher in Korean women (Table 4-2). One of the Korean pregnant women did smoke and approximately 3.8% (Korean women) and 14.1% (Thai women) did drink alcohol during pregnancy. The pre-pregnancy BMI ranged between 17.2 kg/m^2 and 38.8 kg/m^2 in Korea and between 15.4 kg/m^2 and 30.9 kg/m^2 in Thailand, respectively (Table 4-2). The age and (pre-pregnancy) BMI of the non-pregnant Korean women population from KoNEHS were not statistically different from those of pregnant Korean women.

Table 4-2. Characteristics of the target population.

Category	Pregnant women Korea *n* (%)	 Thailand *n* (%)	Non-pregnant women KoNEHS[a] *n* (%)
Total *n*	69	102	249
Age (years) mean±SD (p value)	31.8 ± 3.4 (reference)	27.4 ± 5.2 (<0.000)	32.3 ± 5.8 (0.100)
No response	17	0	0
Stages of pregnancy			
1st trimester (T1)	55	76	-
2nd trimester (T2)	53	96	-
3rd trimester (T3)	59	102	-
(Pre-pregnancy) BMI (kg/m^2)			
p value	(reference)	0.485	0.313
Underweight (<18.5)	7 (13.2)	12 (12.2)	19 (7.6)
Normal weight(18.5 to <23)	32 (60.4)	56 (57.1)	161 (64.7)
Overweight (23 to <25)	6 (11.3)	17 (17.4)	35 (14.1)
Obese (>25)	8 (15.1)	13 (13.3)	34 (13.7)
No response	16	4	0
Present smoking status			
p value	reference	0.373	0.260
Yes	1 (1.9)	0 (0.0)	13 (5.2)
No	52 (98.1)	89 (100)	236 (94.8)
No response	16	13	
Present alcohol consumption			
p value	reference	0.054	**<0.000**
Yes	2 (3.8)	14 (14.1)	214 (85.9)
No	51 (96.2)	85 (85.9)	35 (14.1)
No response	16	3	0
Education			
p value	reference	**<0.000**	**0.000**
Less than high school	0 (0.0)	31 (30.4)	3 (1.2)
High school or equivalent	5 (9.3)	33 (32.4)	54 (21.7)
Undergraduate degree	38 (70.4)	36 (35.3)	177 (71.1)
More than a graduate school	11 (20.4)	2 (2.0)	15 (6.0)
No response	15	0	0

[a] The Korean National Environmental Health Survey (KoNEHS) 2015-2017. For comparison between 'Korean and Thai pregnant women' or 'pregnant and non-pregnant women in Korea', statistical analysis was conducted by the Wilcoxon rank-sum test for continuous variables, age, and pre-pregnancy BMI, and the Fisher's exact test for categorical variables with expected frequency less than 5, i.e., smoking status, alcohol consumption, and education.

4.3.2 Concentration of urinary correction factors for dilution

Descriptive statistics of urinary correction factors for dilution, creatinine, SG, and osmolality, are shown in Table 4-3. In Korean (Thai) women, median of urinary creatinine during T1, T2, and T3 were 97.3 (44.2) mg/dL, 59.9 (41.2) mg/dL, and 57.2 (41.8) mg/dL, respectively. Urinary correction factors at each trimester were not significantly different by age, pre-pregnancy BMI and sampling season, but by nationality in the present population (Table 4-4). Urinary concentrations of creatinine and osmolality were lower at T3 compared to T1 in Korea, while the concentrations were stable during pregnancy among Thai women (Fig. 4-1). Urine creatinine and SG of Korean pregnant women were significantly lower at T3 than those of non-pregnant women from KoNEHS 2015-2017. Compared with the results from previous studies, the trend of variation of urinary creatinine and SG across three trimesters was varied by country (Fig. 4-2).

Table 4-3. Descriptive statistics of urinary creatinine, SG, and osmolality by trimester of gestation.

	Pregnant women				**Non-pregnant women**	
	Korea (*n* = 69)		**Thailand (*n* = 102)**		**Korea (*n* = 249)**	
	arithmetic mean±SD (CV%)	**Median (25h, 75th)**	**arithmetic mean±SD (CV%)**	**Median (25h, 75th)**	**arithmetic mean±SD (CV%)**	**Median (25h, 75th)**
Creatinine (mg/dL)					95.7±64.8 (67.8)	78.8 (45.9, 132.3)
1st trimester (T1)	102.5±65.4 (63.8)	97.3 (49.5, 159.6)	55.7±41.0 (73.7)	44.2 (24.0, 79.8)		
2nd trimester (T2)	81.6±61.5 (75.3)	59.9 (37.0, 98.9)	51.3±34.0 (66.4)	41.2 (25.6, 74.5)		
3rd trimester (T3)	68.8±43.0 (62.5)	57.2 (35.0, 85.4)	56.6±46.4 (81.9)	41.8 (23.8, 78.9)		
Specific gravity (SG, unit less)					1.016±0.008 (0.8)	1.015 (1.009, 1.022)
1st trimester (T1)	1.014±0.008 (0.8)	1.014 (1.007, 1.022)	1.008±0.006 (0.6)	1.007 (1.003, 1.012)		
2nd trimester (T2)	1.013±0.007 (0.7)	1.010 (1.008, 1.020)	1.009±0.006 (0.6)	1.008 (1.004, 1.012)		
3rd trimester (T3)	1.013±0.006 (0.6)	1.010 (1.008, 1.016)	1.009±0.006 (0.6)	1.008 (1.004, 1.012)		
Osmolality (mOsm/Kg)						
1st trimester (T1)	596.9±282.0 (47.2)	654.0 (305.0, 834.0)	384.6±243.0 (63.2)	339.5 (179.0, 509.5)		
2nd trimester (T2)	513.7±250.7 (48.8)	427.0 (331.0, 726.0)	397.4±209.8 (52.8)	383.5 (216.5, 548.3)		
3rd trimester (T3)	496.9±214.4 (43.1)	463.0 (355.0, 636.0)	375.0±235.1 (62.7)	315.5 (196.0, 511.0)		

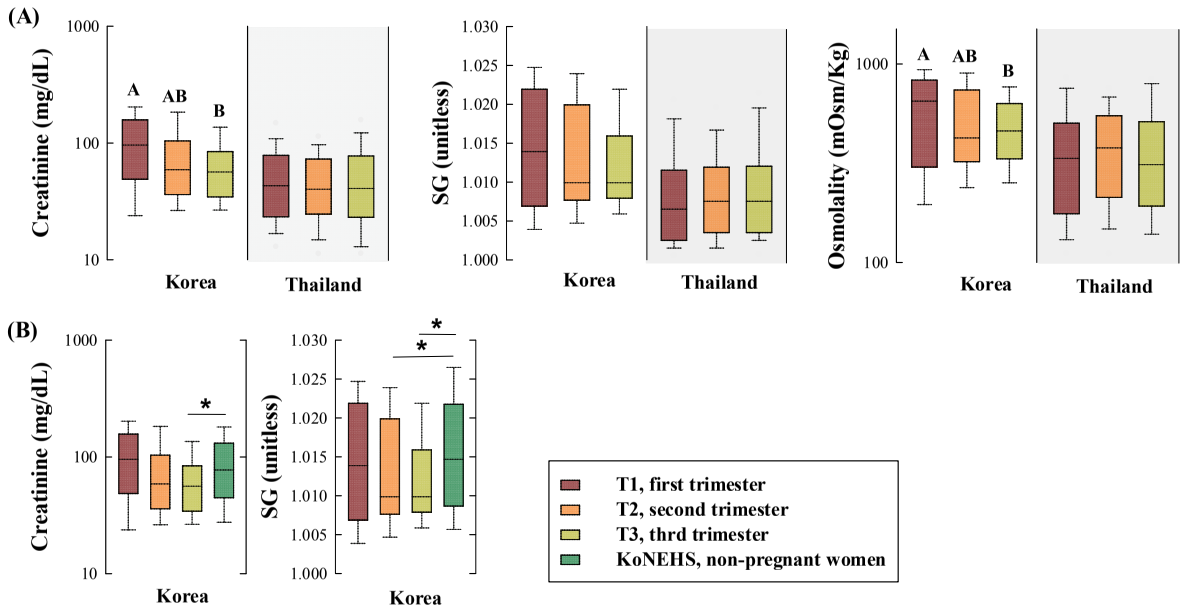


Fig. 4-1. Urine correction factors for dilution, (A) creatinine, specific gravity (SG), and osmolality during pregnancy of Korean (T1: n = 55, T2: n = 53, T3: n = 59), and Thai (T1: n = 76, T2: n = 96, T3: n = 102) pregnant women. Different letters (A and B) represent significant differences across trimesters. To determine the statistical difference among trimesters, the Wilcoxon signed-rank test was conducted. Statistical significance was defined at $p < 0.0167$, after adjustment by the Bonferroni correction (0.05/n, n = 3; number of hypothesis). (B) Comparison urinary correction factors for dilution between pregnant and non-pregnant Korean women (KoNEHS n = 249). Asterisks, *, indicate significant differences between pregnant and non-pregnant women. KoNEHS: The Korean National Environmental Health Survey.

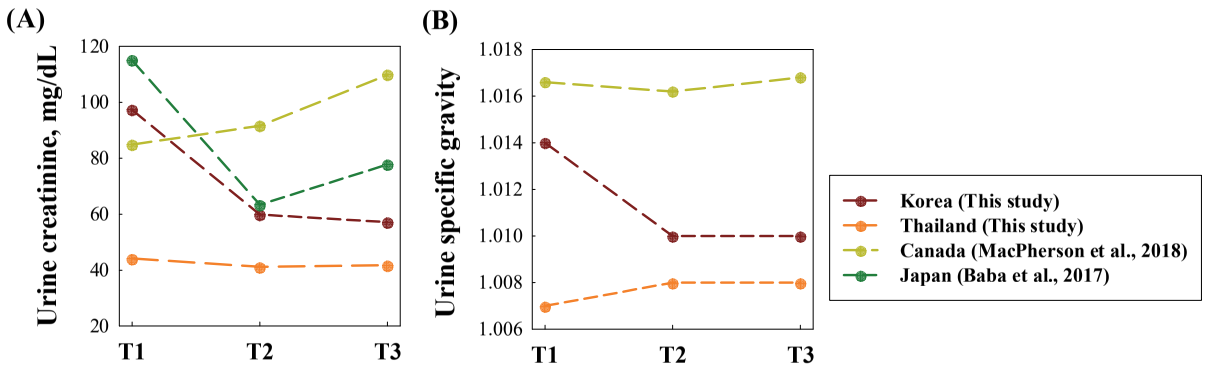


Fig. 4-2. Median concentration of urine correction factors for dilution, (A) creatinine and (B) specific gravity across three trimesters of Canadian (n = 80), Japanese (n = 73), Korean (n = 69), and Thai (n = 102) pregnant women.

Table 4-4. Urine correction factors for dilution across trimesters in pregnant women by demographic characteristics.

