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Biomarkers of arsenic, cadmium, lead, and mercury for human exposure assessment

중금속 4종 인체노출평가에서의 바이오마커 선택과 활용

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Abstract

Biomarkers of arsenic, cadmium, lead, and mercury for human exposure assessment

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Heavy metals and metalloids (referred to as metals) are naturally existing substances and widely distributed in the environment such as soil, water, and air. Among them, selenium, copper, zinc, manganese, etc. are essential elements for maintaining body homeostasis. However, cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As) are highly toxic substances and can cause adverse health outcomes. In addition, exposure sources and pathways are diverse due to their ubiquitous characteristic and they can be exposed as a mixture in daily life.

Biomonitoring can be an important tool to investigate internal exposure. Therefore, national biomonitoring surveys of many countries have measured metals, including Cd, Pb, Hg, and As in urine and/or blood. These data can be utilized in exposure assessment and epidemiological studies. In Korea, the Korean National Environmental Health Survey (KoNEHS) includes urinary Cd, blood Pb, urinary Hg,

and blood Hg measurements to investigate metal exposure in general populations. Although the exposure levels of Cd, Pb, and Hg in the general populations of Korea were gradually decreasing, they were still considerably high compared to other countries. Meanwhile, As was removed from the measurement after KoNEHS I (2009-2011) despite its importance, making it difficult to determine the temporal trend of exposure level and compare it to other populations. Moreover, studies on As exposure biomarkers are scarce in comparison to those on Cd, Pb, and Hg, so studies on biomarkers of As in the general Korean population are required. Consequently, a biomonitoring study focused on As was conducted in this dissertation to fill knowledge gaps in current metal biomonitoring. Furthermore, exposure assessment and epidemiological studies were conducted separately as case studies on how to utilize biomonitoring data.

In the first study, As speciation was performed in urine and blood to reveal the levels of As species and to suggest the proper exposure biomarker of iAs. Samples were collected by the Korean Ministry of Food and Drug Safety (MFDS) from 2017 to 2018. Six As species (i.e., arsenate (As(V)), arsenite (As(III)), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB), arsenocholine (AsC)) were analyzed using ultra-high performance liquid chromatography-inductively coupled plasma mass spectrometry (UPLC-ICP/MS). Concentrations and distribution of As species in the different biological media and related characteristics of the population were investigated. AsB, a non-toxic As compound, was the predominant species in both urine (51.1%) and blood (53.5%). The distributions of inorganic As (iAs) and its metabolites (i.e., MMA, DMA) were significantly different in urine and blood – the proportion of iAs in blood (20.2%)

was higher than in urine (2.18%). Drinking water type and consumption of multigrain rice were associated with increased iAs concentration in urine. Consumption of blue-backed fish was associated with increased AsB concentration in both urine and blood. Methylation efficiency of iAs was higher in females, whereas it decreases in adolescents and smokers. Overall, a high total As level in urine of the Korean population was affected by organic As from seafood, indicating that As speciation is essential for As biomonitoring. In addition, urinary As had a significant association with exposure sources rather than blood As species, suggesting that urine is a more appropriate media in terms of exposure assessment.

In the second study, the biomonitoring-based exposure assessment was conducted using urinary Cd, blood Pb, blood Hg, and urinary iAs measured from the samples from MFDS. Concentrations of Cd, Pb, and Hg were analyzed using inductively coupled plasma mass spectrometry (ICP/MS) and iAs was analyzed using UPLC-ICP/MS. Reverse dosimetry using physiologically-based toxicokinetic (PBTK) modeling was conducted for the estimation. Then, a probabilistic scenario-based exposure assessment was performed to predict exposures to Cd, Pb, Hg, and As from multiple sources and routes (EDI_{SCN}), and compared with biomonitoring-based estimates (EDI_{PBTK}). $EDIs_{PBTK}$ (median) were found to be 0.21-0.72 μg/kg/day for Cd, 0.50-0.97 μg/kg/day for Pb, 0.023-0.053 μg/kg/day for MeHg, and 0.09-3.51 μg/kg/day for iAs. $EDIs_{PBTK}$ of Cd were similar to $EDIs_{SCN}$ in adults and young children, but 1.7-2.8 times higher in the other age groups. EDI_{PBTK} for Pb was 2.3-6.1 times higher in all age groups. In the case of MeHg and iAs, direct comparison between the two methods was not possible because total Hg and total As were targeted in the scenario-based exposure assessment. When two different approaches

were compared, some similarities and differences arise, implying the importance of understanding the strengths and limitations of each approach. For reverse dosimetry, the representativeness of the exposure biomarker, the characteristics of the PBTK model (e.g., model compartments, parameters, and exposure routes), and the assumption of oral exposure might cause estimation uncertainty. Meanwhile, a lack of exposure data (e.g., exposure algorithms, factors, and media concentration) can lead to uncertainties in scenario-based exposure model, implying the need for continuous monitoring of exposure sources and factors. In further studies, the factors that cause these uncertainties need to be improved.

In the third study, the effect of the metal mixture (Cd, Pb, Hg, and iAs) on blood pressure (BP) was investigated in the general population of Korea, including children and adolescents. Both urine and blood samples collected by MFDS were used for the study. For the analysis, several statistical methods were used, including conventional linear regression and novel mixture modeling approaches – Bayesian kernel machine regression (BKMR) and weighted quantile sum (WQS) regression. Populations were divided into adults (≥ 19 yrs) and non-adults (< 19 yrs) for the analysis. In linear regression analysis, UPb and UCd levels were associated with increased DBP in non-adults ($p < 0.040$ and $p < 0.023$, respectively), whereas adults had no association between the metal mixture and BP. In both BKMR and WQS analysis, the overall effect of the metal mixture on BP was significant in non-adults but was less evident in adults. In addition, Pb was the most contributing metal (WQS index = 34.2%) in the urinary mixture, followed by Cd (29.6%), iAs (28.2%), and Hg (8.0%). This study suggests that the non-adults might be more susceptible to the cardiovascular-related health effects of metals than adults, despite the lower

exposure level. In addition, urinary Pb was found to be associated with elevated BP.

Overall, this thesis performed the biomonitoring of four metals in both urine and blood and utilized the data in exposure assessment of metals and epidemiological study. Three studies suggested insights into what / where to measure and how to interpret data in terms of biomonitoring of metals. In future research, it is needed to confirm the results of the present study. Additionally, since the present studies did not focus on the mechanisms of metals in humans, detailed studies on the early biologic effects of metal co-exposure need to be performed.

Keywords: metals, biomonitoring, exposure assessment, physiologically-based toxicokinetic (PBTK) model, combined exposure, blood pressure

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Chapter 1. Introduction

1.1. Toxicity and exposure sources of metals

Heavy metals and metalloids (referred to as metals) are well-known environmental pollutants that have been a global public health problem from the past to the present. Some metals, such as zinc, iron, selenium, manganese, etc. are essential elements that need to maintain the proper physiological functions of humans, however, metals including cadmium (Cd), lead (Pb), mercury (Hg), and arsenic (As) are known as highly toxic to the human body. These toxic metals can cause various health problems, such as cardiovascular, renal, gastrointestinal, musculoskeletal, immunological, reproductive, and hepatic effects (ATSDR, 2007, 2012, 2020, 2021) and also act as endocrine disruptors at the low-levels of exposure (Iavicoli et al., 2009). In particular, inorganic As (iAs) is a known human carcinogen causing skin, urinary bladder, and lung cancer (IARC, 2004; Straif et al., 2009) and impacts populations living in the highly-contaminated region of iAs, such as Bangladesh, Mexico, United States (U.S.), Taiwan (Naujokas et al., 2013). Cd is also classified as a human carcinogen causing lung, prostate, and kidney cancer (IARC, 1993; Waalkes, 2003). Pb and Hg are known to inhibit growth, cognitive function, and neurodevelopment in children (Al-Saleh et al., 2020; ATSDR, 2020; Dórea, 2019; Naranjo et al., 2020). In the case of Hg and As, their toxicities differed depending on their compounds. Methyl Hg (MeHg) is the most important Hg compound due to its high toxicity. Among the As compounds, arsenate (As(III)) has been known to have the highest toxicity (Milstein et al., 2003), but more recent studies suggested that

trivalent methylated As (i.e., monomethylarsonous acid (MMA(III)) and dimethylarsinous acid (DMA(III))) may be more toxic (Styblo et al., 2000; Styblo et al., 2021).

Metals are natural components and widely distributed in the environment, such as soil, air, and water. They are utilized in industry, living environment, agriculture, and medical fields, however, once released into the environment, they can be exposed to the human body via bioaccumulation due to their long half-lives in the environment and living organisms. People can be exposed to metals from various sources, such as foods, dust, soil, air, water, cigarettes, consumer products, and so on. For Cd, people can be exposed to contaminated food and cigarette smoking. Smoking is known as an important exposure route to Cd and it roughly doubles Cd body burden in comparison to not smoking (ATSDR, 2012). For Pb, inhalation is the dominant route in occupational exposure, whereas general populations can be exposed to contaminated food, drinking water, dust, and soil. In Korea, in order of agricultural products (41%), processed foods (33%), seafood (24%), and animal products (2%) contributed to Pb exposure (MFDS, 2016b). For Hg, people are exposed to Hg through food, especially through the consumption of fish and seafood which is the major source of MeHg (Abass et al., 2018). Another source of Hg exposure is dental amalgam, containing elemental Hg. For As, drinking water and contaminated food are the dominant sources of exposure. In detail, populations can be exposed to iAs by drinking water and agricultural products (e.g., grains) from the iAs-contaminated region. The most contributing source of As exposure in Koreans is seafood, which contains organic As compounds (Schmeisser et al., 2006; Taylor et al., 2017).

1.2. Biomonitoring of metals in the general population

1.2.1. National biomonitoring studies in Korea and other countries

Biomonitoring is an important tool to estimate the internal exposure of humans which represents aggregated exposure from multiple pathways and sources. Metals are measured in various biological media, such as urine, blood (i.e., whole blood, plasma, serum, and erythrocyte), hair, nail, toenail, saliva, breastmilk, placenta, bone, teeth, and feces. Among them, urine and blood (whole blood) are widely used media in biomonitoring studies. The half-lives of metals differ from biological media based on toxicokinetic (TK) characteristics of metals (Table 1-1).

Table 1-1. Half-lives of metals in urine and blood

Metal	Half-life	
	Urine	Blood
Cd	> 10 years	100 days
Pb	Unknown[a]	1 month
Hg	1-3 months	1-3 weeks (inorganic), 50 days (MeHg)
As	2-4 days (iAs and its metabolites)	4-6 hours

[a] 1-1.5 month in soft tissue, 25-30 years in bone.

Cd is measured in both urine and blood, where urinary Cd (UCd) reflects long-term exposure and blood Cd (BCd) reflects relatively recent exposure (Suwazono et al., 2009; Vacchi-Suzzi et al., 2016). Shimbo et al. (2000) found that UCd and BCd are highly correlated with each other in the samples of Japanese women, which implies both of them can be employed as biomarkers of Cd exposure.

For Pb, blood Pb (BPb) is known as a good biomarker of exposure (Sommar et al., 2014). Urinary Pb (UPb) is an alternative for biomonitoring of Pb exposure, but toxicokinetic (TK) information is insufficient (Barbosa et al., 2005) and its reproducibility is still unclear (Sallsten et al., 2022; Sommar et al., 2014).

Hg is also measured in both urine and blood and used in biomonitoring studies. Urinary Hg (UHg) is known to include inorganic Hg (iHg) because MeHg slightly excretes in urine (Hong et al., 2012). In blood, 70% of total Hg (BHg) composes of MeHg (Jung et al., 2013a). Therefore, for Hg, speciation is performed in the blood to differentiate the exposure to toxic compounds (i.e., MeHg).

As is mostly measured in urine and the concentration of total As or As species is used as an exposure biomarker. For measuring total As and its species in blood, several studies suggested it as an alternative biomarker of As (Bommarito et al., 2019a; Hall et al., 2006). Bommarito et al. (2019a) proposed As species in plasma might be a good biomarker in terms of the internal dose of the target organ of iAs because the distribution of As species in plasma was different from that of urine but similar to that of urothelial cells.

Several national biomonitoring studies including the U.S. National Health and Nutrition Examination Survey (NHANES), the Canadian Health Measures Survey (CHMS), and the German Environmental Survey (GerES) measures Cd, Pb,

Hg, and As in urine and/or blood. NHANES includes Cd (urine, blood), Pb (urine, blood), total Hg (urine, blood), Hg species (blood), total As (urine), and As species (urine) (CDC, 2021). CHMS includes Cd (urine, blood), Pb (blood), total Hg (blood), Hg species (blood), and As species (urine) (Health Canada, 2019). GerES measures Cd, Pb, Hg, and total As in urine (Schmied et al., 2021). In Korea, the Korea National Health and Nutrition Examination Survey (KNHANES) measures Cd, Pb, and Hg in blood, and the Korean National Environmental Health Survey (KoNEHS) includes Cd (urine), Pb (blood), and Hg (urine, blood) measurements. Urinary total As was measured in the KoNEHS I (2009-2011) but it was excluded from KoNEHS II (2012-2014). According to the KoNEHS III (2015-2017) report, Koreans' exposure levels to metals had a slightly decreasing trend from KoNEHS I (2009-2011). However, they still had considerably higher metal levels than other countries. The median for UCd was 0.422 μg/L, which was much higher than the results from the NHANES 2015-2016 (0.179 μg/L) and CHMS 2016-2017 (0.15 μg/L). The median for BPb was 1.60 μg/dL and approximately two times higher than the populations of the U.S. (0.880 μg/dL) and Canada (0.92 μg/dL). The median for UHg (0.339 μg/L) was higher than the U.S. population (0.140 μg/L) and BHg (2.71 μg/L) was also higher than U.S. populations (0.740 μg/L) and Canadians (0.70 μg/L).

