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Consumer chemicals and kidney function markers: observational studies in the general populations

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강 하 병

Consumer chemicals and kidney function markers: observational studies in the general populations

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To the Faculty of Graduate School of Public Health at
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by
Habyeong Kang

Supervised by Professor Kyungho Choi
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Data approved by:

Domyung Paek _____

Sungkyoon Kim _____

Jung Pyo Lee _____

Hyo-Bang Moon _____

Kyungho Choi _____

Abstract

Consumer chemicals and kidney function markers: observational studies in the general populations

Habyeong Kang

Graduate School of Public Health

Seoul National University

Chronic kidney disease (CKD) is a growing public health concern worldwide. Recently, environmental exposure to chemicals has been suggested as a risk factor to kidney disease. For several chemicals used in consumer products, e.g., phthalates and bisphenol A, epidemiological and experimental evidences on association with kidney disease have been accumulating. However, attempt to expand this research topic to other various chemicals in consumer products such as environmental phenols and organophosphate esters (OPEs) is minuscule yet. In this context, the present study explored possible associations of various chemicals in consumer products with kidney disease in a series of epidemiological observations.

The aim of the first study was to identify chemical determinants of the urinary albumin-to-creatinine ratio (ACR), which is a kidney disease marker, among multiple chemicals including phthalate metabolites, bisphenols, and benzophenones. For this purpose, Korean women (20–45 years old, n = 441) were recruited, and questionnaire survey and urine and blood sample collection were conducted. Firstly, using the variables measured for the participants, the creatinine-adjusted urinary concentration of each urinary chemical was associated with ACR in a linear regression model (single-pollutant model). Then, compounds with a significant association with ACR in the single-pollutant model were added in a multi-pollutant model and

evaluated for their association with ACR. Several compounds measured in the urine showed a significant association with ACR in the single-pollutant model. In the multi-pollutant model, however, only monobutyl phthalate (MBP), monoisobutyl phthalate (MiBP), monobenzyl phthalate (MBzP), and benzophenone-1 (BP-1) showed significant positive associations. The association of MBP and BP-1, which are metabolites of dibutyl phthalate (DBP) and benzophenone-3 (BP-3), respectively, remained significant in a couple of the sensitivity analyses with a different adjustment of urine dilution and in a subpopulation with normal ACR. In summary, among dozens of urinary chemicals, MBP and BP-1 consistently showed a strong association with urinary ACR.

The second study was designed to verify the potential chemical determinants of kidney disease which was identified in the first study using the US general adult population by employing data from the US National Health and Nutrition Examination Survey (NHANES) 2005-2016. Among the 9008 adults, the associations of the urinary chemicals with chronic kidney disease (CKD) and related parameters, i.e., estimated glomerular filtration rate (eGFR) and ACR were assessed. To account for urine dilution, in addition to conventional creatinine adjustment, covariate-adjusted creatinine standardization, which controls for potential confounding by kidney function, was employed. Multi-pollutant models were also constructed to verify the associations observed in the models on individual chemicals. Several chemicals were positively associated with eGFR when the conventional creatinine adjustment was applied, while most of the chemicals were negatively associated with eGFR with the covariate-adjusted standardization method, implying significance of adjustment method for urine dilution. Regardless of the adjustment methods, MBP was positively associated with ACR, and MBzP was also associated with ACR with a marginal significance, which is in line with the observations in the first study. In the multi-pollutant model, MBP, MBzP, and BPA showed significant association with eGFR, and among these three chemicals, MBP were significantly associated with CKD

outcome defined by eGFR and ACR ranges. In summary, among the US general population, MBP, MBzP, and BPA were associated with CKD related parameters, and the association with CKD outcome was most evident for MBP.

In the third study, association of OPE exposure with chronic kidney disease was evaluated employing US general adult population (NHANES 2013–2014). Among 1578 adults, the associations of the urinary OPE metabolites with CKD and related parameters were assessed. As in the second study, both the conventional creatinine adjustment and covariate-adjusted creatinine standardization were applied. Multi-pollutant models were also considered. The urinary bis(2-chloroethyl) phosphate (BCEP) level was negatively associated with eGFR only with the covariate-adjusted standardization method, but not with the conventional creatinine adjustment. In addition, both bis(1,3-dichloro-2-propyl) phosphate (BDCIPP) and di-n-butyl phosphate (DNBP) were positively associated with the ACR, regardless of methods of urine dilution adjustment. These three compounds were also associated with CKD. Following adjusting urine dilution with the covariate-adjusted standardization method, the association became more evident. Moreover, similar results were observed in the secondary analysis with the multi-pollutant models. In summary, among the US general population, several OPEs were identified as potential chemical determinants of CKD.

In the fourth study, effect modification by vitamin D was evaluated on the association between chemical exposure and CKD observed in the second and the third studies. For this purpose, population with relevant measurements were selected from NHANES 2009-2014 (n = 3207) and NHANES 2013-2014 (n = 1069). Effect modification by serum vitamin D level was tested as assessing association between the chemical concentrations and CKD parameters in stratification by vitamin D status (deficiency, insufficiency, and sufficiency). Association between OPE metabolites (i.e., BDCIPP, BCEP, and DNBP) and eGFR was decreased as serum vitamin D level was increased. Also, association of MEP and DNBP with ACR was also decreased with the

increase of serum vitamin D. In a secondary analysis in stratification with sun-exposure time and dietary vitamin D intake, the associations of BDCIPP and BCEP with eGFR were lower in groups where high vitamin D is expected (high sun-exposure time and high vitamin D intake) than in a reference group (low sun-exposure time and low vitamin D intake). On the other hand, effect modification was not obvious for other associations. In summary, vitamin D status was suggested as an effect modifier on the association between exposure to several OPEs and eGFR.

Through a sequence of the studies, chemical risk factors for kidney disease were identified. The multi-pollutant approach could identify major chemical determinants of kidney disease, i.e., MBP, MBzP, BP-1, and BPA, among the various chemicals used in consumer products. Several OPEs were also associated with CKD. Some of these associations could be modified by vitamin D status. The finding in this study will provide basic knowledge for chemical management, and the methodologies applied in this study will help conduct future studies.

Keywords: chronic kidney disease (CKD); renal function; creatinine; albuminuria; glomerular filtration rate (GFR); phthalates; environmental phenols; organophosphate esters (OPEs); vitamin D

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Contents

List of Tables

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

List of Figures

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 1. General introduction: linking chemical exposure and kidney health

1.1 Chronic kidney disease and chemical exposure

Kidney is an excretory organ found in vertebrates. The physiological function of kidney is critical in excretion of waste materials and homeostasis of body fluid. Chronic kidney disease (CKD) is defined as persistent abnormalities of function or structure of kidney over 3 months (Stevens and Levin, 2013). Globally, CKD is a growing concern with over 10% of its prevalence worldwide (Hill et al., 2016). Progress of CKD can lead to end-stage kidney failure, which is hard to be recovered and requires renal replacement therapy. Because of the physiological importance of kidney and irreversibility of end-stage kidney failure, CKD is a severe burden on patients and public health system.

1.1.1. Clinical markers for chronic kidney disease

Following the progress of CKD, the amount of blood filtered in glomerulus is decreased, resulting in decrease of excretion of waste products into urine. Therefore, glomerular filtration rate (GFR) is considered as a clinical measure of kidney function. GFR is quantified as measuring the clearance of exogenous molecules such as inulin, which are freely filtered in the glomerulus but rarely reabsorbed or secreted by the renal tubules (Laterza et al., 2002), However, because of its labor and cost-intensive nature, this method cannot be routinely applied (Laterza et al., 2002). Instead, equations to estimate GFR have been developed. These equations for estimated glomerular filtrations rate (eGFR) mostly employ serum creatinine level as a surrogate of the filtration amount (Levey et al., 2009). To consider differences of individual base levels of creatinine production, demographic factors, i.e.,

age, sex, and race/ethnicity, are include in the equations. Cystatin C has been also considered as a surrogate of the filtration amount to improve the estimation by the creatinine-based equations (Laterza et al., 2002).

Detection of abnormal levels of proteins in urine is also an indicator of kidney disease. In normal renal condition, little amount of proteins is excreted into urine because they are hardly filtered in glomerulus, and renal tubules reabsorb excess proteins filtered from the glomerulus. When renal function is impaired, excess amount of proteins can be detected in urine. Typically, urinary detection of albumin, an abundant protein in blood, is commonly used as an indicator of kidney impairment. To quantify the abnormal urinary excretion of albumin, which is called as albuminuria, urinary albumin concentration divided by urinary creatinine concentration (albumin-to-creatinine ratio, ACR) is used.

1.1.2. Risk factors for kidney disease

Cardiometabolic diseases have been investigated as major risk factors for CKD. Diabetes mellitus is known as the leading cause of CKD and end-stage renal disease worldwide (McClellan and Flanders, 2003). Diabetes mellitus can lead to diabetic nephropathy via problem in blood pressure (Lea and Nicholas, 2002). Metabolic disease (Kurella et al., 2005), and obesity (Iseki et al., 2004) are also well-known risk factors for CKD.

Chemical exposure has also been suggested to play a role in the development of CKD. For example, nephrotoxicity due to cadmium exposure has been relatively well known not only among occupationally exposed workers (Buchet et al., 1980; Jakubowski et al., 1987) but also in the general population (Åkesson et al., 2005; Noonan et al., 2002). In addition, kidney damage has been reported among infants who consumed melamine-contaminated formula (He et al., 2014) and Taiwan workers in melamine-related manufacturing factories (Wu et al., 2015).

Previous review articles summarizing epidemiological studies on chemical exposure and kidney disease are listed in Table 1-1. Metals are the most

investigated chemical group for kidney disease. Cadmium and lead, which are well-known nephrotoxicants, as well as other such as mercury and arsenic have been previously reviewed on their relationship with kidney disease. Because of melamine milk scandal in China in 2008, melamine-induced kidney disease has been studied especially on Chinese children populations. Among other organic pollutants, perfluoroalkyl substances had mostly investigated in various human populations. Not only effects of perfluoroalkyl substances on kidney health but also their pharmacokinetics by kidney status and removal during hemodialysis have been studied. For the chemicals that people are exposed to via use of consumer products can associated with kidney disease. Kataria et al. (2015) reviewed epidemiological studies associating of phthalates, bisphenol and persistent organic pollutants with chronic kidney disease markers.

Table 1-1. Previous reviews (2000-2019) on chemical exposure and kidney disease.

Reference	Chemical	Outcome	Scope
Edwards and Prozialeck (2009)	Cadmium	Diabetes and diabetes-related kidney disease	Epidemiological and animal studies
Byber et al. (2017)	Cadmium and cadmium compounds	Non-neoplastic kidney disease	Epidemiological studies including environmental and occupational exposure
Ekong et al. (2006)	Lead	Kidney effects	Epidemiological studies in general, occupational, and patient populations
Wang et al. (2013)	Melamine	Recovery of melamine-induced kidney disease	Epidemiological studies on children population with melamine induced kidney disease recovery rate reported for a specific period
Wen et al. (2016)	Melamine	Effects on kidney and urinary tract	Pediatric studies using human populations or animal models
Stanifer et al. (2018)	Perfluoroalkyl substances	kidney-related health, including clinical, histologic, molecular, and metabolic outcomes related to kidney disease, or outcomes related to the pharmacokinetic role of the kidneys	Epidemiological, pharmacokinetic, and toxicological studies
Ferrari et al. (2019)	Perfluoroalkyl substances	Kidney function markers and the ability of hemodialysis to remove PFCs from the circulating blood	Epidemiological studies

Table 1-1. (continued)

Reference	Chemical	Outcome	Scope
Kataria et al. (2015)	Phthalates, bisphenol A, perfluoroalkyl substances, dioxins, furans, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls	Albuminuria, eGFR, blood pressure, and serum uric acid	Epidemiological studies
Vervaet et al. (2017)	Cadmium, lead, mercury, melamine, aristolochic acid	Acute kidney injury	Epidemiological, *in vivo* and *in vitro* studies
Weidemann et al. (2016)	Arsenic, cadmium, lead, mercury, uranium, aristolochic acid, mycotoxins, melamine	Chronic kidney disease	Pediatric epidemiological studies
Zheng et al. (2017)	Arsenic, cadmium, lead, mercury, thallium/uranium, fluoride, aflatoxin, melamine, environmental tobacco, bisphenol A, dental procedures, phthalates, perfluorooctanoic acid, and triclosan	Kidney disease and related biomarkers	Pediatric epidemiological studies

1.1.3. Scope of the present study

Though various environmental chemicals can be associated with kidney disease, this study focused on synthetic chemicals used in consumer products. Perfluoroalkyl substances, which are also used in consumer products, were not considered in this study since their associations with kidney disease have been investigated in numerous studies (Ferrari et al., 2019; Stanifer et al., 2018). Only chemicals with short biological half-lives (< several days), e.g., plasticizers and environmental phenols, were considered in this study.

Exposure to chemicals with short half-lives has unique properties; 1) after several hours to several days of exposure, the chemicals are mainly excreted from the human body via urine, so their exposure can be monitored with their urinary biomarkers, 2) because these chemicals are not persistent in the environment, personal exposure can be easily reduced by individual efforts in a short time, and 3) they share common exposure sources leading to high correlation among the chemical exposure.

1.2 Chemical exposure and kidney disease: Findings from previous epidemiology studies

Positive associations of exposure to DEHP, other phthalates or BPA exposure with albuminuria has been reported, implying possible adverse effects of these chemicals on kidney health. For several phthalates such as low-molecular-weight phthalate, DIDP, and DINP, however, association with ACR was not observed (Table 1-2).

For the chemical exposure and eGFR (Table 1-3), associations were not significant or positive in cross-sectional studies (Malits et al., 2018; You et al., 2011). However, in a study monitoring change of eGFR, negative association between urinary BPA level and annual change of eGFR was observed for hypertension or diabetes patients (Hu et al., 2015). the positive association observed in the cross-sectional studies can be explained by reverse causality (Weaver et al. 2016), which is described in **Section 1.2.4.** in detail.

CKD outcome or other related biomarkers have been associated with phthalates or BPA exposure (Table 1-4). In a US children population, urinary levels of phthalates metabolites and BPA are not positively associated with urinary protein to creatinine ratio (Malits et al. 2018). In a Taiwan study, children who consumed food with higher contamination of DEHP showed higher level of N-acetyl β-D-glucosaminidase, which is a marker for renal tubule dysfunction (CF Wu et al., 2015). In China population, patients with higher BPA level showed more frequent CKD development (Hu et al., 2015).

Table 1-2. Studies associating exposure to phthalates or bisphenols with albuminuria

Chemical	Major findings	Country	Target population	Sample size	Study period	Reference
DEHP	Increase of microalbuminuria for higher exposure to DEHP	Taiwan	Children who consumed DEHP-contaminated food	189	2012-2013	CF Wu et al. (2018)
DEHP	Increase of microalbuminuria for higher exposure to DEHP	Taiwan	Children who consumed DEHP-contaminated food	166	2012-2013	Tsai et al. (2016)
Metabolites of high-molecular-weight phthalates and DEHP	Positive association with ACR	US	Children (6-19 yr, NHANES 2009-2010)	667	2009-2010	Trasande et al. (2014)
Metabolites of low-molecular-weight phthalates, DIDP, and DINP	No association with ACR	US	Children (6-19 yr, NHANES 2009-2010)	667	2009-2010	Trasande et al. (2014)
BPA	Increase of ACR for higher level of BPA	US	Children (6-19 yr, NHANES 2009-2010)	667	2009-2010	Trasande et al. (2013)
BPA	Increase of low-grade albuminuria for higher level of BPA	China	Adults (> 40 yr)	3055	2008	Li et al. (2012)

Table 1-2. (continued)

Chemical	Major findings	Country	Target population	Sample size	Study period	Reference
Metabolites of DEHP	Increase of albuminuria for higher level of DEHP metabolites	Italy	Adults with diabetes (>18 yr)	209	2017	Mengozzi et al. (2019)
Metabolites of DEP, BBP, and DEHP	Positive association with ACR	China	Adults (>18 yr)	1663	2012-2014	J Chen et al. (2019)
Metabolites of DBP and DiBP	Negative association with ACR	China	Adults (>18 yr)	1663	2012-2014	J Chen et al. (2019)

Abbreviations: Diethyl phthalate (DEP), benzyl butyl phthalate (BBP), di-n-butyl phthalate (DBP), di-2-isobutyl phthalate (DiBP), di-(2-ethylhexyl) phthalate (DEHP), di-isodecyl Phthalate (DIDP), diisononyl Phthalate (DINP), and bisphenol A (BPA)

Table 1-3. Studies associating exposure to phthalates or bisphenols with estimated glomerular filtration rate (eGFR)

Chemical	Major findings	Country	Target population	Sample size	Study period	Reference
Several metabolites of phthalates	No association or positive association with eGFR	US	Children with mildly or moderately impaired kidney function (1-17 yr)	451	2009-2014	Malits et al. (2018)
Bisphenol A (BPA)	No association with eGFR	US	Children with mildly or moderately impaired kidney function (1-17 yr)	451	2009-2014	Malits et al. (2018)
BPA and triclosan	Positive association with eGFR	US	Adults aged 20 yr or over (NHANES 2003-2006)	NA	2003-2006	You et al. (2011)
BPA	Annual decrease of eGFR for higher level of BPA	China	Hypertension patients with eGFR of 60 or over (20-80 yr)	302	2008-2014	Hu et al. (2015)
BPA	Annual decrease of eGFR for higher level of BPA	China	Diabetes patients with eGFR of 60 or over (35-80 yr)	121	2008-2014	Hu et al. (2015)

Table 1-4. Studies associating exposure to phthalates or bisphenols with CKD and other related biomarkers

Chemical	Major findings	Country	Target population	Sample size	Study period	Reference
Several metabolites of phthalates	No association or negative association with urinary protein-to-creatinine ratio	US	Children with mildly or moderately impaired kidney function (1-17 yr)	436	2009-2014	Malits et al. (2018)
DEHP	Positive association with N-acetyl β-D-glucosaminidase	Taiwan	Children who consumed DEHP-contaminated food	189	2012-2013	CF Wu et al. (2018)
Metabolites of DEP, BBP, and DEHP	Positive association with urinary β_2-microglobulin	China	Adults (>18 yr)	1663	2012-2014	J Chen et al. (2019)
Metabolites of DBP and DiBP	Negative association with urinary β_2-microglobulin	China	Adults (>18 yr)	1663	2012-2014	J Chen et al. (2019)
Metabolites of BBP and DEHP	Positive association with urinary N-acetyl β-D-glucosaminidase	China	Adults (>18 yr)	1663	2012-2014	J Chen et al. (2019)
Metabolites of DMP DBP, and DiBP	Negative association with urinary N-acetyl β-D-glucosaminidase	China	Adults (>18 yr)	1663	2012-2014	J Chen et al. (2019)

Table 1-4. (continued)

Chemical	Major findings	Country	Target population	Sample size	Study period	Reference
BPA	Higher CKD prevalence for higher BPA level	China	Hypertension patients with eGFR of 60 or over (20-80 yr)	302	2008-2014	Hu et al. (2015)
BPA	Higher CKD prevalence for higher BPA level	China	Diabetes patients with eGFR of 60 or over (35-80 yr)	121	2008-2014	Hu et al. (2015)
BPA	No association with urinary protein to creatinine ratio	US	Children with mildly or moderately impaired kidney function (1-17 yr)	436	2009-2014	Malits et al. (2018)
BPA	Increase of hyperuricemia for higher level of BPA	China	Adults (20-80 yr)	482	2008-2014	Hu et al. (2015)
Triclosan and metabolites of DEHP	Positive association with urinary β_2-microglobulin	Korea	Adults (≥19 yr, KoNEHS 2012-2014)	5327-5435	2012-2014	Lim et al. (2019)
Metabolite of DBP	Negative association with urinary β_2-microglobulin	Korea	Adults (≥19 yr, KoNEHS 2012-2014)	5431	2012-2014	Lim et al. (2019)

Abbreviations: Dimethyl phthalate (DMP), diethyl phthalate (DEP), benzyl butyl phthalate (BBP), di-n-butyl phthalate (DBP), di-2-isobutyl phthalate (DiBP), di-(2-ethylhexyl) phthalate (DEHP), and bisphenol A (BPA)

1.2.1. Research gaps and challenges

1) Multiple exposure to the urinary chemicals sharing common exposure sources

Since exposure sources of chemicals used in consumer products are similar, urinary levels of these chemicals usually share relatively high correlation coefficients. Because of this high correlation, even though significant associations are observed between chemical levels and kidney disease, it is hard to identify the real player for the association among the chemicals with statistically significant associations. To overcome this issue, statistical approaches to consider this complex situation with multiple with common sources have been introduced in environmental epidemiology studies (Kim et al., 2017; Lenters et al., 2015). However, this effort is scares for kidney outcome so far.

2) Urine dilution adjustment

When associating urinary biomarkers of exposure and health outcome, urine dilution should be adjusted to account variations in intra-individual hydration status of urine over time. For this purpose, adjustment factors such as urinary creatinine, specific gravity of urine, or urine flow rate are commonly used. However, when the health outcome of interest is kidney disease, adjustment methods should be applied with caution because the adjustment factors are directly affected by kidney status (Bulka et al., 2017; Weaver et al., 2016).

In this situation, urinary creatinine is directly caused by both urine dilution status and kidney status (Fig. 1-1). Such factors directly caused by two independent variables are called as collider. When collider is included in statistical models, collider bias can be introduced possibly leading to confounding (Bulka et al., 2017; O'Brien et al., 2016). To minimize this collider bias, covariate-adjusted standardization is proposed (O'Brien et al., 2016) and applied in an obesity study (Bulka et al., 2017), while this method has never applied to kidney studies.

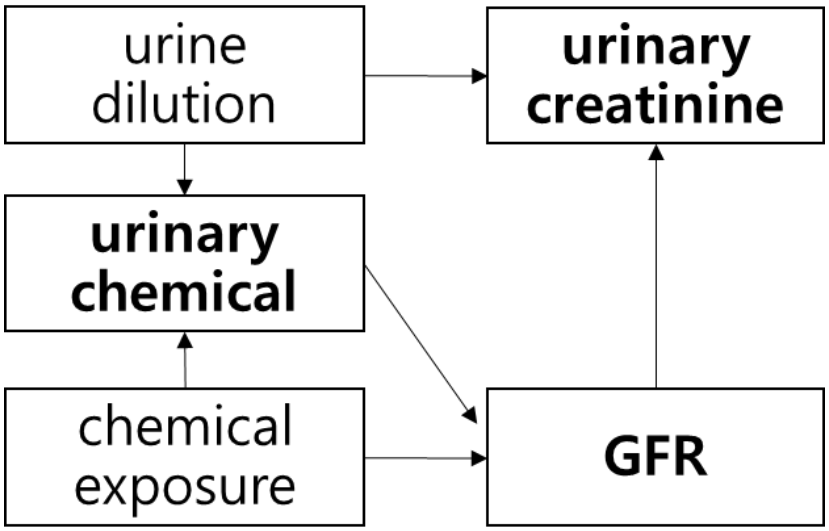

Fig. 1-1. Directed acyclic graph pathway demonstrating urinary creatinine as a collider in the causal relationship between urinary chemical levels and glomerular filtration rate (GFR). Boldface represents variables measured in the studies.

3) Effect modifiers

Exposure to chemicals used in consumer products can be mitigated by personal efforts to reduce exposure to these chemicals, e.g., reducing use of the products with the chemicals, replacing the products into an alternative one without the chemicals, or removing the chemicals in the living environment with frequent cleaning. However, because of ubiquitous contamination of the chemicals in the environment, it is hard to reduce the exposure to zero. Therefore, together with the efforts to reduce the chemical exposure, efforts to identify factors to modify the risk caused by chemical exposure could help reduce chemical-induced kidney disease. Chemical-induced kidney disease are considered to be mediated by oxidative stress and inflammation (Kataria et al., 2015). Therefore, antioxidants or other micronutrients can be candidates for the effect modifier of chemical-induced kidney disease. However, this attempt to find possible effect modifier has not been tried in epidemiological studies so far.

1.3 Chemical exposure and kidney disease: Findings from experimental studies

For chemicals used in consumer products, their nephrotoxicity and related mechanisms have been experimentally investigated in animal (Table 1-5) and *in vitro* models (Table 1-6). Di-(2-ethylhexyl) phthalate (DEHP), a well-known phthalate plasticizer, was revealed to induce epithelial-to mesenchymal transition via AKT and PPAR γ-related signaling pathways (CT Wu et al., 2018). Another phthalate plasticizer, dibutyl phthalate, also induced damage and functional impairment in mouse kidney (Cheng et al. 2019). Genotoxicity was observed in a human renal proximal convoluted tubule cell line exposed to diisononyl cyclohexane-1,2-dicarboxylate (DINCH), which is an alternative plasticizer of DEHP (Vasconcelos et al., 2019).

Bisphenol A (BPA)-induced nephrotoxicity and its mechanism have been investigated in both *in vivo* and *in vitro* models. Following the exposure to BPA, kidney damage and reduced kidney function have been observed in mice and (Olea-Herrero et al., 2014; Tong et al., 2019). Nephrotoxicity of BPA was revealed to be mediated by mitochondrial damage including oxidative stress and apoptosis in a human renal proximal convoluted tubule cell line (Bosch-Panadero et al., 2017), and inflammation was revealed to mediate BPA-induced podocytopathy (Tong et al., 2019).

Organophosphate esters (OPEs), which are used as plasticizers and flame retardants, such as tris(1,3-dichloro-2-propyl) phosphate (TDCIPP) and tris(2-chloroethyl) phosphate (TCEP), also have shown toxic effects, e.g., oxidative stress, proapoptotic effects, and alteration of cell cycle and uptake of molecules, in renal proximal tubule cells (Killiea et al., 2017; Ren et al., 2008; 2009; 2012).