	T1			T2			T3		
	n	**50th (25th, 75th)**	**p**	**n**	**50th (25th, 75th)**	**p**	**n**	**50th (25th, 75th)**	**p**
Creatinine									
Nationality			**0.001**			**<0.000**			**0.006**
Korea	55	97.3 (49.5, 159.6)		53	59.9 (37.0, 98.9)		59	57.2 (35.0, 85.4)	
Thailand	76	44.2 (24.0, 79.8)		96	41.2 (25.6, 74.5)		102	41.8 (23.8, 78.9)	
Age (years)			0.844			0.287			0.423
<30	60	49.3 (27.8, 83.2)		77	49.5 (29.0, 86.0)		81	46.3 (27.7, 89.8)	
≥30	57	60.1 (24.6, 112.7)		60	45.1 (32.0, 80.8)		65	47.7 (30.3, 78.2)	
Pre-pregnancy BMI (kg/m^2)			0.74			0.404			0.661
<23	76	49.6 (25.6, 88.9)		94	47.3 (31.1, 82.4)		102	44.8 (30.2, 78.9)	
≥23	37	58.2 (27.6, 99.4)		40	44.5 (28.4, 87.7)		41	51.1 (23.3, 90.0)	
Sampling season[a]									
Spring (Mar. – May)	2	70.5 (55.3 85.8)	0.891	9	59.9 (26.1, 91.9)	0.582	25	74.8 (43.1, 92.6)	0.221
Summer (Jun. – Aug.)	21	95.6 (28.8, 150.5)	0.878	0	-	-	1	39.0 (27.0, 66.2)	0.231
Fall (Sep. – Nov.)	23	74.7 (35.2, 105.0)	0.567	22	46.6 (32.2, 150.4)	0.711	2	40.4 (39.4, 41.4)	0.374
Winter (Dec. – Feb.)	11	97.3 (61.4, 169.2)	reference	22	67.4 (42.0, 112.2)	reference	21	57.2 (30.2, 79.8)	reference

Table 4-4. (*Continued*)

	T1			T2			T3		
	n	**50th (25th, 75th)**	**p**	**n**	**50th (25th, 75th)**	**p**	**n**	**50th (25th, 75th)**	**p**
SG									
Nationality			**0.004**			**0.001**			**<0.000**
Korea	55	654 (305, 834)		53	427 (331, 726)		59	463 (335, 636)	
Thailand	76	340 (179, 510)		96	383 (217, 548)		102	316 (196, 511)	
Age (years)			0.718			0.380			0.188
<30	60	373 (209, 569)		77	403 (245, 583)		81	366 (229, 619)	
≥30	57	416 (222, 747)		60	385 (277, 580)		65	350 (234, 541)	
Pre-pregnancy BMI (kg/m^2)			0.959			0.111			0.733
<23	76	373 (206, 669)		94	401 (261, 528)		102	353 (230, 541)	
≥23	37	393 (215, 654)		40	389 (219, 571)		41	345 (238, 575)	
Sampling season[a]									
Spring (Mar. – May)	2	-	0.354	9	459 (304, 592)	0.372	25	532 (405, 718)	0.850
Summer (Jun. – Aug.)	21	659 (268, 820)	0.918	0	-	-	11	409 (281, 602)	0.641
Fall (Sep. – Nov.)	23	609 (261, 811)	0.575	22	369 (293, 763)	0.5700	2	-	0.980
Winter (Dec. – Feb.)	11	834 (700, 878)	reference	22	503 (367, 793)	reference	21	413 (284, 512)	reference

Table 4-4. (*Continued*)

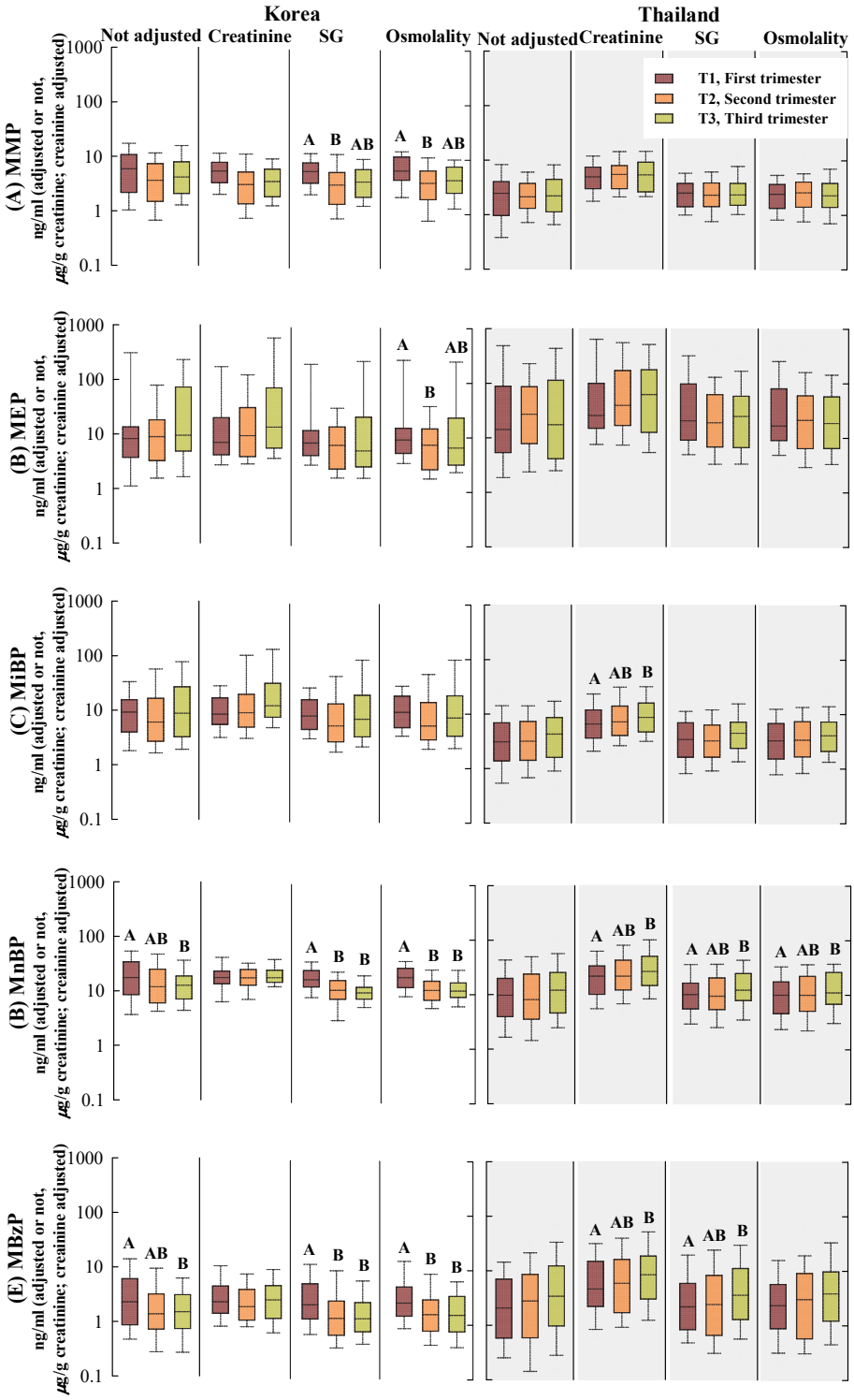
	T1			T2			T3		
	n	**50th (25th, 75th)**	**p**	**n**	**50th (25th, 75th)**	**p**	**n**	**50th (25th, 75th)**	**p**
Osmolality									
Nationality			**0.004**			**0.001**			**<0.000**
Korea	55	654 (305, 834)		53	427 (331, 726)		59	463 (335, 636)	
Thailand	76	340 (179, 510)		96	383 (217, 548)		102	316 (196, 511)	
Age (years)			0.718			0.380			0.188
<30	60	373 (209, 569)		77	403 (245, 583)		81	366 (229, 619)	
≥30	57	416 (222, 747)		60	385 (277, 580)		65	350 (234, 541)	
Pre-pregnancy BMI (kg/m2)			0.959			0.111			0.733
<23	76	373 (206, 669)		94	401 (261, 528)		102	353 (230, 541)	
≥23	37	393 (215, 654)		40	389 (219, 571)		41	345 (238, 575)	
Sampling season[a]									
Spring (Mar. – May)	2	-	0.354	9	459 (304, 592)	0.372	25	532 (405, 718)	0.850
Summer (Jun. – Aug.)	21	659 (268, 820)	0.918	0	-	-	11	409 (281, 602)	0.641
Fall (Sep. – Nov.)	23	609 (261, 811)	0.575	22	369 (293, 763)	0.5700	2	-	0.980
Winter (Dec. – Feb.)	11	834 (700, 878)	reference	22	503 (367, 793)	reference	21	413 (284, 512)	reference

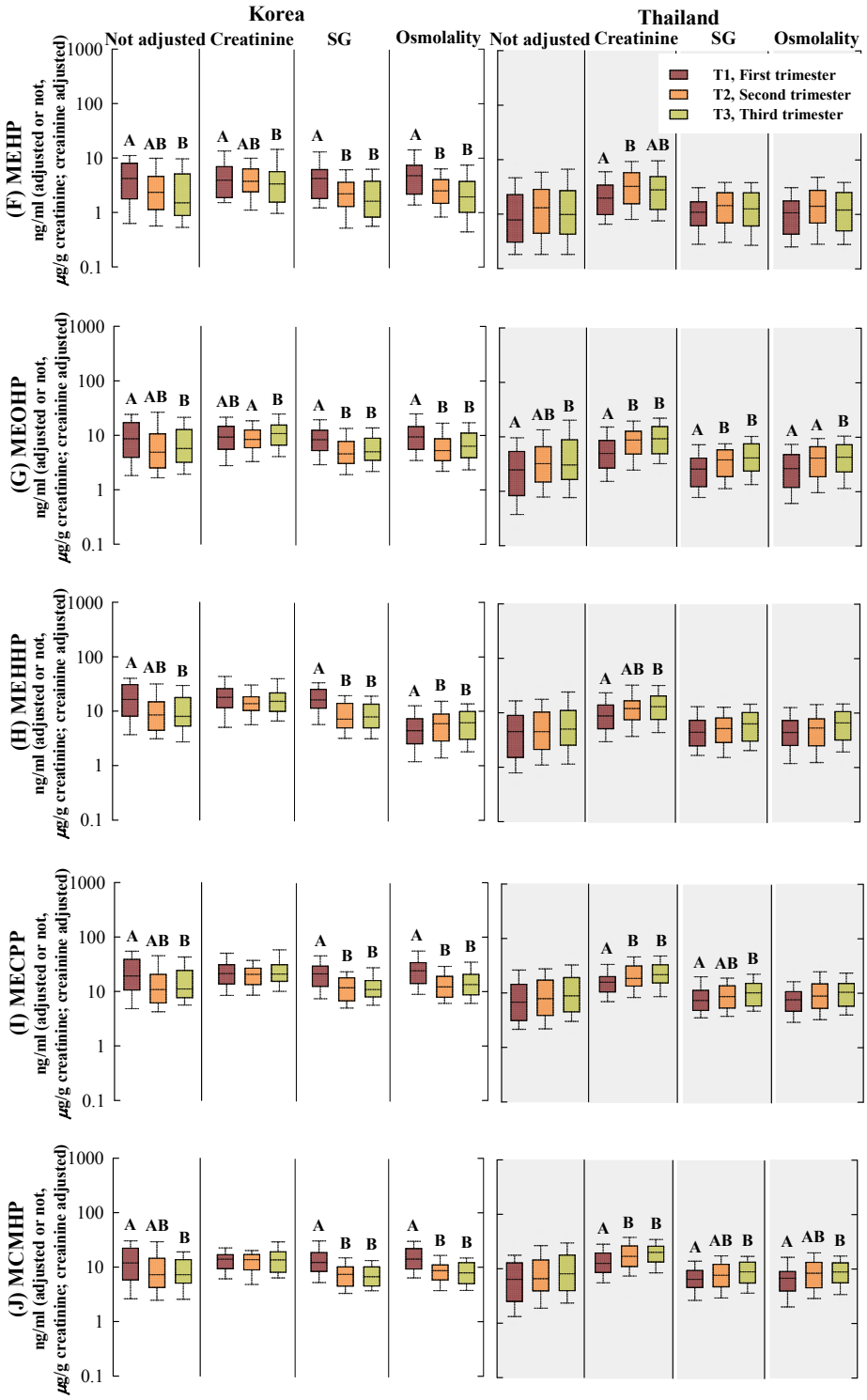
[a]Comparison of urinary correction factors depending on sampling season was conducted only for Korean pregnant women.