Table 1-2. Concentrations of urinary metals in national biomonitoring surveys (μg/L)

Country		Korea						United States								Canada	
Acronym		KoNEHS I	KoNEHS II	KoNEHS III				NHANES								CHMS	
Years		2009-2011	2012-2014	2015-2017				2015-2016				2017-2018				2016-2017	2018-2019
Population		19+ y	19+ y	3-6 y	7-12 y	13-18 y	19+ y	3-5 y	6-11 y	12-19 y	20+ y	3-5 y	6-11 y	12-19 y	20+ y	3-79 y	3-79 y
As (total)[a]	p50	41.7	-	-	-	-	-	8.27	6.09	3.94	5.62	8.19	6.25	3.79	5.72	-	-
	p95	121.9	-	-	-	-	-	40.9	28.1	22.3	56.2	42.4	27.0	33.9	59.7	-	-
As(V)	p50	-	-	-	-	-	-	<LOD[b]	<LOD[b]	<LOD[b]	<LOD[b]	<LOD[b]	<LOD[b]	<LOD[b]	<LOD[b]	<LOD[c]	<LOD[c]
	p95	-	-	-	-	-	-	0.820	<LOD[b]	<LOD[b]	<LOD[b]	<LOD[b]	1.05	0.840	0.870	0.23	0.15
As(III)	p50	-	-	-	-	-	-	<LOD[d]	0.160	0.320	<LOD[d]	<LOD[d]	<LOD[d]	<LOD[d]	<LOD[d]	0.36	0.42
	p95	-	-	-	-	-	-	1.10	1.00	1.35	1.11	1.10	1.01	1.12	1.00	3.5	3.6
MMA	p50	-	-	-	-	-	-	<LOD[e]	0.230	0.340	0.400	<LOD[e]	<LOD[e]	<LOD[e]	<LOD[e]	0.40	0.41
	p95	-	-	-	-	-	-	1.20	1.27	1.51	1.55	1.47	1.32	1.63	1.48	1.7	1.5
DMA	p50	-	-	-	-	-	-	2.62	2.98	2.65	2.95	2.61	2.91	2.73	2.96	3.1	3.5
	p95	-	-	-	-	-	-	10.5	11.3	9.88	12.0	10.8	9.13	10.2	12.0	15	21
AsB	p50	-	-	-	-	-	-	<LOD[f]	<LOD[f]	<LOD[f]	<LOD[f]	<LOD[f]	<LOD[f]	<LOD[f]	<LOD[f]	1.3[*]	1.2[*]
	p95	-	-	-	-	-	-	10.1	13.0	13.1	33.9	12.4	11.6	21.7	48.2	56[j]	63[j]
AsC	p50	-	-	-	-	-	-	<LOD[g]	<LOD[g]	<LOD[g]	<LOD[g]	<LOD[g]	<LOD[g]	<LOD[g]	<LOD[g]	-	-
	p95	-	-	-	-	-	-	0.410	0.380	0.430	0.440	<LOD[g]	<LOD[g]	<LOD[g]	0.330	-	-
Cd	p50	0.570	0.393	0.091	0.231	0.297	0.422	<LOD[h]	<LOD[h]	0.049	0.179	<LOD[h]	<LOD[h]	0.062	0.184	0.16	1.4
	p95	2.39	1.36	0.430	0.735	0.951	1.75	0.078	0.134	0.248	1.08	0.096	0.148	0.206	0.919	1.4	1.1
Hg (total)	p50	0.540	0.400	0.396	0.373	0.421	0.339	<LOD[i]	<LOD[i]	<LOD[i]	0.140	<LOD[i]	<LOD[i]	<LOD[i]	<LOD[i]	-	-
	p95	1.67	1.27	1.30	1.09	1.39	1.42	0.280	0.520	0.610	1.22	0.390	0.570	0.700	1.16	-	-
Pb	p50	-	-	-	-	-	-	0.23	0.25	0.20	0.32	-	-	-	-	-	-
	p95	-	-	-	-	-	-	1.20	0.870	0.730	1.38	-	-	-	-	-	-

[*] Sum of arsenocholine and arsenobetaine, [a] Creatinine corrected concentration (μg/g_{crea}), [b] 0.79 μg/L, [c] 0.14 μg/L, [d] 0.12 μg/L, [e] 0.2 μg/L, [f] 1.16 μg/L, [g] 0.11 μg/L, [h] 0.036 μg/L, [i] 0.13 μg/L

Table 1-3. Concentrations of blood metals in national biomonitoring surveys (μg/L)

Country		Korea						United States								Canada	
Acronym		KoNEHS I	KoNEHS II	KoNEHS III		KNHANES*		NHANES								CHMS	
Years		2009-2011	2012-2014	2015-2017		2016		2015-2016				2017-2018				2016-2017	2018-2019
Population		19+ y	19+ y	13-18 y	19+ y	10-19 y	19+ y	1-5 y	6-11 y	12-19 y	20+ y	1-5 y	6-11 y	12-19 y	20+ y	3-79 y	3-79 y
Cd	p50	-	-	-	-	0.83	0.93	<LOD[b]	0.100	0.130	0.270	<LOD[b]	0.100	0.140	0.270	0.21	0.21
	p95	-	-	-	-	-	-	0.160	0.200	0.330	1.35	0.190	0.220	0.360	1.44	2.8	1.7
Hg (total)	p50	3.05	3.05	1.35	2.71	3.19	3.43	<LOD[c]	0.310	0.340	0.740	<LOD[c]	<LOD[c]	0.310	0.730	0.65	0.77
	p95	9.90	9.05	3.02	8.81	-	-	1.06	1.33	1.89	4.66	0.960	1.71	1.71	4.36	3.7	3.8
iHg	p50	-	-	-	-	-	-	<LOD[d]	<LOD[d]	<LOD[d]	<LOD[d]	<LOD[e]	<LOD[e]	<LOD[e]	<LOD[e]	<LOD[f]	<LOD[f]
	p95	-	-	-	-	-	-	0.270	0.330	0.400	0.500	<LODd	0.280	0.280	0.500	<LOD[f]	<LOD[f]
MeHg	p50	-	-	-	-	-	-	<LOD[g]	0.140	0.160	0.530	<LOD[h]	<LOD[h]	<LOD[h]	0.500	0.23	0.30
	p95	-	-	-	-	-	-	0.830	1.11	1.81	4.42	0.790	1.40	1.52	3.89	1.9	2.6
Pb[a]	p50	1.84	1.97	0.834	1.60	1.67	1.76	0.69	0.550	0.450	0.880	0.620	0.460	0.390	0.850	0.88	0.78
	p95	3.90	4.09	1.52	3.36	-	-	2.76	1.59	1.17	2.89	2.02	1.19	1.09	2.62	2.4	2.3

* Geometric mean, [a] Unit: μg/dL, [b] 0.1 μg/L, [c] 0.28 μg/L, [d] 0.27 μg/L, [e] 0.21 μg/L, [f] 0.22 μg/L, [g] 0.12 μg/L, [h] 0.26 μg/L

1.2.2. Biomonitoring of arsenic and its species

As is chemically classified as a metalloid which has both properties of metal and non-metal. Among As compounds, exposure to iAs has become a public health concern due to its high toxicity. Drinking water is a major route of iAs exposure in some areas where iAs is contained within the earth crust and contaminated groundwater. Organic As compounds (oAs), such as DMA, AsB, AsC, arsenosugars, and arsenolipids are known to be mainly exposed by seafood consumption.

Once absorbed in the body, iAs undergo sequential reduction and methylation reactions to form MMA and DMA (Figure 1-1) which has been considered to be a detoxification mechanism (ATSDR, 2007). Most of iAs and its methylated metabolites are excreted in the urine and DMA is the predominant form among them. In the case of oAs, they are rapidly absorbed and excreted in the urine within a few days (Brown et al., 1990). Furthermore, arsenosugars and arsenolipids can be metabolized into DMA, which is the metabolite of iAs (Schmeisser et al., 2006; Taylor et al., 2017). Accordingly, DMA is known as an inappropriate biomarker of iAs exposure in populations with a high seafood intake (Molin et al., 2012). Given their short half-life and predominant excretion route, many biomonitoring studies have measured As species in urine as biomarkers of recent exposure to As species (ATSDR, 2007).

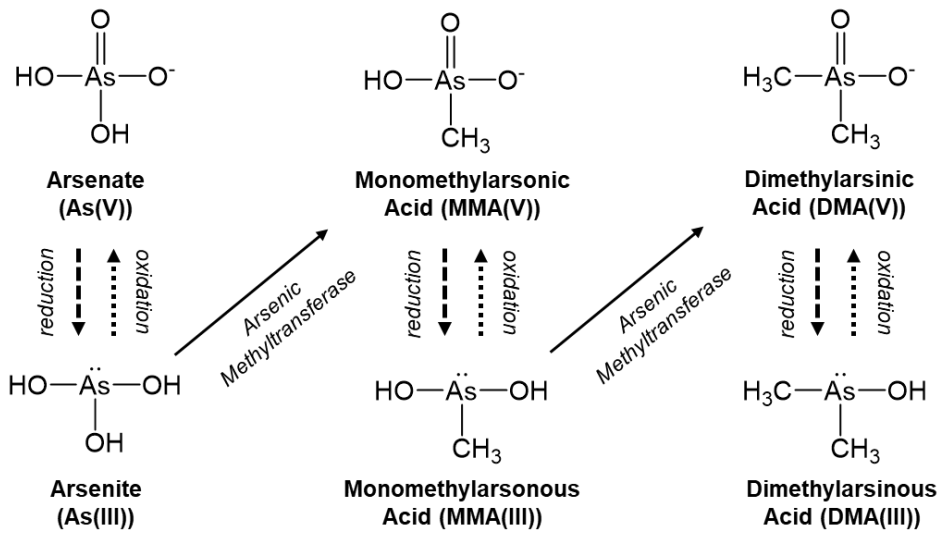

Figure 1-1. Metabolism pathway for the inorganic As and its methylated metabolites.

Meanwhile, recent studies suggested that blood is an alternative media of iAs biomarker. Compared with urine, obtaining blood samples is more difficult and samples require greater care in handling and disposal (Marchiset-Ferlay et al., 2012) and the analytes in the blood are detected at lower levels than in urine. However, blood As metabolites could reflect the internal dose of As and may provide insight into metabolic differences that lead to disparities in susceptibility to health effects (Hall et al., 2006). Bommarito et al. (2019a) found that the distribution of As species in blood could represent those in target organs (Currier et al., 2014). Furthermore, blood does not require dilution adjustments such as creatinine, specific gravity, and osmolality correction, which can induce collider bias in multivariate analysis. However, few studies have utilized urine-blood pairs of samples, where blood markers were compared to urinary ones. Earlier studies were conducted in iAs-contaminated areas, and study subjects were highly exposed to iAs via drinking water (Abuawad et al., 2021; Bommarito et al., 2019a; Hall et al., 2006; Hall et al.,

2007; Mandal et al., 2007). A Japanese study reported the use of blood-urine pairs in the population exposed to low-level iAs, but As speciation was not studied in the blood (Takayama et al., 2021).

Despite the importance of analyzing As species, many Korean studies have used total As in urine (Bae et al., 2017; Kim and Lee, 2011; Lee et al., 2012; Park et al., 2016; Park and Lee, 2013) and some of them found that seafood as the main exposure source (Choi et al., 2010; Khan et al., 2015; Kim et al., 2020). Recent studies measured urinary As species and observed that urinary As mostly consists of DMA and/or AsB (Chung et al., 2016; Hong et al., 2017; Lee et al., 2022; Seo and Hong, 2021) but they targeted adults or residents near mining areas.

1.3. Dose reconstruction of metals

Due to the high concerns about exposure to metals, many regulatory agencies in the world set safe exposure limits or reference values (e.g., reference dose (RfD) of Environmental Protection Agency (EPA) and provisional tolerable intake values of Joint FAO/WHO Expert Committee on Food Additives (JECFA)) (Wong et al., 2022). These values are based on the prevention of non-cancer health effects. For example, the RfD of iAs was set to 0.3 μg/kg/day, which was based on the end point of hyperpigmentation, keratosis, and possible vascular complications of the population exposed to iAs via contaminated well water (Tseng, 1977; Tseng et al., 1968). To identify the risk of metals from the population, intake amounts can be estimated and compared with the reference values. However, metals can be exposed to the general population through multiple exposure routes and sources, and uncertainty may arise from the lack of exposure scenarios, exposure concentrations, and exposure factors.

For that reason, biomonitoring can be utilized for health-based risk assessment because it can reflect internal aggregated doses (Meslin et al., 2022). However, the interpretation of biomarker concentration to assess human health risk is a challenging task because the values cannot directly compare with existing reference values. In this case, converting to external dose from internal dose using TK modeling can be a possible option. For metals, two-compartment TK modeling and physiologically based toxicokinetic (PBTK) modeling can be utilized to reconstruct doses from the biomonitoring data. Both TK and PBTK models represent the whole body as a series of compartments and reflect the absorption, distribution, metabolism, and excretion (ADME) of substances. The two-compartment model

divides the body into two boxes – central and peripheral compartments – with absorption and excretion rate constants. The central compartment consists of the blood and tissue where the rapid distribution of the substance is achieved and the peripheral compartment consists of tissues where the distribution of the substances is slower.

Unlike TK model, the compartments constituting the PBTK model represent the actual organ and tissue, with a unique set of physiological (i.e., tissue volume, blood flow) and physicochemical parameters (i.e., partition coefficients) (EPA, 2006). The structure of a PBTK model depends on the purpose of developing the model and the intention of the modeler (EPA, 2006). Various TK and PBTK models have been developed for Cd, Pb, iHg, MeHg, and iAs (Table 1-4). The complexity of the model structures varies depending on the purpose of the model and most of them are validated using human TK datasets.

Table 1-4. Toxicokinetic models of Cd, Pb, Hg, and As for humans

Metal	Reference
Arsenic (inorganic)	Dede et al. (2018)[a]
	El-Masri and Kenyon (2008)
	(2008); Mann et al. (1996)
Cadmium	Amzal et al. (2009)
	Choudhury et al. (2001)
	Dede et al. (2018)[a]
	Diamond et al. (2003)
	Nordberg and Kjellström (1979)
Lead	Dede et al. (2018)[a]
	EPA (1994)[b]
	Leggett (1993)
	O'Flaherty (1993, 1995, 2000)
	White et al. (1998)
Mercury (inorganic)	Farris et al. (2008)
Mercury (organic)	Carrier et al. (2001a)
	Clewell et al. (1999)
	Smith et al. (1994)

[a] Simplified model of El-Masri and Kenyon (2008), Nordberg and Kjellström (1979), and O'Flaherty (1993), [b] Integrated Exposure Uptake BioKinetic (IEUBK) Model for Lead in Children.

These models were used for the evaluation of potential doses in the target tissue or biomarker levels under various exposure conditions (Chen et al., 2020; Fierens et al., 2016; Leconte et al., 2021; Pouillot et al., 2022; Ruiz et al., 2010; Van Holderbeke et al., 2016). However, limited studies were conducted for investigating the exposure to metals using PBTK models (Abass et al., 2018; Lee et al., 2017b; Lin et al., 2020; Satarug et al., 2013). To our knowledge, only one study (Lee et al., 2017b) was performed for estimating MeHg exposure based on biomonitoring in Koreans.

1.4. Effects of metal mixtures on health outcomes

Metals including Cd, Pb, Hg, and As are identified as highly toxic and widely distributed in the environment. Since environmental media have naturally occurring mixtures of metals, humans are spontaneously exposed to metals as mixtures. In metal mixture, some of them can act additively when they are present together, others can act independently of each other, and others are antagonistic or synergetic (EPA, 2007). Some studies have indicated evidence that metals interact to cause health effects that are different from exposure to each metal alone (Claus Henn et al., 2014). Therefore, it is necessary to understand the potential joint effect of multiple metals on various health outcomes (Yim et al., 2022).