Table 1-5. Animal studies on adverse effects of chemicals in consumer products on kidney

Chemical exposure	Animal model	Major finding	Reference
Di-(2-ethylhexyl) phthalate (DEHP)	Kidney fibrosis mouse model (C57BL/6 male mouse)	Induction of renal fibrosis and nephropathy, induction of epithelial-to-mesenchymal transition mediated by PPARγ-related signaling pathway	CT Wu et al. (2018)
DEHP	Quail (*Coturnix Japonica*)	Heat shock response-mediated kidney injury	Li et al. (2018)
Dibutyl phthalate	KM mouse	Histological damage in kidney, increase of serum creatinine and urea nitrogen, oxidative stress, oxidative stress-mediated alteration of expression of phosphorylated ERK1/2,	Cheng et al. (2019)
Bisphenol A	CD1 mouse	Proteinuria, renal hypertrophy, glomerular hyperfiltration, and change in renal morphology and renal protein expression	Olea-Herrero et al. (2014)
Bisphenol A	Male C57BL/6 mouse	Increase of serum creatinine and blood urea nitrogen, induction of fibrosis, and inflammation-related podocytopathy	Tong et al. (2019)
Benzophenone-2	*Xenopus laevis*	Histological pathology in kidney	Haselman et al. (2016)

Table 1-6. *In vitro* studies on adverse effects of chemicals in consumer products on kidney

Chemical exposure	*In vitro* model	Major finding	Reference
Di-(2-ethylhexyl) phthalate (DEHP) and mono-(2-ethylhexyl) phthalate (MEHP)	NRK-52E cell line (rat renal proximal tubule cell)	Induction of epithelial-to-mesenchymal transition mediated by AKT and PPARγ-related signaling pathways (only for DEHP)	CT Wu et al. (2018)
Diisononyl cyclohexane-1,2-dicarboxylate (DINCH)	HK-2 cell line (human renal proximal convoluted tubule cell)	Genotoxicity	Vasconcelos et al. (2019)
Bisphenol A	Conditionally immortalized mouse podocyte	Hypertrophy apoptosis, alteration of expression of TGF-β1 system and cyclin-dependent kinase inhibitor p27Kip1, collagen IV production, and downregulation of nephrin and podocin	Olea-Herrero et al. (2014)
Bisphenol A	HK-2 cell line	Energy depletion caused by mitochondrial dysfunction, oxidative stress and apoptosis in mitochondria and cytoplasm	Bosch-Panadero et al. (2017)

Table 1-6. (continued)

Chemical exposure	*In vitro* model	Major finding	Reference
Bisphenol A	Immortalized human podocyte	Inflammation-related podocytopathy	Tong et al. (2019)
Bisphenol A	Marc-145 cell line (Rhesus monkey embryo renal epithelial cell)	Oxidative stress, apoptosis, and DNA damage	Yuan et al. (2019)
Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP)	HK-2 cell line	Oxidative stress-mediated toxicity	Killilea et al. (2017)
Tris(2-chloroethyl) phosphate (TCEP)	Primary rabbit renal proximal tubule cell	Alteration of cell cycle regulatory protein expression	Ren et al. (2008)
TCEP	Primary rabbit renal proximal tubule cell	Reduced uptake of ion and non-ion molecules and alteration of uptake related protein expression	Ren et al. (2009)
TCEP	Primary rabbit renal proximal tubule cell	Proapoptotic effect	Ren et al. (2012)

1.4 Study design and objectives

The aim of this study is to evaluate association between exposure to chemicals used in consumer products and kidney disease and find possible effect modifier on these association. This study consists of a series of human observational studies (Fig. 1-3). In the first study (**Chapter 2**), various chemicals were associated with ACR in a Korean women population to identify the major player for the association among the chemicals with high correlation. In the second study (**Chapter 3**), the observation in the first study was replicated in the US general population (NHANES 2005-2016) employing eGFR and ACR as CKD parameters. In the third study (**Chapter 4**), organophosphate esters (OPEs), which have not been assessed for kidney disease before, were associated with CKD parameters employing the US general population (NHANES 2013-2014). Following **Chapter 3 and 4**, vitamin D was investigated as an effect modifier on the association between chemical exposure and CKD in **Chapter 5**.

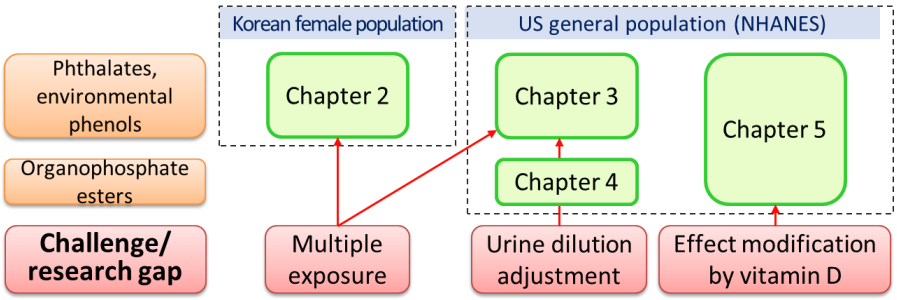

Fig. 1-2. Study designs for associating exposure to chemicals in consumer products and kidney disease.

Chapter 2. Association of phthalate and environmental phenol metabolites with albuminuria: a Korean women population

2.1 Introduction

Chronic kidney disease (CKD) is a global health threat of growing concern, and its prevalence worldwide is estimated at 11–13% (Hill et al., 2016). In Korea, CKD prevalence is estimated at 8.2% among adults, with a higher rate among females (8.9%) than in males (7.5%) (Park et al., 2016). Since its progress to end-stage kidney failure requires renal replacement therapy, which is a great burden on both patients and the health care system, the prevention of CKD is important.

CKD can be developed as a consequence of dysfunction in glomerular filtration. Clinically, the glomerular filtration rate (GFR) is considered as an indicator of kidney function. When GFR is impaired, larger molecules in the blood, such as albumin, may reach the urine. Therefore, the urinary albumin concentration adjusted by the urinary creatinine concentration (albumin-to-creatinine ratio, ACR) measured in the urine can be another marker for kidney function, which could be used alternatively to GFR. Micro- and macro-albuminuria, which are defined as ACR of 30–300 and >300 mg/g, respectively, are considered to reflect an abnormal status of kidney function, although ACR below the 30 mg/g threshold can also be indicative of altered renal function (Danziger, 2008; Knight and Curhan, 2003).

Major risk factors for CKD include various chronic diseases such as metabolic syndrome (Kurella et al., 2005) and diabetes mellitus (Brancati et al., 1997). Chemical exposure has also been suggested to play a role in the development of CKD. For example, nephrotoxicity due to cadmium exposure has been relatively well known not only among occupationally exposed

workers (Buchet et al., 1980; Jakubowski et al., 1987) but also in the general population (Åkesson et al., 2005; Noonan et al., 2002). In addition, kidney damage has been reported among infants who consumed melamine-contaminated formula (He et al., 2014) and Taiwan workers in melamine-related manufacturing factories (Wu et al., 2015).

While studies on chemically induced kidney injury have focused on typical kidney toxins such as cadmium (Järup and Åkesson, 2009) and melamine (Wang et al., 2013), there are growing evidences suggesting that other environmental contaminants, such as non-persistent endocrine disrupting chemicals (EDCs), are associated with adverse kidney function (Kataria et al., 2015). For instance, exposure to di-(2-ethylhexyl) phthalate (DEHP) through the consumption of contaminated milk was associated with micro-albuminuria in children (Tsai et al., 2016; CF Wu et al., 2018). The association of urinary phthalate metabolites with CKD markers such as ACR, estimated glomerular filtration rate (eGFR), and urinary protein-to-creatinine ratio (UPCR) has also been reported in a population of children from the US National Health and Nutrition Examination Survey (NHANES) (Malits et al., 2018; Trasande et al., 2014). Urinary bisphenol A (BPA) levels were also associated with ACR among the US children (Trasande et al., 2013), as well as Chinese adults (Li et al., 2012). A positive association of urinary BPA levels was also reported for eGFR among a general population of the US adult females, but not in the adult males who participated in NHANES 2003–2006 (You et al., 2011).

People are not exposed to a single compound but to multiple compounds from their environment at the same time, through the same living environment. Indeed, many non-persistent EDCs, e.g., phthalates, bisphenols, benzophenones, and parabens, share common exposure sources such as plastic ware and personal care products among many things. In association studies, efforts have been made to consider multiple compounds in statistical models (Kim et al., 2017; Lenters et al., 2015; Patel et al., 2010). Previously, we demonstrated that a significant association between BPA and thyroid hormones observed in a regression model involving one compound ('single-

pollutant model') disappeared when multiple compounds, including DEHP and benzophenone-3 (BP-3), were considered together in the regression model ('multi-pollutant model') (Kim et al., 2017).

For identifying chemical determinants of renal function among humans, only a few studies have employed a multiple-chemical approach or a multi-pollutant model, and most studies were conducted only among children (Malits et al., 2018; Trasande et al., 2013; CF Wu et al., 2018). For example, a significant association of BPA with ACR among the US children was verified by adding BP-3 and triclosan together with BPA in the statistical model (Trasande et al., 2013). Urinary phthalate metabolites and BPA were associated with eGFR and UPCR among the children with CKD (Malits et al., 2018). The interaction of DEHP and melamine on ACR was reported in a population of Taiwan children (CF Wu et al., 2018). However, attempts to consider exposure to multiple chemical groups in association with kidney function are still scarce, especially among a healthy adult population.

In the present study, we aimed to identify major urinary chemicals that are significantly associated with a renal function marker, i.e., ACR, among healthy adult females before menopause. Considering potential interactions among the measured chemicals (e.g., multicollinearity), a multi-pollutant regression model, as well as another sensitivity analysis, were conducted. The results of this study will help find chemical determinants of renal function among healthy adults and develop both epidemiological and experimental studies to validate the role of the identified chemicals.

2.2 Materials and methods

2.2.1. Study participants and sample collection

During 2015–2016, healthy adult females before menopause (n = 516, 20–48 years) were recruited from university hospitals located in Seoul, Incheon, Gyeonggi Province, and Jeju Province of Korea and were assessed for environmental chemical exposure and health outcomes. Among the original population, 33 participants with current pregnancy and another 42 women with missing values were excluded, and a total of 441 participants (20–45 years of age) were included in the final population of this study (Fig. 2-1).

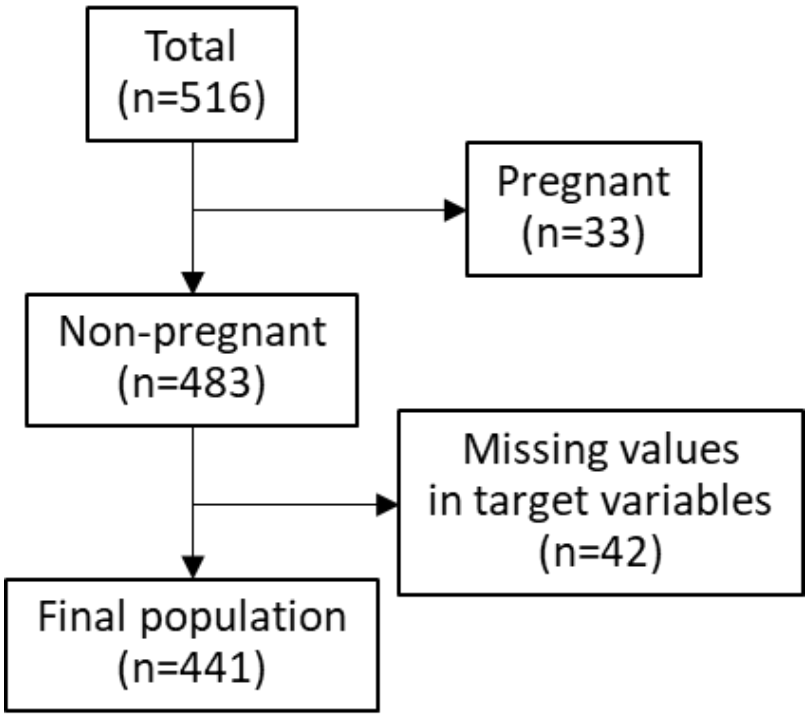

Fig. 2-1. Criteria for selecting study population.

Fasting blood samples were collected in serum separation tubes when each participating woman visited the hospital, i.e., Soonchunhyang University Hospital in Seoul, Inha University Hospital in Incheon, Hallym University Dongtan Sacred Heart Hospital and Korea University Ansan Hospital in Gyeonggi, and The Environmental Health Center of Jeju National University in Jeju. The serum was then separated with centrifugation and was stored at −80 °C until analysis. Spot urine samples were also collected at the time of the hospital visit after >9 h of fasting and were stored at −20 °C until analysis. The research protocol of this study was reviewed and approved by the Institutional Review Board of Seoul National University (IRB No. 1509/001-011).

2.2.2. Quantification of urinary endocrine disrupting chemicals

A total of 32 compounds, which included 17 phthalate metabolites, 5 bisphenols, 3 benzophenones, 5 parabens, and 2 antimicrobials, were analysed in the urine samples (Tables 2-1 and 2-2). Details of the extraction and quantification of EDCs in urine are described in **Section 2.2.2.1.** For adjustment of urine dilution, creatinine and specific gravity (SG) of the urine samples were measured by enzymatic kinetic colorimetry assay (CREA, Roche Diagnostics, Germany) and reflection refractometry (URISYS 2400 Cassette, Roche Diagnostics, Germany), respectively.

Table 2-1. Target phthalates and their urinary metabolites analyzed in this study.

Parent compounds	Chemical formula	Molecular weight (g/mole)	Metabolites	Chemical formula	Molecular weight (g/mole)
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 (DiPrP)	$C_{14}H_{18}O_4$	250.3	Mono-isopropyl phthalate (MiPrP)	$C_{11}H_{12}O_4$	208.2
Di-n-butyl phthalate (DBP)	$C_{16}H_{22}O_4$	278.3	Mono-n-butyl phthalate (MBP)	$C_{12}H_{14}O_4$	222.2
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-pentyl phthalate (DPeP)	$C_{18}H_{26}O_4$	306.4	Mono-n-pentyl phthalate (MPeP)	$C_{13}H_{16}O_4$	236.3
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
Benzyl butyl phthalate (BBP)	$C_{19}H_{20}O_4$	312.4	Monobenzyl phthalate (MBzP)	$C_{15}H_{12}O_4$	256.3
Di-isononyl phthalate (DiNP)	$C_{26}H_{42}O_4$	422.6	Mono-isononyl phthalate (MiNP)	$C_{17}H_{24}O_4$	292.4
Dioctyl phthalate (DOP)	$C_{24}H_{38}O_4$	390.6	Mono(3-carboxypropyl) phthalate (MCPP)	$C_{12}H_{12}O_6$	252.2
			Monooctyl phthalate (MOP)	$C_{16}H_{22}O_4$	278.3
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-carboxypentyl) phthalate (MECPP)	$C_{16}H_{20}O_6$	308.3
			Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	$C_{16}H_{22}O_5$	294.3
			Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP)	$C_{16}H_{22}O_5$	292.3
			Mono-[(2-carboxymethyl)hexyl] phthalate (MCMHP)	$C_{16}H_{20}O_6$	308.3

Table 2-2. Target environmental phenols analyzed in this study.

Compounds		Chemical formula	Molecular weight (g/mole)
Bisphenols	Bisphenol F (BPF)	$C_{13}H_{12}O_2$	200.2
	Bisphenol A (BPA)	$C_{15}H_{16}O_2$	228.3
	Bisphenol B (BPB)	$C_{16}H_{18}O_2$	242.3
	Bisphenol S (BPS)	$C_{12}H_{10}O_4S$	250.3
	Bisphenol AP (BPAP)	$C_{20}H_{18}O_2$	290.4
Benzophenones	Benzophenone-1 (BP-1)	$C_{13}H_{10}O_3$	214.2
	Benzophenone-3 (BP-3)	$C_{14}H_{12}O_3$	228.2
	Benzophenone-8 (BP-8)	$C_{14}H_{12}O_4$	244.2
Parabens	Methyl paraben (MePr)	$C_8H_8O_3$	152.1
	Ethyl paraben (EtP)	$C_9H_{10}O_3$	166.2
	Propyl paraben (PrP)	$C_{10}H_{12}O_3$	180.2
	Butyl paraben (BuP)	$C_{11}H_{14}O_3$	194.2
	Benzyl paraben (BzP)	$C_{14}H_{12}O_3$	228.2
Antimicrobials	Triclosan (TCS)	$C_{12}H_7C_{l3}O_2$	189.5
	Triclocarban (TCC)	$C_{13}H_9C_{l3}N_2O$	315.6

2.2.2.1. Quantification of urinary endocrine disrupting chemicals

1) Extraction of chemicals in urine

Extraction methods of urinary phthalate metabolites are described as follows. Urine samples (500 μL) were spiked with internal standards. Deconjugation was carried out with 0.2 mL of ammonium acetate buffer (1.0 M; pH 4.5) containing β-glucuronidase (2 μL/mL) at 37℃ for 12 hr. After that, the samples were diluted with 1 mL of phosphate buffer and loaded onto solid phase extraction cartridges (ABS ELUT-Nexus, Varian, Walnut Creek, CA, US; 60 mg/3 mL) pre-conditioned with 1.5 mL of acetonitrile and 2 mL of phosphate buffer (pH 2). After the loading, the cartridge was rinsed with 2 mL of formic acid (0.1 M) and 1.2 mL of Mili-Q water and dried under nitrogen. The target compounds were recovered from the dried cartridge with 1.2 mL of acetonitrile and 1.1 mL of ethyl acetate. The extract was concentrated under nitrogen and reconstituted with acetonitrile and Milli-Q water solution (1:9).

Extraction methods of urinary environmental phenols are described as follows. Urine samples (500 μL) were spiked with internal standards. Deconjugation was carried out with 0.25 mL of ammonium acetate buffer (1 M; pH 4.5) containing β-glucuronidase (2 μL/mL) at 37℃ for 10 hr. After that, target analytes were extracted with 3 mL of ethyl acetated by shaking for 60 min. Then, the samples were centrifuged at 4000 rpm for 10 min, supernatants were collected and dried. The extracts were reconstituted in 500 μL of methanol.

2) Instrumental analysis

The target analytes were chromatographic separated by an Agilent 1260 Series HPLC system (Agilent Technologies, Santa Clara, CA) equipped with a Betasil C18 column (Thermo Electron, Bellefonte, PA; 100 mm × 2.1 mm, 5 μm). Liquid chromatography was run with 10 μL of injection volume and 300 μL/min of flowrate (3 μL of injection volume and 200 μL/min of low rate only for parabens and antimicrobials). Identification and quantification of

target compounds were accomplished with an API 4000 electrospray triple quadrupole mass spectrometer (ESI–MS/MS; AB Sciex, Framingham, MA). Details of analytical condition are shown in Table 2-3.

Table 2-3. Instrumental condition of LC-ESI-MS/MS

Compounds	Parameter	Condition
All compounds	**Column**	Betasil C_{18} (2.1 × 100 mm, 5 μm)
	Ionization mode	ESI / negative
Phthalate metabolites	**Mobile phase**	A: 0.1% acetic acid in water
		B: 0.1% acetic acid in acetonitrile
	Gradient mode	Time: 0, 2, 6, 7, 9, 12, 17, 19 and 26
		A (%): 80, 80, 60, 50, 50, 10, 10, 80, and 80
		B (%): 20, 20, 40, 50, 50, 90, 90, 20, and 20
	Flow rate	300 μL/min
	Injection volume	10 μL
Bisphenols and benzophenones	**Mobile phase**	A: methanol
		B: 0.1% ammonium hydroxide in water
	Gradient mode	Time: 0, 2, 5, 7, 10, 14, 14.5, and 20
		A (%): 15, 15, 75, 75, 99, 99, 15, and 15
		B (%): 85, 85, 25, 25, 1, 1, 85, and 85
	Flow rate	300 μL/min
	Injection volume	10 μL
Parabens and antimicrobials	**Mobile phase**	A: methanol
		B: 0.1% formic acid in water
	Gradient mode	Time:0, 6, 9, 11, 13.5, 21, 21.1, and 26
		A (%): 5, 5, 50, 75, 99, 99, 5, and 5
		B (%): 95, 95, 50, 25, 1, 1, 95, and 85
	Flow rate	200 μL/min
	Injection volume	3 μL

3) Quality assurance and quality control

Two procedural blanks and two matrix spiked samples were processed along with the real samples for each batch of analysis. Between every 10-15 samples, midpoint calibration standard was injected to assure instrumental sensitivity. Trace concentrations of (0.76 ng/mL), MiBP (0.49 ng/mL), MECPP (0.12 ng/mL), MEHP (1.04 ng/mL) and BPA (0.21 ng/mL) were detected in procedural blanks. To adjust this, the concentrations in the procedural blanks were subtracted from the concentrations of the participants urine samples. The limits of quantification (LOQs) of phthalate metabolites and environmental phenols varied from 0.01 to 0.1 ng/mL. In the blanks, the recoveries of phthalate metabolites, bisphenols, benzophenones, parabens, and antimicrobials were 83-122%, 97-111%, 69-127%, 107-131%, and 120-147%, respectively. The recoveries of phthalate metabolites, bisphenols, benzophenones, parabens, and antimicrobials in matrix blanks were 62-205%, 89-104%, 63-119%, 83-99%, and 112-131%, respectively.

2.2.3. Health-related measurements

For ACR calculation, albumin and creatinine were measured in the urine samples. For measurement of urinary albumin, immunoturbidimetric assay using an ALBT2 microalbumin kit was used (Roche Diagnostics, Germany). In addition, urinary cotinine was measured using an Immulite 2000 Nicotine Metabolite kit (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Insulin, glucose, triglyceride, and high-density lipoprotein (HDL)-cholesterol serum levels were measured using commercially available kits produced by Roche Diagnostics (Germany). Both systolic and diastolic blood pressure were measured using an automated machine for blood pressure measurement after a rest of 5 minutes or more. Using blood pressure data from the Korean NHANES 2016 (available at https://knhanes.cdc.go.kr/knhanes/eng/index.do), z-scores of systolic and diastolic blood pressures were calculated, stratified by age groups (20–24, 25–29, 30–34, 35–39, 40–44, and 45–49 years). Based on the height and weight of a subject, body mass index (BMI) was calculated

using the following equation.

2.2.4. Statistical analysis

Statistical analyses were carried out only for the compounds with a detection frequency > 70%. For the non-detects, the limit of quantification (LOQ)/$\sqrt{2}$ was used as a proxy value. Correlations among the unadjusted urinary concentrations of the compounds were determined by Spearman's correlation coefficients. DEHP concentration corresponding to the sum of urinary DEHP metabolites ($DEHP_{sum}$) was calculated using the following equation.

$$\mathrm{DEHP_{sum}} = \left[\left(\sum \frac{\text{molar concentration of metabolite}}{\text{FUE of metabolite}}\right) \div 5\right] \times (\text{molecular weight of DEHP})$$

For molar excretion fractions through urine, i.e., FUEs of mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono-(2-carboxymethyl) phthalate (MCMHP), those reported by Koch et al. (2005) were used (5.9, 18.5, 23.3, 15, and 4.2%, respectively). BP-1 is a urinary metabolite of BP-3, but FUEs of BP-1 and -3 corresponding to BP-3 intake are not available. Therefore, the BP-3 concentration corresponding to the sum of urinary BP-1 and BP-3 was estimated using the following equation.

$$\text{BP-3}_{sum} = \text{BP-3 concentration} + (\text{molar concentration of BP-1}) \times (\text{molecular weight of BP-3})$$

For regression models, urinary chemical concentrations were adjusted not only by urinary creatinine concentration but also by SG, to correct urine dilution, using the following equations.

$$\text{Creatinine-adjusted concentration (μg/g creatinine)} = \frac{\text{chemical concentration (ng/mL)}}{\text{creatinine concentration (g/L)}}$$

$$\text{SG-adjusted concentration} = \text{(chemical concentration)} \times [(SG_{age\text{-}group} - 1)/(\text{individual SG} - 1)]$$

$SG_{age\text{-}group}$ represents the arithmetic means of the SGs measured from the urine collected from each age group (20–24, 25–29, 30–34, 35–39, 40–44, and 45–49 years of age). Urinary chemical concentrations and ACRs were log-transformed before the regression analyses.

The association analysis was conducted in two steps. First, in the single-pollutant model, each chemical measured in the urine was included in a linear regression model with two sets of covariates. The first set of covariates (first model) included demographic variables, i.e., age (continuously), region (categorically: Seoul, Gyeonggi and Incheon, and Jeju), education (categorically: high school or less, undergraduate, and graduate school), parity (categorically: 0 and ≥1), and urinary cotinine (categorically: <10, 10–500, and >500 ng/mL). The second set of covariates (second model) included health-related variables, i.e., BMI, serum insulin, fasting glucose, triglyceride, HDL-cholesterol, systolic blood pressure z-score, and diastolic blood pressure z-score (all continuously). Second, a multi-pollutant model was developed with the urinary chemicals that were determined to have a significant association with ACR in the single-pollutant model, following a similar approach as Kim et al. (2017). In developing the multi-pollutant model, $DEHP_{sum}$ was used to represent urinary DEHP metabolites. For two benzophenones, i.e., BP-1 and BP-3, BP-1, was used in the multi-pollutant model, because BP-1 is a major metabolite of BP-3 (Wang and Kannan, 2013).

Because changes in kidney function could influence the concentrations of

urinary creatinine and other chemicals, which may possibly lead to reverse causality in association studies involving urinary chemical measurements (Weaver et al., 2016), a sensitivity analysis was conducted only for the women with ACR < 9.71 mg/g, i.e., those with low-grade albuminuria, and the results were compared with those calculated from the total population. Statistical significance was determined at $p = 0.05$. All statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA).

2.3 Results

2.3.1. Participants' characteristics

Participating women were between 20 and 45 years of age, and a majority (48.3%) were in their thirties (Table 2-4). Most women (78.2%) showed BMI in the normal range (18.5–24.9 kg/m^2), while 5.9 and 3.2% of the women were underweight (<18.5 kg/m^2) and obese (≥30 kg/m^2), respectively. Approximately 2% of the participating women, i.e., 11 and 8 out of 441 women, showed hypertension for systolic and diastolic blood pressure, respectively. Approximately 8.4% of the participants showed urinary ACR > 30 mg/g, i.e., micro/macro-albuminuria, and 27.4% showed urinary ACR in the range of 9.71–30 mg/g, which is considered low-grade albuminuria (Li et al., 2012).

Table 2-4. Characteristics of the participant women.

Characteristics	N (%)
Total	441 (100.0)
Age (year)	
20-29	119 (27.0)
30-39	213 (48.3)
40-49	109 (24.7)
Region	
Seoul	148 (33.6)
Gyeonggi and Incheon	189 (42.9)
Jeju	104 (23.6)
Education	
High school or less	75 (17.0)
Undergraduate school	306 (69.4)
Graduate school	60 (13.6)
Parity[a]	
0	169 (38.3)
1	63 (14.3)
≥ 2	209 (47.4)
Urinary cotinine (ng/mL)	
< 10	394 (89.3)
10-500	24 (5.4)
> 500	23 (5.2)
Body mass index (kg/m^2)	
< 18.5 (underweight)	26 (5.9)
18.5-24.9 (normal weight)	345 (78.2)
25-29.9 (pre-obesity)	56 (12.7)
≥ 30 (obesity)	14 (3.2)
Systolic blood pressure (mmHg)	
< 120 (normal)	298 (67.6)
120-139 (pre-hypertension)	132 (29.9)
≥ 140 (hypertension)	11 (2.5)
Diastolic blood pressure (mmHg)	
< 80 (normal)	359 (81.4)
80-89 (pre-hypertension)	74 (16.8)
≥ 90 (hypertension)	8 (1.8)
Albumin-to-creatinine ratio (mg/g)	
< 9.71 (normal)	283 (64.2)
9.71-30 (low-grade albuminuria)	121 (27.4)
> 30 (micro/macroalbuminuria)	37 (8.4)

[a] Parity was derived from number of children and pregnancy (see Supplementary Material).

2.3.2. Urinary chemical concentrations and their correlation

Among the 34 compounds analyzed in the urine, 17 compounds were detected in >70% of the urine samples (Table 2-5). Monomethyl phthalate (MMP), monoethyl phthalate (MEP), monobutyl phthalate (MBP), monoisobutyl phthalate (MiBP), and monobenzyl phthalate (MBzP) each had a 94.8–99.6% detection frequency, and among these phthalate metabolites, MEP and MBP showed the highest median concentrations of 5.7 and 5.0 ng/mL, respectively. Among the DEHP metabolites, MECPP, MEHHP, MEOHP, and MCMHP were detected in >99% of the samples. Among bisphenols, bisphenol A (BPA) and S (BPS) were detected in >70% of the urine samples. In addition, benzophenone-1 (BP-1), BP-3, methyl- (MePr), ethyl- (EtP), and propyl parabens (PrP), and triclosan were detected in >70% of the samples. Most compounds showed significant positive correlations based on Spearman's correlation analysis, although stronger correlations were generally observed between the compounds within the same chemical category (Fig. 2-2).

Table 2-5. Detection frequencies and levels (ng/mL) of EDCs in the urine samples collected from participating women (n = 441).