Boldface p-value indicates statistical significant ($p < 0.05$) difference of urinary correction factors depending on the demographic factors. For the comparison, ANCOVA was used after adjusting for nationality, age, pre-pregnancy BMI and (or) sampling season. Stages of pregnancy were classified as 1st trimester (T1), 2nd trimester (T2), and 3rd trimester (T3).

4.3.3 Variation of urinary phthalate metabolites by urine correction factors

Urinary phthalate metabolites by each urine correction factors were shown in Fig. 4-3. Levels of several phthalate metabolites, such as MnBP, MBzP, MEHP, MEOHP, MEHHP, MECPP and MCMHP, were significantly variable across the trimesters of gestation and their variations were altered depending on the method used for urine dilution adjustment. The concentrations of MMP, MEP in Thailand and MiBP in Korea were stable during gestation. Only variation of MCPP during pregnancy was held regardless of correction factors in Thai pregnant women.


Korea
Thailand
Not adjusted
Creatinine
SG
Osmolality
T1, First trimester
T2, Second trimester
T3, Third trimester
(A) MMP
(B) MEP
(C) MiBP
(B) MnBP
(E) MBzP
ng/ml (adjusted or not, μg/g creatinine; creainine adjusted)
1000
100
10
1
0.1
A
B
AB


Korea
Thailand
Not adjusted
Creatinine
SG
Osmolality
T1, First trimester
T2, Second trimester
T3, Third trimester
(F) MEHP
(G) MEOHP
(H) MEHHP
(I) MECPP
(J) MCMHP
ng/ml (adjusted or not,
μg/g creatinine; creatinine adjusted)
1000
100
10
1
0.1

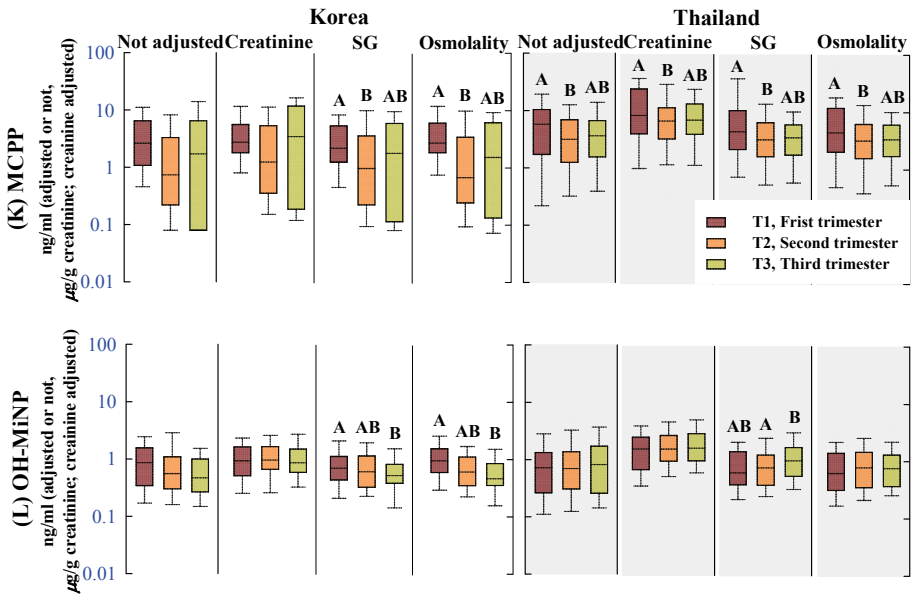


Fig. 4-3. Adjusted urinary phthalates metabolites (A) MnBP, (B) MBzP, (C) MEHHP, (D) MECPP, and (E) MCPP depending on urinary correction factors across trimester of gestation in Korean women (T1: n = 55, T2: n = 53, T3: n = 59, total: n = 69), and Thai pregnant women (T1: n = 76, T2: n = 96, T3: n = 102, total: n = 102). Different letters (A, B) represent significant differences across trimesters determined by Wilcoxon signed-rank test. The significance was defined at $p < 0.0167$ adjusted by the Bonferroni correction.

4.3.4 Intraclass correlation coefficients (ICC) across three trimesters and Spearman's correlation of adjusted urinary concentrations of the phthalate metabolites

During pregnancy, ICCs of SG adjusted phthalate metabolites were <0.4, while those of unadjusted, creatinine adjusted and osmolality adjusted concentrations were mostly higher than those of SG adjusted in Korean pregnant women (Fig. 4-4, Table 4-5). ICCs of unadjusted phthalate metabolites during three trimesters were relatively higher than those of adjustment for creatinine, SG and osmolality in Thai pregnant women. Most of the adjusted phthalate metabolites by different correction factors were highly correlated with one another (Table 4-6). The highest correlated phthalate metabolites levels were adjusted by osmolality and SG in Korea. Among Thai women, urinary phthalate metabolites level adjusted by osmolality and creatinine were highly correlated.

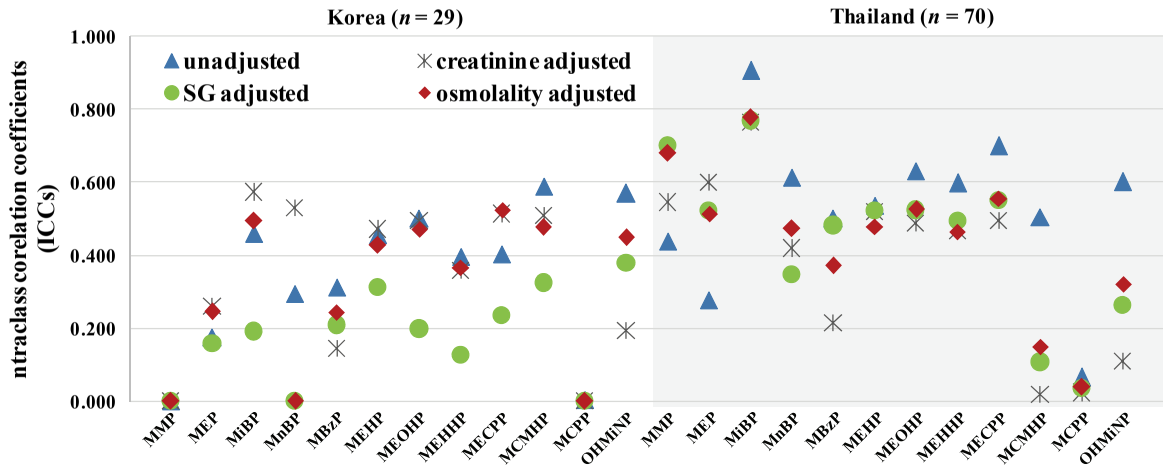


Fig. 4-4. Intraclass correlation coe□cients (ICCs) for phthalates concentrations between three trimester of pregnancy in Korean (n = 29) and Thai (n = 70) pregnant women. We give the negative values to zero.

Table 4-5. Intraclass correlation coefficients (ICCs) of urinary metabolites concentrations measure across three trimesters in Korean and Thai pregnant women.

Parent compound	Metabolites	Korea (*n* = 29)				Thailand (*n* = 70)			
		Unadjusted	Creatinine adjusted	SG adjusted	Osmolality adjusted	Unadjusted	Creatinine adjusted	SG adjusted	Osmolality adjusted
DMP	**MMP**	-0.022	-0.059	-0.088	-0.066	0.437**	0.545**	0.700**	0.680**
DEP	**MEP**	0.176	0.260	0.159	0.246	0.276	0.600**	0.523**	0.514**
DiBP	**MiBP**	0.460*	0.573**	0.191	0.494*	0.905**	0.763**	0.769**	0.778**
DnBP	**MnBP**	0.294	0.528**	-0.192	-0.008	0.613**	0.419**	0.348*	0.473**
BBzP	**MBzP**	0.313	0.145	0.209	0.243	0.502**	0.215	0.482**	0.372*
DEHP	**MEHP**	0.455*	0.471*	0.313	0.428*	0.536**	0.518**	0.522**	0.478**
	MEOHP	0.500*	0.495*	0.198	0.471*	0.630**	0.487**	0.524**	0.525**
	MEHHP	0.396	0.357	0.126	0.367	0.597**	0.465**	0.493**	0.465**
	MECPP	0.404	0.513*	0.236	0.524**	0.699**	0.494**	0.551**	0.556**
	MCMHP	0.588**	0.508*	0.324	0.476*	0.505**	0.017	0.107	0.150
DnOP	**MCPP**	0.004	-0.077	-0.002	-0.054	0.065	0.023	0.035	0.039
DiNP	**OHMiNP**	0.569**	0.193	0.379	0.449*	0.601**	0.108	0.262	0.321*

* $p < 0.05$, ** $p < 0.01$

Table 4-6. Spearman correlation coefficients and its rank between the arithmetic mean of adjusted urinary concentrations of the phthalate metabolites: unadjusted, creatinine adjusted, SG adjusted, or osmolality adjusted.