Cd, Pb, Hg, and As are known to be associated with cardiovascular disease (CVDs) in common. The mechanisms linking the exposure to those metals and CVDs were suggested in various studies, which have been known to include oxidative stress, endothelial dysfunction with impaired nitric oxide (NO) system, inflammation, alteration of cellular ion transporter, and dysregulation of the hormonal system (Boskabady et al., 2018; da Cunha Martins et al., 2018; Houston, 2011; Vaziri, 2008). Several epidemiological studies reported the association between exposure to the metal mixture and CVD-related health outcomes, such as high blood pressure, hypertension, preeclampsia, stroke, coronary heart disease, myocardial infarction, etc. (Bommarito et al., 2019b; Cabral et al., 2021; Castiello et al., 2020; Desai et al., 2021; Kim and Park, 2022; Kupsco et al., 2019; Qu et al., 2022; Wen et al., 2019). Among them, elevated blood pressure is an important modifiable risk factor and the biggest contributor to CVDs. Several studies have

reported the potential effect of the metal mixture on health outcomes including elevated blood pressure (Bulka et al., 2019; Castiello et al., 2020; Howe et al., 2021; Kim and Park, 2022; Kupsco et al., 2019; Liu et al., 2021; Qu et al., 2022; Shih et al., 2021; Wang et al., 2021).

However, studies on the association between metal exposure in non-adult populations and their BP are still limited. Elevated BP during childhood and adolescence is a precursor of hypertension and CVDs in adulthood. Some previous studies have focused on prenatal exposure of children in mother-children pairs. But relatively few studies have examined the effect of metal exposure in childhood and adolescence on blood pressure (Castiello et al., 2020; Desai et al., 2021; Yao et al., 2020). Desai et al. (2021); Yao et al. (2020) investigated the association between metal mixture (Cd, Pb, and Hg) and BP in same-aged groups using NHANES 2007-2016 data. They used Pb, Cd, and Hg in both urine and blood as exposure biomarkers. Each metal had a different association with BP, and even the same metal had inconsistent results depending on which biological media was measured. For example, BPb showed a significant association with DBP whereas UPb had no significant association with any BP measurements. Desai et al. (2021) also analyzed the association between low-level exposure to metals (Cd, Pb, Hg, and As) and BP in children and adolescents (aged 8-17 years old) using NHANES 2009-2016 data. They used urinary Cd, total As, blood Pb, and total Hg as exposure biomarkers. Metal mixture showed an inverse association with diastolic BP (DBP), but not systolic BP (SBP) and pulse pressure (PP).

Recently, several novel statistical methods, such as Bayesian kernel machine regression (BKMR), weighted quantile sum (WQS) regression, and quantile-based

g-computation were developed for analyzing the association between exposure and health outcomes (Bobb et al., 2015; Carrico et al., 2015; Keil et al., 2020). For the general population of Korea, Kim and Park (2022) investigated the association between co-exposure to metals (Cd, Pb, and Hg) in adults. They found a significant positive association between the metal mixture and blood pressure and an increase in the risk of hypertension as BPb concentration increases. But more studies are needed in more diverse populations including more substances.

1.5. Study rationale and objectives

Toxic metals, including Cd, Pb, Hg, and As, have been of interest to researchers for a long time and their concentrations have been measured in various media such as environments, foods, products, and biological samples. For this reason, their exposure and health effects are thought to be well-known; however, some areas still need to be investigated as mentioned in previous sections.

First, biomonitoring data for As species and information on proper exposure biomarkers of iAs are still limited, whereas biomarkers of Cd, Pb, and Hg have been well studied and continuously measured in the Korean national biomonitoring survey. In addition, exposure assessments of metals are mainly performed through conventional approach – scenario-based exposure model. Meanwhile, exposure assessment using biomonitoring is limited although it can reflect integrated internal exposure. In addition, many studies have already investigated the association between metal exposure and health outcomes, but studies on the association between combined exposure to metals and health outcome are relatively limited.

Accordingly, this study consisted of three detailed studies – one is a biomonitoring study that filled the knowledge gap in biomonitoring data focusing on As speciation, and the other two are case studies on the application of biomonitoring (exposure assessment and epidemiological studies). Detailed descriptions of each content are below:

In the first study (Chapter 2), As speciation analysis was performed using urine and blood samples from the general population of Korea. The characteristics

of urine and blood were compared as conventional media and alternative media, respectively, and sociodemographic/characteristics related to the concentrations of As species and methylation efficiency of iAs were also investigated. The objectives of this study were to reveal the levels of As species in the study population and suggest the appropriate media and biomarker for iAs exposure assessment in the low-exposure population for iAs.

In the second study (Chapter 3), biomonitoring-based exposure assessment was performed to determine exposure amounts of four metals (i.e., Cd, Pb, MeHg, and iAs) among the general population of Korea. Reverse dosimetry approach was applied to convert internal dose to external exposure amount using PBTK model. Then, the results were compared with the estimates obtained from scenario-based exposure assessment, the conventional method. The objectives of this study were to discuss the applicability and/or utility of reverse dosimetry compared to the conventional method and areas for improvement of each approach.

In the third study (Chapter 4), the association between combined exposure to metals and blood pressure was investigated in Korean adults and non-adults. Several statistical methods, including multiple linear regression modeling and two novel statistical methods (i.e., BKMR and WQS regression), were used for the analysis. In addition to the association between metal co-exposure and BP, this study also aimed to explore the joint effect and interaction of the metal mixture on BP.

The overview of the study design is shown in Figure 1-2.

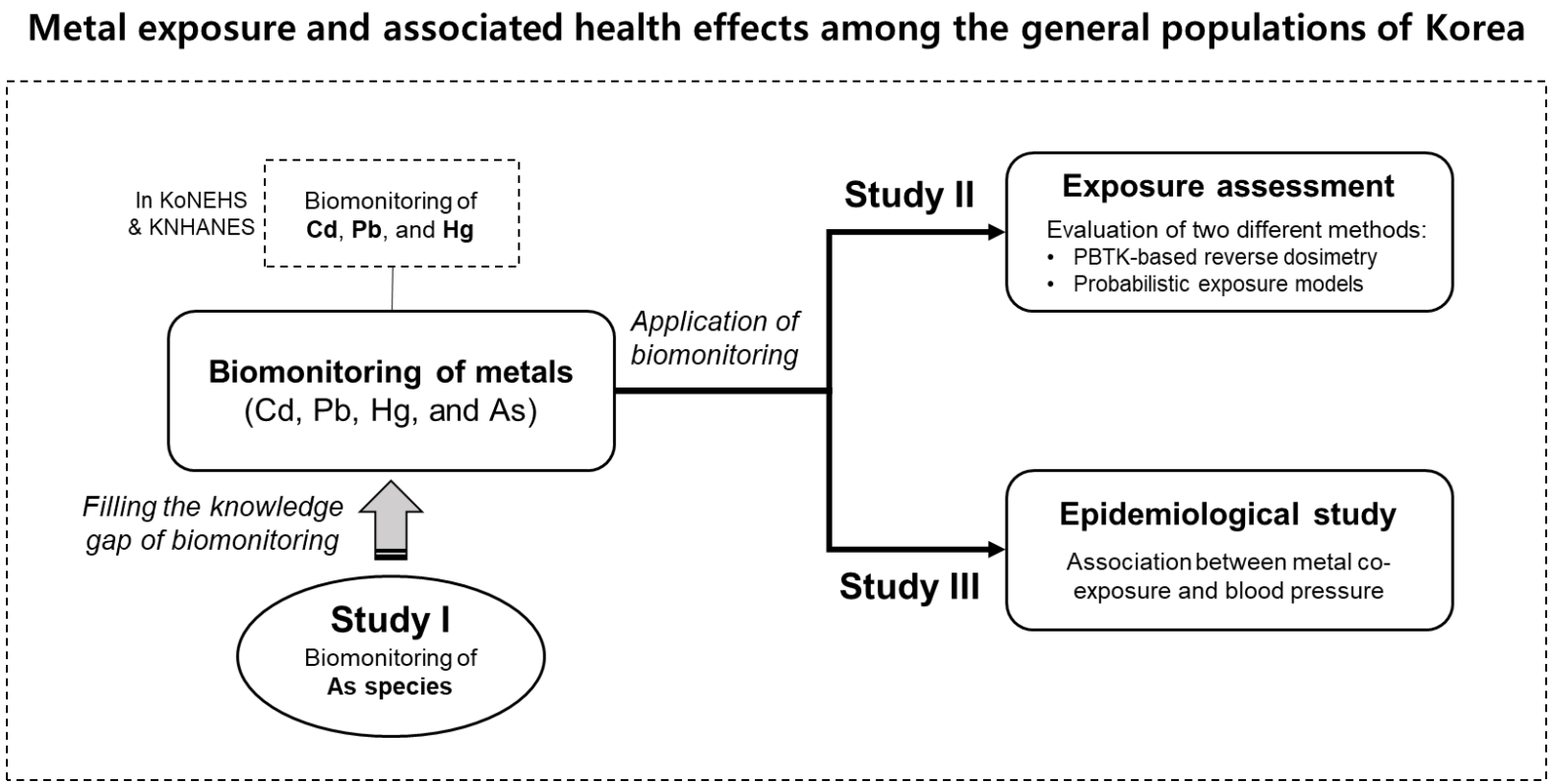

Figure 1-2. Overview of the study design.

Chapter 2. Concentrations and characteristics of urinary and blood metals among the general population of Korea: Biomonitoring of arsenic species

2.1. Introduction

Arsenic (As) is a ubiquitous metalloid in the environment. Populations can be exposed to various sources such as groundwater, air, soil, dust, food, consumer products, and pesticides (ATSDR, 2007; Hughes et al., 2011). Especially, exposure to high levels of inorganic As (iAs) via drinking water is a global problem in several regions. Exposure to iAs and its methylated species (*e.g.*, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)) can cause adverse health effects in various organs in the nervous, respiratory, cardiovascular, immune, endocrine, hepatic, and renal systems (Abdul et al., 2015; ATSDR, 2007; Hong et al., 2014; Naujokas et al., 2013). These findings were observed even in populations with low iAs exposure (Brauner et al., 2014; Farzan et al., 2015; O'Bryant et al., 2011; Zhang et al., 2013).

To date, many biomonitoring studies have used urinary As as biomarkers of recent exposure to iAs (ATSDR, 2007). Many Korean studies have used total As in urine (Bae et al., 2017; Kim and Lee, 2011; Lee et al., 2012; Park et al., 2016; Park and Lee, 2013) and some of them indicated seafood as the main exposure source which contained organic As (oAs), such as DMA, arsenobetaine (AsB), arsenocholine (AsC), arsenolipids and arsenosugars (Choi et al., 2010; Khan et al., 2015; Kim et al., 2020; Navas-Acien et al., 2011; Taylor et al., 2017). Suggestively,

recent studies conducted As speciation (Chung et al., 2016; Hong et al., 2017; Lee et al., 2022; Seo and Hong, 2021), however, those studies recruited adult general populations or residents near mining areas. Information on As speciation is still lacking in the general population of various age groups.

Blood As was reported as an alternative biomarker of iAs although fewer studies have utilized it compared to urine. Moreover, few studies have utilized urine-blood pairs of samples, where blood markers were compared to urinary ones. Plasma (Bommarito et al., 2019a) and both plasma and erythrocytes (Takayama et al., 2021) were analyzed in these cases. Compared with urine, obtaining blood samples is more difficult and samples require greater care in handling and disposal (Marchiset-Ferlay et al., 2012); however, the analytes in blood are detected at lower levels than in urine, and could reflect the internal dose of As. Furthermore, blood As metabolites may provide insight into metabolic differences that lead to disparities in susceptibility to health effects (Hall et al., 2006). Furthermore, blood As does not require dilution adjustments such as creatinine, specific gravity, and osmolality correction, which can induce collider bias in multivariate analysis. Earlier studies were conducted in iAs-contaminated areas, and study subjects were highly exposed to iAs via drinking water (Abuawad et al., 2021; Bommarito et al., 2019a; Hall et al., 2006; Hall et al., 2007; Mandal et al., 2007), with few studies conducted on populations exposed to low levels of iAs. A Japanese study reported the use of blood-urine pairs in the population exposed to low-level iAs, but As speciation was not studied in the blood (Takayama et al., 2021).

Once absorbed in the gastrointestinal tract, iAs undergo sequential reduction and methylation reaction to form MMA and DMA, which has been

considered to be a detoxification mechanism (ATSDR, 2007). However, current studies suggested that the biotransformation of iAs may also be a pathway for activation. MMA and DMA contain either pentavalent As or trivalent As in this process. The methylated trivalent species (i.e., MMA(III), DMA(III)) are more toxic than the methylated pentavalent species (i.e., MMA(V), DMA(V)) and are also more toxic than iAs in pentavalent (As(V)) or trivalent (As(III)) form (Styblo et al., 2000; Styblo et al., 2021). As methylation efficiency has been determined through the relative proportions of iAs, MMA, and DMA (%iAs, %MMA, and %DMA) and it is considered a modifier of As-related health outcomes (Abuawad et al., 2021; Grau-Perez et al., 2017; Li et al., 2021; Lopez-Carrillo et al., 2020; Zhang et al., 2014). Several studies reported that various factors, such as age, sex, ethnicity, alcohol consumption, smoking status, and nutrition can affect iAs methylation efficiency (Balakrishnan et al., 2018; Desai et al., 2020; Gao et al., 2019; Kurzius-Spencer et al., 2017; Shen et al., 2016; Soler-Blasco et al., 2021; Tseng, 2009). Most of these studies were conducted on populations living in iAs polluted areas.

To our knowledge, there is limited information on the composition of As species in urine-blood pairs and what determines the proportions among general populations with low-dose exposure to iAs. In addition, investigations were skewed towards certain locations or adult groups. The purpose of this study is (i) to determine As speciation among the general population in Korea, (ii) to compare the composition of six As species (i.e., As(V), As(III), MMA, DMA, AsB, and AsC) in the urine-blood pairs, and (iii) to investigate the factors associated with As exposure and methylation efficiency.

2.2. Methods

2.2.1. Study populations and sample collections

The Korean Ministry of Food and Drug Safety (MFDS) collected biological samples to measure environmental chemicals in the general Korean population (aged 1–98 years) between 2017 and 2018. Approximately, 2,500 subjects including infants and children, were recruited with stratified sampling units representing the residential distribution of the geographical area. Each subject was requested to provide a pair of urine-blood biospecimens and questionnaire responses for their demographic information, lifestyle, and food consumption. 'Young children' (≤ 6 years old) did not provide blood samples. All procedures were approved by the Institutional Review Board of Chungbuk National University (IRB No. CBNU-201610-SBBR-378-01) and Seoul National University (IRB No. E2003/001-003).