Phthalates		Detection	Median
Parent compounds	**Metabolites**	**frequency**	**(Interquartile rage)**
DMP	MMP	98.6%	1.7 (0.8-3.5)
DEP	MEP	99.6%	5.7 (2.6-15.3)
DiPrP	MiPrP	14.1%	<LOD (<LOD)
DBP	MBP	98.9%	5.0 (2.7-7.8)
DiBP	MiBP	94.8%	2.0 (0.9-3.7)
DPeP	MPeP	7.5%	<LOD (<LOD)
DCHP	MCHP	2.5%	<LOD (<LOD)
DHxP	MHxP	33.3%	<LOD (<LOD -0.1)
BBP	MBzP	97.5%	0.5 (0.2-1.0)
DiNP	MiNP	43.8%	<LOD (ND-0.1)
DOP	MCPP	46.0%	<LOD (<LOD -0.7)
	MOP	25.8%	<LOD (ND-0.1)
DEHP	MEHP	40.1%	<LOD (<LOD-1.4)
	MECPP	100.0%	11.3 (5.4-20.1)
	MEHHP	99.6%	2.6 (1.4-4.1)
	MEOHP	99.3%	1.3 (0.7-2.1)
	MCMHP	99.8%	3.9 (2.3-6.8)
	$DEHP_{sum}$	100.0%	55.0 (29.5-85.4)

Environmental Phenols		Detection	Median
		frequency	**(Interquartile rage)**
Bisphenols	BPF	8.8%	<LOD (<LOD)
	BPA	100.0%	0.5 (0.3-0.9)
	BPB	7.3%	<LOD (<LOD)
	BPS	79.8%	0.1 (0.0-0.2)
	BPAP	0.4%	<LOD (<LOD)
Benzophenones	BP-1	98.4%	1.3 (0.7-2.7)
	BP-3	74.6%	0.4 (<LOD -1.9)
	$BP\text{-}3_{sum}$	99.6%	2.1 (1.0-4.6)
	BP-8	22.9%	<LOD (<LOD)
Parabens	MeP	100.0%	46.1 (13.3-122.7)
	EtP	98.0%	17.7 (4.2-48.9)
	PrP	93.6%	2.4 (0.3-10.5)
	BuP	63.5%	0.1 (<LOD -0.6)
	BzP	1.4%	<LOD (<LOD)
Antimicrobials	TCS	89.6%	0.3 (0.1-0.9)
	TCC	4.1%	<LOD (<LOD)

Interquartile range shows the 25^{th} and 75^{th} percentile values.

LOD: Limit of detection.

Full names of the compounds are shown in the Table 2-1 and 2-2.

Phthalate metabolites								Environmental phenols								
Low molecular weight				DEHP metabolites				Bisphenols		Benzophenones		Parabens			Antimicrobials	
MEP	MBP	MiBP	MBzP	MECPP	MEHHP	MEOHP	MCMHP	BPA	BPS	BP-1	BP-3	MePr	EtP	PrP	TCS	
0.47	**0.44**	**0.24**	**0.35**	**0.56**	**0.53**	**0.49**	**0.55**	**0.34**	**0.33**	0.07	**0.15**	**0.29**	**0.27**	**0.22**	**0.36**	MMP
	0.30	**0.14**	**0.18**	**0.41**	**0.34**	**0.30**	**0.44**	**0.25**	**0.29**	**0.11**	**0.25**	**0.31**	**0.29**	**0.27**	**0.33**	MEP
		0.57	**0.54**	**0.54**	**0.54**	**0.59**	**0.55**	**0.16**	**0.19**	-0.02	**0.11**	**0.29**	**0.18**	**0.22**	**0.27**	MBP
			0.37	**0.47**	**0.35**	**0.30**	**0.35**	0.05	0.05	0.00	0.07	**0.17**	**0.10**	**0.09**	**0.20**	MiBP
				0.51	**0.49**	**0.52**	**0.50**	**0.24**	**0.19**	-0.07	0.08	**0.22**	**0.15**	**0.14**	**0.19**	MBzP
					0.74	**0.71**	**0.83**	**0.38**	**0.35**	0.11	**0.16**	**0.27**	**0.23**	**0.19**	**0.36**	MECPP
						0.91	**0.69**	**0.31**	**0.29**	0.02	0.08	**0.26**	**0.27**	**0.18**	**0.26**	MEHHP
							0.69	**0.27**	**0.24**	0.01	0.05	**0.23**	**0.28**	**0.15**	**0.18**	MEOHP
								0.39	**0.40**	**0.14**	**0.21**	**0.34**	**0.25**	**0.23**	**0.40**	MCMHP
									0.28	**0.12**	**0.18**	**0.21**	**0.15**	**0.18**	**0.33**	BPA
										0.06	**0.19**	**0.16**	**0.16**	**0.12**	**0.22**	BPS
											0.54	0.13	0.07	**0.10**	**0.13**	BP-1
												0.32	**0.12**	**0.23**	**0.27**	BP-3
													0.21	**0.70**	**0.27**	MePr
														0.12	**0.11**	EtP
															0.21	PrP

Spearman's correlation

0 — 1

Fig. 2-2. Spearman's correlation among the urinary concentrations of the target EDCs (n = 441). Boldface value represents $p < 0.05$.

2.3.3. Association with ACR in single-pollutant model

With covariates of general demographic characteristics (Model 1), most of the urinary chemicals, after creatinine adjustment, were determined to be associated with ACR (Table 2-6). With additional covariates of BMI, serum insulin, triglyceride, and systolic/diastolic blood pressure z-scores in the model (Model 2), similar associations with comparable effect sizes were observed. In both models, several metabolites of low molecular weight phthalates (MBP, MiBP, and MBzP) and DEHP, along with BPA, BP-1, BP-3, MePr, and PrP, were determined to be positively associated with ACR.

Table 2-6. Associations between creatinine-adjusted urinary EDC concentrations (μg/g creatinine) and urinary albumin-to-creatinine ratio (ACR) (mg/g) with single chemical in each regression model (n = 441, Single-pollutant model).

Compounds	Model 1[a]		Model 2[b]	
	β (95% CI)	***p*-value**	**β (95% CI)**	***p*-value**
MMP	-0.01 (-0.09, 0.06)	0.704	-0.02 (-0.10, 0.06)	0.622
MEP	0.05 (-0.01, 0.11)	0.076	0.05 (-0.01, 0.11)	0.089
MBP	**0.23 (0.15, 0.30)**	**<0.001**	**0.23 (0.15, 0.30)**	**<0.001**
MiBP	**0.10 (0.05, 0.15)**	**<0.001**	**0.10 (0.05, 0.15)**	**<0.001**
MBzP	**0.15 (0.09, 0.21)**	**<0.001**	**0.16 (0.10, 0.22)**	**<0.001**
MECPP	**0.11 (0.01, 0.22)**	**0.039**	**0.12 (0.01, 0.23)**	**0.034**
MEHHP	**0.26 (0.16, 0.35)**	**<0.001**	**0.27 (0.17, 0.36)**	**<0.001**
MEOHP	**0.17 (0.08, 0.27)**	**<0.001**	**0.19 (0.09 0.28)**	**<0.001**
MCMHP	**0.11 (0.00, 0.23)**	**0.049**	0.11 (0.00, 0.23)	0.050
$DEHP_{sum}$	**0.20 (0.08, 0.33)**	**0.001**	**0.21 (0.08, 0.34)**	**0.001**
BPA	**0.14 (0.06, 0.21)**	**<0.001**	**0.14 (0.06, 0.21)**	**<0.001**
BPS	0.02 (-0.04, 0.07)	0.566	0.02 (-0.03, 0.07)	0.475
BP-1	**0.13 (0.09, 0.18)**	**<0.001**	**0.13 (0.09, 0.18)**	**<0.001**
BP-3	**0.03 (0.00, 0.06)**	**0.032**	**0.03 (0.01, 0.06)**	**0.021**
$BP\text{-}3_{sum}$	**0.08 (0.04, 0.12)**	**<0.001**	**0.09 (0.05, 0.13)**	**<0.001**
MePr	**0.09 (0.03, 0.14)**	**0.001**	**0.09 (0.04, 0.15)**	**0.001**
EtP	0.02 (-0.03, 0.06)	0.500	0.01 (-0.03, 0.06)	0.592
PrP	**0.04 (0.00, 0.07)**	**0.035**	**0.04 (0.00, 0.07)**	**0.035**
TCS	0.01 (-0.03, 0.05)	0.682	0.01 (-0.03, 0.05)	0.645

Boldface value represents $p < 0.05$. Both EDC concentrations and ACR were log-transformed.

[a] Adjusted for age, region, education, parity, and urinary cotinine.

[b] Adjusted for age, region, education, parity, urinary cotinine, body mass index, serum insulin, fasting glucose, triglyceride, high density lipoprotein-cholesterol, systolic blood pressure z-score, and diastolic blood pressure z-score.

2.3.4. Association with ACR in multi-pollutant model

When all the urinary chemicals (creatinine-adjusted) that were determined to be significantly associated with urinary ACR in the single-pollutant model were added in the multi-pollutant model, MBP, MiBP, MBzP, and BP-1 concentrations were significantly associated with urinary ACR (Table 2-7). Among these compounds, the effect size and significance of the association were greatest for MBP and BP-1. Even when MECPP, a major metabolite of DEHP, was included in the multi-pollutant model instead of $DEHP_{sum}$, the results were similar (data not shown).

Table 2-7. Associations between creatinine-adjusted urinary EDC concentrations (μg/g creatinine) and urinary albumin-to-creatinine ratio (ACR) (mg/g) with multiple chemicals in the regression model (n = 441, Multi-pollutant model).

Compounds	β (95% CI)	*p*-value
MBP	**0.15 (0.06, 0.23)**	**<0.001**
MiBP	**0.05 (0.00, 0.10)**	**0.048**
MBzP	**0.09 (0.02, 0.16)**	**0.010**
$DEHP_{sum}$	-0.06 (-0.20, 0.08)	0.379
BPA	0.06 (-0.01, 0.13)	0.108
BP-1	**0.11 (0.07, 0.16)**	**<0.001**
MePr	0.04 (-0.03, 0.10)	0.317
PrP	0.00 (-0.04, 0.04)	0.968

Boldface value represents $p < 0.05$. Both EDC concentrations and ACR were log-transformed. The regression model was adjusted for age, region, education, parity, and urinary cotinine.

With SG-adjusted concentrations, urinary levels of MMP, MBP, and BP-1 were identified as significant determinants of urinary ACR in the multi-pollutant model (Table 2-8). For this analysis, DEHP metabolites were not included because MEHHP and MCMHP showed the opposite directions of association in the single-pollutant model. In both multi-pollutant models with different methods of adjustment, i.e., creatinine and SG adjustment, both MBP and BP-1 were identified consistently as significant determinants of ACR.

Table 2-8. Associations between specific gravity-adjusted urinary EDC concentrations (ng/mL) and urinary albumin-to-creatinine ratio (ACR) (mg/g) with multiple chemicals in the regression model (n = 441, Multi-pollutant model).

Compounds	β (95% CI)	*p*-value
MMP	**-0.14 (-0.22, -0.07)**	**<0.001**
MBP	**0.10 (0.01, 0.19)**	**0.025**
MiBP	0.03 (-0.02, 0.08)	0.208
MBzP	0.07 (0.00, 0.13)	0.050
BP-1	**0.09 (0.05, 0.14)**	**<0.001**

Boldface value represents $p < 0.05$. Both EDC concentrations and ACR were log-transformed. The regression model was adjusted for age, region, education, parity, and urinary cotinine.

2.3.5. Sensitivity analysis with subgroup with 'healthy' kidney function

A sensitivity analysis designed to control the potential influence of altered kidney function on the association between urinary chemicals and ACR showed consistent results (Fig. 2-3). In a quartile analysis with the total population (n = 441), ACR was higher in the higher quartiles (Q2–4) than in the first quartile (Q1) of urinary MBP and BP-1 levels following creatinine and SG adjustment (Fig. 2-3A and 2-3C). For the subgroup with ACR under 9.71 mg/g (n = 283), the effect size was decreased, but the direction and significance of the effect remained the same (Fig. 2-3B and 2-3D).

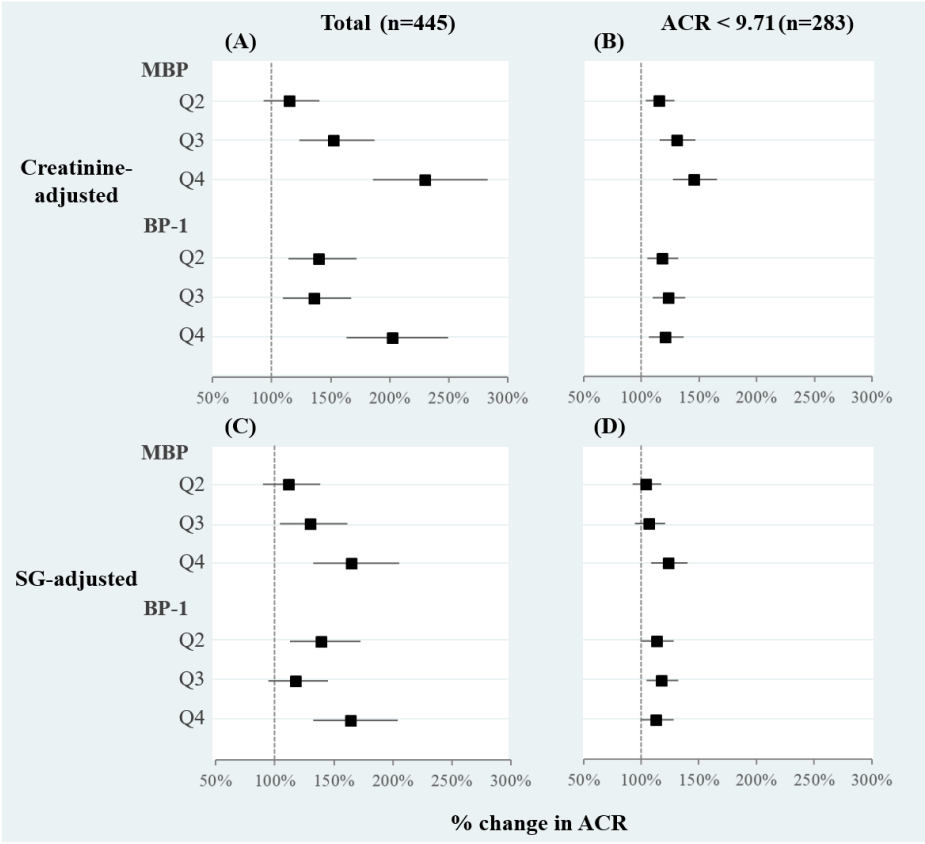

Fig. 2-3. Estimated percent changes in urinary albumin-to-creatinine ratio (ACR) by urinary MBP or BP-1 concentration quartiles. Creatinine-adjusted concentrations of MBP and BP-1 were used for (A) total participants and (B) participants with lower ACR (< 9.71 mg/g). SG-adjusted concentration of MBP and BP-1 were also used for (C) total participants and (D) participants with lower ACR. Estimation of percent changes were adjusted for age, region, education, parity, and urinary cotinine.

2.4 Discussion

Significant associations of urinary MBP and BP-1 with ACR, which were observed among the healthy adult females in the present study, support potential links between chemical exposure and kidney function previously reported in other populations. Our observation is unique, however, in that both multi-pollutant models, which considered multiple chemicals in the regression model, and the sensitivity analysis, with a subgroup with ACR less than low-grade albuminuria, consistently indicated potential adverse renal effects of BP-1 and MBP.

We report, for the first time, a significant association of urinary BP-1 with ACR. It should also be noted that, when $BP\text{-}3_{sum}$ (creatinine-adjusted) was used on behalf of BP-1 in the analytical model, a similar significant association was observed (data not shown). BP-3 has been extensively used as an active ingredient of sunscreen and suggested to disrupt thyroid and sex hormones among the general population (Kim et al., 2017; Scinicariello and Buser, 2013). BP-1 in the urine is generally considered as a major metabolic product of BP-3 but can also present as a consequence of direct exposure to BP-1 (Kim and Choi, 2014). However, the majority of urinary BP-1 among the participants is considered to be derived from the metabolism of BP-3, because BP-1 is prohibited in cosmetic products (including sunscreen) in Korea (Kang et al., 2016). It is interesting that the association with ACR was more evident with BP-1 than with BP-3 in the present population. Because most of the BP-3 is metabolized by the demethylation pathway in humans (Wang and Kannan, 2013), urinary BP-1 may be a better biomarker of BP-3 exposure than urinary BP-3 itself. Our observation that urinary BP-1 levels were approximately 3 times greater than those of BP-3 (Table 2-5) also supports the potential value of urinary BP-1 as an exposure biomarker. Previously, in the general US population, urinary BP-3 did not show a significant association with eGFR (You et al., 2011); however, in that population, urinary BP-1 was not assessed. One cannot ignore a possibility

that BP-1, but not BP-3, has specific mechanisms of action on kidney function. Further epidemiological and experimental studies are warranted to confirm the association of these sunscreen chemicals with kidney function.

A significant association of MBP with the kidney function marker is interesting and noteworthy. An association between urinary MBP levels and kidney function biomarkers has been reported previously in the US populations of children and adolescents; however, it was based on a single-pollutant approach, and similar significant associations were also observed with urinary MMP, MiBP, MzBP, and several DEHP metabolites (Malits et al., 2018; Trasande et al., 2014). Potential effects of DEHP on the renal function and damage are relatively well-known. DEHP-induced kidney dysfunction has been suggested in the children with high levels of DEHP exposure via milk consumption (Tsai et al., 2016). In addition, DEHP caused epithelial-to-mesenchymal transition and renal fibrosis in *in vitro* and *in vivo* experiments (CT Wu et al., 2018). In the present population, however, the association between DEHP exposure and ACR was not significant. How could this observation be explained? Due to strong correlations among the phthalate metabolites, which was also shown in our population (Fig. 2-2), associations of each phthalate metabolite observed based on single-pollutant models may lead to false positives, and potential confounding effects among the phthalate metabolites should be controlled. Indeed, we also observed significant associations of several phthalate metabolites, including DEHP in the single-pollutant model, but not in the multi-pollutant model (Table 2-6, Table 2-7). Notably, a lower level of DEHP exposure in the present study compared to the previous study (Tsai et al., 2016) and different age range from previous studies that focused on younger population (Malits et al., 2018; Tsai et al., 2016; Trasande et al., 2014) may provide other possible explanations for the insignificant association of DEHP metabolites in the multi-pollutant model among the present population.

For BPA, previous studies have reported inconsistent associations, e.g., positive and null associations with kidney dysfunction. Positive associations

of BPA exposure with albuminuria level have been reported in cross-sectional studies on adults (Li et al., 2012) or children population (Trasande et al., 2013). Serum BPA level was also associated with decrease of eGFR in prospective studies on type 2 diabetes patients (Hu et al., 2015; 2016). In contrast, urinary BPA was not associated with eGFR or UPCR among a children population of the US (Malits et al., 2018). Similarly, among children, exposure to dental composites, which are considered to be related to BPA exposure, was not associated with ACR or N-acetyl-β-d-glucosaminidase excretion (Trachtenberg et al., 2014). In the present population, however, the association of BPA was not significant (Table 2-7). Similar to DEHP, the effects of other chemicals which may share common exposure sources with BPA, e.g., benzophenones and phthalates (Kim et al., 2017), may provide a possible explanation for the inconsistent association of BPA with renal function markers. Different population characteristics and measurements for kidney disease among the published studies may be also in part responsible for these discrepant observations.

Consistent significant associations of MBP and BP-1 concentrations in the urine among the subgroup with low ACR (<9.71 mg/g) further strengthen the reliability of our observation. Dysfunction in glomerular filtration can lead to an abnormally low excretion of larger molecules from the blood into the urine, and this can lead to reverse causality in the association between urinary chemical concentrations and kidney function (Weaver et al., 2016). Because of this, chemical concentrations in spot urine have often been challenged as inappropriate biomarkers of exposure when associating with kidney function (Weaver et al., 2016). Despite this argument, the association between chemical concentrations in the urine and markers for kidney function can be suggested with more confidence, when the association was also detected among those with normal kidney function, i.e., with ACR below low-grade albuminuria. Therefore, our consistent observations of association with a reasonable sample size strongly imply a possible causal link of DBP and BP-3 exposure with kidney dysfunction markers among healthy adult females.

Although we repeatedly observed the significant associations between several urinary chemicals and albuminuria in statistical models with different conditions, our observations should be interpreted carefully. This study considered only ACR to assess kidney function. Since progress of kidney disease can be assessed by different markers according to its status, other relevant measurements, e.g., GFR, should be additionally considered in understanding association between chemical exposure and kidney disease. In addition, the participants were asked to fast at least for 8 hours before the biospecimen collection, which can be responsible for relatively low levels of urinary chemical concentrations compared to previous reports from other populations. According to their biological half-lives and exposure sources, effects of fasting on urinary concentration of the chemicals can be different; for example, urinary concentrations of DEHP metabolites can be decreased as the fasting time is increased (Aylward et al., 2011) while that of BPA may not be affected by the fasting time (Stahlhut et al., 2009). Therefore, effects of the fasting, which may be different by the chemicals, should be considered in the interpretation of the observations in this study.

2.5 Summary and implications

In healthy adult females before menopause, a significant association of DBP and BP-3 exposure with ACR, a kidney function marker, was observed. To our knowledge, this is the first study showing an association of urinary BP-1 concentration with kidney function among a human population. In addition, a predominant association of MBP with the renal function marker, among many other phthalate metabolites, outlines the importance of considering chemicals with possible common sources of exposure in epidemiological studies. Confirmation of our observation in other human populations and animal experiments is warranted.

Chapter 3. Association of phthalate metabolites and environmental phenols with CKD: NHANES 2005-2016

3.1 Introduction

Kidney plays significant roles in maintaining homeostasis of our body. Because of the critical roles of kidney, chronic kidney disease (CKD), which can be defined by decrease of glomerular filtration or presence of albuminuria over 3 months (Stevens and Levin, 2013), is a threat to global public health. Considering global prevalence (Hill et al, 2016) and economic burden of CKD (Nugent et al., 2011), it is important to identify and manage modifiable risk factors of CKD.

Environmental exposure to chemicals has been identified as a potential risk factor for developing CKD (Kataria et al., 2015). Metals such as cadmium and lead have been identified as nephrotoxicants in previous epidemiological and experimental studies (Byber et al., 2017; Ekong et al., 2006; Vervaet et al., 2017). Melamine and perfluoroalkyl substance, which are man-made chemicals, have been associated with kidney disease (Stanifer et al., 2018; Watkins et al., 2013). There have been evidences suggesting that plant or fungal natural products such as aristolochic acid and mycotoxins can also induce kidney injury (Nauffal and Gabardi, 2016; Vervaet et al., 2017; Weidemann et al., 2016).

Recently, consumer chemicals, e.g., plasticizers, have been suggested to be associated with kidney disease. Exposure to phthalates, e.g., di-(2-ethylhexyl) phthalate (DEHP), has been associated with albuminuria and decrease of glomerular filtration rate (GFR) in child (Malits et al., 2018; Trasande et al., 2014; CF Wu et al., 2018) as well as adult populations (J Chen et al., 2019). Exposure to bisphenol A (BPA) has been also associated with kidney disease

in child (Li et al., 2012; Trasande et al., 2013) and adult populations (You et al., 2011). In **Chapter 2**, we observed association of several urinary phthalate metabolites, e.g., monobutyl phthalate (MBP) and mono-benzyl phthalate (MBzP), and benzophenone-3 (BP-3) with albuminuria. Experimental studies have supported nephrotoxicity of consumer chemicals hypothesized by from epidemiological data. Adverse renal effects were observed in rodent models exposed to DEHP (CT Wu et al., 2018), dibutyl phthalate (DBP), the parent compound of MBP (Cheng et al., 2019), or BPA (Olea-Herrero et al., 2014; Tong et al., 2019). *In vitro* studies using cell lines derived from kidney cells also revealed nephrotoxicity of DEHP (CT Wu et al., 2018) and BPA (Bosch-Panadero et al., 2017).

Because exposure to consumer chemicals is frequently quantified by measuring urinary concentrations of their parent compounds or metabolites, adjustment of urinary dilution for the concentrations of the chemicals is one of the challenges in association studies involving adverse kidney outcome (Weaver et al., 2016). Urinary creatinine concentration has been frequently used as an adjusting factor for urine dilution. However, since urinary creatinine excretion can be affected when the kidney function, i.e., glomerular filtration, is decreased, urine dilution should be carefully adjusted considering this relationship (Bulka et al., 2017; Weaver et al., 2014; 2016).

In the present study, employing the US adult general population, possible association of exposure to various consumer chemicals, i.e., phthalates, BPA, BP-3, and parabens, with CKD and related parameters, i.e., estimated glomerular filtration rate (eGFR) and albumin-to-creatinine ratio (ACR), was investigated. In addition, an alternative adjustment method for urinary dilution is applied for this association study.

3.2 Materials and methods

3.2.1. Study participants

Dataset from US National Health and Nutrition Examination Survey (NHANES) was employed for this study. Since 1999, NHANES has been conducted as nationwide cross-sectional surveys in 2-year cycles to evaluate the health and nutrition of the US population. For the present analysis, approximately one third of the adult population of NHANES 2005–2016 (n = 31178) were randomly chosen for quantification of phthalate metabolites and environmental phenols in the spot urine samples (n =9580). Among them, participants without eGFR, ACR, or body mass index (BMI) values were excluded. Finally, we employed the data obtained from 9008 participants for this study (Fig. 3-1). NHANES protocols were approved by US National Center for Health Statistics Research Ethics Review Board, and all participants provided written informed consent.

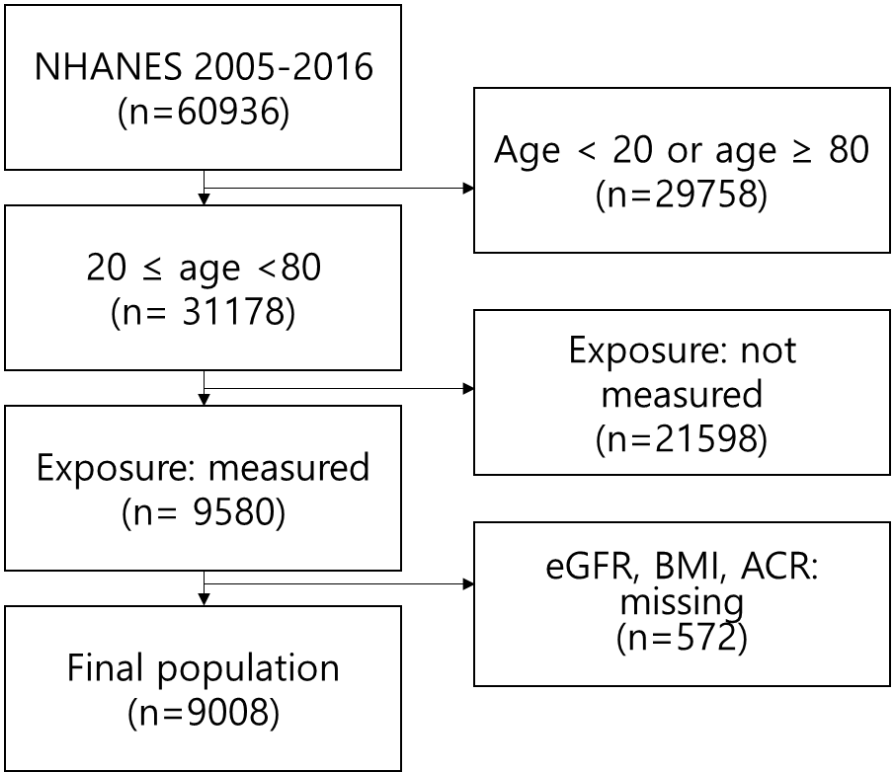

Fig. 3-1. Population selection from NHANES 2005-2016.

3.2.2. Quantification of chemicals in urine

Among the phthalate metabolites and environmental phenols measured across all the survey cycles (2005-2016), we included only compounds that were detected in >75% of the samples: monoethyl phthalate (MEP), MBP, mono-2-isobutyl phthalate (MiBP), MBzP, mono(3-carboxypropyl) phthalate (MCPP), mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono(carboxyisooctyl) phthalate (MCiOP), mono(carboxyisononyl) phthalate (MCiNP), benzophenone-3 (BP-3), bisphenol A (BPA), methyl paraben (MePr), propyl paraben (PrPr).