			Korea (n = 69)						Thailand (n = 102)					
Phthalate	metabolite	coefficient	Un-Cr	Un-Sg	Un-Os	Os-Cr	Sg-Cr	Os-Sg	Un-Cr	Un-Sg	Un-Os	Os-Cr	Sg-Cr	Os-Sg
DMP	MMP	ρ	0.686	0.770	0.798	0.850	0.886	0.903	0.603	0.560	0.721	0.908	0.789	0.872
		rank	1	2	2	3	3	4	1	1	2	4	2	3
DEP	MEP	ρ	0.811	0.847	0.883	0.941	0.944	0.977	0.872	0.857	0.906	0.973	0.951	0.963
		rank	3	3	3	4	4	4	3	3	4	4	4	4
DiBP	MiBP	ρ	0.783	0.819	0.866	0.927	0.923	0.972	0.772	0.731	0.840	0.943	0.883	0.914
		rank	2	3	3	4	4	4	2	2	3	4	3	4
DnBP	MnBP	ρ	0.613	0.702	0.803	0.836	0.823	0.932	0.832	0.805	0.870	0.964	0.907	0.935
		rank	1	2	3	3	3	4	3	3	3	4	4	4
BBzP	MBzP	ρ	0.761	0.808	0.853	0.930	0.913	0.967	0.903	0.885	0.922	0.975	0.958	0.974
		rank	2	3	3	4	4	4	4	3	4	4	4	4
DEHP	MEHP	ρ	0.738	0.781	0.847	0.902	0.908	0.972	0.801	0.757	0.865	0.942	0.898	0.922
		rank	2	2	3	4	4	4	3	2	3	4	3	4
	MEOHP	ρ	0.671	0.737	0.812	0.874	0.854	0.955	0.751	0.693	0.822	0.931	0.837	0.887
		rank	1	2	3	3	3	4	2	1	3	4	3	3
	MEHHP	ρ	0.641	0.716	0.799	0.904	0.859	0.916	0.726	0.671	0.798	0.925	0.843	0.888
		rank	1	2	2	4	3	4	2	1	2	4	3	3
	MECPP	ρ	0.613	0.658	0.762	0.838	0.817	0.933	0.619	0.549	0.733	0.884	0.768	0.840
		rank	1	1	2	3	3	4	1	1	2	3	2	3
	MCMHP	ρ	0.518	0.596	0.702	0.788	0.793	0.930	0.633	0.555	0.732	0.884	0.773	0.834
		rank	1	1	2	2	2	4	1	1	2	3	2	3
DnOP	MCPP	ρ	0.855	0.884	0.917	0.953	0.953	0.983	0.805	0.778	0.837	0.956	0.926	0.938
		rank	3	3	4	4	4	4	3	2	3	4	4	4
DiNP	OHMiNP	ρ	0.689	0.739	0.805	0.890	0.901	0.962	0.765	0.733	0.840	0.940	0.870	0.908
		rank	1	2	3	3	4	4	2	2	3	4	3	4
DiDP	cxMiDP	ρ	0.686	0.770	0.798	0.850	0.886	0.903	0.603	0.560	0.721	0.908	0.789	0.872
		rank	1	2	2	3	3	4	1	1	2	4	2	3
Score sum (total rank)			**19 (6)**	**26 (5)**	**33 (4)**	**41 (3)**	**41 (3)**	**48 (1)**	**27 (5)**	**22 (6)**	**34 (4)**	**46 (1)**	**37 (3)**	**43 (2)**

The coefficients were scored on a scale of 1 to 4, 1 with $\rho \geq 0.6$, 2 with $\rho \geq 0.7$, 3 with $\rho \geq 0.8$, and 4 with $\rho \geq 0.9$. Un: unadjusted, Cr: creatinine adjusted, SG: SG adjusted, Os: osmolality adjusted.

4.4 Discussion

Among Korean pregnant women, urinary concentrations of creatinine and osmolality were significantly lowered at T3 than T1 suggesting more hydrated urine as later stages of pregnancy (Fig. 4-1). Although SG was not significantly decreased during pregnancy, SG of pregnant women at T2 and T3 was significantly lower than that of non-pregnant women from KoNEHS 2015 – 2017. These variations of urine dilution during pregnancy may be partially explained by physiological changes in pregnancy. Increases of glomerular filtration rate and creatinine clearance result in lower serum creatinine (Katharine and Lafayette, 2013; Odutayo and Hladunewich, 2012). At the same time, however, urine volume increases (Maikranz et al., 1989), and consequently, urinary creatinine might decrease. Mean urinary volume was 1.78 L/d (Chen et al., 2018) in Norwegian women, but the median urinary volume was 2 L/d at 20 weeks of gestation (T2) in Norwegian pregnant women (Ye et al., 2009).

Urinary correction factors were decreased during pregnancy in Korean women, but those factors were stable across the trimester of gestation in Thai women (Fig. 4-1). Renal physiological changes during pregnancy would be similar for all women, but differences in lifestyle (water intake) depending on the climate might make these differences of urine dilution during pregnancy. Three urinary correction factors, creatinine, SG, and osmolality, in Thai pregnant women, were significantly lower than those of Korean pregnant

women at all three trimesters following the adjustments for age, pre-pregnancy BMI, and nationality (Table 4-4). The stable and low levels of urine correction factors in Thai pregnant women might be because their baseline of urine was already diluted. The levels of urinary creatinine in Thai were also lower than that of Korean in other age groups of population; mean urinary creatinine levels are 104.0 mg/dL in Thai children (12 – 13 years) (Panuwet et al., 2009), but 134.6 mg/dL and 162.3 mg/dL in Korean children (12 and 13 years, respectively) (Kwon and Na, 2014). Daily drinking water intake was also huge different; 2.05 L/d for 19 y to 35 y of Thai (National Bureau of Agricultural Commodity and Food Standards, 2006), but 1.06 L/d for the 30s of Korean general women (Park et al., 2019). Further studies are warranted to compare important parameters affecting urine correction factors of pregnant women by country.

Variation profiles of urinary dilution during pregnancy could be different between countries. Korean and Japanese pregnant women showed a similar decrease in urine dilution at T2 and T3 than T1 (Fig. 4-2). On the other hand, urinary creatinine concentrations of pregnant women in Canada (n = 80) were slightly increased during pregnancy (median 84.9 mg/dL, 91.6 mg/dL, and 109.8 mg/dL at T1, T2, and T3, respectively) (MacPherson et al., 2018). The base levels of creatinine and SG are different by age, sex, ethnicity, and BMI (Barr et al., 2005; Macpherson et al., 2018). The lifestyle might be the other determinant factor of the urinary hydration status of pregnant women. The median SG of pregnant women was 1.008 in Thai, 1.012 in Korean, 1.015 in

American, 1.016 in Canadian, 1.020 in Puerto Rican, (Ferguson et al., 2015; MacPherson et al., 2018; Watkins et al., 2015). The urinary correction factors were not different by age, pre-pregnancy BMI, and sampling season, but the country in the present study (Table 4-4). Even though the creatinine and osmolality concentrations were not statistically different by sampling season in Korean, those concentrations showed about two orders difference. The reason might be that the sample size of Korean pregnant women is not enough to estimate the statistical significance. Further multiple comparison studies of urinary correction factors depending on the country or other demographic factors are warranted in a large population.

The exposure profile of phthalates metabolites during pregnancy was changed according to the methods of urine correction. Various exposure profiles of MnBP, MBzP, MEHHP, and MECPP showed depending on the urine correction factors (Fig. 4-3). To describe the variation of phthalate concentrations adjusted by only one correction factor across the trimesters of pregnancy may drive an incomplete conclusion. Urine dilution adjustment could affect the results to not only exposure estimation but also risk assessment and association studies. Recent literature shows that associations between chemical exposure and oxidative stress biomarkers in pregnant women were different based on the method used for urine dilution adjustment (Ferguson et al., 2019). These results imply that understanding urine dilution profiles during pregnancy is important to study exposure biomarkers in pregnant women.

ICCs of unadjusted DEHP metabolites were mostly higher than those of creatinine, SG, and osmolality adjusted concentrations in both countries (Fig. 4-4). These findings might suggest that variations of urine correction factors could affect concentrations of adjusted phthalate metabolites. Even though urinary correction factors of Thai pregnant women were relatively stable, ICCs of unadjusted phthalate metabolites were higher than those of adjusted concentrations except MMP and MEP. These results suggest that the variations of urinary correction factors could make the uncertainty propagation in exposure assessment of chemicals for pregnant women with urine samples. It, therefore, is necessary to understand the changes in urinary dilutions by trimesters for a reduction in exposure misclassification of pregnant women.

Adjusted phthalate metabolites concentrations by creatinine, SG and osmolality were highly correlated with each other ($\rho \geq 0.7$) (Table 4-5). For a comparison of differently adjusted phthalate concentrations, it is more reliable to compare among only adjusted concentrations (or only unadjusted concentrations) than between unadjusted and adjusted concentrations. The highest correlated phthalate metabolites levels were adjusted by osmolality and SG in Korea ($\rho \geq 0.9$) and by osmolality and creatinine in Thai. Urinary SG was depending on the weight of urinary solutes. Relatively heavy molecules like albumins will be major factors of urinary SG than small molecules like creatinine in urine. Osmolality is the number of dissolved particles per unit of water in the urine. Because both the number and weight

of particles were considered in osmolality, SG (or creatinine) and osmolality adjusted concentrations may be highly correlated.

Limited sample size and missing values of covariates should be considered as a limitation of the present study. Reduced statistical power resulting from a small sample size may lead to difficulties in detecting important demographic factors related to urine correction factors during pregnancy. Pregnant women recruited from the hospitals in capital cities were biased, thus findings may lack generalizability. The present study, however, provided new information on variations of urine dilution throughout pregnancy by country with three different urine correction factors, creatinine, SG and osmolality. Adjusted concentrations of phthalates metabolites by each correction factor, were compared by ICCs and correlations coefficients.

In summary, our observations showed that urinary dilution during pregnancy due to physiological change was variable. Different lifestyles depending on countries may affect the changes in urinary dilution during pregnancy. Phthalate metabolites during pregnancy showed various exposure profiles depending on the urine correction factors. ICCs of unadjusted phthalate metabolites concentrations across three trimesters were relatively higher than those of the adjusted one. These results suggest that the variations of urinary correction factors may make the uncertainty propagation in exposure assessment of chemicals for pregnant women with urine samples. It, therefore, is necessary to understand the changes in urinary correction factors for dilution by trimesters and the country for a reduction in exposure

misclassification of pregnant women. It also needs to be careful at understanding the exposure biomarker during pregnancy in terms of the changes in urine volume or a series of characteristics related to the absorption, distribution, metabolism, and excretion (ADME) of chemicals in the body.

Chapter 5 Summary and conclusions

To evaluate the risk of exposure to plasticizers and characteristic exposure profiles in women of reproductive age, a series of studies carried out. Associations between exposure to plasticizers and uterine fibroids, the most common disease in women of reproductive age, were investigated using a case-control study in Chapter 2. We described general exposure profiles of major plasticizers across three trimesters in pregnant women in Chapter 3. We tried to explain the various exposure profiles of plasticizers across trimesters with variations of urinary dilution status during pregnancy. We described exposure profiles of urinary correction factors, such as creatinine, SG, and osmolality, across three trimesters of gestation in Chapter 4. This series of studies suggest new insight into risk and exposure management of emerging plasticizers in women of reproductive age.