A total of 2,025 spot urine samples were available, among which 598 whole blood samples were selected to match after consideration of distributions of age and sex. Sample collection and management were based on the operating manual of the MFDS (MFDS, 2013b). Midstream voided urine was collected and participants were asked to receive about 2/3 of the plastic specimen container. The collected samples were immediately dispensed into three 15 mL PP tubes and we received one of them from MFDS. 1.2 mL of each was dispensed into a 2 mL PP tube on the day the samples were provided. For blood, 4 mL of whole blood was collected into a 10 mL BD vacutainer ® EDTA tube (Becton Dickinson, USA), and 1 mL of each was

dispensed into two 2 mL PP cryovials. We received one tube from MFDS for analysis. The samples were stored at -80 ℃ until analysis.

2.2.2. Chemical analysis

Chemical reagents

As(V) and As(III) standard solutions (1,000 μg/mL for AA and ICP) were purchased from SPEX CertiPrep, Inc. (Metuchen, NJ, USA). The MMA (99.5%) standard was purchased from Chemservice (West Chester, PA, USA). DMA (cacodylic acid, ≥ 98.0%) and AsB (≥ 95.0%) standards, sodium 1-butane sulfonate (98%), malonic acid (99%), and tetramethylammonium hydroxide pentahydrate (≥ 97.0%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Arsenocholine bromide (95%) was purchased from FUJIFILM Wako Pure Chemicals (Osaka, Japan). Nitric acid (ultrapure grade) was purchased from ECO Research Inc. (Cheonan, South Korea). Methanol (HPLC grade) and acetonitrile (HPLC grade) were purchased from Honeywell Research Chemicals (Muskegon, MI, USA). The triply distilled water (Milli-Q® Advantage A10 Water Purification System, Merck Millipore) was used throughout the analysis.

Sample preparation

Sample preparation was performed as previously described with some modifications (Hata et al., 2007; Ito et al., 2011; MFDS, 2018). Blood and urine samples were vortexed after thawing. For blood, 200 μL of samples were diluted to a range of 10-25 mL with a mobile phase. Then, the samples were centrifuged at 6,000 ×*g* for 20 min and filtered through a syringe filter (Phenex-RC membrane, 0.2 μm, Phenomenex, Torrance, CA, USA) before injection. For urine, the thawed samples were centrifuged (6,000 ×*g*, 20 min), and 1000 μL of the supernatants were

transferred into a polytetrafluoroethylene (PTFE) tube. Then, the transferred sample was diluted to a range of 10-25 mL with a mobile phase and filtered through a syringe filter before injection.

The creatinine in the urine was determined using an Enzymatic Roche Coba 8000 analyzer (Roche Diagnostics, Indianapolis, IN, USA).

Instrumental analysis

All analytes - As(V), As(III), MMA, DMA, AsB, and AsC were determined using an ACQUITY™ UPLC™ H-Class PLUS (Waters, Milford, MA, USA) coupled to a NexION® 2000 inductively coupled plasma mass spectrometer (PerkinElmer, Waltham, MA, USA) equipped with a dynamic reaction cell (DRC), and a radio frequency (RF) of 1.6 kW power was selected. The argon (Ar, ≥ 99.99%) nebulizer gas flow was optimized at 0.96–1.04 mL/min, and the oxygen (O_2, ≥ 99.99%) DRC gas flow was optimized at 0.5 mL/min. The signal at *m/z* 91 (AsO) was monitored. Detailed information is provided in Supplementary Table S2-1. The HPLC conditions were optimized with some modifications from the method supposed by MFDS (MFDS, 2018). Chromatographic separation was performed using a CAPCELL PAK® C18 MG II (4.6 mm i.d. × 250 mm, 5 μm, Shiseido Ltd., Tokyo, Japan), and the sample injection volume was 40 μL. The column temperature was 20℃. The mobile phase was composed of 10 mM sodium 1-butane sulfonate, 4 mM malonic acid, 4 mM tetramethylammonium hydroxide pentahydrate, and 0.05% methanol and adjusted to pH 2.7 with 10% nitric acid. The flow rate was 0.75 mL/min.

Quality assurance and quality control (QA/QC)

Analytical quality assurance was performed by analyzing the triplicates, reference material, and blanks. Triplicates of three different concentrations (i.e., low, medium, and high) of analytes were evaluated for intra- and inter-day variations (3 days) by spiking analytical standards into urine and blood (Table S2-2). For urine, the accuracy of the validated method ranged from 81.3% to 110.7%. The intra-day precision (%RSD) ranged from 0.4% to 11.5% and inter-day precision ranged from 3.3% to 19.0%, respectively. For blood, the accuracy of the validated method ranged from 90.6% to 112.9%. The intra-day precision ranged from 0.7% to 9.1% and inter-day precision ranged from 2.6% to 15.5%, respectively. To evaluate the accuracy of the analytical method, a standard reference material (SRM) for As species (As species in frozen human urine, SRM 2669, National Institute of Standard and Technology, USA) was analyzed (n = 3). In SRM 2669 level I, the average concentrations represented 114.6%, 89.7%, 103.3%, and 114.5% of the certified values and the %RSDs were 12.4%, 10.6%, 3.6%, and 0.85% for iAs, MMA, DMA, and AsB, respectively. In SRM 2669 level II, the average concentrations represented 101.7%, 87.0%, 95.9%, 128.9%, and 96.7% of the certified values and the %RSDs were 6.9%, 2.4%, 1.3%, 1.4%, and 10.2% for iAs, MMA, DMA, AsB, and AsC, respectively. Quality control (n = 206) samples including known amounts of the standards (matrix-spiked samples) were analyzed every 10-15 samples. The accuracy and %RSD of each As species ranged from 99.3 to 104.4% and from 8.06 to 12.2%, respectively. Since there is currently no SRM for As species in whole blood available, matrix-spiked samples (n = 62) were analyzed every 10-15 samples. The accuracy and %RSD of each As species ranged from 96.2 to 104.4% and from 6.50

to 10.6%, respectively. Background contamination was monitored using procedural, solvent, and matrix blanks for urine and blood analyses. The calibration curves ranged from 1.0 to 1000 μg/L for urine and from 0.5 to 50 μg/L for blood. The coefficients of determination (R^2) were > 0.999. The limits of detection (LOD) of all analytes were 0.3 μg/L in urine and 0.17 μg/L in blood. The LODs were determined by a signal-to-noise (S/N) ratio of 3:1 (MFDS, 2013a).

2.2.3. Adjustment of urine dilution

For adjusting urine dilution, estimates of urinary concentration were used after the standardization of each urinary creatinine measurement (n = 2,025) with the corresponding age, sex, and BMI of the participant (Bulka et al., 2017; O'Brien et al., 2016). Firstly, we excluded some outlying measurements according to the criteria (urinary creatinine level: < 30 mg/dL or > 300 mg/dL) (Barr et al., 2005). Then, the log-transformed urinary creatinine concentration was regressed on the covariates (i.e., age, sex, and BMI) that are known to affect urinary creatinine levels in the study population. Subsequently, a ratio between the fitted urinary creatinine concentration ($\widehat{C_{crea}}$) and the measured urinary creatinine concentration (C_{crea}) was multiplied by the chemical concentration in urine as below:

$$Covariate-adjusted\ \ standardized\ \ concentration = Chemical\ \ concentration\ \ in\ \ urine\ \times \frac{\widehat{C_{crea}}}{C_{crea}}$$

2.2.4. Statistical analysis

For statistical analysis, concentrations below the LOD were imputed as LOD/√2 (Hornung and Reed, 1990b). When calculating concentrations of ∑As and iAs and the proportions of each As species, individual compounds less than LODs were treated as zero.

In the population with moderate to high seafood consumption, the sum of iAs, MMA, and DMA could not properly reflect the exposure to iAs. Especially, DMA concentrations can be contributed to oAs in seafood and its products. Thus, we calibrated iAs, MMA, and DMA concentrations using the statistical method in the previous study (Jones et al., 2016). In brief, AsB concentrations were used as a marker of exposure to arsenic species from seafood consumption. The calibrated iAs, MMA, and DMA concentrations were obtained by regressing the measured iAs, MMA, and DMA concentrations on AsB concentrations using three separate log-log regression models. We added the residuals of each model to the mean of the corresponding As species, estimated from participants with low AsB levels. Low AsB exposure was defined as < 1 μg/L in urine, and < 0.34 μg/L in blood. The same value as in previous studies (Jones et al., 2016; Soler-Blasco et al., 2021) was used for urine, and the concentration of the quartile corresponding to urine in our population was used for blood.

The calculations of proportions were expressed in two ways: (i) divided by the sum of concentrations of all As species (∑As = As(V) + As(III) + MMA + DMA + AsB + AsC), and (ii) divided by the sum of concentrations of iAs and its metabolites (As(V) + As(III) + MMA + DMA). Since the percentage of As species

(iAs, MMA, DMA) over the sum of iAs, MMA, and DMA has been used to determine iAs metabolism efficiency (Pierce et al., 2013), we used the calibrated concentrations when calculating them. Since separate As(III) and As(V) might not be reliable due to possible oxidation of the As species between sampling and analysis (Feldmann et al., 1999); therefore, iAs was used for As(V) + As(III) in statistical analyses. The proportions of As species were compared between urine and blood samples using Wilcoxon rank tests.

The relationship between urine and blood As species were investigated using Spearman's rank correlation. Then, multiple linear regression models were used to examine the determinants and relationship of each As species in blood and urine, respectively. Explanatory variables were associated with As exposure and methylation (Bae et al., 2017; Bommarito et al., 2019a; Lindberg et al., 2008; Shen et al., 2016) including sex, age group, BMI, electricity charge, smoking status, and alcohol consumption frequency. Among them, the electricity charge of a household was an indirect question about household income level. Potential exposure sources such as drinking water and seafood consumption were included. BMI was categorized according to the World Health Organization (WHO) Asia-Pacific classification: overweight BMI 23–24.9 kg/m^2 and obese BMI ≥ 25 kg/m^2 (WHO, 2000). Type of drinking water was classified as 'Purified water', 'Bottled water', 'Tap water', and 'Others'. 'Others' included simple piped water, self-supplied water, and springs, and we integrated them into one variable due to the small number of responses (< 3% of subjects). Consumption of seafood products was classified as 'Yes' if the food item was eaten within the last one month in the questionnaire, and 'No' if not. Concentrations of As species in urine and blood were log-transformed to

a normal distribution. The effect size of the covariates on the levels of urine and blood As species after adjustment for the covariates was expressed in the standardized regression coefficients (β). We used the measured concentrations of As species for the aforementioned analysis. In addition, the methylation efficiency of iAs was also determined using multiple linear regression models. At this time, %iAs, %MMA, and %DMA in urine and blood were calculated using method (ii) and were treated as outcome variables.

R (version 4.1.0) was used for all statistical analyses, and the statistical significance was set at $p < 0.05$.

2.3. Results

2.3.1. Characteristics of the study population

The proportions of adult samples are as follows: 53.9% of urine and 48.5% of blood samples. Overall, the study subjects had normal BMI (53.3% of urine and 43.9% of blood), never or rarely drank alcohol (38.8% of urine and 48.1% of blood), and were non-smokers (87.6% of urine and 92.3% of blood). Most of the population drank purified water (51.8% of urine and 48.2% of blood) and more than 70% of the population ate seafood products (Table 2-1).

Table 2-1. Demographic and exposure characteristics of the population (*n* (%))

	Urine	Blood	Urine + blood
Age group (years)	2,025 (100.0)	598 (100.0)	537 (100.0)
≤ 6	283 (14.0)	-	-
7–12	236 (11.7)	99 (16.6)	91 (16.9)
13-18	283 (14.0)	100 (16.7)	91 (16.9)
19-64	1092 (53.9)	290 (48.5)	263 (49.0)
≥ 65	131 (6.5)	109 (18.2)	92 (17.1)
Sex	2,025 (100.0)	598 (100.0)	537 (100.0)
Male	1040 (51.4)	300 (50.2)	279 (52.0)
Female	985 (48.6)	298 (49.8)	258 (48.0)
BMI (kg/m^2)	1,930 (100.0)	578 (100.0)	518 (100.0)
Normal (< 23)	1,029 (53.3)	254 (43.9)	228 (44.0)
Overweight (23-24.9)	367 (19.0)	143 (24.7)	126 (24.3)
Obese (≥ 25)	534 (27.7)	181 (31.3)	164 (31.7)
Alcohol consumption	1,744 (100.0)	597 (100.0)	536 (100.0)
Never or seldom	677 (38.8)	287 (48.1)	254 (47.4)
≤1/month	373 (21.4)	117 (19.6)	105 (19.6)
2-4/month	380 (21.8)	105 (17.6)	94 (17.5)
≥ 2/week	314 (18.0)	88 (14.7)	83 (15.5)
Electricity charge (KRW/month)	2,020 (100.0)	598 (100.0)	537 (100.0)
< 30,000	575 (28.5)	156 (26.1)	139 (25.9)
≥ 30,000, < 50,000	746 (36.9)	229 (38.3)	200 (37.2)
≥ 50,000	572 (28.3)	186 (31.1)	175 (32.6)
Unknown	127 (6.3)	27 (4.5)	23 (4.3)
Smoking status	1,740 (100.0)	596 (100.0)	536 (100.0)
Non-smoker	1,524 (87.6)	550 (92.3)	492 (91.8)
Smoker	216 (12.4)	46 (7.7)	44 (8.2)
Type of drinking water	2,017 (100.0)	598 (100.0)	537 (100.0)
Purified water	1,045 (51.8)	288 (48.2)	256 (47.7)
Bottled water	447 (22.2)	127 (21.2)	118 (22.0)
Tap water	412 (20.4)	138 (23.1)	122 (22.7)
Others	113 (5.6)	45 (7.5)	41 (7.6)

Table 2-1. *(continued)*

Grain consumption			
White rice	1,924 (100.0)	568 (100.0)	509 (100.0)
No	230 (12.0)	85 (15.0)	70 (13.8)
Yes	1,694 (88.0)	483 (85.0)	439 (86.2)
Multigrain rice	1,929 (100.0)	570 (100.0)	511 (100.0)
No	317 (16.4)	83 (14.0)	77 (15.1)
Yes	1612 (83.6)	487 (85.4)	434 (84.9)
Seafood product consumption			
Blue-backed fishes	1,936 (100.0)	572 (100.0)	513 (100.0)
No	403 (20.8)	123 (21.5)	107 (20.9)
Yes	1,533 (79.2)	449 (78.5)	406 (79.1)
Other fishes	1,935 (100.0)	572 (100.0)	513 (100.0)
No	141 (7.3)	37 (6.5)	36 (7.0)
Yes	1,794 (92.7)	535 (93.5)	477 (93.0)
Laver	1,932 (100.0)	570 (100.0)	511 (100.0)
No	169 (8.7)	57 (10.0)	52 (10.2)
Yes	1,763 (91.3)	513 (90.0)	459 (89.8)
Other seaweeds	1,934 (100.0)	572 (100.0)	513 (100.0)
No	187 (9.7)	53 (9.3)	48 (9.4)
Yes	1,747 (90.3)	519 (90.7)	465 (90.6)
Other seafood	1,936 (100.0)	572 (100.0)	513 (100.0)
No	489 (25.3)	167 (29.2)	151 (29.4)
Yes	1,447 (74.7)	405 (70.8)	362 (70.6)

2.3.2. Arsenic species in urine and blood

The detection rates of MMA, DMA, AsB, and AsC were 97.2%, 99.3%, 95.0%, and 45.8% in urine and 73.0%, 92.5%, 96.0%, and 73.5% in blood, respectively. For As(V) and As(III) constituting iAs, the detection rates were 69.0% and 76.5% in urine, and 89.1% and 91.2% in blood, respectively. Since the detection rate of urinary AsC is relatively low, this species was excluded from further analysis. The levels of DMA and AsB in the blood were one to two orders of magnitude lower than those in urine (Table 2-2). The calibrated concentrations of iAs, MMA, and DMA were lower in all As species, especially for DMA concentrations (median (p95): 23.4 (93.8) and 9.34 (36.4) in measured concentration and calibrated concentrations, respectively). More results are shown in Table S2-3.