The analytical methods for the quantification of phthalate metabolites and environmental phenols in the urine have been described in detail elsewhere (CDC 2018; 2019). Briefly, phthalate metabolites and environmental phenols in urine samples were extracted with solid phase extraction and quantified by high performance liquid chromatography tandem mass spectrometry. The limits of detection (LODs), which varied across the survey cycles, ranged from 0.2 to 1.232 ng/mL and from 0.1 to 1 ng/mL for phthalate metabolites and environmental phenols, respectively.

3.2.3. Measurement of chronic kidney disease

Serum and urinary creatinine were measured by the Jaffe rate methods, and urinary albumin was measured by a fluorescent immunoassay. To determine CKD, eGFR and ACR values were used. For the eGFR calculation, we applied the CKD-EPI formula (Levey et al., 2009):

$$\begin{aligned} &\mathrm{eGFR}_{\text{CKD-EPI}}\ (\text{mL/min/1.73 m}^2) \\ &\quad = 141 \times \min\left(\frac{\mathrm{Scr}}{\kappa}, 1\right)^{\alpha} \times \max\left(\frac{\mathrm{Scr}}{\kappa}, 1\right)^{-1.209} \times 0.993^{\mathrm{Age}} \\ &\quad \times 1.018\ [\text{if female}] \times 1.159\ [\text{if black}] \end{aligned}$$

in this formula, Scr denotes the creatinine level in serum (mg/dL). κ is 0.7 and 0.9, and α is −0.329 and −0.411 for females and males, respectively. The ACR was calculated dividing urinary albumin concentration by urinary creatinine concentration. We defined CKD as an ACR > 30 mg/g or an eGFR < 60 mL/min/1.73 m^2 according to an extended CKD definition by Murphy et al. (2016).

3.2.4. Adjustment of urine dilution

Two adjustment methods of the urine dilution were applied. Firstly, a conventional method (denoted as the 'traditional creatinine-adjustment'), where the urinary chemical concentration was divided by the urinary creatinine concentration of each spot urine sample, was used. In the second method, because urinary creatinine level can be affected by primary outcomes of this study, i.e., GFR, a novel method of covariate-adjusted creatinine standardization (Bulka et al., 2017; O'Brien et al., 2016) was applied (denoted as a 'novel creatinine-adjustment'). The novel creatinine-adjustment method follows two steps. First, among the participants aged from 20 to 79 years old (n = 31178, Table 3-1), a linear regression model was fitted as regressing log-transformed urinary creatinine on age, sex, race/ethnicity, BMI, and eGFR, which chronically affect urinary creatinine level. Second, urinary chemical concentration was standardized by a ratio between the fitted urinary creatinine concentration ($\widehat{\mathrm{Ucr}}$) and the measured urinary creatinine concentration (Ucr) (O'Brien et al., 2016):

$$\text{Covariate-adjusted standardized chemical concentration} = [\text{chemical concentration in urine}] \times \frac{\widehat{\mathrm{Ucr}}}{\mathrm{Ucr}}$$

Table 3-1. Regression coefficients for predicting log-transformed urinary creatinine concentration (mg/dL) in the general US population (n = 31178)[a].

Predictors	β (95% CI)	*p*-value
Intercept	5.679 (5.578, 5.78)	<0.0001
Sex (male: 1; female: 2)	-0.384 (-0.4,5 -0.363)	<0.0001
Age (year, continuous)	-0.012 (-0.013, -0.011)	<0.0001
Race (non-Hispanic Black:1; others: 0)	0.4174 (0.381, 0.427)	<0.0001
BMI (kg/m^2, continuous)	0.017 (0.016, 0.019)	<0.0001
eGFR ($mL/min/1.73\ m^2$, continuous)	-0.005 (-0.006, -0.005)	<0.0001

[a] Among the all participants of NHANES 2005-2016, participants with age from 20-79 years old were included, and participants with current pregnancy or with missing values were excluded.

3.2.5. Statistical analysis

Chemical concentrations below the LOD were substituted with LOD/$\sqrt{2}$. Both chemical concentrations and ACR in the urine were log-transformed prior to the statistical analyses. Complex sampling design was accounted by the survey sampling and analysis procedures in SAS 9.4 (SAS institute, Cary, NC, USA). Correlation among the urinary chemical concentrations were examined with Spearman's correlation coefficients. The associations between the urinary chemical concentrations and the eGFR or ACR were evaluated in two steps following a similar approach as **Chapter 2**. First, each chemical concentration was included in a linear regression (Single-pollutant model). Second, urinary chemicals that showed significant associations with eGFR or ACR in the single-pollutant models were included in a regression model to construct a multi-pollutant model. To treat effects of DEHP as a variable in the multi-pollutant model, the sum of urinary DEHP metabolites ($DEHP_{sum}$) was calculated as accounting molar excretion fractions of the DEHP metabolites (Koch et al., 2005; section 2.2.4). In both of the single- and multi-pollutant models, the associations were adjusted for potential covariates including sex, age (continuous), race/ethnicity (Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, and other groups), survey cycle (categorical), BMI (continuous), smoking history (<100 cigarettes and ≥100 cigarettes), poverty income ratio (PIR; ≤1.85, >1.85 to ≤3.50, and >3.50), and self-reported physical activity (inactive/moderate and vigorous). Missing values for PIR (n =733, 8.1%) were imputed by the value estimated from the linear regression model with demographic determinants, i.e., sex, age, and race/ethnicity. The association between the chemical concentrations and CKD (dichotomous) was investigated with logistic regression models with the same adjusting variables. P-values less than 0.05 were considered as statistically significant. All statistical analyses were performed using SAS 9.4.

3.3 Results

3.3.1. CKD status and other characteristics of the participating population

The 12.0% of the general US adult was classified as CKD defined by eGFR and ACR range (Table 3-2). ACR was increased and eGFR was decreased as the age group is getting older. The non-Hispanic white group had the lowest eGFR among the race/ethnicity groups. In the study population, 30.6% exhibited BMI values of normal range (<25 kg/m^2).

Table 3-2. Demographic and socio-behavioral characteristics and current chronic kidney disease status of the study population.

Characteristics	N (%)	Weighted %[a]	eGFR (mL/min/1.73 m^2)[b]	*p*-value[c]	ACR (mg/g)[b]	*p*-value[c]
Total	9008 (100)	100.0	97.1 (82.2-111.7)		6.5 (4.3-11.2)	
Sex						
Male	4473 (49.7)	49.4	97.1 (82.4-112)	0.006	5.4 (3.7-9.4)	<0.001
Female	4535 (50.3)	50.6	97.1 (81.8-111.1)		7.5 (5.1-12.9)	
Age (year)						
20-39	3221 (35.8)	37.4	112 (98.7-122.7)	<0.001	5.7 (3.8-9.3)	<0.001
40-59	3234 (35.9)	40.7	94.7 (82.2-105.7)		6.4 (4.4-10.8)	
60-79	2553 (28.3)	21.9	77.7 (65.3-90)		8.3 (5.3-15.9)	
Race/ethnicity						
Mexican American	1462 (16.2)	8.5	111.7 (97-123.5)	<0.001	6.9 (4.7-12.9)	<0.001
Other Hispanic	912 (10.1)	5.7	104.9 (91.2-118.6)		6.7 (4.3-11.6)	
Non-Hispanic White	3732 (41.4)	67.5	93.3 (79.1-107.1)		6.4 (4.2-10.8)	
Non-Hispanic Black	1960 (21.8)	11.0	103.2 (87.1-119.9)		6.3 (4-12.4)	
Other groups	942 (10.5)	7.3	104 (87.9-116)		7.0 (4.5-11.6)	
BMI (kg/m^2)						
<25	2566 (28.5)	30.6	100.5 (85.9-114.2)	<0.001	6.7 (4.4-11.4)	<0.001
≥25 to <30	2986 (33.1)	32.7	94.5 (80.1-109)		5.8 (4-9.4)	
≥30	3456 (38.4)	36.7	96.3 (81-111.7)		6.9 (4.5-13)	
Lifetime smoking						
<100 cigarettes	4972 (55.2)	54.6	98.1 (83.2-112.6)	<0.001	6.4 (4.2-10.7)	0.006
≥100 cigarettes	4036 (44.8)	45.4	96.1 (80.7-110.4)		6.6 (4.4-11.8)	

Table 3-2. (continued)

Characteristics	N (%)	Weighted %[a]	eGFR (mL/min/1.73 m^2)[b]	*p*-value[c]	ACR (mg/g)[a]	*p*-value[c]
Poverty income ratio						
≤1.85	3742 (41.5)	29.5	104 (86.3-117.9)	<0.001	7.1 (4.5-13.2)	<0.001
>1.85 to ≤ 3.50	2509 (27.9)	27.4	98.2 (83-113.6)		6.6 (4.4-11.2)	
>3.50	2757 (30.6)	43.0	92.7 (79.7-105.1)		6.1 (4.2-10)	
Physical activity						
Inactive or moderate	5702 (63.3)	59.0	94.9 (79.8-110.2)	<0.001	7.2 (4.7-12.7)	<0.001
Vigorous	3306 (36.7)	41.0	99.8 (86-113.9)		5.6 (3.8-9.2)	
Current CKD[d]						
No	7670 (85.1)	88.0	98.4 (84.7-112.2)	<0.001	5.9 (4.1-9.2)	<0.001
Yes	1338 (14.9)	12.0	78.8 (54.6-104.5)		45.4 (17.9-101.5)	

[a] Percent values were weighted to account for the complex survey design.
[b] Median (25th–75th percentile).
[c] *p*-value calculated from one-way analysis of variance.
[d] Those with albumin-to-creatinine ratio (ACR) above 30 mg/g or estimated glomerular filtration rate (eGFR) below 60 mL/min/1.73 m^2 were defined as patients with chronic kidney disease (CKD).

3.3.2. Chemical concentrations in urine

All the target compounds were detected in more than 90% of the samples. Among the phthalate metabolites, MEP exhibited the highest concentration (unadjusted geometric mean of 57.2 ng/mL), followed by MECPP, MBP, and MEHHP (unadjusted geometric mean of 16.0, 11.2 and 10.4 ng/mL, respectively; Table 3-3). Among the environmental phenols, MePr exhibited the highest concentration (unadjusted geometric mean of 50.8 ng/mL), followed by BP-3, PrPr, and BPA (unadjusted geometric mean of 21.6, 6.7 and 1.5 ng/mL, respectively; Table 3-3).

Regardless of the adjustment methods, strong correlations were observed among the urinary concentrations DEHP metabolites (correlation coefficients ranging 0.901-0.967). Metabolites of low-molecular-weight phthalate (i.e., MEP, MBP, MiBP, and MBzP) shared modest correlations (coefficients ranging 0.147-0.524). BPA was also correlated with most of the phthalate metabolites while the correlations with other environmental phenols were weak (Fig. 3-2).

Table 3-3. Detection and distribution of target phthalate metabolites and environmental phenols in urine of the general US population (n = 9008).

Compound	Creatinine adjustment	Detection frequency	Geometric mean	Percentile		
				25th	50th	75th
MEP	Unadjusted	99.9%	57.2	18.7	50.5	161.2
	Traditional		59.3	20.4	49.7	145.3
	Novel		56.7	19.4	48.0	139.5
MBP	Unadjusted	98.1%	11.2	5.7	12.5	25.1
	Traditional		11.6	6.8	11.5	19.7
	Novel		11.1	6.5	11.2	18.9
MiBP	Unadjusted	98.1%	6.4	3.3	6.9	13.5
	Traditional		6.6	4.1	6.5	10.8
	Novel		6.3	3.8	6.3	10.6
MBzP	Unadjusted	97.8%	4.8	2.1	5.1	12.0
	Traditional		5.0	2.6	5.0	9.8
	Novel		4.8	2.4	4.8	9.5
MCPP	Unadjusted	92.1%	2.0	0.9	2.0	4.6
	Traditional		2.1	1.0	1.9	3.8
	Novel		2.0	1.0	1.8	3.7
MECPP	Unadjusted	99.8%	16.0	7.0	15.2	32.9
	Traditional		16.6	8.3	14.4	28.6
	Novel		15.8	7.9	13.9	27.0
MEHHP	Unadjusted	99.5%	10.4	4.3	10.0	22.2
	Traditional		10.8	5.2	9.4	18.8
	Novel		10.3	4.8	9.0	18.1
MEOHP	Unadjusted	99.1%	6.3	2.8	6.1	13.3
	Traditional		6.6	3.3	5.7	11.3
	Novel		6.3	3.1	5.6	10.7
MCiOP	Unadjusted	98.3%	10.3	3.8	9.0	25.9
	Traditional		10.7	4.0	8.8	25.5
	Novel		10.2	3.8	8.4	24.2
MCiNP	Unadjusted	95.4%	2.4	1.2	2.3	4.6
	Traditional		2.5	1.3	2.2	4.0
	Novel		2.4	1.2	2.1	3.9

Table 3-3. (continued)

Compound	Creatinine adjustment	Detection frequency	Geometric mean	Percentile		
				25th	50th	75th
BP-3	Unadjusted	96.3%	21.6	4.7	17.3	81.1
	Traditional		22.3	4.8	16.6	81.8
	Novel		21.4	4.8	16.3	77.2
BPA	Unadjusted	92.0%	1.5	0.7	1.5	3.0
	Traditional		1.6	0.9	1.5	2.6
	Novel		1.5	0.8	1.4	2.6
MePr	Unadjusted	99.2%	50.8	13.1	51.2	190.6
	Traditional		52.6	12.7	55.1	215.7
	Novel		50.3	13.0	53.2	188.7
PrPr	Unadjusted	95.3%	6.7	1.0	6.3	40.6
	Traditional		6.9	1.0	6.9	45.7
	Novel		6.6	1.0	6.5	41.0

Units are in ng/mL for unadjusted and novel adjustment and in ng/mg creatinine fo
r traditional adjustment.

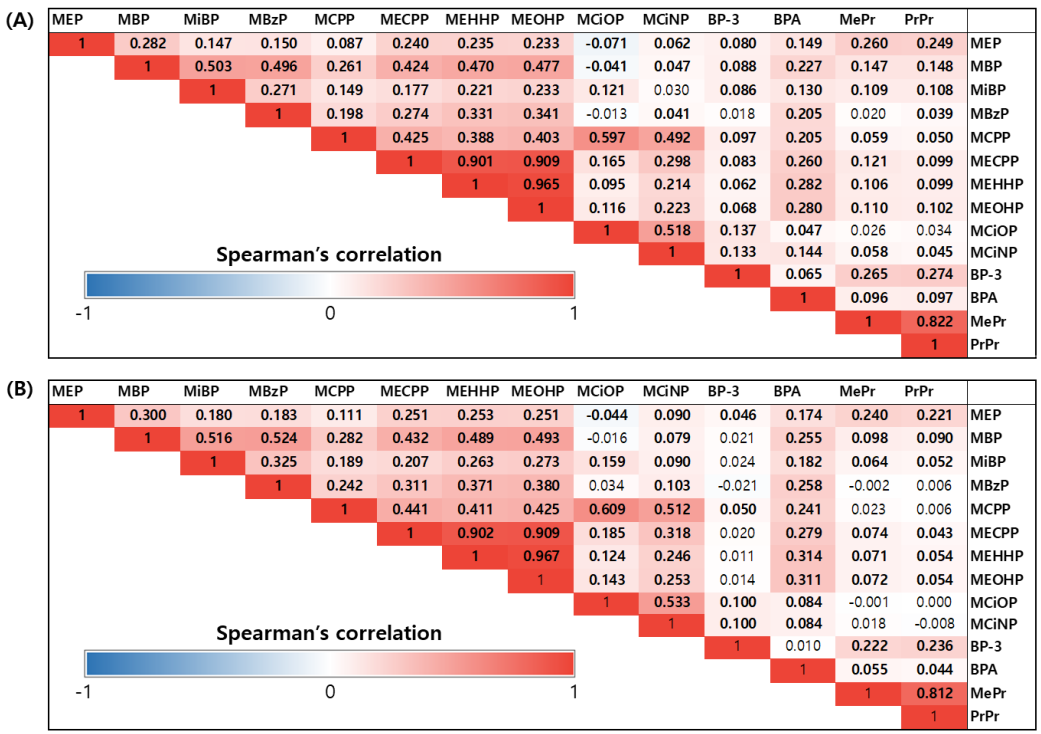

Fig. 3-2. Spearman's correlation among the urinary phthalate metabolites and environmental phenols by (A) method of traditional creatinine adjustment or novel creatinine adjustment (n = 9008). Bonferroni correction was used to calculate *p*-value. Coefficients in boldface represent $p < 0.05$.

3.3.3. Association with eGFR or ACR in single-pollutant model

When the traditional adjustment was used for controlling urine dilution, several compounds showed positive association with the eGFR. However, when the novel adjustment was applied, the positive association disappeared, and most of the compounds showed negative associations with the eGFR (Table 3-4).

Table 3-4. Association of urinary phthalate metabolites and environmental phenols with estimated glomerular filtration rate (mL/min/1.73 m^2) with single chemical in each regression model (n = 9008, Single-pollutant model).

Compound	Traditional creatinine-adjustment (ng/mg creatinine)		Novel creatinine-adjustment (ng/mL)	
	β (95% CI)	***p*-value**	**β (95% CI)**	***p*-value**
MEP	**0.59 (0.24, 0.94)**	**0.001**	-0.22 (-0.57, 0.13)	0.206
MBP	0.23 (-0.20, 0.66)	0.297	**-1.77 (-2.16, -1.37)**	**<0.001**
MiBP	**0.80 (0.18, 1.32)**	**0.010**	**-1.51 (-2.03, -0.98)**	**<0.001**
MBzP	0.13 (-0.23, 0.49)	0.488	**-1.38 (-1.75, -1.01)**	**<0.001**
MCPP	0.14 (-0.28, 0.57)	0.505	**-1.22 (-1.64, -0.81)**	**<0.001**
MECPP	0.50 (0.00, 1.00)	0.052	**-1.28 (-1.78, -0.78)**	**<0.001**
MEHHP	0.27 (-0.21, 0.74)	0.266	**-1.28 (-1.7, -0.81)**	**<0.001**
MEOHP	-0.01 (-0.52, 0.50)	0.976	**-1.64 (-2.15, -1.12)**	**<0.001**
MCiOP	0.33 (-0.07, 0.73)	0.106	**-0.75 (-1.15, -0.35)**	**<0.001**
MCiNP	**1.53 (0.98, 2.08)**	**<0.001**	-0.14 (-0.67, 0.40)	0.608
BP-3	0.18 (-0.03, 0.40)	0.094	-0.17 (-0.38, 0.04)	0.110
BPA	**0.45 (0.04, 0.85)**	**0.030**	**-1.39 (-1.77, -1.00)**	**<0.001**
MePr	0.25 (-0.04, 0.55)	0.092	**-0.38 (-0.67, -0.08)**	**0.012**
PrPr	0.11 (-0.12, 0.34)	0.337	**-0.27 (-0.50, -0.04)**	**0.024**

The association was adjusted for NHANES cycle, sex, age, race/ethnicity, poverty income ratio, smoking history, physical activity, and BMI.

Regardless of the adjustment methods, only urinary MBP showed significant association with ACR ($\beta = 0.03$). In addition, urinary MBzP level was associated with ACR with a marginal significance ($\beta = 0.03$ and $p = 0.052$; Table 3-5).

Table 3-5. Association of urinary phthalate metabolites and environmental phenols with urinary albumin-to-creatinine ratio (mg/g) with single chemical in each regression model (n = 9008, Single-pollutant model).

Compound	Traditional creatinine-adjustment (ng/mg creatinine)		Novel creatinine-adjustment (ng/mL)	
	β (95% CI)	***p*-value**	**β (95% CI)**	***p*-value**
MEP	0.01 (-0.01, 0.03)	0.598	0.01 (-0.01, 0.03)	0.580
MBP	**0.03 (0.01, 0.05)**	**0.017**	**0.03 (0.01, 0.06)**	**0.018**
MiBP	0.02 (-0.01, 0.06)	0.169	0.02 (-0.01, 0.06)	0.158
MBzP	0.03 (0.00, 0.05)	0.052	0.03 (0.00, 0.05)	0.052
MCPP	0.00 (-0.02, 0.02)	0.962	0.00 (-0.02, 0.02)	0.996
MECPP	0.00 (-0.03, 0.03)	0.925	0.00 (-0.03, 0.03)	0.960
MEHHP	-0.01 (-0.04, 0.01)	0.370	-0.01 (-0.04, 0.02)	0.399
MEOHP	-0.01 (-0.04, 0.01)	0.321	-0.01 (-0.04, 0.01)	0.350
MCiOP	-0.01 (-0.03, 0.01)	0.201	-0.01 (-0.03, 0.01)	0.222
MCiNP	-0.01 (-0.04, 0.02)	0.680	-0.01 (-0.04, 0.02)	0.710
BP-3	-0.01 (-0.02, 0.01)	0.225	-0.01 (-0.02, 0.01)	0.229
BPA	0.00 (-0.03, 0.03)	0.905	0.00 (-0.03, 0.03)	0.939
MePr	0.01 (0.00, 0.03)	0.110	0.01 (-0.00, 0.03)	0.108
PrPr	0.00 (-0.02, 0.01)	0.629	0.00 (-0.02, 0.01)	0.644

The association was adjusted for NHANES cycle, sex, age, race/ethnicity, poverty income ratio, smoking history, physical activity, and BMI.

3.3.4. Association with eGFR in multi-pollutant model

In the multi-pollutant model including chemical variables showing significant association with eGFR in the single-pollutant models, MBP, MBzP, and BPA were significantly associated with eGFR. Associations between other compounds and eGFR became insignificant (Table 3-6).

Table 3-6. Association of urinary phthalate metabolites and environmental phenols with estimated glomerular filtration rate (mL/min/1.73 m^2) with multiple chemical in each regression model (n = 9008, Multi-pollutant model).

Compound	β (95% CI)	*p*-value
MBP	**-0.74 (-1.36, -0.13)**	**0.018**
MiBP	-0.40 (-1.07, 0.27)	0.236
MBzP	**-0.74 (-1.20, -0.28)**	**0.005**
MCPP	-0.58 (-1.18, 0.01)	0.055
$DEHP_{sum}$	-0.41 (-1.00, 0.18)	0.171
MCiOP	-0.11 (-0.66, 0.44)	0.693
BPA	**-1.03 (-1.42, -0.64)**	**<0.001**
MePr	-0.21 (-0.66, 0.25)	0.371
PrPr	-0.10 (-0.45, 0.26)	0.581

The association was adjusted for NHANES cycle, sex, age, race/ethnicity, poverty income ratio, smoking history, physical activity, and BMI.

3.3.5. Association with CKD classification

Analysis with dichotomous CKD outcome showed similar results (Fig. 3-3). When the urine dilution was adjusted with the traditional adjustment, MBP, MBzP, and BPA was not associated with CKD. With the novel adjustment, however, monotonous trends between the chemical concentrations and odds ratios (ORs) for CKD appeared more evident, and the OR for the highest quartile (Q4) of MBP became significant (OR = 1.37). The association with CKD was also significant when MBP variable was treated as a continuous variable (OR = 1.13).

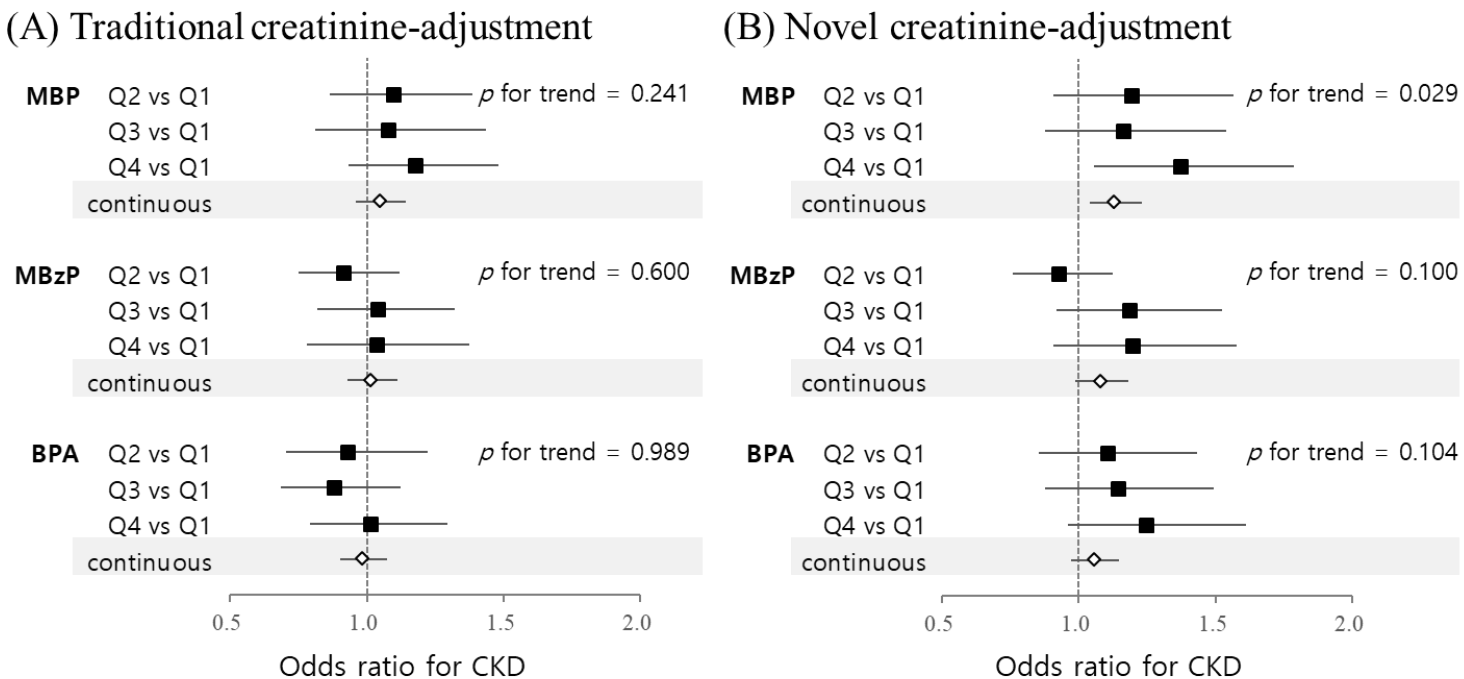

Fig. 3-3. Adjusted* odds ratios (■ for quartile groups and ◇ for continuous variables) and their 95% confidence intervals (bar) for chronic kidney disease (CKD) by quartiles or continuous increase of urinary phthalate metabolites and environmental phenols with (A) the traditional creatinine-adjustment and (B) the novel creatinine adjustment using covariate-adjusted standardization (n = 9008).
*Adjusted for NHANES cycle, sex, age, race/ethnicity, poverty income ratio, smoking history, physical activity, and BMI.

3.4 Discussion

Significant association of MBP with CKD and related parameters observed in the general US population in the present study indicates potential adverse effects of MBP exposure on CKD. Associations of MBzP and BPA with albuminuria also suggest their possible relation with kidney disease. Our observation is notable in that the chemical risk factors were identified following multi-pollutant approach.

In the **Chapter 2**, we identified MBP and MBzP as risk factors for albuminuria (Table 2-7). In this study, we replicated our previous observation in Korean female population and further validated possible links between exposure to the phthalates and kidney disease using eGFR. In an experimental study, mice exposed to DBP showed symptoms of kidney damage, e.g., histological renal damage, increase of serum creatinine, increase of serum urea nitrogen, and oxidative stress in kidney (Cheng et al., 2019) supporting our observation of MBP in the present study. However, there has been no study on benzyl butyl phthalate (BBP). Experimental studies would support our observation of MBzP.