In Chapter 2, we identified, for the first time, several alternative plasticizers, such as DEHTP, DPrHpP, DINCH, TDCIPP, and TBOEP, which were associated with uterine fibroids among women of reproductive age. These positive associations of new plasticizers could be supported by previous experimental and epidemiological studies. Sex hormone-related disruptive effects of new plasticizers might be related to uterine fibroids through estrogen receptor binding or increase of estrogen and progesterone levels. While the results of this study should be validated by further experimental and epidemiological studies, our observations suggest that

several alternative plasticizers may not be safer alternatives to more conventional plasticizers.

In Chapter 3, we showed exposure profiles of major phthalates and an alternative plasticizer across three trimesters in pregnant women of Korea and Thailand. Our observations showed that phthalate exposure profile during gestation is variable and also different by country. Compared to later stages of pregnancy, DnBP, BBzP, and DEHP metabolites were higher in the first trimester among Korean women. This finding suggests that the measurement of spot urine collected in later stages of pregnancy may underestimate the extent of phthalate exposure among Korean pregnant women. Moreover, we found that the frequency of detection for a DINCH metabolite was up to two-thirds of the population and recommend long-term surveillance for the use of alternative plasticizers. Considering the vulnerability of growing fetuses, further studies are warranted to identify major sources of exposure to these plasticizers during pregnancy.

In chapter 4, our observations showed that urinary hydration status during pregnancy due to physiological change was variable. Nationality or lifestyle may affect the changes in urinary dilution status during pregnancy. The correlations between unadjusted and adjusted phthalate concentrations during pregnancy were less than those of each adjusted phthalate concentrations. These results suggest that the variations of urinary correction factors may make the uncertainty propagation in exposure assessment of chemicals for pregnant women with urine samples. It is necessary to understand the changes

in urinary correction factors for dilution by trimesters and nationality for a reduction in exposure misclassification of pregnant women. In addition, it needs to be careful at understanding the exposure biomarker during pregnancy in terms of the changes in urine volume or a series of characteristics related to the absorption, distribution, metabolism, and excretion (ADME) of chemicals in the body.

Limited sample size and missing values of covariates should be considered as a major limitation of the present study. Reduced statistical power resulting from a small sample size may lead to difficulties in detecting variability among the trimesters. In addition, a lack of information on potential exposure sources and pathways for the study populations should be noted. Finally, participants (both pregnant and non-pregnant women) recruited from the hospitals were biased, thus findings may lack generalizability. However, we determined the association between exposure to new plasticizers and reproductive health outcomes, uterine fibroids. The present study also provided new information on temporal variations of phthalates exposure throughout pregnancy and explained the variation with urine dilution status. The results of this study will facilitate follow up studies focusing on exposure assessment for plasticizers among pregnant women, and potential health outcomes associated with plasticizer exposure during this critical window of susceptibility.

References

Adibi, J.J., Whyatt, R.M., Williams, P.L., Calafat, A.M., Camann, D., Herrick, R., Nelson, H., Bhat, H.K., Perera, F.P., Silva, M.J., Hauser, R., 2008. Characterization of phthalate exposure among pregnant women assessed by repeat air and urine samples. Environ. Health. Perspect. 116, 467-473.

Anderson, W.A., Castle, L., Scotter, M.J., Massey, R.C., Springall, C., 2001. A biomarker approach to measuring human dietary exposure to certain phthalate diesters. Food Addit. Contam. 18, 1068–1074.

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.

Barr, D.B., Wilder, L.C., Caudill, S.P., Gonzalez, A.J., Needham, L.L., and Pirkle, J.L., 2005. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ. Health Perspect. 113, 192-200.

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.

Bhat, V.S., Durham, J.L., Ball, G.L., English, J.C., 2014. Derivation of an oral reference dose (RfD) for the nonphthalate alternative plasticizer 1,2-cyclohexane dicarboxylic acid, di-isononyl ester (DINCH). J. Toxicol. Environ. Health B Crit. Rev. 17, 63–94.

Boeniger, M.F., Lowry, L.K., and Rosenberg, J., 1993. Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. Am. Ind. Hyg. Assoc. J. 54, 615-627.

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.

Bornehag, C.G., Carlstedt, F., Jönsson, B.A., Lindh, C.H., Jensen, T.K., Bodin, A., Jonsson, C., Janson, S., Swan, S.H., 2015. Prenatal phthalate exposures and anogenital distance in Swedish boys. Environ. Health Perspect. 123, 101–107.

Braun, J.M., Smith, K.W., Williams, P.L., Calafat, A.M., Berry, K., Ehrlich, S., Hauser, R., 2012. Variability of urinary phthalate metabolite and bisphenol A concentrations before and during pregnancy. Environ. Health Perspect. 120, 739–745.

Bui, T.T., Giovanoulis, G., Cousins, A.P., Magnér, 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.

Cardozo, E.R., Clark, A.D., Banks, N.K., Henne, M.B., Stegmann, B.J., Segars, J.H., 2012. The estimated annual cost of uterine leiomyomata in the United States. Am J Obstet Gynecol. 206, 211 e211-219.

Carignan, C.C., Minguez-Alarcon, L., Williams, P.L., Meeker, J.D., Stapleton, H.M., Butt, C.M., Toth, T.L., Ford, J.B., Hauser, R., Team, E.S., 2018. Paternal urinary concentrations of organophosphate flame retardant metabolites, fertility measures, and pregnancy outcomes among couples undergoing in vitro fertilization. Environ Int. 111, 232-238.

Castorina, R., Butt, C., Stapleton, H.M., Avery, D., Harley, K.G., Holland, N., Eskenazi, B., Bradman, A., 2017. Flame retardants and their metabolites in the homes and urine of pregnant women residing in California (the CHAMACOS cohort). Chemosphere. 179, 159-166.

Chen, S.L., Dahl, C., Meyer, H.E., and Madar, A.A., 2018. Estimation of Salt Intake Assessed by 24-Hour Urinary Sodium Excretion among Somali Adults in Oslo, Norway. Nutrients 10.

Cheung, K.L., Lafayette, R.A., 2013. Renal physiology of pregnancy. Adv. Chronic Kidney Dis. 20, 209-214.

Christensen, K.L., Makris, S.L., Lorber, M., 2014. Generation of hazard indices for cumulative exposure to phthalates for use in cumulative risk assessment. Regul. Toxicol. Pharmacol. 69, 380–389.

Correia-Sá, L., Schütze, A., Norberto, S., Calhau, C., Domingues, V.F., Koch, H.M., 2017. Exposure of Portuguese children to the novel non-phthalate plasticizer di-(isononyl)-cyclohexane-1,2-dicarboxylate (DINCH). Environ. Int. 102, 79–86.

CPSIA, 2008. Consumer Product Safety Improvement Act of 2008, Public Law 110–314, 110th Congress (Available online at https://www.cpsc.gov/s3fs-public/pdfs/blk_pdf_cpsia.pdf (accessed Jun 3, 2019)).

Dewalque, L., Charlier, C., Pirard, C., 2014. Estimated daily intake and cumulativebrisk assessment of phthalate diesters in a Belgian general population. Toxicol. Lett. 231, 161–168.

Directive 2005/84/EC of the European Parliament and of the Council (2005) Vol 344, Official Journal of the European Union (Available online at http://eur-ex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:344:0040:0043:EN:PDF (accessed Jun 3, 2019)).

Du, J., Li, H., Xu, S., Zhou, Q., Jin, M., Tang, J., 2019. A review of organophosphorus flame retardants (OPFRs): occurrence, bioaccumulation, toxicity, and organism exposure. Environ Sci Pollut Res Int. 26, 22126-22136.

EFSA (European Food Safety Authority), 2005. Opinion of the scientific panel on food additives, flavourings, processing aids and material in contact with food (AFC) on a request from the commission related to di-butylphthalate (DBP) for use in food contact materials. EFSA J. 242, 1–17.

EFSA (European Food Safety Authority), 2005b. Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on a request from the Commission related to Di-isononylphthalate (DINP) for use in food contact materials. EFSA J. 244, 1–18.

Engel, A., Buhrke, T., Kasper, S., Behr, A.C., Braeuning, A., Jessel, S., Seidel, A., Volkel, W., Lampen, A., 2018. The urinary metabolites of DINCH have an impact on the activities of the human nuclear receptors ERalpha, ERbeta, AR, PPARalpha and PPARgamma. Toxicol Lett. 287, 83-91.

Ferguson, K.K., McElrath, T.F., Ko, Y.A., Mukherjee, B., Meeker, J.D., 2014. Variability in urinary phthalate metabolite levels across pregnancy and sensitive windows of exposure for the risk of preterm birth. Environ. Int. 70, 118–124.

Ferguson, K.K., Meeker, J.D., Cantonwine, D.E., Chen, Y.H., Mukherjee, B., McElrath, T.F., 2016. Urinary phthalate metabolite and bisphenol A associations with ultrasound and delivery indices of fetal growth. Environ. Int. 94, 531–537.

Ferguson, K.K., Lan, Z., Yu, Y., Mukherjee, B., McElrath, T.F., and Meeker, J.D., 2019. Urinary concentrations of phenols in association with biomarkers of oxidative stress in pregnancy: Assessment of effects independent of phthalates. Environ. Int. 131, 104903.

Fisher, M., Arbuckle, T.E., Mallick, R., LeBlanc, A., Hauser, R., Feeley, M., Koniecki, D., Ramsay, T., Provencher, G., Bérubé, R., Walker, M., 2015. Bisphenol A and phthalate metabolite urinary concentrations: daily and across pregnancy variability. J. Expo. Sci. Environ. Epidemiol. 25, 231–239.

Flake, G.P., Andersen, J., Dixon, D., 2003. Etiology and pathogenesis of uterine leiomyomas: a review. Environ Health Perspect. 111, 1037-1054.

Fromme, H., Gruber, L., Schuster, R., Schlummer, M., Kiranoglu, M., Bolte, G., Völkel, W., 2013a. Phthalate and di-(2-ethylhexyl) adipate (DEHA) intake by German infants based on the results of a duplicate diet study and biomonitoring data (INES 2). Food Chem. Toxicol. 53, 272–280.

Fromme, H., Lahrz, T., Kraft, M., Fembacher, L., Dietrich, S., Sievering, S., Burghardt, R., Schuster, R., Bolte, G., Völkel, W., 2013b. Phthalates in German daycare centers: occurrence in air and dust and the excretion of their metabolites by children (LUPE 3). Environ. Int. 61, 64–72.

Fromme, H., Schütze, A., Lahrz, T., Kraft, M., Fembacher, L., Siewering, S., Burkardt, R., Dietrich, S., Koch, H.M., Völkel, W., 2016. Non-phthalate plasticizers in German daycare centers and human biomonitoring of DINCH metabolites in children attending the centers (LUPE 3). Int. J. Hyg. Environ. Health 219, 33–39.

Fu, Z., Zhao, F., Chen, K., Xu, J., Li, P., Xia, D., Wu, Y., 2017. Association between urinary phthalate metabolites and risk of breast cancer and uterine leiomyoma. Reprod Toxicol. 74, 134-142.