Table 2-2. Distribution of As species levels in urine and blood

Media	As species	Dilution adjustment method	*n*	DR (%)	Mean ± SD (μg/L)	Median (μg/L)	p95 (μg/L)	Range (μg/L)
Urine	∑As	Unadjusted	2,025	-	101.8 ± 141.6	62.3	299.4	< LOD[a] – 2542.2
		CAS	1,886		103.3 ± 134.8	62.9	314.2	< LOD[a] – 2535.9
	iAs	Unadjusted	2,025	-[b]	1.79 ± 1.88	1.40	4.40	< LOD[a] – 40.2
		CAS	1,886		1.77 ±1.93	1.41	4.00	< LOD[a] – 55.1
	MMA	Unadjusted	2,025	97.2	2.04 ± 2.20	1.53	5.00	< LOD – 48.5
		CAS	1,886		1.96 ±2.40	1.56	4.00	< LOD – 64.4
	DMA	Unadjusted	2,025	99.3	33.1 ± 33.8	23.4	93.8	< LOD – 418.7
		CAS	1,886		33.1 ± 32.2	23.2	92.5	< LOD – 370.8
	AsB	Unadjusted	2,025	95.0	63.3 ± 128.3	27.3	224.6	< LOD – 2476.7
		CAS	1,886		64.8 ± 122.2	29.0	244.1	< LOD – 2470.5
	AsC	Unadjusted	2,025	45.8	-[c]	< LOD	6.75	< LOD – 154.6
		CAS	1,886		-[c]	< LOD	7.01	< LOD – 222.7
Blood	∑As		598	-	7.11 ± 4.91	5.87	16.5	0.56 – 39.7
	iAs			-[b]	1.33 ± 1.42	1.17	2.27	< LOD[a] – 27.2
	MMA			73.0	0.37 ± 0.40	0.27	0.95	< LOD – 4.92
	DMA	-		92.5	0.88 ± 0.67	0.76	2.19	< LOD – 4.37
	AsB			96.0	4.22 ± 4.31	2.87	12.0	< LOD – 34.0
	AsC			73.5	0.39 ± 0.33	0.30	1.05	< LOD – 3.21

Abbreviations: ∑As, sum of As species (= As(V) + As(III) + MMA + DMA + AsB + AsC); iAs, inorganic As (= As(V) + As(III)); As(V), arsenite; As(III), arsenate; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; AsB, arsenobetaine; AsC, arsenocholine; DR, detection rate; SD, standard deviation; p95, 95th percentile
LODs: 0.3 μg/L for urine, 0.17 μg/L for blood
[a] When ∑As and iAs were calculated, individual compounds <LODs were treated as zero to prevent incorrect results, and subjects with ∑As or iAs concentrations of zero were designated as <LOD.
[b] DRs of As(V) and As(III) were 69.0% and 76.5% in urine, and 89.1% and 91.2% in blood, respectively.
[c] Data was not calculated because DR was too low to provide a valid result.

The most abundant As species was AsB (51.1%), followed by DMA (40.5%), MMA (2.42%), and iAs (2.18%) in urine, whereas, in blood, the order was: AsB (53.5%), iAs (20.2%), DMA (11.7%), AsC (4.53%), and MMA (4.33%) (Figure 2-1A and Table S2-4). As species distribution was significantly different between urine and blood ($p < 0.0001$), although not for AsB ($p = 0.297$). In summary, %iAs and %MMA were higher in blood than those in urine. The proportion of AsC (%AsC) in blood was 4.53%, whereas close to zero in urine. Regarding iAs and its metabolites, the proportions of %iAs, %MMA, and %DMA were 5.31%, 5.90%, and 88.6% in urine and 52.5%, 12.1%, and 33.0% in blood, respectively. The proportions, calculated using the calibrated concentrations, of %iAs, %MMA, and %DMA were 9.16%, 8.90%, and 81.5% in urine and 54.5%, 15.1%, and 27.4% in blood, respectively (Figure 2-1B and Table S2-4).

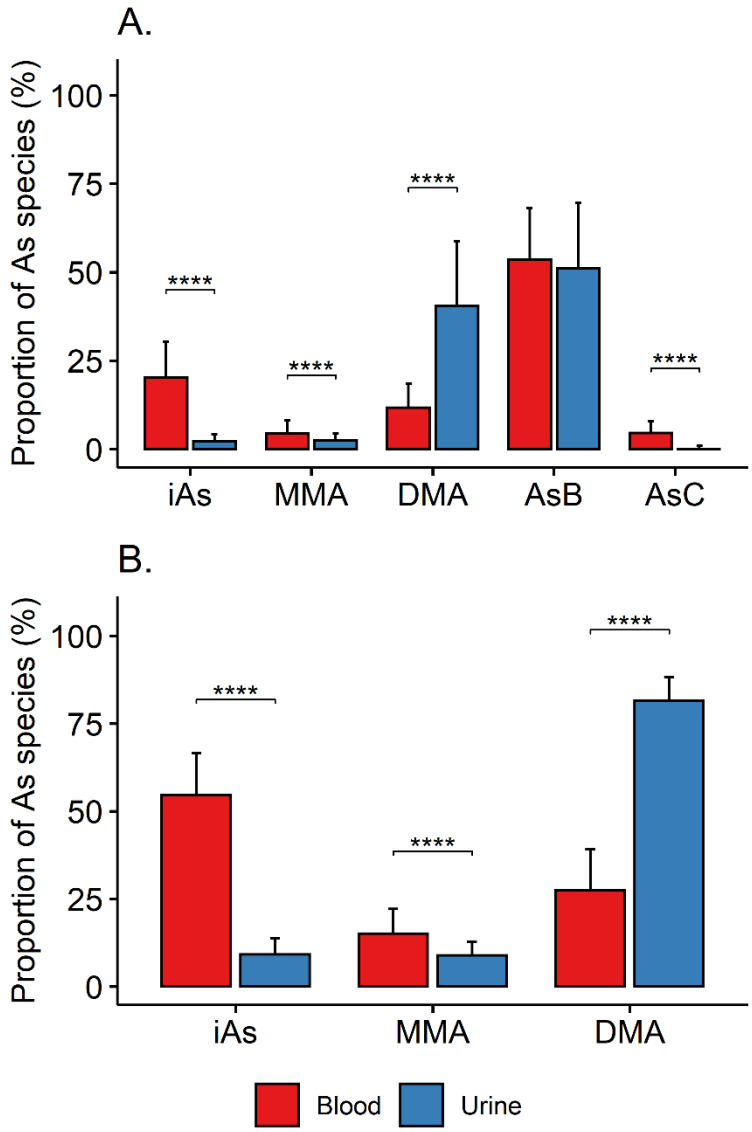

Figure 2-1. Bar plot depicting the proportions of arsenic (As) species in urine and blood. Bars and error bars represent the median and the 75th percentile, respectively. (A) is divided by the sum of concentrations of all As species (∑As), and (B) is divided by the sum of concentrations of iAs and its metabolites (iAs + MMA + DMA). The measured and calibrated concentrations were used for (A) and (B), respectively. ****$p < 0.0001$ based on Wilcoxon rank test. *Abbreviations:* iAs, inorganic arsenic; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; AsB, arsenobetaine; AsC, arsenocholine

2.3.3. Determinants associated with As species levels in urine and blood

Table 2-3 and 2-4 show the potential covariates associated with the levels of As species in urine and blood, respectively. After the adjustment for the covariates, adults including the elderly (≥ 65 years) showed higher urinary ∑As (β = 0.332, p = 0.047 for adults; β = 0.271, p = 0.003 for elderly), blood ∑As (β = 0.242, p < 0.001 for adults; β = 0.346, p < 0.001 for elderly), and AsB (β = 0.262, p < 0.001 for adults; β = 0.331, p < 0.001 for elderly) than the reference group – young children (≤ 6 years) for urine, children (7–12 years) for blood. Adolescents (13–18 years) had significantly higher urinary iAs (β = 0.276, p = 0.028) and the elderly had higher urinary AsB (β = 0.218, p = 0.019) than the reference group. Females had lower urinary levels of iAs (β = -0.091, p = 0.002) and MMA (β = -0.145, p < 0.001) than males. Obese subjects showed relatively lower urinary iAs (β = -0.091, p = 0.002) and MMA levels (β = -0.077, p = 0.009). Subjects who frequently drink alcohol (≥ 2 times/week) had higher urinary AsB (β = 0.081, p = 0.022) than subjects who never or rarely drink. Subjects who had the highest economic status (≥ 50,000 KRW/month) showed higher urinary ∑As (β = 0.062, p = 0.041) than others. Smokers had relatively lower urinary ∑As levels (β = -0.061, p = 0.028) than non-smokers. Interestingly, consumption of tap water and other types of water was associated with an increase in urinary iAs (β = 0.085, p = 0.002 for tap water; β = 0.087, p = 0.001 for others) and MMA (β = 0.055, p = 0.043 for tap water; β = 0.078, p = 0.003 for others). Subjects who drank bottled water showed lower urinary DMA levels (β = -0.082, p = 0.002) than purified water. Consumption of multigrain rice was associated with an increase in urinary iAs (β = 0.069, p = 0.009). As for seafood consumption,

blue-backed fishes were associated with the increase of urinary ∑As (β = 0.082, p = 0.003), AsB (β = 0.056, p = 0.049), blood ∑As (β = 0.140, p = 0.001), and AsB (β = 0.123, p = 0.005); While laver was associated with the increase of urinary DMA (β = 0.065, p = 0.018), other seaweeds were associated with the increase of urinary iAs (β = 0.055, p = 0.044). The models for blood iAs, MMA, DMA, and AsC were not statistically significant (data not shown).

Table 2-3. Effects of covariates on urinary As species (n = 1,507)

Covariates	Urinary As species (μg/L)[a, b]									
	∑As		iAs		MMA		DMA		AsB	
	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value	β	*p*-value
Age group (years)										
7–12	0.177	0.140	0.116	0.338	0.055	0.647	-0.001	0.995	0.163	0.180
13–18	0.098	0.433	**0.276**	**0.028**	0.200	0.109	-0.065	0.604	0.086	0.494
19–64	**0.332**	**0.047**	0.203	0.229	0.048	0.775	-0.002	0.989	0.303	0.074
≥ 65	**0.271**	**0.003**	0.149	0.108	0.119	0.195	0.114	0.220	**0.218**	**0.019**
Sex										
Female	0.008	0.761	**-0.125**	**<0.001**	**-0.145**	**<0.001**	0.031	0.274	0.007	0.815
BMI (kg/m^2)										
Overweight (23-24.9)	0.028	0.320	-0.007	0.810	0.024	0.396	0.023	0.418	0.049	0.090
Obese (≥ 25)	0.023	0.438	**-0.091**	**0.002**	**-0.077**	**0.009**	-0.004	0.919	0.050	0.093
Alcohol consumption										
≤ 1/month	-0.006	0.860	-0.017	0.602	-0.019	0.554	-0.036	0.274	0.023	0.476
2-4/month	0.030	0.366	-0.010	0.764	-0.036	0.293	-0.038	0.272	0.052	0.125
≥ 2/week	0.051	0.146	-0.033	0.352	-0.057	0.103	-0.043	0.223	**0.081**	**0.022**
Electricity charge (KRW/month)										
≥ 30,000, < 50,000	0.037	0.219	0.016	0.604	0.024	0.422	0.029	0.351	0.026	0.395
≥ 50,000	**0.062**	**0.041**	0.006	0.832	0.024	0.430	0.026	0.395	0.048	0.122
Unknown	0.003	0.916	-0.013	0.642	0.009	0.748	-0.052	0.061	0.019	0.492
Smoking status										
Smoker	**-0.061**	**0.028**	-0.021	0.464	-0.035	0.208	-0.052	0.063	-0.052	0.068
Type of drinking water										
Bottled water	-0.017	0.520	-0.009	0.731	-0.043	0.108	**-0.082**	**0.002**	-0.009	0.750
Tap water	-0.012	0.660	**0.085**	**0.002**	**0.055**	**0.043**	-0.008	0.763	-0.011	0.674

Table 2-3. *(continued)*

Others	-0.007	0.785	**0.087**	**0.001**	**0.078**	**0.003**	-0.008	0.757	-0.027	0.302
Grain consumption										
White rice										
Yes	-0.034	0.194	-0.019	0.464	-0.030	0.241	-0.011	0.665	-0.028	0.288
Multigrain rice										
Yes	0.015	0.567	**0.069**	**0.009**	0.028	0.274	0.043	0.098	0.010	0.707
Seafood consumption										
Blue-backed fishes										
Yes	**0.082**	**0.003**	0.009	0.740	0.004	0.878	0.053	0.061	**0.056**	**0.049**
Other fishes										
Yes	0.048	0.086	**-0.056**	**0.047**	-0.048	0.083	0.018	0.513	0.033	0.247
Laver										
Yes	0.029	0.287	-0.001	0.959	0.024	0.365	**0.065**	**0.018**	-0.004	0.873
Other seaweeds										
Yes	0.009	0.754	**0.055**	**0.044**	0.040	0.140	0.043	0.116	0.009	0.743
Other seafood										
Yes	0.038	0.167	0.015	0.592	0.019	0.479	-0.008	0.781	0.031	0.259

Abbreviations: ∑As, sum of As species (= As(V) + As(III) + MMA + DMA + AsB + AsC); iAs, inorganic As (= As(V) + As(III)); As(V), arsenite; As(III), arsenate; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; AsB, arsenobetaine; AsC, arsenocholine

β: Standardized regression coefficients of each blood As species after adjustment for covariates (i.e., age, sex, BMI, electricity charge, smoking status, alcohol consumption, type of drinking water, consumption of grains, and seafood items).