Previously, serum BPA level was associated with decrease of eGFR in prospective studies on type 2 diabetes patients (Hu et al., 2015; 2016), and association between BPA and albuminuria was also reported in cross-sectional studies on children population (Trasande et al., 2013) and adults (Li et al., 2012). On the contrary, BPA concentration in urine was not associated with eGFR or proteinuria in a US children population (Malits et al., 2018). In our previous study on Korean women, BPA was associated with albuminuria only in the single-pollutant model (**Chapter 2**). Such inconsistent results on the association between BPA exposure and kidney disease may be understood by differences in study populations, measurements for kidney disease, and statistical conditions. The studies measuring eGFR as a parameter of kidney disease are in line with our observation showing association of BPA exposure with decreased eGFR (Hu et al., 2015; 2016) with an exception in a study on a

children population (Maltis et al., 2018). On the other hand, for ACR, studies showed controversial results. The significant association between BPA level and ACR was observed in children populations (Li et al., 2012; Trasande et al., 2013), but the statistical models of these studies did not incorporate urinary phthalate metabolites, which possibly share common exposure sources with BPA (Fig. 2-2 and 3-2). Considering the positive association between BPA and ACR in the single-pollutant model became in significant in the multi-pollutant model in Chapter 2 (Tables 2-6 and 2-7), the positive associations with ACR may be confounded by exposure to phthalates, which was not controlled in the previous studies. A previous animal study supports the association between BPA exposure and eGFR observed in the present study; BPA exposure induced increase of serum creatinine level in mice (Tong et al., 2019) though proteinuria was also caused by BPA exposure in another mouse study (Olea-Herrero et al., 2014).

We have reported a significant association between benzophenone-1 (BP-1), which is a urinary metabolite of a UV filter BP-3, and ACR (**Chapter 2**), while BP-3 was associated with none of eGFR and ACR in the present study. As discussed in **Chapter 2**, both BP-1 and BP-3 can be urinary biomarkers of BP-3 exposure, but BP-1 may be a better urinary biomarker of BP-3 exposure than BP-3 in urine because BP-3 is mostly metabolized into BP-1 by the demethylation in human body (Wang and Kannan, 2013). Additional assessment of BP-1 would help clarify the association of these UV filters with kidney disease.

Our observation is of noteworthy considering that none of phthalate metabolites and environmental phenols were identified as potential chemical determinants of CKD in a recent environmental-wide association study (EWAS) employing NHANES data (Lee et al., 2020). This EWAS approach in the previous study was useful to identify possible nephrotoxicants in that plenty of chemicals (n = 262) were simultaneously considered in a series of statistical models using data from a well-designed national survey, and the associations were validated across the NHANES survey cycles (1999-2016).

However, this approach poses limitations. Firstly, effects of kidney outcome on urine dilution was not considered in the creatinine adjustment. Also, although EWAS treated a number of chemicals, multiple chemical exposure was not considered in the statistical models. In the present study, we revealed that inappropriate adjustment of urine dilution could result in distorted association between urinary chemical concentrations and eGFR (Table 3-4). Indeed, the previous EWAS research reported inversed association of 21 urinary chemicals with reduced eGFR. These unexpected and insignificant results for phthalate metabolites and BPA in the previous study might be due to lack of consideration of relation of GFR with urine dilution. Our application of the novel creatinine-adjustment and consideration of multiple compounds successfully identified major chemical determinants which could not be captured in the previous screening approach.

This study has limitations. First, the association observed in this study does not indicate causal relationship because of cross-sectional nature of this study design. Second, because of their short biological half-lives, single measurements of the urinary chemicals do not directly represent long-term exposure, which is responsible for chronic effects on kidney. Therefore, the observations in this study should be interpreted with caution.

3.5 Summary and implications

In the US general adult population, we suggested possible links between exposure to consumer chemicals, i.e., DBP, BBP, and BPA, and CKD. The predominant associations of MBP and MBzP with CKD parameters observed in this study are meaningful as supporting our previous observations in a Korean female population (**Chapter 2**) and suggest consideration of multiple chemicals sharing exposure sources is important in epidemiological studies. In addition, as applying a novel method of urinary creatinine adjustment, we demonstrated significance of considering influence of kidney outcome on urine dilution. Further studies in other populations and experimental understanding of mechanism of the nephrotoxicity of the compounds to support the associations observed in this study are warranted.

Chapter 4. Association of metabolites of organophosphate esters (OPEs) with CKD: NHANES 2013-2014

4.1 Introduction

Organophosphate esters (OPEs) are a group of chemicals that have been increasingly used to replace traditional flame retardants such as polychlorinated biphenyls and polybrominated diphenyl ethers. For this purpose, OPEs are used as additives in furniture, electronics, and building materials (Kemmlein et al., 2003; Stapleton et al., 2012). In addition, OPEs have been used as plasticizers in other consumer products such as children's products and cosmetic products (Mendelsohn et al., 2016; Stapleton et al., 2011). Foodstuffs can also be contaminated with OPEs (Poma et al., 2018). Since OPEs are not chemically bound to these products or materials, they may leach from the consumer products or building materials and enter the environment including indoor dust and air (Saillenfait et al., 2018). Therefore, people are exposed to OPEs not only by the use of consumer products (Ingle et al., 2019; Mendelsohn et al., 2016) or the consumption of foods (Poma et al., 2018) but also via dust or air (Xu et al., 2016, Xu et al., 2019).

Although the metabolism of OPEs in human is not thoroughly understood, most OPEs are known to be metabolized into their diester metabolites and excreted through the urine (Saillenfait et al., 2018). Increasing number of human biomonitoring studies indicate that exposure to OPEs among humans is widespread worldwide (Saillenfait et al., 2018). In the general population of the US, diester metabolites of several OPEs such as triphenyl phosphate (TPHP), tris(1,3-dichloro-2-propyl) phosphate (TDCIPP), tris(2-chloroethyl) phosphate (TCEP), and tri-n-butyl phosphate (TNBP) have been detected in >80% of urine samples (Ospina et al., 2018). The biomonitoring of OPEs

exposure includes but not limited to diphenyl phosphate (DPHP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), bis(2-chloroethyl) phosphate (BCEP), and di-n-butyl phosphate (DNBP). Hydroxylated metabolites, as well as diester metabolites of OPEs, have also been frequently detected in the urine samples of a Japanese children population (Bastiaensen et al., 2019) and an Australian children population (He et al., 2018).

Several adverse effects have been suggested for OPEs, in both experimental and human observational studies. In zebrafish and *in vitro* models, exposure to OPEs, including TPHP and TDCIPP, caused the disruption of thyroid and sex hormones (Kim et al., 2015; Liu et al., 2012, Liu et al., 2019). Indeed, TPHP and TDCIPP in house dust have been associated with sperm quality or hormone levels among the US adult male population (Meeker and Stapleton, 2010). Adverse effects on development and behavior have also been observed in early life stage zebrafish (Dishaw et al., 2014). Urinary metabolites of OPEs have also been associated with allergic symptoms among Japanese children (Araki et al., 2018).

Chronic kidney disease (CKD) has caused a substantial public health and economic burden worldwide with its global prevalence of 11–13% (Hill et al., 2016). In addition to major clinical risk factors of hypertension and diabetes, many environmental chemicals have been linked to CKD. Several metals and melamine are among the most well-known nephrotoxins (Stanifer et al., 2018; Wang et al., 2013). Recently, another frequently used chemicals, such as phthalates, benzophenones, and perfluoroalkyl substances, have also been associated with adverse kidney outcomes (**Chapter 2**; Kataria et al., 2015; Stanifer et al., 2018). For OPEs, however, little is known about their toxic potentials on kidney, except limited experimental data on TDCIPP or TCEP suggesting adverse renal outcomes, such as adverse effects on renal proximal tubule cells (Killilea et al., 2017; Ren et al., 2008; 2009; 2012). The association of OPEs exposure with kidney function has never been assessed in human populations. This is partially due to the limited availability of biomonitoring data for OPEs among general human populations.

For association studies that involve adverse effects on the kidneys, one of the challenges is to adjust urinary dilution for the concentrations of the urinary exposure biomarkers. This is due to the fact that the urinary creatinine concentration, which has been the most frequently used factor for adjusting urinary dilution, can be influenced when the kidney function is compromised. The excretion of small molecules, such as creatinine, through the urine can be influenced by kidney function, i.e., glomerular filtration rate. Therefore, the methods of adjustment for urine dilution should be carefully selected, especially when urinary exposure biomarkers are associated with kidney function (Bulka et al., 2017; Weaver et al., 2014; 2016).

In the present study, a possible link between OPEs exposure and CKD was assessed among general adult population. For this purpose, the analytical data available from the US National Health and Nutrition Examination Survey (NHANES) 2013–2014 (Ospina et al., 2018) were used, and the associations of OPEs exposure with CKD and related parameters, i.e., estimated glomerular filtration rate (eGFR) and urinary albumin-to-creatinine ratio (ACR), were examined. In addition, an alternative method of urinary dilution adjustment is proposed for this association study. The results of this study will provide an insight into the possible effects of these frequently used flame retardants on kidney function among the general population and stimulate further follow-up studies.

4.2 Materials and methods

4.2.1. Study participants

Dataset from NHANES of the US was used for this study. NHANES is a nationwide population-based survey of the US, which has been conducted since 1999 to assess the health and nutritional status of the US population. For the present study, the data from NHANES 2013–2014 survey were used. About a third of the participants of NHANES 2013–2014 were randomly chosen and measured for OPE metabolites in the stored spot urine samples (n = 2666). Among them, we chose only adults between 20 and 79 years of age, and excluded women who were then pregnant, as well as participants with missing values in eGFR, ACR, or body mass index (BMI). Finally, the data obtained from 1578 participants were employed for this study (Fig. 4-1) NHANES was approved by the US National Center for Health Statistics Research Ethics Review Board, and all participants provided informed consent.

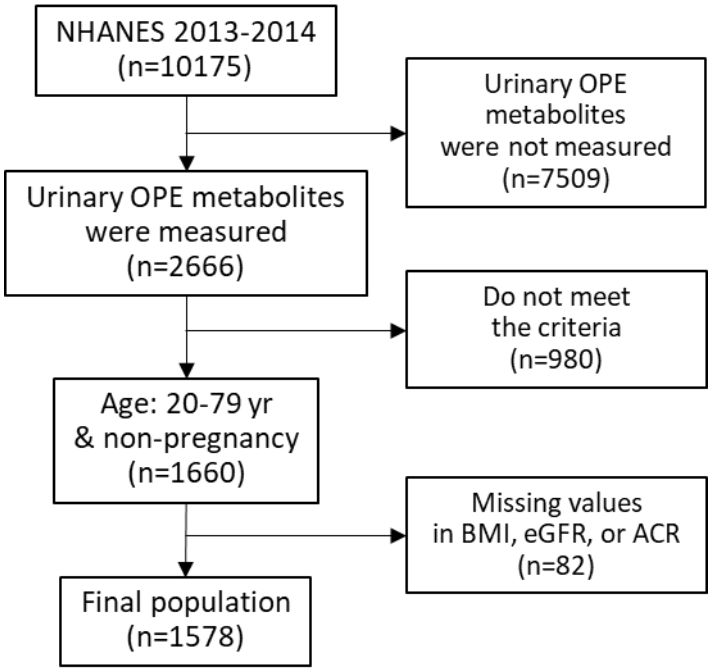

Fig. 4-1. Procedure for selecting study population from NHANES 2013-2014.

4.2.2. OPE metabolites and creatinine in urine

The analytical procedure for the quantification of OPE metabolites in the urine has been described in detail elsewhere (Jayatilaka et al., 2017; Ospina et al., 2018). Briefly, 400 μL of urine was used, and the extraction was performed via the enzymatic hydrolysis of urinary conjugation and a subsequent solid phase extraction (60 mg Strata XAW polymeric SPE packing with 1.5 mL liquid space, Phenomenex, Torrance, CA, USA). The target analytes in the extracts were separated via reversed-phase high-performance liquid chromatography (Agilent 1290, Agilent Technologies, Santa Clara, CA, USA). Afterwards, the analytes were quantified via isotope dilution-electrospray ionization tandem mass spectrometry detection (AB Sciex 5500 Qtrap mass spectrometer, Applied Biosystems, Foster City, CA, USA). The limits of detection (LODs) were 0.16, 0.11, 0.08, and 0.05 ng/mL for DPHP, BDCIPP, BCEP, and DNBP, respectively. The creatinine in the urine was determined using an Enzymatic Roche Coba 6000 analyzer (Roche Diagnostics, Indianapolis, IN, USA).

4.2.3. Measurement of chronic kidney disease

The creatinine in the serum was determined using a Beckman UniCel DxC800 Synchron (Beckman, Fullerton, CA, USA). The albumin in the urine was measured via a fluorescent immunoassay (SequoiaTurner 450 Digital Fluorometer, Block Scientific, Holbrook, NY, USA).

We used eGFR and ACR values as parameters to determine CKD. To calculate the eGFR, the following CKD-EPI formula (Levey et al., 2009) was applied:

$$\mathrm{eGFR}_{\text{CKD-EPI}}\ (\mathrm{mL/min/1.73\ m^2}) = 141 \times \min\left(\frac{\mathrm{Scr}}{\kappa}, 1\right)^{\alpha} \times \max\left(\frac{\mathrm{Scr}}{\kappa}, 1\right)^{-1.209} \times 0.993^{\mathrm{Age}} \times 1.018\ [\text{if female}] \times 1.159\ [\text{if black}]$$

In this formula, Scr denotes the serum creatinine level (mg/dL), κ is 0.7 for females and 0.9 for males, and α is −0.329 for females and −0.411 for males. The ACR was calculated as the urinary albumin/creatinine ratio. Participants with an ACR above 30 mg/g or an eGFR below 60 mL/min/1.73 m^2 were classified as CKD patients, according to an extended definition of CKD (Murphy et al., 2016).

4.2.4. Adjustment of urine dilution

For adjusting the urine dilution, firstly, a conventional method (the urinary chemical concentration divided by the urinary creatinine concentration of each spot urine sample [denoted as the 'traditional creatinine-adjustment']) was used. In addition, to control potential confounding by compromised kidney function on creatinine excretion, an alternative method of covariate-adjusted standardization (Bulka et al., 2017; O'Brien et al., 2016) was employed (denoted as a 'novel creatinine-adjustment'). The novel creatinine-adjustment method consisted of two steps. First, the log-transformed urinary creatinine was regressed on the covariates that are known to affect chronic urinary creatinine levels, among the total study population. Second, a ratio between the fitted urinary creatinine concentration ($\widehat{\mathrm{Ucr}}$) and the measured urinary creatinine concentration (Ucr) was used to standardize the urinary chemical concentration as follows (O'Brien et al., 2016):

$$\text{Covariate-adjusted standardized chemical concentration} = [\text{chemical concentration in urine}] \times \frac{\widehat{\mathrm{Ucr}}}{\mathrm{Ucr}}$$

To fit the urinary creatinine concentration, a linear regression model was built with the relevant covariates, i.e., age, sex, race/ethnicity, BMI, and eGFR, which was derived from the participants aged from 20 to 79 years old and without current pregnancy, but regardless of their OPE metabolite measurements (n = 4852, Table 4-1).

Table 4-1. Regression coefficients for predicting log-transformed urinary creatinine concentration (mg/dL) in the general US population (n = 4852)[a].

Predictors	β (95% CI)	*p*-value
Intercept	5.453 (5.227, 5.678)	<0.0001
Sex (male: 1; female: 2)	-0.377 (-0.432, -0.323)	<0.0001
Age (year, continuous)	-0.011 (-0.013, -0.010)	<0.0001
Race (non-Hispanic Black:1; others: 0)	0.417 (0.359, 0.475)	<0.0001
BMI (kg/m^2, continuous)	0.018 (0.012, 0.024)	<0.0001
eGFR ($mL/min/1.73\ m^2$, continuous)	-0.004 (-0.005, -0.003)	<0.0001

[a] Among the all participants of NHANES 2013-2014, participants with age from 20-79 years old were included, and participants with current pregnancy or with missing values were excluded.

4.2.5. Statistical analysis

Only compounds that were detected in >75% of the samples were included in the statistical analyses. For the non-detected samples, LOD/$\sqrt{2}$ was substituted. Prior to the statistical analyses, both OPEs metabolite levels and the ACR in the urine were log-transformed. To account for the complex sampling design, the statistical analyses were performed with the survey sampling and analysis procedures in SAS 9.4 (SAS institute, Cary, NC, USA). In addition, Spearman's correlation coefficients among the urinary concentrations of the OPE metabolites, with different urine dilution adjustments, were calculated. The associations of the OPE metabolite concentrations with eGFR or ACR were examined in linear regression models. Then, following the approach of **Chapter 2 and 3**, multi-pollutant models were developed to assess the associations of multiple OPE metabolites with eGFR or ACR. The linear associations between the OPE metabolites and either the eGFR or ACR were adjusted for potential covariates. The covariates included sex, age (continuous), race/ethnicity (Mexican American, other Hispanic, non-Hispanic White, non-Hispanic Black, and other groups), BMI (continuous), smoking history (<100 cigarettes and ≥100 cigarettes), poverty income ratio (≤1.85, >1.85 to ≤3.50, and >3.50), and self-reported physical activity (inactive/moderate and vigorous). For poverty income ratio, the values provided by NHANES were used, which were derived by dividing family income by the poverty guideline (Johnson et al., 2013). To preserve the sample size, missing values for poverty income ratio (n = 121, 7.7%) were imputed by the value estimated from the linear regression model with demographic determinants of the ratio (i.e., sex, age, and race/ethnicity). A logistic regression was also used to investigate the association between the OPE metabolite concentrations and the CKD (dichotomous) with the same adjusting variables in the model.

To visualize non-linear relationship between the levels of urinary OPE metabolites and the outcome variables, restricted cubic spline regression models were constructed using the mgcv package of the R software. In the

spline regression models, only the single chemical was included with the same set of covariates of the linear models. Three knots were used for the log-transformed OPE metabolites levels. The knots were located at the 25th, 50th, and 75th percentiles.

4.3 Results

4.3.1. CKD status and other characteristics of the participating population

Among the general US adult population, 14.5% exhibited eGFR or ACR which could be classified as CKD (Table 4-2). The older group tended to exhibit lower eGFR and higher ACR. Among the race/ethnicity groups, the non-Hispanic white group showed the lowest eGFR. Smoking was associated with lower eGFR and higher ACR, but such a pattern was not observed for drinking. The 30.3% of the general US adults were revealed to have the normal range of BMI values (<25 kg/m^2).

Table 4-2. Demographic and socio-behavioral characteristics and current chronic kidney disease status of the study population.

Characteristics	N (%)	Weighted %[a]	eGFR (mL/min/1.73 m^2)[b]	*p*-value[c]	ACR (mg/g)[b]	*p*-value[c]
Total	1578 (100)	100	98.6 (81.9-113.1)		6.8 (4.7-13.1)	
Sex						
Male	765 (48.5)	48.8	97.8 (80.8-113.1)	0.895	5.9 (4.0-11.1)	<0.001
Female	813 (51.5)	51.2	99.2 (82.6-113.1)		7.6 (5.5-14.5)	
Age (year)						
20-39	561 (35.6)	38.7	113.5 (100.2-123.3)	<0.001	5.9 (4.0-10.0)	<0.001
40-59	605 (38.3)	39.2	98.7 (84.6-108.2)		6.8 (4.6-12.5)	
60-79	412 (26.1)	22.2	78.5 (66.1-91.4)		9.1 (5.7-19.5)	
Race/ethnicity						
Mexican American	219 (13.9)	9.42	106.4 (90.8-118.8)	<0.001	7.1 (4.9-13.0)	0.790
Other Hispanic	142 (9.00)	6.01	103.2 (86.4-114.6)		7.1 (5.0-13.9)	
Non-Hispanic White	649 (41.1)	64.8	93.5 (77.2-106.7)		6.8 (4.8-13.1)	
Non-Hispanic Black	314 (19.9)	11.3	100.2 (82.0-119.3)		6.7 (4.2-13.1)	
Other groups	254 (16.1)	8.45	101.2 (87.6-113.3)		6.6 (4.6-12.5)	
BMI (kg/m^2)						
<25	489 (31.0)	30.3	102.1 (88.0-115.3)	<0.001	7.0 (4.9-11.5)	<0.001
≥25 to <30	495 (31.4)	31.1	95.3 (79.3-111.6)		6.3 (4.4-11.5)	
≥30	594 (37.6)	38.7	97.3 (80.1-112.8)		7.2 (4.8-16.4)	
Lifetime smoking						
<100 cigarettes	883 (56.0)	56.8	100.2 (83.2-115.1)	0.001	6.7 (4.5-11.6)	0.006
≥100 cigarettes	695 (44.0)	43.2	96.9 (80.1-11.2)		7.1 (4.8-15.2)	

Table 4-2. (continued)

Characteristics	N (%)	Weighted %[a]	eGFR (mL/min/1.73 m^2)[b]	*p*-value[c]	ACR (mg/g)[a]	*p*-value[c]
Poverty income ratio						
≤1.85	777 (49.2)	37.7	100.6 (83.7-115.5)	<0.001	7.2 (4.9-15.0)	<0.001
>1.85 to ≤3.50	364 (23.1)	23.7	98.2 (82.9-113.6)		6.9 (4.7-14.3)	
>3.50	437 (27.7)	38.6	95.1 (80.6-109.0)		6.1 (4.4-10.4)	
Physical activity						
Inactive or moderate	962 (61.0)	58.6	96.4 (79.1-112.4)	<0.001	7.6 (5.1-15.2)	<0.001
Vigorous	616 (39.0)	41.4	100.9 (85.9-114.4)		5.9 (4.0-10.0)	
Current CKD[d]						
No	1326 (84.0)	85.5	100.6 (86.8-114.4)	<0.001	6.2 (4.4-9.6)	<0.001
Yes	252 (16.0)	14.5	76.1 (54.1-102.9)		46.9 (18.5-107.8)	

[a] Percent values were weighted to account for the complex survey design.
[b] Median (25th–75th percentile).
[c] *p*-value calculated from one-way analysis of variance.
[d] Those with albumin-to-creatinine ratio (ACR) above 30 mg/g or estimated glomerular filtration rate (eGFR) below 60 mL/min/1.73 m^2 were defined as patients with chronic kidney disease (CKD).

4.3.2. OPE metabolite concentrations in urine

Among the nine OPE metabolites originally analyzed in the NHANES 2013-2014, only four compounds, i.e., DPHP, BDCIPP, BCEP, and DNBP, were detected in more than 75% of the samples. Among them, DPHP exhibited the highest unadjusted geometric mean (0.71 ng/mL), followed by BDCIPP, BCEP, and DNBP (0.69, 0.38 and 0.16 ng/mL, respectively; Table 4-3).

Without adjusting the urine dilution, the Spearman's correlation coefficients among the OPE metabolites in the urine ranged from 0.34 to 0.50. After controlling for urine dilution by traditional or novel creatinine-adjustment, the correlation coefficients decreased, and DNBP exhibited close to null or weak correlations with the rest of the other OPE metabolites (Fig. 4-2).

Table 4-3. Detection and distribution of organophosphate ester metabolites in urine of the general US population (n = 1578).

Compound	Creatinine adjustment	Detection frequency	Geometric mean	Percentile		
				25th	50th	75th
DPHP	Unadjusted	89.9%	0.71	0.31	0.71	1.43
	Traditional		0.67	0.20	0.75	2.18
	Novel		0.71	0.39	0.64	1.18
BDCIPP	Unadjusted	90.7%	0.69	0.27	0.69	1.76
	Traditional		0.73	0.35	0.70	1.42
	Novel		0.68	0.32	0.68	1.41
BCEP	Unadjusted	87.4%	0.38	0.14	0.35	0.83
	Traditional		0.40	0.19	0.35	0.73
	Novel		0.38	0.18	0.32	0.70
DNBP	Unadjusted	78.6%	0.16	0.06	0.21	0.34
	Traditional		0.17	0.10	0.19	0.32
	Novel		0.16	0.09	0.18	0.29

Units are in ng/mL for unadjusted and novel adjustment and in ng/mg creatinine for traditional adjustment.

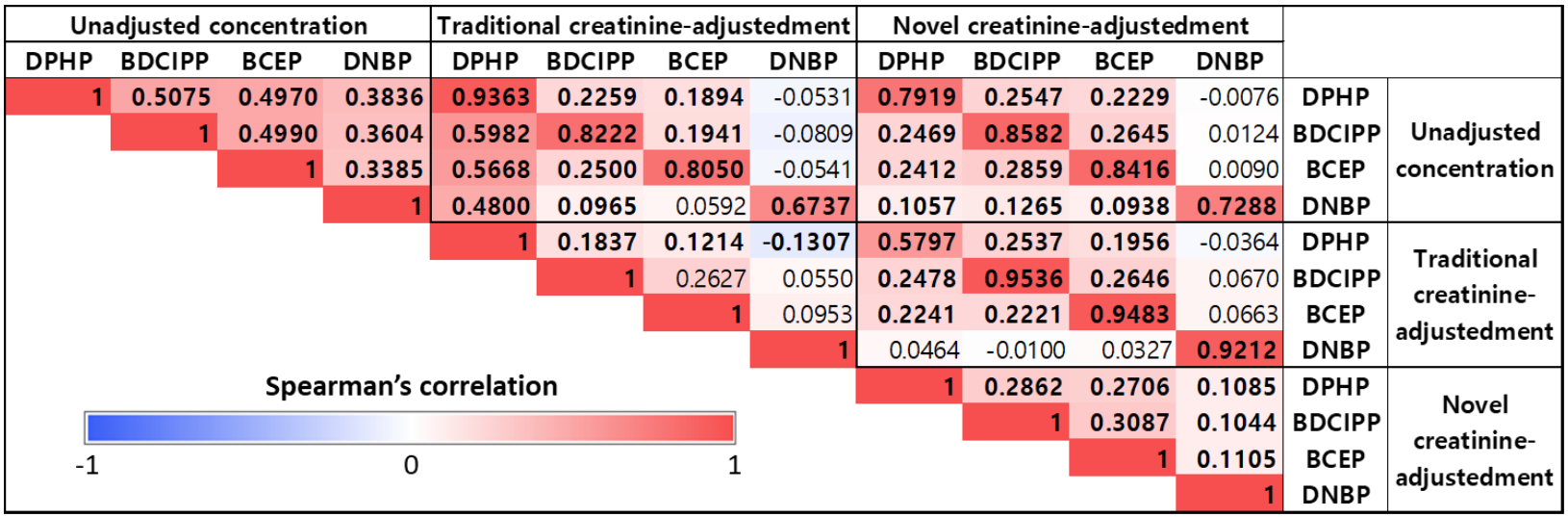

Fig. 4-2. Spearman's correlation among the urinary metabolites of organophosphate esters by method of urinary dilution adjustment (n = 1578). Bonferroni correction was used to calculate *p*-value. Coefficients in boldface represent $p < 0.05$.

4.3.3. Association with CKD parameters in single-pollutant model

When the traditional creatinine-adjustment was applied, no metabolites showed significant association with the eGFR (Table 4-4). However, when the novel creatinine-adjustment was used for controlling urine dilution, urinary BDCIPP and BCEP levels showed negative associations with the eGFR ($\beta = -1.13$ and -1.26, respectively). Similarly, in the natural cubic spline regression models, the negative association became more pronounced when the novel creatinine-adjustment was used (Fig. 4-3), except for DPHP.

Table 4-4. Association of urinary metabolites of organophosphate esters with estimated glomerular filtration rate (mL/min/1.73 m^2) in the general US population (n = 1578, Single-pollutant model).