Gao, C.J., Liu, L.Y., Ma, W.L., Ren, N.Q., Guo, Y., Zhu, N.Z., Jiang, L., Li, Y.F., Kannan, K., 2016. Phthalate metabolites in urine of Chinese young adults: concentration, profile, exposure and cumulative risk assessment. Sci. Total Environ. 543, 19–27.

Giovanoulis, G., Alves, A., Papadopoulou, E., Cousins, A.P., Schütze, A., Koch, H.M., Haug, L.S., Covaci, A., Magnér, J., Voorspoels, S., 2016. Evaluation of exposure to phthalate esters and DINCH in urine and nails from a Norwegian study population. Environ. Res. 151, 80–90.

Gomez Ramos, M.J., Heffernan, A.L., Toms, L.M.L., Calafat, A.M., Ye, X., Hobson, P., Broomhall, S., Mueller, J.F., 2016. Concentrations of phthalates and DINCH metabolites in pooled urine from Queensland, Australia. Environ. Int. 88, 179–186.

Guo, Y., Alomirah, H., Cho, H.S., Minh, T.B., Mohd, M.A., Nakata, H., Kannan, K., 2011. Occurrence of phthalate metabolites in human urine from several Asian countries. Environ Sci Technol. 45, 3138-3144.

Hauser, R., Meeker, J.D., Duty, S., Silva, M.J., Calafat, A.M., 2006. Altered semen quality in relation to urinary concentrations of phthalate monoester and oxidative metabolites. Epidemiology 17, 682–691.

Heavner, D.L., Morgan, W.T., Sears, S.B., Richardson, J.D., Byrd, G.D., and Ogden, M.W., 2006. Effect of creatinine and specific gravity normalization techniques on xenobiotic biomarkers in smokers' spot and 24-h urines. J. Pharm. Biomed. Anal. 40, 928-942.

Hoffman, K., Lorenzo, A., Butt, C.M., Adair, L., Herring, A.H., Stapleton, H.M., Daniels, J.L., 2017. Predictors of urinary flame retardant concentration among pregnant women. Environ Int. 98, 96-101.

Hornungm, R.W., Reed, L.D., 1990. Estimation of Average concentration in the presence of Nondetectable Values. Appl. Occup. Environ. Hyg. 5, 6.

Hsieh, C.J., Chang, Y.H., Hu, A., Chen, M.L., Sun, C.W., Situmorang, R.F., Wu, M.T., Wang, S.L., TMICS Study Group, 2019. Personal care products use and phthalate exposure levels among pregnant women. Sci. Total Environ. 648, 135–143.

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.

Huang, Y.F., Pan, W.C., Tsai, Y.A., Chang, C.H., Chen, P.J., Shao, Y.S., Tsai, M.S., Hou, J.W., Lu, C.A., Chen, M.L., 2017. Concurrent exposures to nonylphenol, bisphenol A, phthalates, and organophosphate pesticides on birth outcomes: a cohort study in Taipei, Taiwan. Sci. Total Environ. 607–608, 1126–1135.

Itoh, H., Yoshida, K., Masunaga, S., 2007. Quantitative identification of unknown exposure pathways of phthalates based on measuring their metabolites in human urine. Environ. Sci. Technol. 41, 4542–4547.

James-Todd, T.M., Chiu, Y.H., Messerlian, C., Minguez-Alarcon, L., Ford, J.B., Keller, M., Petrozza, J., Williams, P.L., Ye, X., Calafat, A.M., Hauser, R., EARTH Study Team, 2018. Trimester-specific phthalate concentrations

and glucose levels among women from a fertility clinic. Environ. Health 17, 55.

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., 2018a. 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., Eom, S., Kim, H.J., Lee, J.J., Choi, G., Choi, S., Kim, S., Kim, S.Y., Cho, G., Kim, Y.D., Suh, E., Kim, S.K., Kim, S., Kim, G.H., Moon, H.B., Park, J., Kim, S., Choi, K., Eun, S.H., 2018b. Association between maternal exposure to major phthalates, heavy metals, and persistent organic pollutants, and the neurodevelopmental performances of their children at 1 to 2years of age – CHECK cohort study. Sci. Total Environ. 624, 377–384.

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., Evans, S., Miodovnik, A., Barrett, E.S., Thurston, S.W., Calafat, A.M., Swan, S.H., 2014. Prenatal phthalate exposures and neurobehavioral development scores in boys and girls at 6–10 years of age. Environ. Health Perspect. 122, 521–528.

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.

Koch, H.M., Angerer, J., 2007. Di-iso-nonylphthalate (DINP) metabolites in human urine after a single oral dose of deuterium-labelled DINP. Int. J. Hyg. Environ. Health 210, 9–19.

Koch, H.M., Bolt, H.M., Preuss, R., Angerer, J., 2005. New metabolites of di(2-ethylhexyl)phthalate (DEHP) in human urine and serum after single oral doses of deuterium-labelled DEHP. Arch. Toxicol. 79, 367–376.

Koch, H.M., Lorber, M., Christensen, K.L., Pälmke, C., Koslitz, S., Brüning, T., 2013a. Identifying sources of phthalate exposure with human biomonitoring: results of a 48h fasting study with urine collection and personal activity patterns. Int. J. Hyg. Environ. Health 216, 672–681.

Koch, H.M., Rüther, M., Schütze, A., Conrad, A., Pälmke, C., Apel, P., Brüning, T., Kolossa-Gehring, M., 2017. Phthalate metabolites in 24-h urine samples of the German Environmental Specimen Bank (ESB) from 1988 to 2015 and a comparison with US NHANES data from 1999 to 2012. Int. J. Hyg. Environ. Health 220, 130–141.

Koch, H.M., Schütze, A., Pälmke, C., Angerer, J., Brüning, T., 2013b. Metabolism of the plasticizer and phthalate substitute diisononyl-cyclohexane-1,2-dicarboxylate (DINCH) in humans after single oral doses. Arch. Toxicol. 87, 799–806.

Koniecki, D., Wang, R., Moody, R.P., Zhu, J., 2011. Phthalates in cosmetic and personal care products: concentrations and possible dermal exposure. Environ. Res. 111, 329–336.

Kwon, S., N, Y., 2014. Concentration of Serum and Urine Creatinine in Children and Adolescents. Korean J. Clin. Lab. Sci. 46, 117-123.

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.

Lee, G., Kim, S., Kho, Y., Kim, S., Lee, S., Choi, G., Park, J., Worakhunpiset, S., Moon, H.B., Okanurak, K., Geounuppakul, M., Tangtitawong, J., Wetsutthanon, K., Trisurat, D., Choi, K., 2019. Urinary levels of phthalates and DINCH metabolites in Korean and Thai pregnant women across three trimesters. Sci. Total Environ. 134822.

Lee, H., Lee, J., Choi, K., Kim, K.T., 2019a. 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, I., Alakeel, R., Kim, S., Al-Sheikh, Y.A., Al-Mandeel, H., Alyousef, A.A., Kho, Y., Choi, K., 2019b. Urinary phthalate metabolites among children in Saudi Arabia: occurrences, risks, and their association with oxidative stress markers. Sci. Total Environ. 654, 1350–1357.

Levy, G., Hill, M.J., Plowden, T.C., Catherino, W.H., Armstrong, A.Y., 2013. Biomarkers in uterine leiomyoma. Fertil Steril. 99, 1146-1152.

Li, J., Xia, W., Wu, C., Zhao, H., Zhou, Y., Wei, J., Ji, F., Luan, H., Xu, S., Cai, Z., 2019. Variations of phthalate exposure and metabolism over three trimesters. Environ. Pollut. 251, 137–145.

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.

Lin, S., Ku, H.Y., Su, P.H., Chen, J.W., Huang, P.C., Angerer, J., Wang, S.L., 2011. Phthalate exposure in pregnant women and their children in central Taiwan. Chemosphere 82, 947–955.

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.

Machtinger, R., Berman, T., Adir, M., Mansur, A., Baccarelli, A.A., Racowsky, C., Calafat, A.M., Hauser, R., Nahum, R., 2018. Urinary concentrations of phthalate metabolites, bisphenols and personal care product chemical biomarkers in pregnant women in Israel. Environ. Int. 116, 319–325.

MacPherson, S., Arbuckle, T.E., and Fisher, M., 2018. Adjusting urinary chemical biomarkers for hydration status during pregnancy. J. Expo. Sci. Environ. Epidemiol. 28, 481-493

Maikranz, P., Holley, J.L., Parks, J.H., Lindheimer, M.D., Nakagawa, Y., and Coe, F.L., 1989. Gestational hypercalciuria causes pathological urine calcium oxalate supersaturations. Kidney Int, 36, 108-113.

Mankidy, R., Wiseman, S., Ma, H., Giesy, J. P., 2013. Biological impact of phthalates. Toxicol Lett. 217, 50-58.

Martinez-Arguelles, D.B., Papadopoulos, V., 2016. Prenatal phthalate exposure: epigenetic changes leading to lifelong impact on steroid formation. Andrology 4, 573–584.

Martino-Andrade, A.J., Liu, F., Sathyanarayana, S., Barrett, E.S., Redmon, J.B., Nguyen, R.H., Levine, H., Swan, S.H., TIDES Study Team, 2016. Timing of prenatal phthalate exposure in relation to genital endpoints in male newborns. Andrology 4, 585–593.

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.

Middleton, D.R., Watts, M.J., Lark, R.M., Milne, C.J., and Polya, D.A., 2016. Assessing urinary flow rate, creatinine, osmolality and other hydration adjustment methods for urinary biomonitoring using NHANES arsenic, iodine, lead and cadmium data. Environ. Health 15, 68.

National Bureau of Agricultural Commodity and Food Standards, 2006. Food consumption data of Thailand. Thailand: The national bureau of agricultural commodity and food standards (ACFS).

Navarro, A., Yin, P., Monsivais, D., Lin, S.M., Du, P., Wei, J.J., Bulun, S.E., 2012. Genome-wide DNA methylation indicates silencing of tumor suppressor genes in uterine leiomyoma. PLoS One. 7, e33284.

Nehring, A., Bury, D., Kling, H.W., Weiss, T., Bruning, T., and Koch, H.M., 2019. Determination of human urinary metabolites of the plasticizer di(2-ethylhexyl) adipate (DEHA) by online-SPE-HPLC-MS/MS. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 1124, 239-246.

O'Brien, K.M., Upson, K., and Buckley, J.P., 2017. Lipid and Creatinine Adjustment to Evaluate Health Effects of Environmental Exposures. Curr. Environ. Health Rep. 4, 44-50.

O'Brien, K.M., Upson, K., Cook, N.R., and Weinberg, C.R., 2016. Environmental Chemicals in Urine and Blood: Improving Methods for Creatinine and Lipid Adjustment. Environ. Health Perspect. 124, 220-227.

Odutayo, A., and Hladunewich, M., 2012. Obstetric nephrology: renal hemodynamic and metabolic physiology in normal pregnancy. Clin. J. Am. Soc. Nephrol. 7, 2073-2080.

Panuwet, P., Prapamontol, T., Chantara, S., and Barr, D.B., 2009. Urinary pesticide metabolites in school students from northern Thailand. Int. J. Hyg Environ. Health 212, 288-297.