Reference: ≤ 6 years (age group), male (sex), normal (BMI), never or seldom (alcohol consumption), < 30,000 KRW/month (electricity charge), non-smoker (smoking status), purified water (type of drinking water), no (white rice, multigrain rice, blue-backed fishes, other fishes, laver, other seaweeds, other seafood)

[a] Urinary As species were corrected using covariate-adjusted standardized (CAS) method.

[b] Concentrations were log-transformed.

Table 2-4. Effects of covariates on blood As species (n = 553)

Covariates	Blood As species (μg/L) [a,b]			
	∑As		AsB	
	β	*p*-value	β	*p*-value
Age group (years)				
13–18	-0.025	0.643	-0.005	0.919
19–64	**0.242**	**<0.001**	**0.262**	**<0.001**
≥ 65	**0.346**	**<0.001**	**0.331**	**<0.001**
Sex				
Female	-0.010	0.821	-0.013	0.762
BMI (kg/m^2)				
Overweight (23–24.9)	0.011	0.799	0.009	0.823
Obese (≥ 25)	0.020	0.665	0.010	0.829
Alcohol consumption				
≤ 1/month	0.070	0.143	0.072	0.132
2-4/month	**0.108**	**0.030**	0.085	0.088
≥ 2/week	-0.026	0.578	0.003	0.957
Electricity charge (KRW/month)				
≥ 30,000, < 50,000	-0.019	0.695	0.030	0.526
≥ 50,000	0.033	0.506	0.028	0.566
Unknown	0.062	0.209	0.029	0.564
Smoking status				
Smoker	-0.012	0.787	-0.012	0.785
Type of drinking water				
Bottled water	0.014	0.750	0.017	0.691
Tap water	0.041	0.352	0.024	0.587
Others	0.002	0.959	-0.006	0.887
Grain consumption				
White rice				
Yes	-0.056	0.195	-0.065	0.134
Multigrain rice				
Yes	0.046	0.263	0.025	0.545
Seafood consumption				
Blue-backed fishes				
Yes	**0.140**	**0.001**	**0.123**	**0.005**
Other fishes				
Yes	-0.017	0.687	0.043	0.321
Laver				
Yes	0.037	0.380	-0.050	0.239

Table 2-4. *(continued)*

Other seaweeds				
Yes	0.072	0.081	0.035	0.412
Other seafood				
Yes	**-0.087**	**0.041**	0.042	0.312

Abbreviations: ∑As, sum of As species (= As(V) + As(III) + MMA + DMA + AsB + AsC); iAs, inorganic As (= As(V) + As(III)); As(V), arsenite; As(III), arsenate; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; AsB, arsenobetaine; AsC, arsenocholine

β: Standardized regression coefficients of each blood As species after adjustment for covariates (i.e., age, sex, BMI, electricity charge, smoking status, alcohol consumption, type of drinking water, consumption of grains and seafood items).

Reference: 7-12 years (age group), male (sex), normal (BMI), never or seldom (alcohol consumption), < 30,000 KRW/month (electricity charge), non-smoker (smoking status), purified water (type of drinking water), no (white rice, multigrain rice, blue-backed fishes, other fishes, laver, other seaweeds, other seafood)

[a] The results of iAs, MMA, DMA, and AsC were excluded because the linear models were not statistically significant for them.

[b] Concentrations were log-transformed

2.3.4. Associations between As species in urine and blood

Regarding the correlations of As species between urine and blood, ∑As ($\rho = 0.48$, $p < 0.001$) and AsB ($\rho = 0.53$, $p < 0.001$) were moderate, but MMA and DMA ($\rho = 0.15$, $p < 0.001$; $\rho = 0.25$, $p < 0.001$) were weak positive. No correlations were found between urine and blood for iAs ($\rho = 0.066$, $p = 0.16$) (Figure 2-2). After adjusting for other covariates, ∑As, MMA, DMA, and AsB still had significant associations between blood and urine. For iAs, no significant association was observed (b [95% CI]: 0.07 [-0.03, 0.17], $p = 0.018$) between blood and urine (Table 2-5).

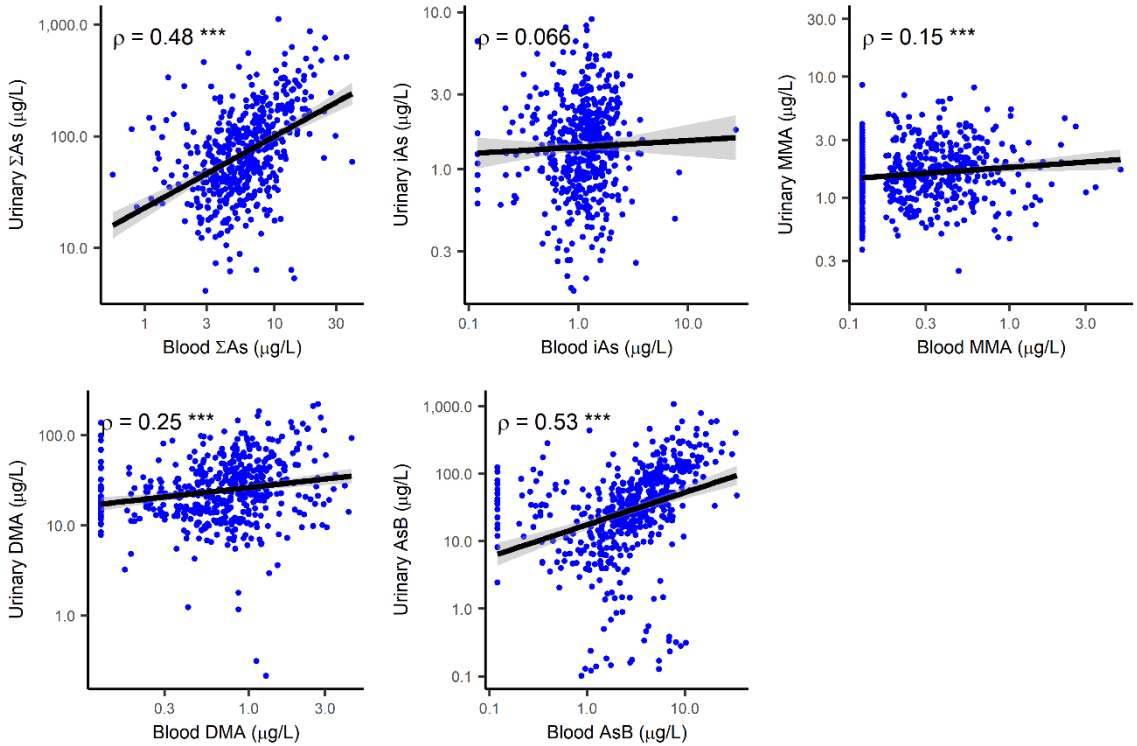

Figure 2-2. Scatter plot illustrating the correlations of urine and blood As levels. ρ indicates Spearman correlation coefficient. *Abbreviations:* iAs, inorganic arsenic = As(III) + As(V); MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; AsB, arsenobetaine; All urinary arsenic analytes here were used after dilution adjustment. *** $p < 0.001$

Table 2-5. Association of blood and urinary As species after adjusting other covariates (n = 458)

Blood As species (μg/L)[b]	Urinary As species (μg/L)[a,b]									
	∑As		iAs		MMA		DMA		AsB	
	b (95% CI)	*p*-value	b (95% CI)	*p*-value	b (95% CI)	*p*-value	b (95% CI)	*p*-value	b (95% CI)	*p*-value
∑As	**0.52 (0.39, 0.65)**	**<0.001**	-	-	-	-	-	-	-	-
iAs	-	-	0.07 (-0.03, 0.17)	0.18	-	-	-	-	-	-
MMA	-	-	-	-	**0.09 (0.03, 0.16)**	**0.005**	-	-	-	-
DMA	-	-	-	-	-	-	**0.18 (0.09, 0.28)**	**<0.001**	-	-
AsB	-	-	-	-	-	-	-	-	**0.34 (0.10, 0.38)**	**<0.001**

Abbreviations: 95% CI, 95% confidence intervals; ∑As, sum of As species (= As(V) + As(III) + MMA + DMA + AsB + AsC); iAs, inorganic As (= As(V) + As(III)); As(V), arsenite; As(III), arsenate; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; AsB, arsenobetaine; AsC, arsenocholine

b: regression coefficients after adjustment for other covariates(i.e., log(blood As species), age, sex, BMI, electricity charge, smoking status, alcohol consumption, type of drinking water, consumption of grains and seafood items)

[a] Urinary As species were adjusted using covariate-adjusted standardized (CAS) method.

[b] Concentrations were log-transformed.

2.3.5. Determinants associated with As methylation efficiency

Figure 2-3 and Table S2-5 show that the proportions of methylated As species differed according to demographic characteristics (i.e., age, sex, BMI, alcohol consumption, electricity charge, and smoking status). Age, sex, and smoking status were significant factors in urine. Adolescents had the highest %iAs (b [95% CI] = 3.13 [0.64, 5.62], p = 0.014), %MMA (b [95% CI] = 3.14 [1.25, 5.09], p = 0.001), and lowest %DMA (b [95% CI] = -6.51 [-10.4, 2.60], p = 0.001) among age groups. Females had lower %iAs (b [95% CI] = -1.79 [-3.26, -0.31], p = 0.018), %MMA (b [95% CI] = -1.15 [-2.29, -0.02], p = 0.047) and higher %DMA (b [95% CI] = 2.99 [0.67, 5.30], p = 0.012) than males. Smokers had higher %MMA (b [95% CI] = 3.16 [1.19, 5.13], p = 0.002) and lower %DMA (b [95% CI] = -4.62 [-8.63, -0.60], p = 0.024) than nonsmokers, but had no significant difference in %iAs compared with nonsmokers. BMI, alcohol consumption and electricity charge were not significant factors. Models using the blood data were not statistically significant (data now shown).

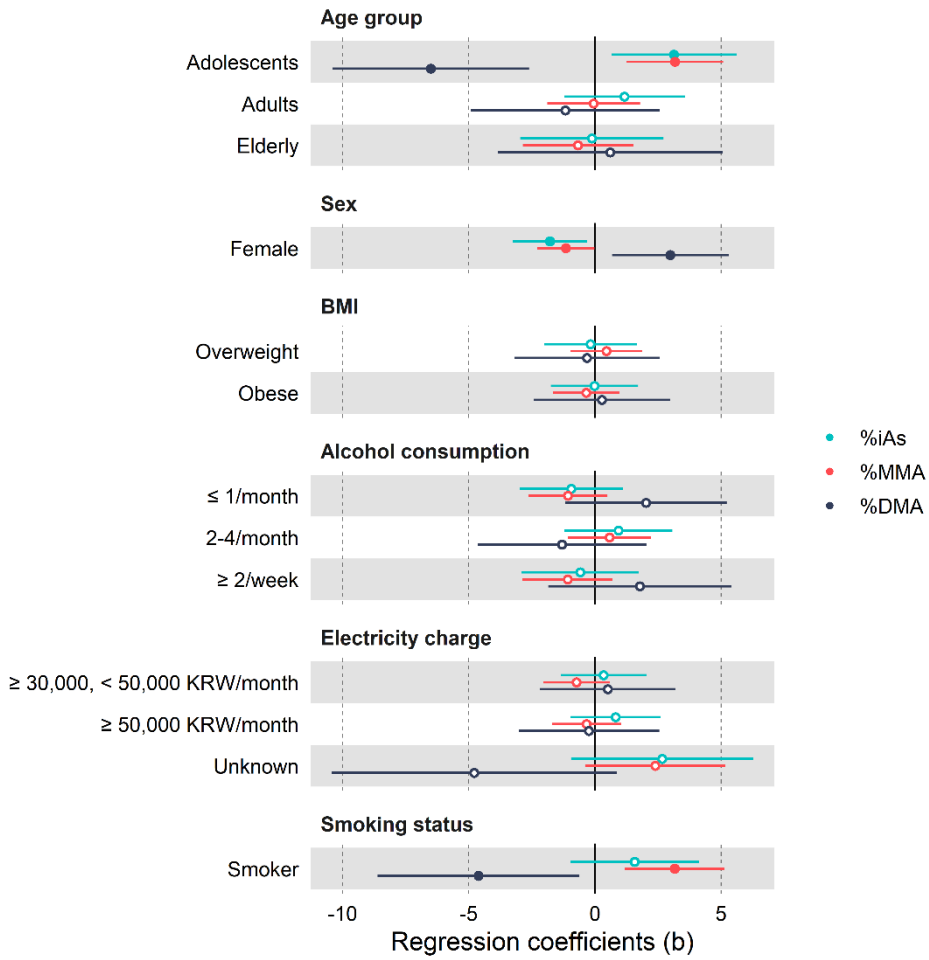

Figure 2-3. Coefficient plots illustrating the iAs methylation efficiency based on urinary proportions of iAs and its metabolites depending on different demographic characteristics. Dots and error bars represent regression coefficients (b) and 95% CI of the multiple linear regression between proportions of As species (%iAs, %MMA, and %DMA) and covariates (i.e., the proportion of blood As species, age group, sex, BMI, electricity charge, smoking status, alcohol consumption, type of drinking water, consumption of grains, and seafood items). *Abbreviations:* %iAs, the proportion of inorganic arsenic; %MMA, the proportion of monomethylarsonic acid; %DMA, the proportion of dimethylarsinic acid; *Reference:* 7-12 years (age group), male (sex), normal (BMI), never or seldom (alcohol consumption), < 30,000 KRW/month (electricity charge), non-smoker (smoking status).

2.4. Discussion

Urinary and blood As biomarkers among the general Korean public

In the present study, we measured As species both in urine and blood samples collected from a general Korean population with low exposure to iAs. For urinary As species, the concentrations were similar or comparable to those in earlier studies targeting the general population. The urinary As(V), As(III), MMA, DMA, and AsB levels (median: 0.48, 0.84, 1.53, 23.4, 27.3 μg/L) detected in the present study was similar to those of Japanese adults (0.4, 1.5, 1.8, 24.1, 21.5 μg/L) (Takayama et al., 2021). In National Health and Nutrition Survey (NHANES) 2017-2018 (CDC, 2022), As(V) was detected in less than 10% of the US population, and the 95th percentile of As(V) (0.88 μg/L) was higher than that of this study. As(III), MMA, and AsB were detected in less than half of the study population, but the 75th percentiles (0.36, 0.67, and 3.48 μg/L, respectively) were similar to those of our study. Furthermore, the level of DMA (2.90 μg/L) was lower than in our study. The urinary AsC was also detected in less than 10% of the population, and the 95th percentile of AsC (0.22 μg/L) was much lower than in the present study (67.5 μg/L). Compared with other studies carried out in highly contaminated regions (Bommarito et al., 2019a), our urinary measurements were lower for iAs and MMA (3.5 and 3.9 μg/L). Whereas, our measurements were higher for DMA (19.4 μg/L), which is likely associated with the high seafood consumption of Koreans. DMA is known as the metabolite of iAs, but it is also the metabolite of arsenosugars and arsenolipids, which are abundantly contained in marine organisms (Schmeisser et al., 2006; Taylor et al., 2017). The high level of urinary DMA in this study might reflect the exposure to DMA and other

oAs (i.e., arsenosugars and arsenolipids) through seafood intake (Choi et al., 2010; Schmeisser et al., 2006). Indeed, urinary AsB concentration in this study was comparable to the Japanese population (Takayama et al., 2021) and greater than that of the US population (CDC, 2022), indicating this study population consumes a lot of seafood.