Compound	Traditional creatinine-adjustment (ng/mg creatinine)		Novel creatinine-adjustment (ng/mL)	
	β (95% CI)	***p*-value**	**β (95% CI)**	***p*-value**
DPHP	-0.45 (-1.08, 0.18)	0.150	-0.28 (-1.24, 0.68)	0.549
BDCIPP	0.06 (-0.95, 1.08)	0.897	-1.13 (-2.22, -0.05)	0.042
BCEP	-0.29 (-1.38, 0.81)	0.586	-1.26 (-2.36, -0.17)	0.027
DNBP	0.53 (-0.76, 1.83)	0.392	-1.10 (-2.40, 0.20)	0.091

The association was adjusted for sex, age, race/ethnicity, poverty income ratio, smoking history, physical activity, and BMI.

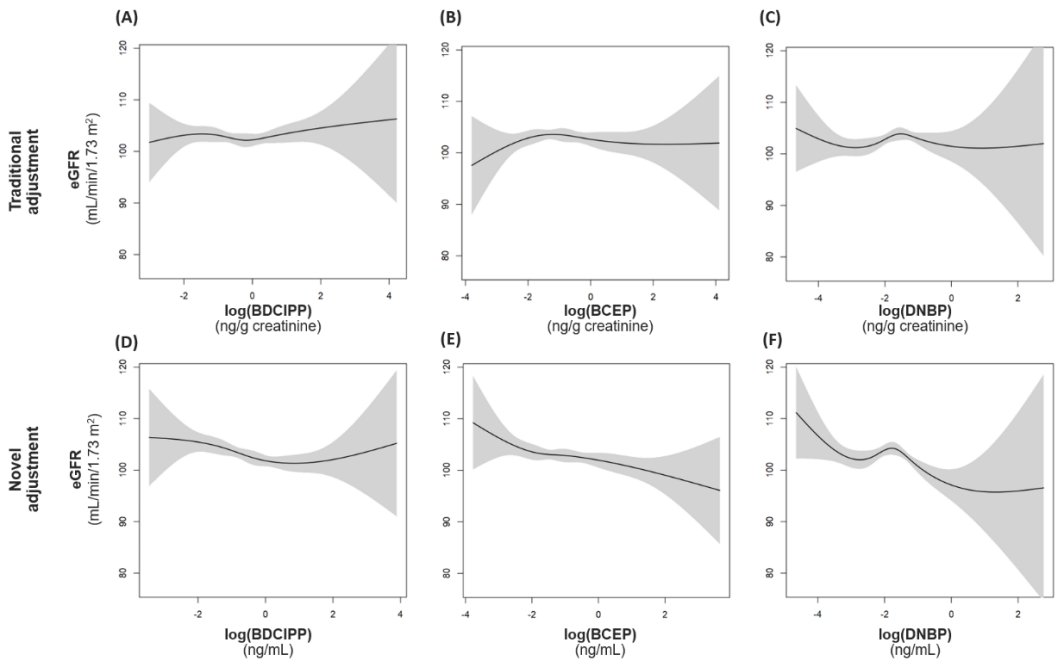

Fig. 4-3. Predicted spline curves for the associations between urinary metabolites of organophosphate esters and estimated glomerular filtration rate (eGFR) based on restricted cubic spline regression models with the traditional creatinine-adjustment [(A) BDCIPP, (B) BCEP, and (C) DNBP] or the novel creatinine-adjustment [(D) BDCIPP, (E) BCEP, and (F) DNBP] (n = 1578). The shaded areas represent 95% confidence intervals. Three knots were used and located at the 25th, 50th, and 75th percentiles. The model was adjusted for sex, age, race/ethnicity, poverty income ratio, smoking history, physical activity, and BMI.

For urinary ACR, most OPE metabolites were significantly associated, regardless of the method of urine dilution adjustment (Table 4-5). Urinary BDCIPP and DNBP levels were positively associated with urinary ACR ($\beta = 0.07$ for both chemicals). In addition, urinary BCEP levels showed a marginally significant association with ACR ($\beta = 0.06$).

Table 4-5. Association of urinary metabolites of organophosphate esters with urinary albumin-to-creatinine ratio (mg/g) in the general US population (n = 1578, Single-pollutant model).

Compound	Traditional creatinine-adjustment (ng/mg creatinine)		Novel creatinine-adjustment (ng/mL)	
	β (95% CI)	***p*-value**	**β (95% CI)**	***p*-value**
DPHP	0.00 (-0.03, 0.03)	0.914	0.02 (-0.04, 0.08)	0.495
BDCIPP	0.07 (0.01, 0.12)	0.018	0.07 (0.01, 0.12)	0.017
BCEP	0.06 (-0.01, 0.13)	0.078	0.06 (-0.01, 0.13)	0.080
DNBP	0.07 (0.00, 0.14)	0.048	0.07 (0.00, 0.14)	0.045

The association was adjusted for sex, age, race/ethnicity, poverty income ratio, smoking history, physical activity, and BMI.

4.3.4. Association between OPE metabolites and CKD classification

A similar direction of association was observed for CKD (Fig. 4-4). With the traditional creatinine-adjustment, the odds ratios (ORs) for the higher quartiles (Q2, Q3, or Q4) of each metabolite against the lowest quartile (Q1) tended to be higher than 1, but the ORs were mostly not significant. When the novel creatinine adjustment was applied, however, a monotonous trend of dose-response appeared to be more evident, and the ORs for Q4 of DPHP, BDCIPP, BCEP, and DNBP increased (OR = 1.56, 1.60, 1.66, and 1.58, respectively).

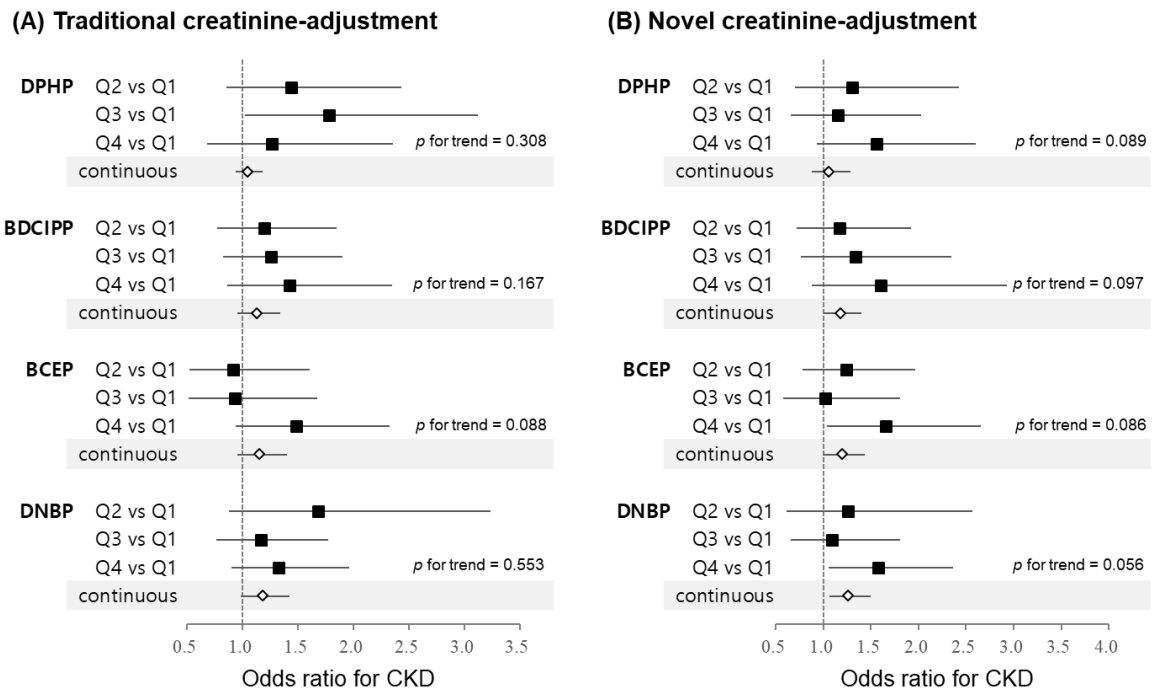

Fig. 4-4. Adjusted* odds ratios (■ for quartile groups and ◇ for continuous variables) and their 95% confidence intervals (bar) for chronic kidney disease (CKD) by quartiles or continuous increase of urinary metabolites of organophosphate esters with (A) the traditional creatinine-adjustment and (B) the novel creatinine adjustment using covariate-adjusted standardization (n = 1578). *Adjusted for sex, age, race/ethnicity, poverty income ratio, smoking history, physical activity, and BMI.

4.3.5. Secondary analysis with multi-pollutant model

BDCIPP, BCEP, and DNBP, which showed positive association with eGFR, ACR, or CKD, were added into the statistical models. With these compounds added to the multi-pollutant models, BDCIPP was determined to be a significant determinant of ACR ($\beta = 0.05$), and DNBP was determined to be a significant determinant of the CKD (OR = 1.24) (Table 4-6).

Table 4-6. Association between metabolites of organophosphate esters and estimated glomerular filtration rate (mL/min/1.73 m^2), log-transformed albumin-to-creatinine ratio (mg/g), or chronic kidney disease (n = 1578, Multi-pollutant model).

Compounds	Estimated glomerular filtration rate		Albumin-to-creatinine ratio		Chronic kidney disease	
	β (95% CI)	*p*-value	β (95% CI)	*p*-value	Odds ratio (95% CI)	*p*-value
BDCIPP	-0.80 (-1.85, 0.25)	0.127	0.05 (0.01, 0.10)	0.026	1.14 (0.98, 1.32)	0.097
BCEP	-1.02 (-2.15, 0.10)	0.072	0.05 (-0.02, 0.11)	0.165	1.15 (0.96, 1.38)	0.130
DNBP	-0.92 (-2.15, 0.30)	0.130	0.06 (-0.01, 0.14)	0.097	1.24 (1.04, 1.47)	0.019

Urinary OPE metabolite levels were adjusted with the novel creatinine-adjustment. The association was adjusted for sex, age, race/ethnicity, poverty income ratio, smoking history, physical activity, and BMI. Linear regression model was applied for both eGFR and ACR. Logistic regression model was applied for chronic kidney disease.

4.4 Discussion

Significant associations between several OPE metabolites in urine and CKD, which were observed in the representative adult population of the US, suggest that OPEs are potential chemical determinants of kidney function. Following the novel creatinine-adjustment of the urinary dilution, BCEP showed a negative association with the eGFR (Table 4-4 and Fig. 4-3), and both BDCIPP and DNBP exhibited positive associations with the ACR (Table 4-5). Our observation is partially supported by existing reports from *in vitro* experiments, which demonstrated adverse effects of TDCIPP and TCEP exposure on proximal tubule cells (Killilea et al., 2017; Ren et al., 2008; 2009; 2012). In the primary rabbit renal proximal tubule cells, TCEP exhibited cytotoxicity, cell cycle regulatory change, pro-apoptotic effects, and dysfunction in ion and non-ion transports (Ren et al., 2008; 2009; 2012). TDCIPP was also documented to cause cytotoxicity in a renal proximal tubule cell line via oxidative stress (Killilea et al., 2017). Inflammation is suggested as one of the underlying mechanisms of chemical induced nephrotoxicity, e.g., melamine, cyanuric acid, aristolochic acid, or bisphenol A in experimental animals of rat (Wang et al., 2018), mouse (Tong et al., 2019), and zebrafish (Ding and Chen, 2012), and mouse podocyte cell line (Tong et al., 2019). Indeed, inflammation has been suggested as a mechanism of hepatotoxicity of TDCIPP (Liu et al., 2016), supporting a hypothesis that TDCIPP exposure may cause nephrotoxicity via inflammation-mediated pathways.

A significant association of DNBP with the ACR (Table 4-5) is noteworthy and requires experimental support, because neither *in vivo* nor *in vitro* studies are available for the effects of TNBP on kidney function to date. When considering the minuscule correlations of DNBP with BDCIPP or BCEP in the urine, DNBP might be an independent chemical risk factor for CKD in general adult populations. Because both the eGFR and the ACR generally reflect glomerular function, rather than tubular function (X Chen et al., 2019), further investigations on glomerular function and their underlying

mechanisms using appropriate *in vivo* or *in vitro* models are warranted for confirmation of this observation.

In contrast, the null association of DPHP with CKD is interesting. In a number of experimental studies, TPHP or its specific metabolite has been reported to exhibit sex and thyroid hormone disruption (Kim et al., 2015; Liu et al., 2012), as well as estrogen receptor affinity (Kojima et al., 2013; 2016), even though its toxicities on the kidneys have not been characterized. This null association, however, should not be interpreted as the lack of an association between TPHP exposure and adverse kidney function because DPHP is not a specific exposure biomarker of TPHP. DPHP is one of the major urinary metabolites of not only TPHP but also other compounds, such as 2-ethylhexyl diphenyl phosphate, bisphenol A bis(diphenyl phosphate), and resorcinol bis(diphenyl) phosphate (Saillenfait et al., 2018; Van den Eede et al., 2016).

Significant associations of OPE metabolites with kidney function were also confirmed by a secondary analysis employing a multivariable analysis that included the multiple OPE metabolites (Table 4-6). The inclusion of multiple chemical stressors in a regression model is essential, because people are simultaneously exposed to the given chemicals. In the present study, considering possible exposure to multiple OPEs (Fig. 4-2), we constructed multi-pollutant models as following the approach in previous studies (**Chapter 2 and 3**). The results of the multi-pollutant models (Table 4-6) are generally comparable to those derived from statistical models employing single chemicals (Table 4-4, Table 4-5), thus supporting the associations of BDCIPP, BCEP, or DNBP with kidney function among the general population.

Changes in association by the method of urine dilution adjustment (Table 4-4 and Fig. 4-3) clearly demonstrate that the method of urinary dilution adjustment can obscure associations, especially when the outcomes of interest are related to kidney function. Urinary dilution has been frequently adjusted by urinary creatinine concentrations in many association studies. Since kidney

function can directly influence urine hydration or creatinine excretion, however, the use of creatinine-adjustment for urine dilution may induce a collider stratification bias leading to possible confounding (Bulka et al., 2017; O'Brien et al., 2016). The collider issue can be addressed to a certain extent by including the fitted urinary creatinine concentration ($\widehat{Ucr}$) in the urine dilution adjustment factor (Table 4-1). Indeed, in the present population, the eGFR was significantly correlated with 1/Ucr, the traditional creatinine-adjustment factor, but not with $\widehat{Ucr}/Ucr$. Therefore, the novel method of creatinine-adjustment that was used in the present study can be applied to association studies between urinary analytes and kidney function outcomes, to minimize possible risks of bias.

Consistent association with ACR regardless of the creatinine-adjustment methods (Table 4-5) is not surprising because ACR and glomerular filtration rate (GFR) are not always correlated with each other (Boer and Steffe, 2007). In addition, for classification of CKD, ACR is commonly considered as a marker of kidney damage (Polkinghorne, 2014), while GFR directly represents function of glomerulus. Since creatinine excretion can be affected by reduced glomerular function rather than by kidney damage in general, different method of creatinine adjustment may pose less significant impact on ACR.

The importance of adjustment methods for urinary dilution has been previously reported in an association between urinary arsenic levels and obesity (Bulka et al., 2017). The authors hypothesized that obesity status may influence urinary creatinine levels because obesity, or body fat mass, is correlated with muscle mass (Gerchman et al., 2009; Janssen et al., 2000), and muscle mass produces creatinine (Barr et al., 2005). Supporting this hypothesis, the authors reported an inverse association between urinary arsenic levels and obesity statuses when the urine dilution was adjusted only with urinary creatinine levels. However, when the urinary creatinine levels were adjusted for covariates, including age, sex, race/ethnicity, and BMI, the negative association disappeared. This example clearly demonstrates the

importance of the adjustment method for urinary dilution, especially when the health outcomes of interest are associated with the adjusting factor.

Several limitations of this study should be considered in interpretation of the observed associations. First, because of short biological half-lives of OPEs and their great temporal variation in individual exposure, single measurements of OPE metabolites may not represent long-term exposure. Second, because of the cross-sectional nature, causal association cannot be concluded. Especially for outcomes related to kidney function, reverse causality can often occur, because reduced glomerulus filtration may decrease urinary excretion of chemicals and therefore increase chemical levels in the blood (Weaver et al., 2016). However, our observations are still meaningful because the positive associations seen between urinary OPE metabolites and CKD would suggest even stronger positive associations if the assumption of reverse causality would have occurred among the study population (Jin et al., 2018). Finally, considering the use of OPEs, other chemicals sharing common exposure sources are potential confounders which could not be controlled in this study. Phthalates, widely used plasticizers, were associated with kidney disease in the previous chapters (**Chapter 2 and 3**). Melamine, a well-known nephrotoxicant, can be used as flame retardants (Liu and Wang, 2009; Thirumal et al., 2010). These possible confounders should be considered in the future studies.

4.5 Summary and implications

Our results suggest possible links between OPE exposure and kidney function among the general US adult population. As a cross-sectional study, the present study has an intrinsic limitation which makes the identification of causality difficult. However, with a reasonable sample size that represents the general US adult population, the results of this study provide an important hypothesis concerning the adverse health outcomes of OPEs on kidney function at the current levels of exposure, which can be tested in other populations. In addition, we demonstrated that the significance and direction of the association, if it is between urinary analytes and the outcomes related to kidney function, can be influenced by the method of urinary dilution adjustment, and we also proposed a new method of adjustment. Further confirmation in other human populations and mechanistic supports with experimental studies should be followed.

Chapter 5. Vitamin D status on the association between chemical exposure and chronic kidney disease

5.1 Introduction

Chronic kidney disease (CKD) has been a health threat all over the world (Hill et al., 2016). Progression of CKD can lead to associated morbidity and mortality. Considering health consequences and socioeconomic burden of CKD, identifying its modifiable risk factors and attenuating the effects of the risk factors are critical.

Vitamin D is well-known to have a role in mineral metabolism and bone health. Ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) are precursors of vitamin D. Vitamin D3 can be synthesized in skin by solar ultraviolet type B (UVB) exposure. Foods and supplements are other sources of vitamin D, i.e., vitamin D2 and D3. In liver, vitamin D2 and D3 are metabolized into 25-hydroxyvitamin D2 [25(OH)D2] and 25-hydroxyvitamin D3 [25(OH)D3], respectively, which are predominant forms of vitamin D in serum. 25(OH)D2 and 25(OH)D3 are finally converted into an active form 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] in renal tubule (Fig. 5-1; Zhu et al., 2015).

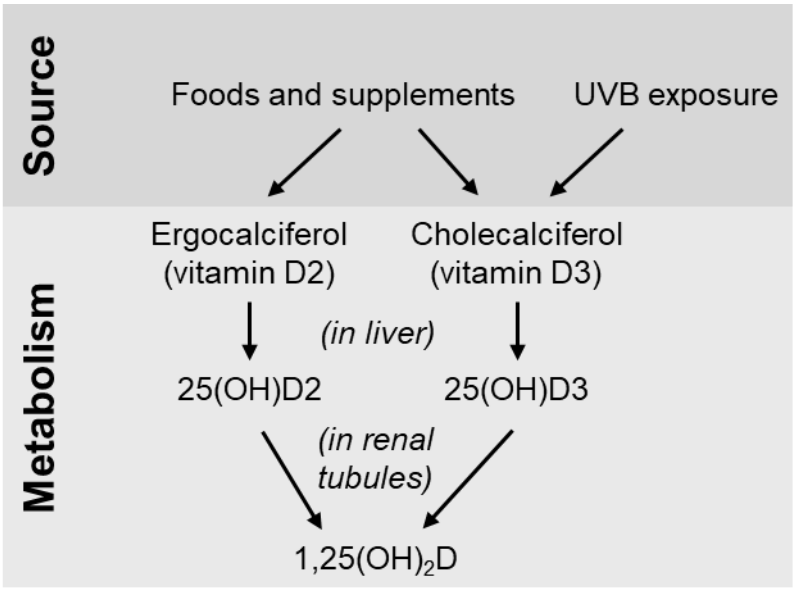

Fig. 5-1. Sources and metabolism of vitamin D.

Kidney is related with vitamin D not only in its metabolism but also in the progression of kidney disease while there have been recently debates on effects of vitamin D supplementation on CKD (Brogan et al., 2020; de Boer et al., 2019; 2020; Peiris, 2020). Serum vitamin D level has been associated with hypertension (Gunta et al., 2013), which is a major risk factor for CKD, and their inverse association was confirmed in a meta-analysis of prospective and cress-sectional studies (Burgaz et al., 2011). In an 8-week randomized clinical trial, sun exposure group showed significant decrease of diastolic blood pressure as well as increase of serum vitamin D level compared to control group (Joh et al., 2020). A meta-analysis showed vitamin D therapy can reduce proteinuria in CKD patients (de Borst et al., 2013), and vitamin D therapy was associated with survival improvement in patients with renal replacement therapy (Kim and Kim, 2014). Sun exposure was inversely associated with mortality of patients with end stage renal disease in a case-crossover study (Yoon et al., 2019). Experimental studies have also demonstrated renoprotective effects of vitamin D (Kim and Kim, 2014).

Several pathways have been suggested as underlying mechanisms of the renoprotective action of vitamin D. Protective effects of vitamin D on kidney disease are considered to be mediated by local renin-angiotensin system (RAS) and nuclear factor (NF)-κB pathway, which are involved in gene expressions regarding inflammation, fibrogenesis, and oxidative stress (Li, 2010). In chemical-induced kidney injury rat models, nephropathy was attenuated in rats by paricalcitol, a vitamin D analog, via anti-inflammatory, antifibrotic (Park et al., 2010a; 2010b). Paricalcitol treatment also inhibited renal inflammatory infiltration as targeting NF-κB (Tan et al., 2008).

Various environmental chemicals, such as phthalates, environmental phenols, organophosphate esters (OPEs) and perfluoroalkyl substances, have been linked with adverse kidney outcomes in previous epidemiological studies (Kataria et al., 2015; Stanifer et al., 2018; **Chapter 2, 3, and 4**). Experimental studies have revealed that the oxidative stress and inflammation are underlying mechanisms of kidney outcomes induced by exposure to

chemicals. For example, oxidative stress was associated with adverse kidney effects induced by dibutyl phthalate (DBP) in mouse (Cheng et al., 2019). Tris(1,3-dichloro-2-propyl) phosphate (TDCIPP)-induced cytotoxicity in proximal tubule cells were attenuated by antioxidant treatment (Killiea et al., 2017). Inflammation-related pathway was suggested as an underlying mechanism of bisphenol A (BPA)-induced podocytopathy in mouse (Tong et al., 2019).

Despite of potential of vitamin D to affect kidney disease associated with chemical exposure, any epidemiological studies regarding chemical exposure and kidney disease have not been conducted to evaluate related effect of vitamin D so far. In the present study, we evaluated effect modification by vitamin D on the association between chemical exposure and CKD as employing US adult population where we observed significant association of exposure to phthalates, environmental phenols, and OPEs and CKD (**Chapter 3 and 4**).

4.2 Materials and methods

4.2.1. Study participants

For phthalates metabolites and environmental phenols, dataset from NHANES 2009-2014 was used. For OPE metabolites, dataset from NHANES 2013-2014 was used. Among the participants with measurement of phthalate metabolites and environmental phenols or OPEs in the spot urine samples, adults between 20 and 59 years of age were included. Then, we excluded participants without vitamin D intake and sun-exposure time and with missing values in eGFR, ACR, body mass index (BMI), or serum vitamin D concentration. Finally, the final populations of 3027 were selected for phthalate metabolites and environmental phenols (Fig. 5-2A), and the final populations of 1069 were selected for OPE metabolites (Fig. 5-2B).

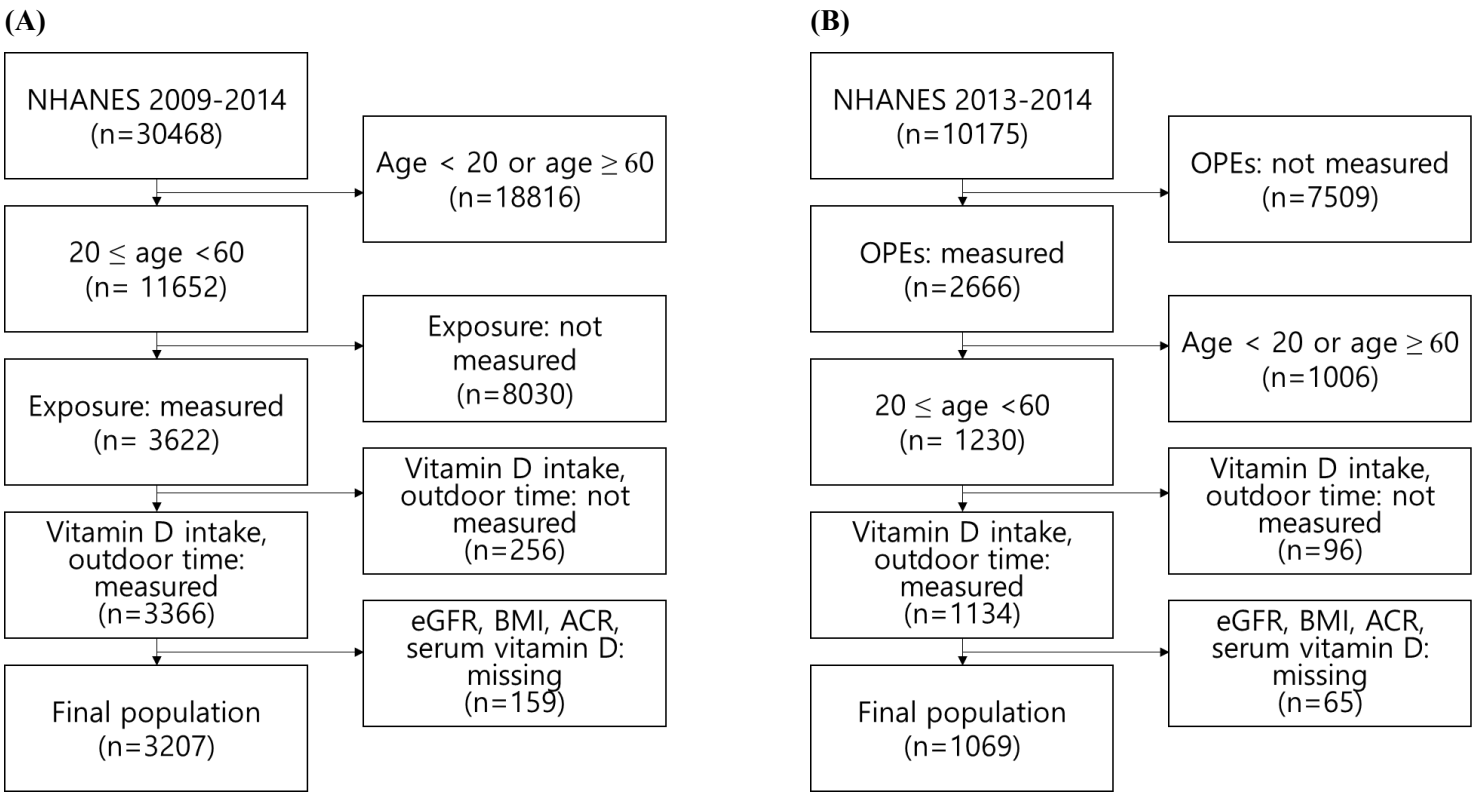

Fig. 5-2. Procedure for selecting study population for (A) phthalate metabolites, environmental phenols, and (B) organophosphate esters (OPEs).

5.2.2. Quantification of chemicals in urine

The same set of phthalate metabolites and environmental phenols which were considered in **Chapter 3**, was included in this study. Diphenyl phosphate (DPHP), bis(1,3-dichloro-2-propyl) phosphate (BDCIPP), bis(2-chloroethyl) phosphate (BCEP), and di-n-butyl phosphate (DNBP), which were investigated for the association with CKD in **Chapter 4**, were also considered in this study. The analytical procedures for the quantification of the chemicals were described in **Section 3.2.2 and 4.2.2**.