Park, C., Choi, W., Hwang, M., Lee, Y., Kim, S., Yu, S., Lee, I., Paek, D., Choi, K., 2017. Associations between urinary phthalate metabolites and bisphenol A levels, and serum thyroid hormones among the Korean adult population-Korean National Environmental Health Survey (KoNEHS) 2012–2014. Sci. Total Environ. 584–585, 950–957.

Park, C., Hwang, M., Kim, H., Ryu, S., Lee, K., Choi, K., and Paek, D., 2016. Early snapshot on exposure to environmental chemicals among Korean adults-results of the first Korean National Environmental Health Survey (2009-2011). Int. J. Hyg. Environ. Health 219, 398-404.

Park, E., Lee, Y., Kim, M., Choi, M., 2019. Daily Water Consumption and its Contribution to Calcium Intake in Korean Adults. Korean J. Community Nutr. 24, 18-23.

Parker, W.H., 2007. Etiology, symptomatology, and diagnosis of uterine myomas. Fertil Steril. 87, 725-736.

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.

Richard, W. Hornung, L.D.R., 1990. Estimation of Average Concentration in the Presence of Nondetectable Values. Appl. Occup. Environ. Hyg. 5, 46-51.

Rodríguez-Carmona, Y., Ashrap, P., Calafat, A.M., Ye, X., Rosario, Z., Bedrosian, L.D., Huerta-Montanez, G., Vélez-Vega, C.M., Alshawabkeh, A., Cordero, J.F., Meeker, J.D., Watkins, D., 2019. Determinants and characterization of exposure to hthalates, DEHTP and DINCH among pregnant women in the PROTECT birth cohort in Puerto Rico. J. Expo. Sci. Environ. Epidemiol. https://doi.org/10.1038/ s41370-019-0168-8.

Sauve, J.F., Levesque, M., Huard, M., Drolet, D., Lavoue, J., Tardif, R., and Truchon, G., 2015. Creatinine and specific gravity normalization in biological monitoring of occupational exposures. J. Occup. Environ. Hyg. 12, 123-129.

Schutze, 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., Brüning, T., Koch, H.M., 2014. Entering markets and bodies: increasing levels of the novel plasticizer Hexamoll DINCH in 24 h urine samples from the German Environmental Specimen Bank. Int. J. Hyg. Environ. Health 217, 421–426.

Serrano, S.E., Braun, J., Trasande, L., Dills, R., Sathyanarayana, S., 2014. Phthalates and diet: a review of the food monitoring and epidemiology data. Environ. Health 13, 43.

Servaes, K., Voorspoels, S., Lievens, J., Noten, B., Allaerts, K., Van De Weghe, H., Vanermen, G., 2013. Direct analysis of phthalate ester biomarkers in urine without preconcentration: method validation and monitoring. J. Chromatogr. A 1294, 25–32.

Shapiro, G.D., Dodds, L., Arbuckle, T.E., Ashley-Martin, J., Fraser, W., Fisher, M., Taback, S., Keely, E., Bouchard, M.F., Monnier, P., Dallaire, R., Morisset, A., Ettinger, A.S., 2015. Exposure to phthalates, bisphenol A and metals in pregnancy and the association with impaired glucose tolerance and estational diabetes mellitus: the MIREC study. Environ. Int. 83, 63–71.

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.

Shin, H.M., Bennett, D.H., Barkoski, J., Ye, X., Calafat, A.M., Tancredi, D., and Hertz-Picciotto, I., 2019. Variability of urinary concentrations of phthalate metabolites during pregnancy in first morning voids and pooled samples. Environ. Int. 122, 222-230.

Silva, M.J., Jia, T., Samandar, E., Preau, J.L., Jr., Calafat, A.M., 2013. Environmental exposure to the plasticizer 1,2-cyclohexane dicarboxylic acid, diisononyl ester (DINCH) in U.S. adults (2000-2012). Environ Res. 126, 159-163.

Silva, M.J., Wong, L.Y., Samandar, E., Preau, J.L., Calafat, A.M., Ye, X., 2017. Exposure to di-2-ethylhexyl terephthalate in a convenience sample of U.S. adults from 2000 to 2016. Arch Toxicol. 91, 3287-3291.

Silva, M.J., Wong, L.Y., Samandar, E., Preau, J.L., Jr., Jia, L.T., Calafat, A.M., 2019. Exposure to di-2-ethylhexyl terephthalate in the U.S. general population from the 2015-2016 National Health and Nutrition Examination Survey. Environ Int. 123, 141-147.

Sohn, J., Kim, S., Koschorreck, J., Kho, Y., Choi, K., 2016. Alteration of sex hormone levels and steroidogenic pathway by several low molecular

weight phthalates and their metabolites in male zebrafish (Danio rerio) and/or human adrenal cell (H295R) line. J. Hazard. Mater. 320, 45–54.

Soliman, A.M., Yang, H., Du, E.X., Kelkar, S.S., Winkel, C., 2015. The direct and indirect costs of uterine fibroid tumors: a systematic review of the literature between 2000 and 2013. Am J Obstet Gynecol. 213, 141-160.

Soma-Pillay, P., Nelson-Piercy, C., Tolppanen, H., and Mebazaa, A., 2016. Physiological changes in pregnancy. Cardiovasc. J. Afr. 27, 89-94.

Søeborg, T., Frederiksen, H., Andersson, A.M., 2012. Cumulative risk assessment of phthalate exposure of Danish children and adolescents using the hazard index approach. Int. J. Androl. 35, 245–252.

Stapleton, H.M., Sharma, S., Getzinger, G., Ferguson, P.L., Gabriel, M., Webster, T.F., Blum, A., 2012. Novel and high volume use flame retardants in US couches reflective of the 2005 PentaBDE phase out. Environ Sci Technol. 46, 13432-13439.

Stewart, E.A., Cookson, C.L., Gandolfo, R.A., Schulze-Rath, R., 2017. Epidemiology of uterine fibroids: a systematic review. BJOG. 124, 1501-1512.

Sun, J., Zhang, M.R., Zhang, L.Q., Zhao, D., Li, S.G., Chen, B., 2016. Phthalate monoesters in association with uterine leiomyomata in Shanghai. Int J Environ Health Res. 26, 306-316.

Sun, X., Li, J., Jin, S., Li, Y., Liu, W., Zhao, H., Zhou, Y., Jiang, Y., Liu, H., Xia, W., Cai, Z., Xu, S., Shen, X., 2018. Associations between repeated measures of maternal urinary phthalate metabolites during pregnancy and cord blood glucocorticoids. Environ. Int. 121, 471–479.

Sun, Y., Gong, X., Lin, W., Liu, Y., Wang, Y., Wu, M., Kannan, K., Ma, J., 2018. Metabolites of organophosphate ester flame retardants in urine from Shanghai, China. Environ Res. 164, 507-515.

Swan, S.H., Main, K.M., Liu, F., Stewart, S.L., Kruse, R.L., Calafat, A.M., Mao, C.S., Redmon, J.B., Ternand, C.L., Sullivan, S., Teague, J.L., Study for Future Families Research Team, 2005. Decrease in anogenital distance among male infants with prenatal phthalate exposure. Environ. Health Perspect. 113, 1056–1061.

Swan, S.H., Sathyanarayana, S., Barrett, E.S., Janssen, S., Liu, F., Nguyen, R.H., Redmon, J.B., TIDES Study Team, 2015. First trimester phthalate exposure and anogenital distance in newborns. Hum. Reprod. 30, 963–972.

Trabert, B., Chen, Z., Kannan, K., Peterson, C.M., Pollack, A.Z., Sun, L., Buck Louis, G.M., 2015. Persistent organic pollutants (POPs) and fibroids: results from the ENDO study. J Expo Sci Environ Epidemiol. 25, 278-285.

US EPA 1987. Integrated Risk Information System (IRIS). Chemical Assessment Summary. Di(2-Di(2-ethylhexyl)phthalate (DEHP); CASRN 117-81-7 (Available online at https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/ 0014_summary.pdf#nameddest=rfd (accessed Jun 3, 2019)).

US EPA 1988. Integrated Risk Information System (IRIS). Chemical Assessment Summary. Butyl benzyl phthalate; CASRN 85-68-7 (Available online at https:// cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0293_summary.pdf (accessed Jun 3, 2019)).

Valvi, D., Casas, M., Romaguera, D., Monfort, N., Ventura, R., Martinez, D., Sunyer, J., Vrijheid, M., 2015. Prenatal phthalate exposure and childhood growth and blood pressure: evidence from the Spanish INMA-Sabadell birth cohort study. Environ. Health Perspect. 123, 1022–1029.

van der Veen, I., de Boer, J., 2012. Phosphorus flame retardants: properties, production, environmental occurrence, toxicity and analysis. Chemosphere. 88, 1119-1153.

Warembourg, C., Basagaña, X., Seminati, C., de Bont, J., Granum, B., Lyon-Caen, S., Manzano-Salgado, C.B., Pin, I., Sakhi, A.K., Siroux, V., Slama, R., Urquiza, J., Vrijheid, M., Thomsen, C., Casas, M., 2019. Exposure to phthalate metabolites, phenols and organophosphate pesticide metabolites and blood pressure during pregnancy. Int. J. Hyg. Environ. Health 222, 446–454.

Watkins, D.J., Sánchez, B.N., Téllez-Rojo, M.M., Lee, J.M., Mercado-García, A., Blank-Goldenberg, C., Peterson, K.E., Meeker, J.D., 2017.

Phthalate and bisphenol A exposure during in utero windows of susceptibility in relation to reproductive hormones and pubertal development in girls. Environ. Res. 159, 143–151.

Weuve, J., Hauser, R., Calafat, A.M., Missmer, S.A., Wise, L.A., 2010. Association of exposure to phthalates with endometriosis and uterine leiomyomata: findings from NHANES, 1999-2004. Environ Health Perspect. 118, 825-832.

WHO, 2003. Diethyl phthalate. Concise International Chemical Assessment Document 52. World Health Organization (WHO)/Concise International Chemical Assessment Document (CICAD) (Available online at http://www.who.int/ipcs/publications/cicad/en/cicad52.pdf (accessed Jun 3, 2017)).

Weiss, J.M., Gustafsson, Å., Gerde, P., Bergman, Å., Lindh, C.H., Krais, A.M., 2018. Daily intake of phthalates, MEHP, and DINCH by ingestion and inhalation. Chemosphere 208, 40–49.

Wormuth, M., Scheringer, M., Vollenweider, M., Hungerbühler, K., 2006. What are the sources of exposure to eight frequently used phthalic acid esters in Europeans?. Risk Anal. 26, 803–824.

Yang, Q., Mas, A., Diamond, M.P., Al-Hendy, A., 2016. The Mechanism and Function of Epigenetics in Uterine Leiomyoma Development. Reprod Sci. 23, 163-175.