For blood As species, there are sparse reports to compare with. Blood ∑As in the present study (5.87 μg/L) was higher than the total As of U.S. mothers (1.4 μg/L) (Claus Henn et al., 2016) and Japanese adults (3.49 μg/L) (Takayama et al., 2021). On the contrary, ∑As concentrations in our study (range: 0.56-39.6 μg/L) were lower than total As concentrations of Bangladeshi (4.8-67.9 μg/L) and Indian (5.56-57.0 μg/L) residents in As-contaminated regions (Hall et al., 2007; Mandal et al., 2007). However, it should be interpreted with caution because they did not separate As species in blood. In the present study, the most abundant species in blood was AsB, a non-toxic form of oAs.

Table 2-6. Urinary concentrations of As species among different studies [median (p95), μg/L]

Country	Population	Age (yrs)	N	Sampling year	ΣAs	iAs	As(V)	As(III)	MMA	DMA	AsB	AsC	Reference
Korea	General population	1-98	1886	2017-2018	62.3 (299.4)	1.40 (4.40)	0.48 (2.09)[a]	0.84 (3.02)[a]	1.53 (5.00)	23.4 (93.8)	27.3 (224.6)	<LOD (6.75)	This study
Korea		19+	2044	2010-2011	82.9 (407.1)	3.0 (19.1)	-	-	1.7 (5.6)	25.3 (83.1)	43.8 (338.0)	-	Lee et al. (2022)
Canada		3-79	2531	2018-2019	-	-	<LOD[b] (0.15)	0.42 (3.6)	0.41 (1.5)	3.5 (21)	1.2 (63)[c]		Health Canada (2021)
Japan		19+	109[d]	2016	85.2 (299)[e]	-	0.4 (1.2)	1.5 (4.4)	1.8 (5.8)	24.1 (78.1)	21.5 (146)	-	Takayama et al. (2021)
Spain[f,g]		19+	1017	2003-2008	28.9[c]	0.27	-	-	0.28	5.54	16.4	-	Soler-Blasco et al. (2021)
US		3+	2860[d]	2017-2018	5.71 (61.5)[c]	-	<LOD[h] (0.880)	<LOD[h] (1.01)	<LOD[h] (1.47)	2.90 (11.4)	<LOD[h] (39.5)	<LOD[h] (0.220)	CDC (2022)
Bangladesh[g]	Population exposed to iAs via drinking water	19+	1606 (visit 1)	2008-2011	26.5 (376)	2.5 (52.2)	0.6 (21.8)	0 (28.9)	1.3 (30.3)	22.5 (297)	-	-	Gao et al. (2019)
			1445 (visit 2)		35.2 (325)	2.4 (39.1)	0.9 (22.5)	0 (16.7)	1.7 (21.5)	35.2 (325)	-	-	
China[f,i]		18+	126 (hypertension)	-	138.0	11.8	-	-	20.4	99.7	-	-	Li et al. (2015)
			386 (non-hypertension)		104.7	7.9	-	-	13.2	79.4	-	-	
Mexico[j]		5-88	232	-	27.7 (34.4)	3.5 (4.3)	-	-	3.9 (4.3)	19.4 (26.5)	-	-	Bommarito et al. (2019a)
UK		18+	207	2011-2013	1.6	-	0.3	0.3	0.7	4.0	6.9	-	Middleton et al. (2016)

Abbreviations: ∑As, sum of As species (= As(V) + As(III) + MMA + DMA + AsB + AsC); iAs, inorganic As (= As(V) + As(III)); As(V), arsenite; As(III), arsenate; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; AsB, arsenobetaine; AsC, arsenocholine

[a] Presented for comparison with other studies, [b] 0.14 μg/L, [c] Sum of AsB and AsC, [d] n = 109 for ΣAs, MMA, DMA, and AsB, 46 for As(V), and 106 for As(III), [e] Total arsenic measured using ICP/MS, [f] Geometric mean, [g] Pregnant women, [h] 0.79 μg/L for As(V), 0.12 μg/L for As(III), 0.2 μg/L for MMA, 1.16 μg/L for AsB, and 0.11 μg/L for AsC, [i] Creatinine adjusted concentration ($\mu g/g_{crea.}$), [j] Median (IQR)

Covariates associated with As species in urine and blood

In urine, the levels of As species were associated with age, sex, BMI, smoking status, types of drinking water, consumption of multigrain rice, blue-backed fishes, laver, and other seaweeds (Table 2-3). Meanwhile, the levels of blood As species were associated with the age and consumption of blue-backed fishes and other seaweeds (Table 2-4).

In our population, adolescents had increased concentrations of urinary iAs than the reference group (≤ 6 years). The same trend was also observed when analyzing using other age groups as a reference (data not shown). Interestingly, our results (more exposure to iAs among adolescents) came after adjustment for rice intake, which is known to be the primary contributor to iAs exposure in Koreans (Seo et al., 2016). It implies an additional source of exposure in the subpopulation. According to the report on integrated exposure assessment of As in Korea (MFDS, 2021), agricultural products and processed foods (*e.g.*, noodles, bread, snacks, teas, beverages, etc.) were the primary- and second-largest contributors to iAs exposure, respectively (MFDS, 2021). But we only considered some kinds of grains (white rice, multigrain rice) and seafood in the present study. In addition, we could speculate on exposure via drinking water because 24% of adolescents responded to not drinking purified- or bottled water in our data. iAs level of drinking water is known to be associated with the residence area (e.g., rural, urban, mining, industrial area, etc.) (Hong et al., 2017; Park et al., 2016), however, there was no information about it in the questionnaire. Therefore, adolescents had high levels of urinary iAs, indicating that they were likely exposed to iAs from other exposure sources which were not considered in our study. Hence, further research is needed to investigate possible

dietary and environmental sources of iAs exposure in adolescents. Adults and the elderly had higher concentrations of AsB in urine and/or blood. These findings are supported by previous studies that established AsB as the major As species present in seafood (Taylor et al., 2017), and that the contribution of seafood to As exposure is highest in adults and elderly (62.9% and 59.8%, respectively) among all ages (MFDS, 2016a, 2021).

As mentioned above, the type of drinking water was also associated with increased urinary iAs and MMA (Table 2-3). In Korea, most of the groundwater did not exceed As levels of the current drinking water standard (< 10.0 μg/L) although some regions still exhibited 10.0 μg/L depending on their geographical characteristics (Park et al., 2016). Multigrain rice was associated with increased urinary iAs and seafood was associated with increased DMA and AsB levels in urine and blood (Table 2-3 and 2-4). Especially, blue-backed fish commonly increased ∑As and AsB levels in urine and blood among seafood items. Those results are consistent with the previous studies (Bae et al., 2017; Choi et al., 2010; Cubadda et al., 2017; Heinrich-Ramm et al., 2002; Mantha et al., 2017; Molin et al., 2012; Seo et al., 2016; Soler-Blasco et al., 2021).

In contrast, other fishes and other seafood showed negative associations with iAs species in urine and ∑As in blood, respectively. White rice or some seafood items did not show significant associations. The lack of available data, such as concentrations of As species in food items and nutrient factors (Kurzius-Spencer et al., 2017) might affect the results. In addition, the food intake data was not captured by the food frequency questionnaire (FFQ). Therefore, further study is needed to confirm the associations between food intakes and As concentrations with more

detailed dietary consumption data from other sources.

Sex, BMI, and alcohol consumption had significant associations with some As species in urine and blood. Although we can speculate that some social activities such as dining and drinking might be associated with more exposure to oAs, it is not clear why other factors such as sex, BMI, and alcohol consumption are associated with As species levels. One possibility is that iAs metabolism, sex, and BMI may be significant factors in iAs methylation (Abuawad et al., 2021; Lindberg et al., 2008; Lindberg et al., 2007). Further studies are needed to investigate the levels of As species in food items and dietary habits depending on those factors.

Associations between As species in urine and blood

In our study, AsB showed a moderate correlation between urine and blood (Figure 2-2), which indicates that blood AsB levels immediately reflect urinary AsB levels. In humans, AsB is rapidly excreted in urine as the unmetabolized form (Brown et al., 1990). For MMA and DMA, there were weak positive correlations between urine and blood and there was no significant correlation between iAs in urine and blood (Figure 2-2). Those tendencies were maintained after statistical adjustment for other covariates (Table 2-5). On the contrary, a Mexican study showed strong correlations between urine and blood plasma in MMA ($\rho = 0.60$) and DMA ($\rho = 0.61$), and a weak correlation between urine and blood plasma in iAs ($\rho = 0.13$) (Bommarito et al., 2019a). Firstly, this seems to indicate relatively different magnitudes of exposure to iAs. For instance, levels of iAs ranged from 3.1 to 215.2 μg/L in the drinking water of the Mexican study. Although we did not measure water iAs in the present study, exposure to iAs via drinking water is under relatively safe control, which can be

supported by indirect data: < 2.0 μg/L of total As was present in 88.0-89.0% of groundwater in Korea (Park et al., 2016), and 74% of people drink purified and bottled water in the present study. In addition, this difference is likely due to different sample types of whole blood and plasma. As species are known to accumulate in red blood cells (RBCs) by binding to hemoglobin and the binding affinity varies among As species (Guo et al., 2021; Shen et al., 2013).

Distribution of As species in urine and blood

In the present study, we found that the distributions of iAs, MMA, DMA, and AsC were significantly different between blood and urine (Figure 2-1A). This may be linked to the rapid excretion of blood AsB into urine before metabolization (Brown et al., 1990). For iAs and other metabolites (i.e., %MMA, and %DMA), our results were supported by earlier studies: higher %iAs and %MMA and lower %DMA in blood relative to in urine (Bommarito et al., 2019a; Hall et al., 2007) (Figure 2-1B). One of the reasons for the relatively high %DMA in urine and high %iAs in the blood might be due to the rapid clearance of iAs in the body. It is known that the distribution of iAs, MMA, and DMA in the blood and tissues is higher for iAs and lower for DMA compared to urine (Currier et al., 2016; Kenyon et al., 2005). In addition, blood iAs may undergo additional methylation before renal excretion because iAs methylation through As methyltransferase (AS3MT) (Currier et al., 2016; Hughes et al., 2010) encoded by the *AS3MT* gene, which is abundant in the liver and kidney (Engstrom et al., 2011). Our observation indicates that blood As species might reflect the internal dose to target tissues and organs better than urine.

The present study shows lower %iAs and %MMA, and higher %DMA in

urine compared to blood (Figure 2-1B and Table S2-4). Comparable proportions of As species in urine or blood were reported in Spanish, Taiwan, and US populations with low-level exposure to iAs (Balakrishnan et al., 2018; Grau-Perez et al., 2017; Soler-Blasco et al., 2021; Su et al., 2012). However, they are distinguishable from Mexico and Bangladesh studies with higher-level exposure to iAs (Abuawad et al., 2021; Bommarito et al., 2019a). Medians were 12.4% for iAs, 14.8% for MMA, and 71.0% for DMA in urine while 23.7% for iAs, 27.5% for MMA, and 46.0% for DMA in blood among the Mexican population, which were similar to the Bangladeshi results. These proportions were lower than our values for iAs, MMA, higher in urine for DMA in urine, and higher for iAs and MMA in blood. To our speculation, these differences might depend on the magnitude of contamination of either iAs in the environment or oAs in seafood - low levels of iAs exposure via drinking water and high levels of oAs exposure via seafood for instance. It has been reported that urinary DMA might reflect the exposure to DMA itself and other oAs, such as arsenosugars and arsenolipids because some oAs can be metabolized into DMA in the human body (Choi et al., 2010; Schmeisser et al., 2006). Our study and (Soler-Blasco et al., 2021) calibrated iAs, MMA, and DMA concentrations to account for the As from seafood consumption, but the assumption might be valid for data from other populations with low-level exposure to iAs. Further studies are required to confirm this speculation.

According to a Bangladeshi study, high exposure to iAs can lead to negative effects on iAs metabolism by inhibition of the methyltransferases, where %DMA production was inhibited as iAs increased to approximately 50 μg/L As (sum of iAs, MMA, and DMA) in the urine (Lindberg et al., 2008). We do not have data to explain how blood and urine As species proportion changes with exposure to iAs through

inhibition of iAs methylation, and how these change upon significant exposure to oAs via seafood consumption. This should be elucidated by an experimental mechanism study.

Despite the lack of data on the mechanisms determining the distribution of As species in urine and blood, this study found that low-level exposure to iAs is better detected in blood than urine, suggesting that blood iAs could be used as an exposure biomarker under these circumstances. We also demonstrated that the sum of iAs, MMA, and DMA may not be an effective exposure biomarker in the general population when iAs levels in drinking water are low and oAs levels in seafood are high, which is supported by a U.S. study (Navas-Acien et al., 2011).