5.2.3. Measurement of chronic kidney disease

Estimated glomerular filtration rate (eGFR) and urinary albumin-to-creatinine ratio (ACR) were used as parameters to determine CKD. Procedures to calculate eGFR and ACR were described in **Section 3.2.3 and 4.2.3.**

5.2.4. Vitamin D-related measures

Serum concentrations of 25-hydroxyvitamin D2 [25(OH)D2] and 25-hydroxyvitamin D3 [(25(OH)D3) were measured in by ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) as described in detail elsewhere (CDC, 2010). Sum of 25(OH)D2 and 25(OH)D3, which are the predominant circulating forms of vitamin D and reliable measures of vitamin D status, was considered as serum vitamin D concentration. Vitamin D status was categorized into deficiency (< 50 nmol/L), insufficiency (≥ 50 and <72.5 nmol/L), and sufficiency (≥ 72.5 nmol/L) following a recommendation (Holick et al., 2011).

Amount of vitamin D (vitamin D2 + D3) intake from foods and beverages was estimated from 24-hour dietary recall interview. Amount of vitamin D intake from dietary supplements was also collected by 30-day dietary supplement use survey. Intake amounts of vitamin D obtained from those two data were summarized to calculate daily intake of vitamin D from all dietary

sources. Intake of vitamin D was categorized into low vitamin D intake (< 15 mcg/day) and high vitamin D intake (≥ 15 mcg/day) following a recommendation (Holick et al., 2011).

In the questionnaire, time to spend outdoors between 9AM and 5PM was asked for weekday and weekend separately. To evaluate sunlight exposure time, weekly sun-exposure time was calculated as summarizing outdoor times on weekday and weekend during the daytime. Since there is no relevant recommendation, sun-exposure time was categorized into two groups (< median and ≥ median, respectively).

5.2.5. Statistical analysis

For the non-detected samples, LOD/$\sqrt{2}$ was substituted as concentrations of the chemical. Following the findings from **Chapter 3 and 4**, the covariate-adjusted creatinine standardization was applied to correct urine dilution (**Section 3.2.4 and 4.2.4**). the Prior to the statistical analyses, urinary chemical concentrations and ACR were log-transformed. The associations between the chemical concentrations and the kidney disease markers were evaluated in linear regression models. To assess effect modification by serum vitamin D status on the association between chemical exposure and CKD, the associations between chemical concentrations and CKD were evaluated in the stratification by vitamin D status (deficiency, insufficiency, and sufficiency). To assess effect modification by sunlight exposure time and dietary vitamin D intake on the association, the associations were also evaluated in the stratification by groups defined by sun-exposure time and vitamin D intake level. The statistical analyses were performed with SAS 9.4 (SAS institute, Cary, NC, USA), and complex sampling design was accounted by the survey sampling and analysis procedures.

5.3 Results

5.3.1. Characteristics of the participants

Among the general US adult population with age between 20 and 59, 26.2 - 27.7% and 37.1 - 39.3% were classified as vitamin D deficiency and insufficiency, respectively (Tables 5-1 and 5-2). Dietary vitamin D intake was less than 15 mcg/day for 73.9 - 76.5% of the population. The older group tended to exhibit higher serum vitamin D level and dietary vitamin D intake. Among the race/ethnicity groups, the non-Hispanic Black group showed the lowest serum vitamin D level and dietary vitamin D intake.

Table 5-1. Demographic and socio-behavioral characteristics, current chronic kidney disease status, serum vitamin D status, and dietary vitamin D intake of the study population (NAHENS 2009-2014).

Characteristics	N (%)	Weighted %[a]	eGFR (mL/min per 1.73 m^2)[b]	ACR (mg/g)[b]	Serum vitamin D (nmol/L)[b]	Dietary vitamin D intake (mcg/day)[b]	Sun-exposure time (min/wk)[b]
Total	3207 (100)	100.0	102.7 (89.4-115.5)	6.0 (4.0-9.9)	64.0 (49.2-80.9)	5.9 (2.6-14.1)	491 (227-1073)
Sex							
Male	1598 (49.8)	49.7	101.1 (88.6-115.8)	5.0 (3.5-8.4)	62.0 (49.2-77.4)	5.9 (2.7-12.9)	657 (320-1591)
Female	1609 (50.2)	50.3	103.7 (90.9-115.2)	7.0 (4.8-11.4)	66.3 (49.1-85.7)	6.0 (2.5-16.1)	398.2 (196-821)
Age (year)							
20-29	824 (25.7)	24.7	116 (103.2-126.7)	5.4 (3.7-9.0)	59.2 (45.7-76.0)	4.4 (2.1-9.0)	571 (263-1199)
30-39	810 (25.3)	23.7	108 (94.4-117.5)	6.0 (3.9-9.8)	61.3 (45.5-76.0)	5.2 (2.4-12.0)	417 (223-938)
40-49	797 (24.9)	25.7	101.4 (88.6-109.8)	6.0 (4.2-9.2)	65.9 (51.6-80.7)	7.1 (2.9-15.9)	536 (228-1119)
50-59	776 (24.2)	25.8	90 (79.4-100.6)	6.9 (4.4-11.6)	69.8 (53.8-90.2)	8.4 (3.2-21.6)	473 (220-946)
Race/ethnicity							
Mexican American	468 (14.6)	9.7	115.2 (102.7-125.1)	6.5 (4.3-11.3)	52.7 (42.1-63.2)	4.9 (2.7-8.9)	479 (201-1389)
Other Hispanic	322 (10.0)	6.9	107 (94.2-120.8)	6.1 (3.9-10.2)	57.3 (45.2-69.2)	4.4 (2.5-9.8)	462 (183-1164)
Non-Hispanic White	1335 (41.6)	64.2	99.1 (87.3-111.1)	5.9 (4.0-9.4)	71.8 (58.0-87.6)	6.7 (2.9-16.1)	526 (233-1051)
Non-Hispanic Black	652 (20.3)	11.5	108.8 (93.6-122.3)	5.9 (3.9-10.6)	40.2 (30.1-55.2)	4.5 (1.8-10.5)	519 (207-1066)
Other groups	430 (13.4)	7.7	107.7 (94.4-117.7)	6.7 (4.3-12)	52.7 (39.5-68.2)	5.6 (2.2-12.5)	413 (187-839)
BMI (kg/m^2)							
<25	995 (31.0)	31.6	105.2 (93.3-117.3)	6.5 (4.3-10.6)	70.0 (53.1-88.4)	6.1 (2.5-13.9)	529 (230-1066)
≥25 to <30	1045 (32.6)	33.3	99.8 (87.6-113.3)	5.1 (3.6-8.3)	66.6 (52.3-82.9)	6.4 (2.7-14.9)	526 (233-1076)
≥30	1167 (36.4)	35.1	102.9 (89.2-115.8)	6.5 (4.3-11.2)	59.1 (43.6-72.8)	5.4 (2.6-13.0)	469 (208-1069)
Lifetime smoking							
<100 cigarettes	1287 (40.1)	40.5	102.9 (89-115.9)	5.9 (3.9-9.5)	63.2 (48.8-79.4)	6.2 (2.9-14.5)	470 (225-945)
≥100 cigarettes	1920 (59.9)	59.5	102 (89.5-114.9)	6.1 (4.1-10.4)	65.3 (49.5-83.3)	5.4 (2.1-13.7)	539 (229-1226)

Table 5-1. (continued)

Characteristics	N (%)	Weighted %[a]	eGFR (mL/min per 1.73 m^2)[b]	ACR (mg/g)[b]	Serum vitamin D (nmol/L)[b]	Dietary vitamin D intake (mcg/day)[b]	Sun-exposure time (min/wk)[b]
Poverty income ratio							
≤1.85	1486 (46.3)	34.0	108.8 (93.9-120.9)	6.2 (4.1-11.1)	56.7 (41.6-72.8)	4.7 (2.1-10.3)	590 (224-1248)
>1.85 to ≤ 3.50	790 (24.6)	26.1	104.4 (91.2-115.8)	6.0 (3.9-9.6)	62.2 (48.2-79.4)	5.0 (2.2-12.0)	472 (218-1250)
>3.50	931 (29.0)	39.9	97.2 (86.6-109)	5.7 (4.0-9.1)	71.7 (56.5-87.6)	7.9 (3.5-20.5)	476 (232-879)
Physical activity							
Inactive or moderate	1845 (57.5)	55.6	102.1 (89.3-115.3)	6.4 (4.4-10.8)	61.8 (46.8-78.6)	5.7 (2.5-13.5)	410 (182-832)
Vigorous	1362 (42.5)	44.4	103.2 (89.5-115.7)	5.4 (3.7-8.9)	67.2 (52.6-83.4)	6.2 (2.7-14.7)	651 (349-1418)
Current CKD[c]							
No	2927 (91.3)	92.6	102.7 (89.9-115.4)	5.6 (3.9-8.6)	64.3 (49.7-81.3)	5.9 (2.6-14.2)	510 (228-1076)
Yes	280 (8.7)	7.4	104.1 (79.1-117.1)	55.2 (35.3-117.6)	60.6 (41.1-76.8)	5.2 (2.4-11.4)	467 (151-955)
Serum vitamin D status							
Deficiency	1134 (35.4)	26.2	110.5 (96.4-122.4)	6.5 (4.3-11.6)	38.1 (30.1-44.9)	3.4 (1.6-6.3)	409 (134-941)
Insufficiency	1148 (35.8)	37.1	103.3 (92.0-115.6)	5.7 (3.9-9.6)	61.2 (55.8-66.6)	5.8 (2.7-12.9)	485 (229-1078)
Sufficiency	925 (28.8)	36.6	97.2 (83.6-108.9)	5.9 (4.0-9.0)	87.3 (78.9-100.6)	10.6 (4.1-24.5)	574 (258-1171)
Dietary vitamin D intake							
< 15 mcg/day	2546 (79.4)	76.5	104.4 (90.7-117.3)	5.8 (4.0-9.9)	59.8 (45.5-75.2)	4.1 (2.0-7.4)	526 (228-1154)
≥ 15 mcg/day	661 (20.6)	23.5	97.6 (86.5-109.1)	6.6 (4.1-9.9)	79.9 (65.2-99.6)	25.7 (19.3-39.2)	470 (221-936)

[a] Percent values were weighted to account for the complex survey design.
[b] Median (25th–75th percentile).
[c] Those with albumin-to-creatinine ratio (ACR) above 30 mg/g or estimated glomerular filtration rate (eGFR) below 60 mL/min/1.73 m^2 were defined as patients with chronic kidney disease (CKD).

Table 5-2. Demographic and socio-behavioral characteristics, current chronic kidney disease status, serum vitamin D status, and dietary vitamin D intake of the study population (NHANES 2013-2014).

Characteristics	N (%)	Weighted %[a]	eGFR (mL/min per 1.73 m^2)[b]	ACR (mg/g)[b]	Serum vitamin D (nmol/L)[b]	Dietary vitamin D intake (mcg/day)[b]	Sun-exposure time (min/wk)[b]
Total	1069 (100)	100	102.7 (89.9-115.2)	6.3 (4.3-10.7)	63.4 (48.4-79.4)	6.0 (2.7-15.5)	494 (229-1063)
Sex							
Male	535 (50.0)	50.6	102.0 (90.2-116.5)	5.4 (3.8-8.6)	61.4 (47.2-74.7)	6.0 (2.8-13.9)	765 (340-1603)
Female	534 (50.0)	49.4	103.7 (89.7-114.6)	7.3 (5.2-12.8)	64.6 (49.6-83.5)	6.0 (2.5-18.6)	389 (116-751)
Age (year)							
20-29	261 (24.4)	26.5	115.1 (104.2-124.7)	6.0 (3.9-9.3)	57.9 (46.7-71.1)	5.1 (2.3-12.8)	692 (245-1300)
30-39	258 (24.1)	23.3	104.8 (92.8-116.4)	5.7 (4.1-11.2)	63.5 (47.3-74.3)	5.7 (2.7-13.5)	500 (165-942)
40-49	286 (26.8)	24.9	99.1 (88.6-109.8)	6.5 (4.4-10.0)	65.4 (51.5-81.2)	5.7 (2.2-13.4)	476 (219-1020)
50-59	264 (24.7)	25.3	91.7 (76.5-101.7)	7.0 (4.9-14.0)	68.8 (46.4-85.7)	9.2 (3.7-23.1)	404 (229-1058)
Race/ethnicity							
Mexican American	149 (13.9)	10.3	112.9 (102.7-123.5)	6.5 (4.6-11.7)	53.7 (43.8-64.2)	4.4 (2.4-10.3)	662 (209-1689)
Other Hispanic	96 (9.0)	6.6	110.4 (100.6-120.4)	7.0 (4.8-13.1)	58.7 (46.9-69.2)	6.3 (2.3-12.2)	434 (215-1055)
Non-Hispanic White	456 (42.7)	63.9	97.7 (84.9-110.3)	6.2 (4.4-10.6)	68.8 (53.5-85.6)	7.1 (3.0-17.5)	488 (232-961)
Non-Hispanic Black	197 (18.4)	11.0	105.3 (92.0-122.1)	5.6 (3.8-11.0)	44.2 (29.9-63.9)	3.8 (1.8-9.5)	651 (235-1632)
Other groups	171 (16.0)	8.2	107.0 (95.5-117.5)	6.2 (4.2-10.3)	57.2 (43.2-70.2)	6.6 (2.0-15.2)	341 (112-729)
BMI (kg/m^2)							
<25	340 (31.8)	30.9	105.6 (92.1-118.4)	6.8 (4.7-10.5)	67.9 (50.7-85.4)	6.2 (2.8-16.3)	513 (225-1047)
≥25 to <30	327 (30.6)	30.8	100.0 (86.0-112.4)	5.7 (4.1-8.6)	64.7 (50.5-76.6)	6.2 (2.6-14.6)	560 (239-1138)
≥30	402 (37.6)	38.3	102.4 (90.8-114.7)	6.7 (4.3-13.5)	59.2 (45.0-73.2)	5.7 (2.6-15.2)	462 (181-951)
Lifetime smoking							
<100 cigarettes	618 (57.8)	57.9	103.9 (88.7-116.6)	6.1 (4.2-9.5)	62.4 (48.8-81.1)	6.5 (2.8-16.6)	417 (225-890)
≥100 cigarettes	451 (42.2)	42.1	101.6 (90.6-113.2)	6.8 (4.6-12.6)	64.7 (47.3-77.2)	5.3 (2.4-13.7)	646 (236-1359)

Table 5-2. (continued)

Characteristics	N (%)	Weighted %[a]	eGFR (mL/min per 1.73 m^2)[b]	ACR (mg/g)[b]	Serum vitamin D (nmol/L)[b]	Dietary vitamin D intake (mcg/day)[b]	Sun-exposure time (min/wk)[b]
Poverty income ratio							
≤1.85	448 (41.9)	32.5	107.7 (96.0-120.4)	6.8 (4.7-12.5)	55.9 (43.9-68.4)	5.0 (2.2-11.3)	496 (163-1331)
>1.85 to ≤ 3.50	308 (28.8)	28.2	103.0 (89.8-116.3)	6.0 (4.2-10.8)	63.6 (49.1-78.3)	5.7 (2.4-13.6)	586 (235-1076)
>3.50	313 (29.3)	39.3	96.8 (82.9-109.3)	6.0 (4.2-9.8)	71.5 (53.9-85.9)	7.5 (3.1-20.5)	452 (230-951)
Physical activity							
Inactive or moderate	563 (52.7)	51.6	103.1 (91.7-114.9)	7.0 (4.8-13.2)	60.9 (46.4-77.7)	5.6 (2.7-13.5)	367 (115-807)
Vigorous	506 (47.3)	48.4	102.2 (87.8-115.8)	5.7 (4.0-9.1)	65.0 (50.5-81.5)	6.4 (2.8-17.4)	715 (350-1341)
Current CKD[c]							
No	959 (89.7)	89.9	102.7 (90.6-115.8)	5.9 (4.2-8.8)	63.4 (48.8-78.8)	6.0 (2.7-15.6)	510 (230-1067)
Yes	110 (10.3)	10.1	103.0 (64.7-114.2)	52.5 (33.1-114.5)	61.6 (44.3-84.6)	5.3 (2.4-13.2)	448 (188-893)
Serum vitamin D status							
Deficiency	354 (33.1)	27.7	107.5 (96.3-119.7)	6.6 (4.3-12.1)	38.7 (30.0-45.2)	3.7 (1.9-7.2)	416 (158-950)
Insufficiency	429 (40.1)	39.3	104.2 (92.5-117.6)	5.9 (4.1-9.2)	61.4 (56.0-67.0)	5.7 (2.7-13.8)	516 (223-1096)
Sufficiency	286 (26.8)	33.1	96.5 (80.7-107.4)	7.0 (4.9-10.8)	87.7 (79.5-103.6)	11.9 (4.7-27.0)	527 (246-1050)
Dietary vitamin D intake							
< 15 mcg/day	810 (75.8)	73.9	104.0 (92.3-116.5)	6.4 (4.2-11.1)	58.9 (44.5-72.2)	4.1 (2.0-7.2)	540 (231-1173)
≥ 15 mcg/day	259 (24.2)	26.1	98.8 (82.8-112.5)	6.1 (4.6-10.3)	75.8 (60.3-94.7)	25.8 (20.0-45.0)	405 (205-836)

[a] Percent values were weighted to account for the complex survey design.
[b] Median (25th–75th percentile).
[c] Those with albumin-to-creatinine ratio (ACR) above 30 mg/g or estimated glomerular filtration rate (eGFR) below 60 mL/min/1.73 m^2 were defined as patients with chronic kidney disease (CKD).

5.3.2. Correlation among vitamin D-related measures

Correlation among the vitamin D-related measures, i.e., serum vitamin D level, sun-exposure time, and dietary vitamin D intake, were evaluated in the study population (Table 5-3). Serum vitamin D level was positively correlated with dietary vitamin D intake (ρ = 0.147 - 0.347). Serum vitamin D level also showed a significant positive correlation with sun-exposure time though the correlation coefficient was relatively small (ρ = 0.081). Sun-exposure time and dietary vitamin D intake showed a weak negative correlation (ρ = −0.032 - −0.050), but it was not statistically significant (p = 0.068 - 0.103).

Table 5-3. Correlations among vitamin D-related measures in the general US population.

	NHANES 2009-2014 (n = 3207)		NHANES 2013-2014 (n = 1069)	
	Sun-exposure time	**Dietary vitamin D intake**	**Sun-exposure time**	**Dietary vitamin D intake**
Serum vitamin D	0.081 (<0.001)	0.147 (<0.001)	0.081 (0.008)	0.347 (<0.001)
Sun-exposure time		-0.032 (0.068)	-	-0.050 (0.103)

Data represent Spearman's correlation coefficients ρ and their p values in the brackets.

Serum vitamin D concentrations by sun-exposure time and vitamin D intake level were shown in Table 5-4. Group 1 (reference group, sun-exposure time < medians and vitamin D intake < 15 mcg/day) showed the lowest serum vitamin D concentration followed by group 2 (sun-exposure time ≥ medians and vitamin D intake < 15 mcg/day), group 3 (sun-exposure time < medians and vitamin D intake ≥ 15 mcg/day), and group 4 (sun-exposure time ≥ medians and vitamin D intake ≥ 15 mcg/day). Group 3 and 4 showed similar serum vitamin D levels.

Table 5-4. Serum vitamin D concentration (nmol/L) by sun-exposure time and vitamin D intake level.

	N	Average	Percentile		
			25th	50th	75th
NHANES 2009-2014 (n = 3207)					
Group 1	1228	58.7	42.9	57.2	72.1
Group 2	1318	63.9	48.6	62.3	77.5
Group 3	351	83.4	64.7	78.6	101.2
Group 4	310	83.1	65.7	80.7	97.0
NHANES 2013-2014 (n = 1069)					
Group 1	391	57.3	42.6	55.9	67.9
Group 2	419	62.6	46.2	63.6	76.1
Group 3	149	79.7	58.6	80.5	94.7
Group 4	110	82.1	61.1	72.8	94.4

Group 1: sun-exposure time < median and vitamin D intake < 15 mcg/day; group 2: sun-exposure time ≥ median and vitamin D intake < 15 mcg/day; group 3: sun-exposure time < median and vitamin D intake ≥ 15 mcg/day; group 4: sun-exposure time ≥ median and vitamin D intake ≥ 15 mcg/day.

5.3.3. Association between chemical concentrations and CKD parameters

In the populations for the study of **Chapter 5**, we observed significant association between urinary chemical concentrations and CKD parameters (Table 5-5) which is similar with the results of **Chapter 3 and 4**. Among the target compounds in NHANES 2009-2014 population, metabolites of monobutyl phthalate (MBP), monobenzyl phthalate (MBzP), di-(2-ethylhexyl) phthalate (DEHP) and BPA were negatively associated with eGFR. monoethyl phthalate (MEP) and monoisobutyl phthalate (MiBP) were positively associated with ACR. Among the target compounds in NHANES 2013-2014, BDCIPP, BCEP, and DBNP were negatively associated with eGFR, and DBNP was positively associated with ACR.

Table 5-5. Association of urinary chemical concentration with estimated glomerular filtration rate (eGFR, mL/min/1.73 m^2) or urinary albumin-to-creatinine ratio (ACR, mg/g) in the general US population (Single-pollutant model).

Compound	eGFR		log(ACR)	
	β (95% CI)	*p*-value	β (95% CI)	*p*-value
NHANES 2009-2014 (n = 3207)				
MEP	0.32 (-0.29, 0.93)	0.298	0.05 (0.01, 0.08)	**0.007**
MBP	-0.92 (-1.40, -0.43)	**<0.001**	0.02 (-0.01, 0.06)	0.217
MiBP	-0.68 (-1.45, 0.09)	0.081	0.05 (0.00, 0.11)	**0.049**
MBzP	-1.21 (-1.87, -0.55)	**<0.001**	0.03 (-0.02, 0.07)	0.248
MCPP	-0.75 (-1.51, 0.01)	0.053	0.00 (-0.04, 0.03)	0.758
MECPP	-0.89 (-1.65, -0.14)	**0.022**	0.02 (-0.03, 0.06)	0.504
MEHHP	-1.00 (-1.70, -0.30)	**0.006**	0.01 (-0.03, 0.06)	0.606
MEOHP	-1.32 (-2.12, -0.52)	**0.002**	0.01 (-0.03, 0.06)	0.564
MCiOP	-0.34 (-1.08, 0.40)	0.362	0.01 (-0.02, 0.04)	0.575
MCiNP	0.08 (-0.93, 1.09)	0.872	0.00 (-0.04, 0.05)	0.989
BP-3	-0.30 (-0.66, 0.06)	0.099	-0.02 (-0.04, 0.00)	0.088
BPA	-1.20 (-1.83, -0.57)	**<0.001**	0.03 (-0.03, 0.08)	0.356
MePr	-0.38 (-0.90, 0.13)	0.142	0.01 (-0.01, 0.03)	0.285
PrPr	-0.16 (-0.54, 0.21)	0.388	0.00 (-0.02, 0.02)	0.856
NHANES 2013-2014 (n = 1069)				
DPHP	-0.93 (-2.20, 0.34)	0.139	0.03 (-0.04, 0.10)	0.386
BDCIPP	-1.51 (-2.94, -0.10)	**0.038**	0.06 (-0.01, 0.12)	0.082
BCEP	-1.41 (-2.66, -0.16)	**0.030**	0.07 (-0.01, 0.15)	0.065
DNBP	-1.55 (-3.09, -0.01)	**0.048**	0.10 (0.01, 0.17)	**0.037**

The association was adjusted for sex, age, race/ethnicity, NHANES cycle, poverty income ratio, smoking history, physical activity, and BMI. Concentrations of OPE metabolites were adjusted by the covariate-adjusted standardization method.

Chemicals which were significantly associated with CKD parameters in the single-pollutant models were assessed in multi-pollutant models (Table 5-6). In the multi-pollutant models, association between BPA and eGFR remained significant ($p = 0.031$), and the association between MBzP and eGFR was marginally significant ($p = 0.089$). For ACR, the association of MiBP disappeared while significance of MEP remained marginally significant ($p = 0.091$). In the multi-pollutant model of OPEs, all the associations of BDCIPP, BCEP, and DNBP with eGFR became insignificant possibly due to their high correlation (data now shown).

Table 5-6. Association of urinary chemical concentration with estimated glomerular filtration rate (eGFR, mL/min/1.73 m^2) or urinary albumin-to-creatinine ratio (ACR, mg/g) in the general US population (Multi-pollutant model, NHANES 2009-2014).

Compound	eGFR		log(ACR)	
	β (95% CI)	***p*-value**	**β (95% CI)**	***p*-value**
MEP	-	-	0.03 (0.00, 0.06)	0.091
MBP	0.25 (-0.55, 1.05)	0.533	-	-
MiBP	-	-	-0.02 (-0.06, 0.03)	0.453
MBzP	-0.64 (-1.39, 0.10)	0.089	-	-
$DEHP_{sum}$	-0.13 (-1.00, 0.74)	0.763	-	-
BPA	-0.63 (-1.20, -0.06)	0.031	-	-

The association was adjusted for sex, age, race/ethnicity, NHANES cycle, poverty income ratio, smoking history, physical activity, and BMI. Concentrations of OPE metabolites were adjusted by the covariate-adjusted standardization method.

5.3.4. Effect modification by serum vitamin D concentration

For the chemicals showing significant associations with CKD parameters, the association were evaluated in stratification by serum vitamin D status (Fig. 5-3). The associations of BDCIPP, BCEP, or DNBP with eGFR were strongest in the vitamin D deficiency group but were decreased in the insufficiency and sufficiency groups. The associations of MEP and DNBP with ACR were also attenuated by vitamin D status. However, there was no trend in the association of MBzP and BPA with eGFR by the vitamin D status.

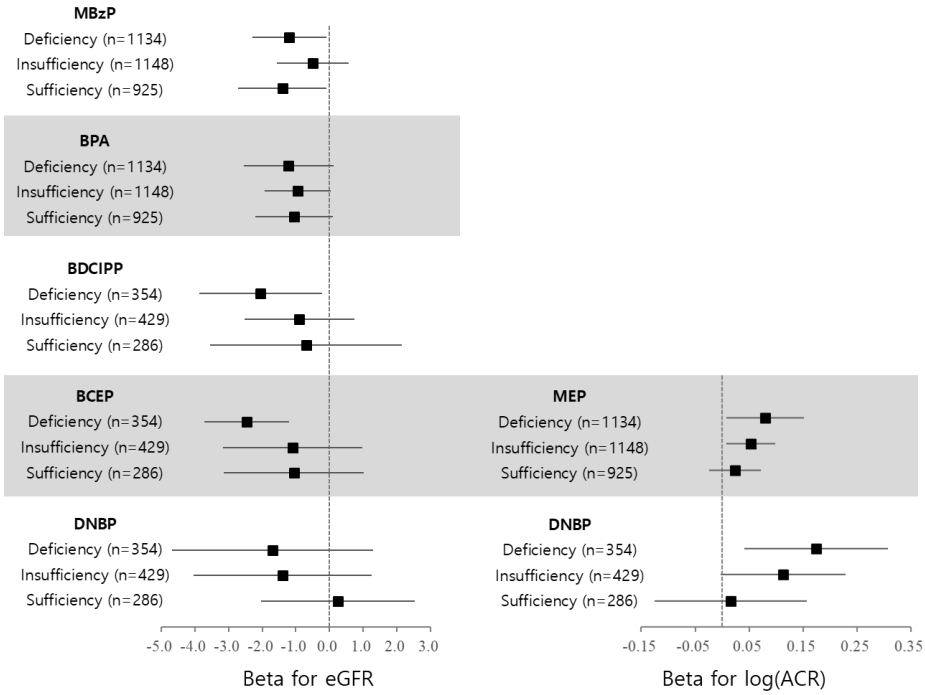

Fig. 5-3. Association of urinary chemical concentrations with estimated glomerular filtration rate (mL/min/1.73 m^2) and urinary albumin-to-creatinine ratio (mg/g) by serum vitamin D status (deficiency, insufficiency, and sufficiency) in the general US population. The association was adjusted for sex, age, race/ethnicity, NHANES cycle, poverty income ratio, smoking history, physical activity, and BMI. Concentrations of OPE metabolites were adjusted by the covariate-adjusted standardization method.