Ye, X., Pierik, F.H., Angerer, J., Meltzer, H.M., Jaddoe, V.W., Tiemeier, H., Hoppin, J.A., Longnecker, M.P., 2009. Levels of metabolites of organophosphate pesticides, phthalates, and bisphenol A in pooled urine specimens from pregnant women participating in the Norwegian Mother and Child Cohort Study (MoBa). Int. J. Hyg. Environ. Health. 212 (5), 481–491.

Yeh, H.C., Lin, Y.S., Kuo, C.C., Weidemann, D., Weaver, V., Fadrowski, J., Neu, A., and Navas-Acien, A., 2015. Urine osmolality in the US population: implications for environmental biomonitoring. Environ. Res. 136, 482-490.

Zarean, M., Keikha, M., Feizi, A., Kazemitabaee, M., Kelishadi, R., 2019. The role of exposure to phthalates in variations of anogenital distance: a systematic review and meta-analysis. Environ. Pollut. 247, 172–179.

Zeman, F.A., Boudet, C., Tack, K., Floch Barneaud, A., Brochot, C., Péry, A.R., Oleko, A., Vandentorren, S., 2013. Exposure assessment of phthalates in French pregnant women: results of the ELFE pilot study. Int. J. Hyg. Environ. Health 216, 271–279.

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.

Zhao, H., Li, J., Zhou, Y., Zhu, L., Zheng, Y., Xia, W., Li, Y., Xiang, L., Chen, W., Xu, S., Cai, Z., 2018. Investigation on metabolism of Di(2-ethylhexyl) phthalate in different trimesters of pregnant women. Environ. Sci. Technol. 52, 12851–12858.

Zhao, Y., Shi, H.J., Xie, C.M., Chen, J., Laue, H., Zhang, Y.H., 2015. Prenatal phthalate exposure, infant growth, and global DNA methylation of human placenta. Environ. Mol. Mutagen. 56, 286–292.

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.

국문 초록(Abstract in Korean)

가임기 여성의 프탈레이트 및 그 대체 물질의 노출 특성과 생식 건강 영향

가임기 여성의 내분비계교란물질(endocrine disrupting chemicals, EDCs)의 노출은 노출과 건강 영향 측면에서 중요하다. 개인생활용품 사용을 통해 노출되는 EDCs 의 경우, 여성은 남성보다 개인생활용품을 더 자주, 그리고 더 많은 양을 사용하므로 그 노출량이 여성에서 훨씬 크다. EDCs 노출은 생식에 직접적인 영향을 줄 수도 있다. 특히 가장 민감한 시기라고 할 수 있는 임신기의 EDCs 노출은 태아의 초기 발달과 태어난 이후 평생에 걸쳐 건강에 영향을 미칠 수 있다. 그러므로 가임기 여성을 대상으로, 특히 노출과 건강 영향에 민감한 시기인 임신중 EDCs 의 노출과 영향을 평가하고 관리하는 것은 중요하다.

가소제로 사용되는 프탈레이트는 항안드로겐성 및 약한 에스트로겐성을 보이는 대표적인 EDCs 이며, 내분비계 교란을 통해 사람의 건강에 영향을 미친다. 그 독성 때문에 여러 국가에서는 프탈레이트의 사용을 제한하고 있으며, 이로 인해 대체 가소제의 사용이 점점 증가하고 있다. 프탈레이트의 내분비교란성을 고려했을 때, 구조적으로 유사한 대체 가소제 역시 내분비계 교란을 일으킬 가능성이 있지만, 관련된 건강 영향 연구는 매우 부족한 실정이다. 특히나 대체 가소제의 바이오모니터링 연구는 대부분 유럽국가를 대상으로만 수행되었으며, 아시아 지역에서의 보고는 미미하다.

본 연구는 가임기 여성에서 프탈레이트 및 대체 물질의 생식건강 영향을 평가하고, 특히 화학물질 노출에 민감한 시기인 임신기의 가소제

노출 특성을 파악하고 평가하기 위해 수행되었다. 프탈레이트 및 대체물질 노출의 여성 생식 건강 측면에서 중요성을 파악하기 위해, 자궁근종 환자 대조군 연구를 수행하였다. 이를 위해 가임기 여성을 모집하여 자궁근종 진단을 위한 부인과 초음파 검사를 수행하였다. 검사 결과를 바탕으로 32 명의 환자와 79 명의 대조군을 선택하고 소변시료에서 프탈레이트와 대체 가소제, 유기인계난연제의 대사체 수준을 측정하였다. 가장 민감한 시기인 임신기의 가소제 노출 특성을 파악하기 위해, 한국과 태국에서 각 81 명과 102 명의 임산부를 모집하였다. 임신 분기별 각 1 회, 즉 임신중 총 3 회 수집된 소변시료에서 총 15 종 프탈레이트의 24 종의 대사체를 측정하였다. 임신기 소변 시료를 이용한 노출평가에서, 임신중 소변의 특성 변화를 이해하기 위해 소변보정지표인 크레아티닌, 비중, 삼투질농도를 측정하여 그 변이를 기술하였다. 전체 연구는 세 부분으로 나누어 다음과 같이 수행하였다.

첫번째 연구는 여성 생식 건강 측면에서 프탈레이트를 포함한 신규 가소제류의 노출의 중요성을 자궁근종 사례연구로 확인하기 위하여 수행되었다. 대체 가소제인, i-isononyl phthalate (DINP) 와 di(2-propylheptyl) phthalate (DPrHpP) 대사체가 소변중 75% 이상 검출되었고, 가소제로도 사용되는 유기인계난연제의 대사체중에는 diphenyl phosphate (DPHP), 2-ethylhexyl phenyl phosphate (EHPHP), 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP)가 80% 이상 검출된것으로 보아, 우리나라 가임기 여성에서 대체가소제가 널리 사용되고 있음을 의미한다. 대조군보다 환자군에서 DEHP 대사체 농도의 총합이 유의하게 높았고, (2-ethylhexyl) terephthalate (DEHTP), DPrHpP, DINCH, tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), tris(2-butoxyethyl) phosphate (TBOEP)의 노출이 자궁근종에 대한 오즈비를 증가시키는 것으로 나타났다. 이러한 연관성에 대한 생물학적인 기전으로는 DEHTP, DINCH, TDCIPP, TPOEP 물질의 estrogenic 한

성질이 자궁근종을 일으키는데 기여했을 것으로 생각된다. 이 연구는 대체 가소제류와 자궁근종간의 연관성을 보고한 첫 연구이다.

두번째 연구는 임신기 프탈레이트와 대체 가소제의 노출 양상을 기술하고 위해도가 큰 주요한 물질을 찾기 위해 수행되었다. 한국 임산부에서 프탈레이트 대사체 중 몇 종의 농도는 임신 시기에 따라 큰 변화를 보였지만, 상대적으로 태국 임산부에서는 그 변이가 작았다. 태국 임산부의 diethyl phthalate (DEP)와 dioctyl phthalate (DnOP) 대사체의 농도가 한국 임산부에서보다 2 – 3 배 높았으며, 노출원을 고려했을 때, 태국 임산부의 화장품사용과 관련이 있을 것으로 보인다. 대체 가소제인 di-(iso-nonyl)-cyclohexane-1,2-dicarboxylate (DINCH) 대사체 검출빈도는 한국에서 67.4%, 태국에서 44.9%였다. 프탈레이트 및 대체 가소제의 위해성평가 결과, 한국에서는 11.9%, 태국에서는 5.3%의 산모가 위해 지수 1 을 초과하였으며, 두 국가 모두에서 di(2-ethylhexyl) phthalate (DEHP), di-n-butyl phthalate (DnBP), di-2-isobutyl phthalate (DiBP)의 노출이 주요 요인인 것으로 나타났다. 이러한 결과는 가소제 노출 특성은 임신기간에 따라, 또한 국가에 따라 분명한 차이가 있음을 나타낸다.

세번째 연구에서는 임신기 소변의 특성 변화를 이해하고, 임신으로 인한 생리학적 변화가 노출평가에 갖는 중요성을 강조하였다. 두번째 연구에서 수집한 임산부 소변시료에서 소변 묽기 보정 지표 세가지를 측정하여 그 변이를 기술하였다. 한국 산모에서는 크레아티닌과 삼투질 농도가 임신 전기보다 후기에서 낮아졌는데, 이는 임신 후반기로 갈수록 소변이 더 묽어진다는 것을 의미한다. 그러나 태국 임산부에서는 임신 3 분기 동안 소변 묽기보정 인자의 수준이 일정했다. 캐나다, 일본 산모연구 결과와 함께 고려했을 때, 임신중 소변 묽기보정 인자의 변화가 국가 또는 생활 방식에 따라 다르게 나타날 수 있음을 의미한다.

임신중 프탈레이트 노출량은 소변 묽기 보정을 한 농도간 높은 연관성을 보이며, 이에 비해 묽기 보정 전후간 연관성은 낮게 나타났다. 소변 보정지표의 선택에 따라 임신 3 분기 프탈레이트 농도 변화 양상의 통계적 유의성에 차이를 나타냈다. 이 결과는, 임신중 체내 생리학적 변화로 인한 소변 보정 지표의 변화가 임산부의 화학물질 노출평가의 불확실성을 크게 만들 수 있음을 의미한다. 그러므로 임신기 소변중 오염물질의 측정을 통한 노출평가에서 임신 분기별, 국가별로 소변 묽기보정 인자의 변화 양상을 이해해야 한다. 또한 임신중 소변량의 변화나 체내 화학물질의 '흡수, 분배, 대사, 배출'과 관련된 일련의 특성 변화를 함께 고려하여 임신중 노출 바이오마커를 해석해야 할 것이다.

프탈레이트를 포함한 가소제를 중심으로, 가임기 여성 집단에서의 EDCs 노출과 관련된 여성 생식 건강 영향과 노출 특성을 관찰한 세 단면 연구를 통해, 대표적인 여성 생식 질환인 자궁근종과 연관성을 보이는 신규 가소제 물질을 파악하였다. 또한, 가장 민감한 임신기의 프탈레이트류 노출 양상이 임신 분기에 따라 다양하며, 임신중 소변 묽기가 변하는 양상이 국가별로 다양하다는 것을 확인하였다. 이 결과는 임산부의 소변 샘플을 사용한 바이오모니터링 연구에서, 연구 설계 및 결과 해석 과정에서 노출 및 관련 인자의 변이를 고려해야 함을 시사한다. 또한, 대체 가소제의 증가하는 사용 추세로 미루어, 향후 다양한 신규 가소제에 대한 지속적인 노출 및 건강 영향 평가가 필요할 것이다. 본 연구 결과는 적은 수의 표본을 통해 도출되었기 때문에, 더 큰 표본수의 인구집단을 대상으로 하는 후속 연구 혹은 실험연구를 통한 보완되어야 할 것이다. 본 연구는 민감인구에서 EDCs 의 노출을 정확히 평가하고, 관리하는데 기여할 수 있을 것이다. 이를 통해 사회 전반적으로 EDCs 노출을 줄여나가는 노력에 도움이 될 수 있을 것으로 기대한다.

표제어: 프탈레이트, 대체 가소제, 임산부, 임신, 소변묽기보정, 자궁근종
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