5.3.6. Effect modification by sun-exposure time and dietary vitamin D intake

The association between OPE metabolites and CKD parameters were examined in the stratification by factors affecting serum vitamin D level, i.e., sun-exposure time and dietary vitamin D intake (Fig. 5-4). Group 2 (high sun-exposure time), 3 (high vitamin D intake), and 4 (inner joint of group 2 and 3) showed weaker association of BDCIPP with eGFR than that of group 1 (low sun-exposure time and low vitamin D intake). The association of BCEP with eGFR was also weaker in group 4 than in group 1. Group 3 showed lower association between BCEP and eGFR, but this pattern was not observed for group 4. For the associations of MEP and DNBP with ACR, group 4 showed the highest association in the both cases.

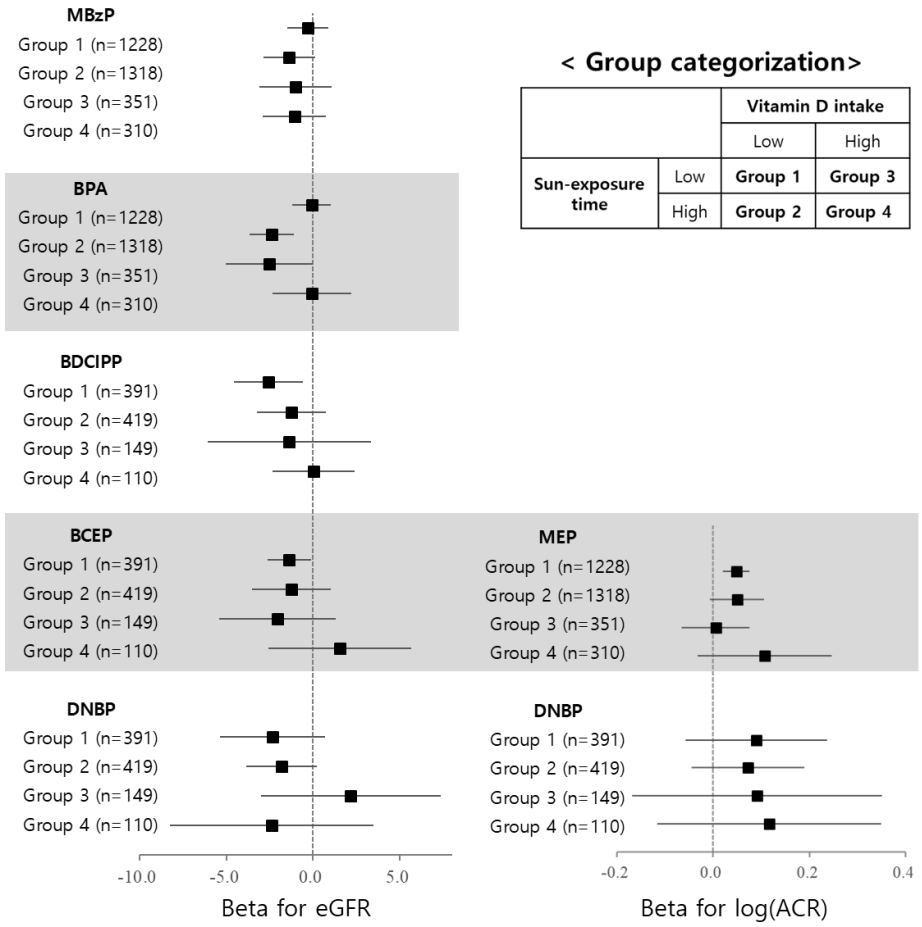

Fig. 5-4. Association of urinary chemical concentrations with estimated glomerular filtration rate (mL/min/1.73 m^2) or urinary albumin-to-creatinine ratio (mg/g) by sun-exposure time and vitamin D intake level in the general US population. The association was adjusted for sex, age, race/ethnicity, NHANES cycle, poverty income ratio, smoking history, physical activity, and BMI. Concentrations of OPE metabolites were adjusted by the covariate-adjusted standardization method. Group 1: sun-exposure time < median and vitamin D intake < 15 mcg/day; group 2: sun-exposure time ≥ median and vitamin D intake < 15 mcg/day; group 3: sun-exposure time < median and vitamin D intake ≥ 15 mcg/day; group 4: sun-exposure time ≥ median and vitamin D intake ≥ 15 mcg/day.

5.4 Discussion

We observed that association between urinary chemicals and eGFR can be modified by vitamin D status (Fig. 5-3), implying possible roles of vitamin D in the chemical exposure-related etiology of CKD. Personal efforts to reduce chemical exposure such as frequent house cleaning or hand washing (Gibson et al., 2019; Sears et al., 2020), use of chemical-free products (Dembsey et al., 2019; Stubbings et al., 2018), and use of appropriate protective equipment (Lee et al., 2018) may mitigate chemical exposure-related risks. Given ubiquitous exposure to consumer chemicals, however, identification of effect modification factors to attenuate the risk by exposure to these chemicals can provide another strategy to prevent CKD in parallel with reduction of exposure to the chemicals.

Inflammation, oxidative stress, and fibrogenesis have been suggested as mechanisms of renoprotective effects of vitamin D (Kim and Kim, 2014; Li, 2010). Treatments of vitamin D or its analogs ameliorated kidney effects in previous studies with chemical-induced kidney disease animal models (He et al., 2011; Park et al., 2010a; 2010b; Xiao et al. 2009), and suppression of inflammation or fibrosis was suggested as the underlying mechanisms (Park et al., 2010a; 2010b). The effect modification by vitamin D on association of OPE metabolites can be understood based on the mechanism of renoprotective effects of vitamin D. An *in vitro* study showed TDCIPP-induced cytotoxicity in renal proximal tubule cells were attenuated by antioxidant treatment (Killiea et al., 2017), supporting that antioxidant capacity of vitamin D could be an underlying mechanism of its effect modification on the association of OPE metabolites with eGFR or ACR observed in the present study. Moreover, inflammation was suggested as one of the mechanisms of hepatotoxicity of TDCIPP (Liu et al., 2016), implying the effect modification by vitamin D could be mediated by its anti-inflammatory effects.

Among the association of phthalate metabolites and BPA with CKD parameters, only the association between MEP and ACR was attenuated by

monotonous trend of serum vitamin D status (Fig. 5-3). There has been no experimental study on MEP-related nephrotoxicity so far. However, considering structurally similarity between the parent compounds of MEP and MBP, i.e., diethyl phthalate (DEP) and DBP, effects of antioxidant on DBP-induced nephrotoxicity observed in a previous mouse experiment (Cheng et al., 2019) give a clue to our observation on MEP. Observations that association of MBzP or BPA with eGFR were not modified by vitamin D status are not clearly understood considering that their potential of oxidative stress or inflammation observed in human populations (Ferguson et al., 2011; 2015; 2016; Yang et al., 2009). Further studies are needed to test possible hypothesis explaining our observations (e.g., nephrotoxic mechanism of MBzP and BPA other than oxidative stress or inflammation).

The observation of the analysis with serum vitamin D status (Fig. 5-3) was partly replicated by the secondary analysis with groups stratified by combination of sun-exposure time and vitamin D intake (Fig. 5-4). For example, effect modification by serum vitamin D status on the association of BDCIPP or BCEP was in line with that observed in the secondary analysis. However, effect modification was not obvious for DNBP and MEP in the secondary analysis. These less obvious results of the secondary analysis could be explained by reliability of the measurement of vitamin D status. Generally, serum vitamin D [25(OH)D2 and 25(OH)D3] level is considered as a reliable clinical measure for vitamin D status (Seamans and Cashman, 2009). On the other hand, sources of vitamin D, i.e., amount of UVB exposure and dietary intake of vitamin D, were measured in a less reliable manner. Sun-exposure time measured in this study was based on questionnaire survey and could not quantitatively consider the participants' behaviors to protect UV exposure (e.g., use of sunscreen, hat, or long-sleeved clothing) and their regional factors (e.g., UV index or sunshine time). Dietary vitamin D intake was surveyed based on the participants' recall in short periods of time (24 hr for foods and beverages and 30 days for supplements), which may cause recall bias and may not represent long-term vitamin D intake. These measurements errors could

be a reason why we observed less obvious results with the measures for vitamin D sources compared to those with serum vitamin D level.

This study has several limitations. First, as mentioned above, amounts of UVB exposure and dietary vitamin D intake were measured less reliably, which may result in measurement errors. Measurement error can be also expected in the concentrations of OPE metabolites considering their short biological half-lives. If the errors are random, however, introduction of the measurements errors leads the estimate toward null value. Therefore, the possible measurements errors do not make our results unconvincing. Second, because of cross-sectional nature of this study, reverse causality should be noticed. CKD status can affect participants' behaviors related to vitamin D supplementation or sunlight exposure, which consequently leads to serum vitamin D status. Therefore, our observation warrants confirmation in prospective studies.

5.5 Summary and implications

Associations between urinary chemical and CKD parameters, i.e., eGFR and ACR, were modified by serum vitamin D status, and this observation was partly replicated in a secondary analysis with sun-exposure time and dietary vitamin D intake. Underlying mechanisms should be investigated in experimental studies, and the hypothesis generated in this study warrants further confirmation in other populations.

Chapter 6. Conclusion

Through the series of observational studies, we could identify potential chemical risk factors for chronic kidney disease among the various consumer chemicals. The results in these studies should be understood based on the limitations of the study designs. Basically, the studies were cross-sectional observational studies, which impose potential confounding and reverse causality. Although we applied an improved method for urinary creatinine-adjustment, we could not verify its accuracy due to lack of gold standard in urine dilution (e.g., 24-hr urine collection). All the chemical measurements were single measurement, which can have high variability because of their short-biological half-lives. Here are main findings of the studies.

6.1 Urinary metabolites of several low-molecular-weight phthalates were identified as risk factors for CKD

In this study, urinary levels of metabolites of low-molecular-weight phthalates, e.g., monobutyl phthalate (MBP) and mono-benzyl phthalate (MBzP), were repeatedly associated with chronic kidney disease. Among the Korean women, MBP showed consistent strong association with albuminuria across the various statistical conditions such as consideration of multiple exposure, urine dilution methods, and subpopulation analysis. MBzP also showed significant association with albuminuria in the multi-pollutant model (**Chapter 2**). In the US NHANES population, the association of MBP and MBzP with albuminuria was observed again, and these compounds were also associated with eGFR even in the multi-pollutant model suggesting their potential adverse effects on kidney (**Chapter 3**). In the **Chapter 5**, MEP was associated with ACR in a reduced population due to additional consideration of vitamin D-related variables. The association of MEP with ACR was attenuated with serum vitamin D status.

6.2 DEHP exposure was not a major risk factor for CKD in our study population

Significant association of DEHP exposure with kidney disease markers were reported previously; in this study, however, association of DEHP exposure with kidney disease appeared to be insignificant with the consideration multiple exposure of the chemicals sharing common exposure sources. In the Korean women population, the association between DEHP metabolites and albuminuria was only significant in the single-pollutant models suggesting possible confounding by other phthalates such as MBP and MBzP (**Chapter 2**). In the US NHANES population, the significant association between DEHP metabolites and eGFR observed in the single-pollutant models also disappeared in the multi-pollutant model (**Chapter 3**). Overall, the associations of DEHP exposure with kidney disease markers observed previously may be artificial results due to confounding by co-exposure to other phthalates, which was not controlled in the previous studies. However, given experimental evidences suggesting nephrotoxicity of DEHP (Li et al., 2018; Wu et al., 2018), further studies are needed to understand the role of DEHP in the etiology of chronic kidney disease.

6.3 Exposure to benzophenone-3 may be linked to CKD, but further studies on its biomarker are needed

Benzophenone-3 (BP-3) is used as UV-filters in sunscreen products. Since BP-3 can be metabolized into benzophenone-1 (BP-1), and both BP-1 and -3 are excreted into urine after the exposure to BP-3, urinary levels of these two chemicals are considered as biomarkers of BP-3 exposure. Among the Korean women, urinary BP-1 showed consistent strong association with albuminuria across the different statistical conditions while the association of BP-3 with

albuminuria was relatively low (**Chapter 2**). We postulated that urinary BP-1 is better biomarker for BP-3 exposure than urinary BP-3, so urinary BP-3 does not clearly represent BP-3 exposure. Urinary BP-3 was associated with neither ACR nor eGFR among the US adults while we could not assess this association for urinary BP-1 due to lack of measurement (**Chapter 3**). Considering BP-1 itself is also used in consumer products and there are knowledge gaps regarding exposure to and metabolism of BP-1 and -3, more sophisticated designs to distinguish between BP-1 and BP-3 exposure are warranted.

6.4 Bisphenol A was associated with eGFR not with ACR

There have been controversial observations on the association between BPA exposure and kidney disease previously. In the present studies, BPA was associated with eGFR (**Chapter 3** and **5**) while not associated with ACR (**Chapter 2**, **3**, and **5**). The association between BPA and eGFR was not attenuated by serum vitamin D status (**Chapter 5**), which suggests that underlying mechanism of BPA effects on CKD may not be related with oxidative stress or inflammation. This hypothesis should be confirmed by experimental studies or other human studies.

6.5 Several OPEs were associated with CKD, which was attenuated by vitamin D status

Urinary metabolites of OPEs, i.e., BDCIPP, BCEP, and DNBP, were associated with CKD as well as ACR and eGFR (**Chapter 4**). Some of these associations were attenuated by vitamin D status (**Chapter 5**) suggesting vitamin D treatment as a measure to prevent OPE-related kidney disease. As various OPEs have been used in consumer products, biomonitoring of OPEs has been extended to wider range of OPE compounds in recent studies

(Bastiaensen et al., 2019; Li et al., 2020). Since information on human exposure to OPEs and their effects on kidney disease is not yet sufficient, additional biomonitoring studies on OPEs and observational studies on OPEs and CKD with extended OPE compounds are warranted.

6.6 A novel urine dilution adjustment demonstrated significance of considering influence of GFR on urinary creatinine excretion

Since urinary creatinine level is influenced by glomerular filtration rate, traditional creatinine-based methods for urine dilution adjustment was improved using the covariate-adjusted standardization method. With this novel method, we could observe more clear association between urinary chemicals and chronic kidney disease (Chapter 3 and 4). Although we could not verify this accuracy using a gold standard, we demonstrated the usefulness of this novel adjustment method based on the observational results and previous knowledge on causal pathway of kidney disease associated with chemical exposure. Wider applications of this method are expected in the field of environmental epidemiology on kidney outcome.

6.7 Concluding remarks

Prevalence of CKD is increasing, imposing a personal and social burden. Considering the importance of CKD in public health and wide-spread human exposure to synthetic chemicals, follow-up studies investigating chemical risk factors for kidney disease are warranted. The finding in this study will provide basic knowledge for chemical management, and the methodologies applied in this study will help conduct future studies.

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국문 초록(Abstract in Korean)

생활화학물질 노출과 신장 기능 지표: 일반인구집단에서의 관찰 연구

서울대학교 보건대학원
환경보건학과 환경보건학 전공
강 하 병

만성신장질환(CKD)는 전세계적으로 급증하고 있는 질환으로 최근 환경오염물질의 노출과의 연관성이 보고되고 있다. 프탈레이트 가소제나 비스페놀 A(BPA)와 같은 생활제품에 사용되는 물질들이 신장질환과 연관될 수 있다는 증거들이 역학연구와 실험연구를 통해 보고되고 있다. 하지만 이러한 연구들은 일부 물질들에만 국한되어 있고, 함께 생활제품을 통해 노출될 수 있는 기타 환경성페놀류나 유기인계 에스터화합물(OPEs)에 대한 연구는 전무한 상황이다. 이 연구에서는 생활제품 사용을 통해 함께 노출될 수 있는 여러 화학물질과 신장질환 간의 연관성을 일련의 인구집단 연구를 통해 확인하였다.

첫번째 연구는 신장질환의 지표인 뇨중 알부민-크레아티닌 비율(ACR)과 연관성을 가지는 화학물질을 확인하기 위하여 20-45 세의 한국 여성 441 명을 모집하였다. 이들 여성들에게서 소변과 혈액 시료를 수집하고 설문조사를 수행하였다. 연구 참여자들에게서 측정된 변수들을 이용하여 크레아티닌으로 보정된 소변 중 화학물질과 ACR 간의 연관성을 선형회귀모형으로 확인하였다(단일물질 모형). 또한 단일물질 모형에서 유의한 연관성이 나타난 물질들을 하나의 선형회귀모형에 독립변수로 포함시켜 다중물질 모형을 구성하였다. 단일물질 모형에서 여러 화학물질이 ACR 과의 유의한 연관성을 보였다. 그러나 유의한 연관성을 보인 물질들을 포함한 다중물질 모형에서는 모노뷰틸 프탈레이트(MBP)와 모노이소뷰틸 프탈레이트(MiBP), 모노벤질 프탈레이트(MBzP), 벤조페논-1(BP-1)가 ACR 과의 유의한 연관성을 보였다. 디뷰틸

프탈레이트(DBP)와 벤조페논-3(BP-3)의 대사체인 MBP 와 BP-1 은 신장이 건강한 참여자들로 제한한 분석과 비중 보정을 사용한 분석에서도 비슷하게 나타나 MBP 와 BP-1 이 뇨중 ACR 의 증가의 중요한 위험인자로 제안된다.

두번째 연구에서는 미국 국민건강영양조사(NHANES) 2005-2016 년 자료를 활용하여 첫번째 연구에서 확인된 화학물질과 신장질환 지표 간의 연관성을 미국 일반인구 집단에서 재확인하였다. 총 9008 명의 성인을 대상으로 소변 중 화학물질과 CKD 및 관련 지표들 간의 연관성을 확인하였다. 신기능 변화로 인한 편향(bias)을 제거하기 위하여 일반적인 크레아티닌 보정법과 함께 신기능이 반영된 개선된 크레아티닌 보정법을 적용하여 소변 묽기를 보정하였다. 또한 다중물질 모형에서도 연관성을 확인하였다. 통계분석 결과, 일반적인 크레아티닌 보정법을 적용하였을 때에 일부 화학물질이 eGFR 과 양의 연관성을 보인 반면에, 개선된 방법을 적용하였을 때에는 대부분의 물질이 eGFR 과 음의 연관성을 보여 크레아티닌 보정 방법이 중요함을 확인하였다. 크레아티닌 보정 방법과 무관하게 MBP 가 ACR 과 양의 연관성을 보였고 MBzP 는 ACR 과 약하게 양의 연관성을 보여서 첫번째 연구에서 관찰한 결과를 재현하였다. 다중물질 모형에서 MBP, MBzP, BPA 가 eGFR 과 양의 연관성을 보였고, 이 물질들 중에서 MBP 가 CKD 와의 연관성이 가장 크게 나타나 CKD 의 중요한 위험인자임이 재확인되었다.

세번째 연구에서는 OPEs 의 노출과 CKD 간의 연관성을 미국일반인구 집단(NHANES 2013-2014)을 이용하여 확인하였다. 총 1578 명의 성인을 대상으로 소변 중 OPE 대사체와 CKD 및 관련 지표들 간의 연관성을 확인하였다. 두번째 연구와 동일하게 일반적인 크레아티닌 보정법과 개선된 크레아티닌 보정법을 적용하였다. 통계모형 분석 결과, 새로운 크레아티닌 보정이 적용된 뇨중 bis(2-chloroethyl) phosphate(BCEP)의 농도는 추정사구체여과율(eGFR)과 음의 연관성을 보였고 뇨중 bis(1,3-dichloro-2-propyl) phosphate(BDCIPP)과 di-n-butyl phosphate(DNBP)는 소변 묽기 보정방법에 무관하게 ACR 과 양의 연관성을 보였다. CKD 와의 연관성을 로지스틱회귀분석으로 확인하였을 때 위의 세 가지 물질은 모두 유의한 연관성을 보였다. 이러한 연관성은 새로운 크레아티닌 보정 방법을 적용하였을 때 더 크게 나타났다.

다중물질 모형을 구성하였을 때에도 세가지 물질에 대해 나타난 연관성이 재현되었다.

네번째 연구에서는 세번째 연구에서 관찰된 화학물질 노출과 신장질환 간의 연관성에 비타민 D 가 영향을 줄 수 있는지 확인하는 분석을 수행하였다. NHANES 2009-2015(n=3207)와 2013-2014(n = 1069) 인구를 대상으로 혈청 중 비타민 D 수준이 소변 중 화학물질과 CKD 간의 연관성에서 효과변경인자로 작용할 수 있는 지 확인하였다. 혈청 중 비타민 D 가 증가할 수록 OPE 대사체(BDCIPP, BCEP, DNBP)와 eGFR 간의 연관성은 줄어들었다. 또한 MEP 또는 DNBP 와 ACR 간의 연관성도 비타민 D 가 증가할수록 감소하였다. 햇빛 노출 시간과 비타민 D 섭취량을 이용하여 층화한 분석에서도 BDCIPP 또는 BCEP 와 eGFR 간의 연관성이 대조 집단(낮은 햇빛 노출 시간 및 낮은 비타민 D 섭취량)에 비해 비타민 D 가 높을 것으로 기대되는 집단(높은 햇빛 노출 시간 및 높은 비타민 D 섭취량)에서 낮게 관찰되었지만 다른 연관성들에 대해서는 효과변경이 명확하지 않았다.

이 연구에서는 일련의 인구집단 관찰을 통하여, 신장질환의 위험인자로 작용할 수 있는 화학물질들을 확인하였다. 다중노출 모형을 이용하여 생활제품 사용을 통해 노출될 수 있는 여러 화학물질 중 MBP, MBzP, BP-1, BPA 가 신장질환과의 연관성에서 중요할 수 있음을 제시하였고, 일부 OPE 물질들이 CKD 와 연관될 수 있음을 확인하였다. 이러한 화학물질 노출과 CKD 와의 연관성에서 비타민 D 수준이 효과변경인자로 작용할 수 있음을 확인하였다. 이 연구에서 확인한 결과들은 화학물질 관리에 도움이 되는 기초자료로 활용될 수 있으며, 연구에서 사용한 방법론들은 향후 관련 연구에서 적용될 수 있을 것이다.

주요어: 만성신장질환; 신기능; 크레아티닌; 알부민뇨; 사구체여과율; 프탈레이트; 환경성페놀류; 유기인계 에스터화합물; 비타민 D

학번: 2015-30658

감사의 글

서른 즈음에, 학교를 떠나며.

제가 박사학위를 받는 2020 년 8 월은 만으로 서른살이 되는 달입니다. 스무살인 2009 년에 서울대학교에 입학한 뒤 20 대를 줄곧 서울대학교에서 학생신분으로 보냈고, 생각이 많아지는 서른 즈음에 학교를 떠나게 되었습니다. 너무 오래 머물면서 지겹기도 했고 빨리 떠나고 싶은 마음이 점점 커졌지만, '추억 보정'일까요? 막상 떠날 시간이 다가오니 나가는 발걸음이 쉽게 떨어지지 않을 것 같습니다. 지난 11 년 반 동안에 서울대학교에서 배우고 얻은 것의 상당 부분이 이 논문에 녹아 있다고 생각합니다. 그 과정에서 여러 좋은 인연들을 만나 도움을 많이 받았습니다. 한 분 한 분을 떠올려보면 이런 분들과 함께할 수 있었던 것은 큰 행운이라 생각합니다. 여러 도움을 주신 분들께 이 지면을 빌어서 감사의 말씀을 드리려고 합니다.

먼저 학위 과정 동안 지도교수로서 학술적인 지도뿐만 아니라 인생을 살아가는 데에 있어서도 조언을 아끼지 않으신 최경호 교수님께 감사의 말씀을 드립니다. 학부생 인턴부터 석사와 박사과정까지 교수님의 지도와 영향을 받아 매 단계 성장할 수 있었습니다. 논문의 심사위원을 흔쾌히 맡아 주시고 심사과정에서 적극적인 조언을 해 주신 백도명 교수님, 김성균 교수님, 이정표 교수님, 문효방 교수님께도 감사의 말씀을 드립니다. 학교 안밖에서의 교육과 연구 환경은 융합 학문을 공부하는 데에 최적의 조건이었습니다. 위의 다섯 분의 교수님을 포함한 보건대학원 및 공동연구진 교수님들로부터 환경보건에 접근하는 다양한 관점과 방법론을 배울 수 있었습니다. 다양한 배경을 가진 동료들과 함께 공부한 경험과 그 인연 자체도 큰 자산이 될 거라 생각합니다. 이러한 환경을 함께 만들어 주신 여러 교수님들과 연구진들께 감사드립니다.

저희가 하는 연구는 연구자들의 노력으로만 이루어지지 않습니다. 연구가 진행될 수 있게 뒷받침하는 시스템이 있습니다. 아침에 기분 좋게 인사해주시는 경비원님부터, 학생들이 오기 전에 새벽부터 건물을 정리해주시는 청소원 아주머님, 문의사항에 항상 밝게 답해주시는 조교님, 그리고 어려운 문제를 해결해주시는 행정선생님들까지 모두가 연구를 뒷받침하는 시스템입니다. 8 년 반 동안 보건대학원 건물에서 겪었던

문제들을 떠올려 보면, 문제를 해결하는 지점에 항상 이러한 분들이 계셨습니다. 새벽에 문이 고장나서 연구실에 갇혔다가 경비원님의 도움으로 탈출했던 기억도 생각나고 주말에 초저온냉동고 온도에 이상이 생겨 조교님과 급히 통화했던 기억도 떠올리게 됩니다. 이 건물에서의 추억들을 떠올릴 때면 이러한 분들도 추억과 함께 기억되며 그리워질 것 같습니다.

십년이 넘는 기간 동안 이해가 되지 않는 이상한 전공을 공부한 아들을 뒷바라지 해 주신 부모님께도 감사의 말씀을 드립니다. 앞으로는 좀 더 이해할 수 있는 연구 결과를 보여드릴 수 있으면 좋겠습니다. 그리고 석박사 학위 기간 내내 여자친구이자 지금은 아내로서 저의 모든 부분을 지지해준 세령이에게 앞으로의 길도 지지해달라는 미안한 부탁과 함께 지금까지의 고마움을 전합니다.

마지막으로 금전적인 지원에 대한 감사를 빼놓을 수 없습니다. 학위 과정 동안 수행한 대부분의 연구는 세금을 바탕으로 이루어졌습니다. 또한 공부를 하는 과정에서 몇몇 장학재단의 도움을 받을 수 있었습니다. 연구비에 내포된 공공성과 장학금을 지원해주신 분들의 마음을 잊지 않겠습니다.

학부에서 자연과학을 배웠던 제가 보건대학원에서 응용학문을 공부하게 되면서 여러 관점에서의 생각이 변하게 되었습니다. 그 중 하나가 과학의 가치중립성입니다. 흔히 과학은 가치중립적이어야 한다는 이야기를 합니다. 그러나 보건학은 연구자의 가치가 개입될 수밖에 없는 과학이라는 것이 제 생각입니다. 보건학은 사회 구성원의 보편적인 건강이라는 가치를 추구하는 학문이고, 시스템 측면에서 건강을 증진시키는 것이 중요하다는 가치판단을 기본으로 합니다. 보건학 연구는 연구자의 가치관이 투영되기 쉽고 사회 구성원의 판단에 직접 영향을 미칠 수 있기 때문에 연구를 계획하고 수행할 때에는 그릇되지 않은 가치관을 가지는 것이 중요합니다. 그러면서도 보건학은 연구 결과를 바탕으로 진리를 탐구하는 과학의 성격을 갖고 있습니다. 선한 가치관을 바탕으로 보건학 연구를 수행하면서 과학의 진리에 다가갈 때에는 가치중립적으로 결론을 내리는 보건학자가 될 수 있도록 노력하겠습니다.