Table 2-7. Proportions (%) of As species in urine and blood among different studies [median (IQR)]

Country	Population	Age (yrs)	N	Urine			Blood			Reference
				%iAs	%MMA	%DMA	%iAs	%MMA	%DMA	
Korea	General population	1-98	1886	9.16 (5.33-13.8)	8.90 (5.74-12.7)	81.5 (73.5-88.2)	54.5 (43.8-66.6)	15.1 (0.00[a]-22.1)	27.4 (18.6-39.2)	This study
Spain		19+	1017	4.3 (4.0-4.5)	4.4 (4.2-4.6)	88.2 (87.6-88.7)	-	-	-	Soler-Blasco et al. (2021)
Taiwan		Elementary school students	202	4.49 ± 0.29	4.89 ± 0.33	90.2 ± 0.65	-	-	-	Su et al. (2012)
US		45-84	264	4.2 (2.0-6.7)	10.3 (6.5-14.9)	85.5 (79.2-89.8)	-	-	-	Balakrishnan et al. (2018)
US		<20	174	-	-	-	63.4 (51.2-74.0)[b]	63.4 (6.3-14.9)[b]	63.4 (18.6-33.5)[b]	Grau-Perez et al. (2017)
Bangladesh[c,d,e]	Population exposed to iAs via drinking water	20-65	527	14.0 ± 4.2/ 13.9 ± 4.7	14.9 ± 4.4/ 11.1 ± 3.7	71.1 ± 6.7/ 75.1 ± 6.6	27.0 ± 3.6/ 27.2 ± 4.2	44.8 ± 4.3/ 43.9 ± 4.5	28.1 ± 5.2/ 28.9 ± 5.0	Abuawad et al. (2021)
		20-65	342	17.2 ± 4.4/ 17.9 ± 6.2	16.0 ± 5.0/ 12.0 ± 4.3	66.8 ± 7.3/ 70.1 ± 8.0	29.5 ± 4.2/ 29.8 ± 4.4	40.5 ± 5.1/ 37.6 ± 5.7	30.0 ± 5.1/ 32.7 ± 5.7	
		14-16	708	13.7 ± 5.33/ 15.2 ± 9.98	11.9 ± 3.43/ 10.6 ± 3.53	74.4 ± 6.87/ 74.2 ± 10.1	-	-	-	
Bangladesh[f,g]		19+	1606 (visit 1)	8.5 (0, 24.0)	4.9 (0, 13.2)	85.7 (66.6, 100)	-	-	-	Gao et al. (2019)
			1445 (visit 2)	6.6 (0, 25.4)	4.8 (0, 11.6)	87.9 (67.3, 99.5)	-	-	-	
China[h]		18+	126 (hypertension)	8.51	14.8	72.4	-	-	-	Li et al. (2015)
			386 (non-hypertension)	7.59	12.6	76.2	-	-	-	
Mexico		5-88	232 (242)	12.4 (8.0)	14.8 (7.7)	71.0 (13.5)	23.7 (21.6)[b]	27.5 (9.1)[b]	46.0 (16.7)[b]	Bommarito et al. (2019a)

Note: %iAs, the proportion of inorganic arsenic; %MMA, the proportion of monomethylarsonic acid; %DMA, the proportion of dimethylarsinic acid

[a] Detection rate was <25%, [b] Measured in plasma, [c] Mean (SD), [d] Presented three different studies - FACT (n = 527), FOX (n = 342), and MANAS (n = 708), [e] Males/females, [f] Median (p5, p95), [g] Pregnant women, [h] Geometric mean

Determinants associated with As methylation efficiency

In the present study, age was the significant determinant of methylation efficiency after adjusting demographic characteristics and exposure sources that can affect As exposure (Figure 2-3 and Table S2-5). Adolescents showed the highest %iAs and %MMA and lowest %DMA among other age groups. Age is considered an important factor in iAs methylation efficiency, but the results are not consistent compared to the previous studies. According to a Bangladeshi study, conducted with populations highly exposed to iAs, children seemed to have better methylation efficiency than adults (Chowdhury et al., 2003); however, a recent meta-analysis found that children's methylation efficiency was lower than that of adults, which could be attributed to metabolic variations in the body of a growing child (Shen et al., 2016). Another Bangladeshi study found that %iAs and %MMA were positively associated with age in children and adolescents, whereas %DMA was adversely associated with age in children and adolescents (Lindberg et al., 2008). Sex was also related to iAs methylation efficiency in our population (Figure 2-3 and Table S2-5). Females had lower %iAs and higher %DMA than males, which indicates better methylation efficiency in females. It was consistent with those of other studies (Lindberg et al., 2008; Lindberg et al., 2007). The sex effect could be explained by the fact that iAs methylation is aided by one-carbon metabolism and is strongly reliant on choline, whose production is aided by estrogen (Shen et al., 2016). Furthermore, smoking was associated with a reduction in methylation efficiency (Figure 2-3 and Table S2-5) which is in agreement with previous studies (Shen et al., 2016; Tseng, 2009). Smoking has been linked to lower levels of nutrients such as vitamin B_{12} (methylcobalamin) and folate, which are linked to methylation

efficiency. Tobacco also contains substances that can interfere with methylation enzymes (Shen et al., 2016).

Strengths, limitations, and implications of the present study

This study has several limitations. First, the present study did not specify the valence between MMA and DMA compounds although many other studies do not determine their oxidation state because it is known that trivalent compounds (i.e., MMA(III), DMA(III)) can be easily oxidized in biological samples (Gong et al., 2001). Since the trivalent form of As is known to be more toxic than the pentavalent form, further study is needed to overcome these limitations. Second, the present study lacks information on some explanatory variables associated with As methylation, such as kidney function and nutritional status. Since the kidney is the target site of iAs methylation and is known to be the target organ of iAs, adjustment for kidney function could improve the strength of the analysis related to urinary As (Weidemann et al., 2015). In addition, methylation of iAs occurs through one-carbon metabolism, which is associated with various nutrients such as choline, folate, and vitamin B_{12} (Desai et al., 2020; Kurzius-Spencer et al., 2017). Since iAs methylation is a complex process and various factors are intercorrelated, the present study should be interpreted with caution. Finally, more detailed information on food consumption would be beneficial. The addition of detailed information on food intake could further inform the correlation between exposure amounts through food intake and As species levels in the body. Nevertheless, our study still has several strengths such as the analysis of As speciation in a large population covering almost all age groups and As speciation studies in urine-blood pairs.

We hypothesize that blood and urinary As species show different characteristics. Especially, higher %iAs in blood and %DMA in urine indicate blood might reflect tissue distribution of iAs and its metabolites better than urine, which indicates the potential biomarkers of internal dose. However, it seems that the levels of urinary iAs, MMA, %iAs, and %MMA could be explained by demographic and exposure factors rather than blood. This might be due to the rapid clearance of iAs in blood and low iAs exposure levels of our study population. Thus, urinary As species might be a useful biomarker of exposure and metabolism in our case. Most biomonitoring or epidemiological research still focused on populations that were highly exposed to iAs, however, more investigations should be conducted in low-exposed areas.

Chapter 3. Exposure assessment of cadmium, lead, mercury, and arsenic: Reverse dosimetry using biomonitoring data among the general population of Korea

3.1. Introduction

Metals are widely distributed in the environment. Metals including Cd, Pb, Hg, and As are consistently present in foods, dust, soil, air, water, cigarettes, and various consumer products and are exposed to general populations in their daily life (ATSDR, 2007, 2012, 2020, 2021; Tchounwou et al., 2012). Due to their high toxicity, these metals are a global public health problem and are recognized as risk factors for various adverse health outcomes, such as cardiovascular, renal, gastrointestinal, hematological, musculoskeletal, and hepatic effects (ATSDR, 2007, 2012, 2020, 2021) and also play as endocrine disrupting chemicals (Iavicoli et al., 2009).

Exposure assessment of metal is mainly performed using a scenario-based approach in which estimated daily intake (EDI) can be calculated using the equations obtained through the development of exposure scenarios. This approach is less expensive and time-consuming, however, it is an indirect estimation and has inherent uncertainties caused by a lack of information on source concentration and exposure factors (Husøy et al., 2020; Yoon et al., 2022).

Biomonitoring is a useful tool for estimating internal aggregated doses and can be utilized for health-based risk assessment (Meslin et al., 2022). Biomonitoring of metals is mostly conducted in both urine and blood (whole blood). Cd is measured

in both urine and blood, where urinary Cd (UCd) reflects long-term exposure and blood Cd (BCd) reflects relatively recent exposure (Suwazono et al., 2009; Vacchi-Suzzi et al., 2016). For Pb, blood Pb (BPb) is the most common biomarker of exposure (Sommar et al., 2014). Urinary Pb (UPb) is an alternative for biomonitoring of Pb exposure, but toxicokinetic (TK) information is insufficient compared to BPb (Barbosa et al., 2005). Hg is also measured in both urine and blood. Urinary Hg (UHg) is known to be composed of inorganic Hg (iHg), whereas blood Hg (BHg) contains both iHg and organic Hg (e.g., MeHg). As is mostly measured in urine and the concentration of total As or As species is used as an exposure biomarker.

However, the interpretation of biomarker concentration to assess human health risk is a challenging task because the values cannot compare with existing safe exposure limits (e.g., reference dose (RfD), reference concentration (RfC), tolerable daily intake (TDI), etc.). Therefore, “reverse dosimetry” approach was suggested to convert biomarker concentration (internal dose) into external dose (Tan et al., 2007). Physiologically-based toxicokinetic (PBTK; or physiologically-based pharmacokinetic, PBPK) model can be used as an effective tool to derive the relationship between internal and external doses and several models have already been developed for metals (Carrier et al., 2001a; Carrier et al., 2001b; Dede et al., 2018; El-Masri and Kenyon, 2008; Nordberg and Kjellström, 1979; O'Flaherty, 1993, 2000). But a small number of studies have performed the biomonitoring-based exposure assessment for metals using PBTK models (Abass et al., 2018; Lee et al., 2017b; Lin et al., 2020).

Therefore, the present study estimated the exposure amounts of metals using a biomonitoring-based (PBTK-based reverse dosimetry) approach and

compared the value with a scenario-based approach among the general population of Korea. The objectives of the present study were: i) to compare the biomonitoring-based and scenario-based approaches; ii) to discuss the strength and limitations of the two approaches in terms of exposure assessment of metals; iii) to suggest the area to improve uncertainties for a more accurate intake estimation of metals.

3.2. Methods

3.2.1. Study population and sample collection

The Korean Ministry of Food and Drug Safety (MFDS) collected biological samples for measuring hazardous chemicals in the general Korean population (aged 1-98 years) from 2017 to 2018. Almost 2,500 subjects were recruited considering their residential distribution of geographical area. The present analysis included a total of 2,100 available spot urine and/or whole blood samples. The details of the project and sample collection method have been described in section 2.2.1.

3.2.2. Chemical analysis

Cd, Pb, and Hg

For Cd, Pb, and Hg, sample preparation was performed as previously described with some modifications (NIER, 2008). Firstly, blood and urine samples were vortexed after thawing. For blood, 100 μL of the samples were transferred to polytetrafluoroethylene (PTFE) microwave tube. Then, 100 μL of nitric acid (HNO_3, 65%, ultrapure grade) and 100 μL of hydrogen peroxide (H_2O_2, 30%, ultrapure grade) were added and the samples were digested using a microwave digester ultraWAVE (Milestone, Sorisole, BG, Italy). The digested samples were diluted to 10 mL with 1% HNO_3 (including 500 μg/L Yttrium (Y)) and filtered through a syringe filter (Phenex-RC membrane, 0.2 μm, Phenomenex, Torrance, CA, USA) before injection. For urine, the thawed samples were centrifuged (6,000 ×*g*, 20 min), and 1000 μL of the supernatants were transferred into a PP tube. Then, the transferred sample was diluted to a range of 20-50 mL with a 1% HNO_3 (including 500 μg/L Y) and filtered through a syringe filter before injection.

The analytes were determined using a NexION® 2000 inductively coupled plasma mass spectrometer (PerkinElmer, Waltham, MA, USA). A radio frequency (RF) of 1.6 kW power was selected and the plasma gas flow was optimized at 15 L/min. The signal *m/z* 111 (Cd), 208 (Pb), 202 (Hg), and 89 (Y) was monitored. Analytical quality assurance was performed based on the protocol of United States Pharmacopeia (USP) chapters <232> with some modifications. Analytical quality assurance and quality control (QA/QC) was performed by analyzing the six replicates at the LOD level, triplicates, and blanks. Six replicates at LOD

concentrations and triplicates of four different concentrations of the analytes were evaluated for every batch by spiking analytical standards into urine and blood. The accuracy and precision (%RSD) of the validated method met the criteria within the range of 70-150% and within 20%, respectively. Background contamination was monitored using procedural, solvent, and matrix blanks for urine and blood analyses. The coefficients of determination (R^2) were > 0.999. The limits of detection (LOD) were 0.01, 0.05, and 0.01 μg/L for UCd, BPb, and BHg, respectively.

Inorganic As

The concentration of iAs was the sum of As(V) and As(III) in urine, which was analyzed using ultra-high-performance liquid chromatography with inductively coupled plasma mass spectrometry (UPLC-ICP-MS) equipped with a dynamic reaction cell (DRC). The detailed method of sample preparation, instrumental analysis, and QA/QC are described in section 2.2.2. and Choi et al. (2022). In brief, the thawed samples were centrifuged (6,000 ×*g*, 20 min), and 1000 μL of the supernatants were transferred into a PTFE tube. Then, the transferred sample was diluted to a range of 10-25 mL with a mobile phase and filtered through a syringe filter before injection. Oxygen (O_2, ≥ 99.99%) was used for DRC gas and the signal at *m/z* 91 (AsO) was monitored. Chromatographic separation was performed using a CAPCELL PAK® C18 MG II (4.6 mm i.d. × 250 mm, 5 μm, Shiseido Ltd., Tokyo, Japan), and the mobile phase was composed of 10 mM sodium 1-butane sulfonate, 4 mM malonic acid, 4 mM tetramethylammonium hydroxide pentahydrate, and 0.05% methanol and adjusted to pH 2.7 with 10% nitric acid. The limits of detection (LOD) of all analytes were 0.3 μg/L.

The urinary creatinine concentration was determined using an Enzymatic Roche Coba 8000 analyzer (Roche Diagnostics, Indianapolis, IN, USA).

3.2.3. PBTK models of the present study

We reviewed PBTK models for Cd, Pb, Hg, and iAs in previous literature and reports and selected suitable ones based on whether the model was reproducible, relevant to humans, included oral route, and used in other studies (Carrier et al., 2001a; Dede et al., 2018; El-Masri and Kenyon, 2008; MFDS, 2011). The structures of the selected models are shown in Figure 3-1. Model simulations were performed using Berkeley Madonna (version 8.3.23.0, University of California, Berkeley, USA).

Cd PBTK model

MFDS (2011) developed Cd PBTK model based on the model of Nordberg and Kjellström (1979), with the parameters optimized using physiological data from Koreans. The model describes blood (plasma, erythrocytes, and metallothionein), intestine, liver, kidney, and other tissue, and excretion via urine and feces (Figure 3-1A). The Cd PBTK model of this study is a life-stage model with age- and sex-specific physiological parameters. The model can simulate the accumulated Cd amounts in the kidney and urinary excretion of Cd in a lifetime. Most of the kinetic and physiological parameters were taken from the original models, except for the body weight (BW), logistic constants of growth function (L, K), and age adjustment factor (Wa_S). These parameters were obtained or optimized from the measurements of our study subjects. The used and modified parameters are presented in Table S3-1. Additionally, to evaluate the implemented models, we fitted the models with human data (Figure S3-1 and S3-2).

Pb PBTK model

Dede et al. (2018) developed Pb PBTK model by simplifying the bone compartment of the previous model (O'Flaherty, 1993, 1995, 2000). The model describes the GI tract, liver, kidney, blood, bone, well-perfused (WP) tissues, poorly-perfused (PP) tissues, and excretion via urine and bile (Figure 3-1C). Most of the kinetic and physiological parameters were taken from the original models, but BW and hematocrit ratio (HCT) differed depending on age and sex. The used and modified parameters are presented in Table S3-2 and the implemented model was evaluated using human data (Figure S3-3).

Hg PBTK model

Carrier et al. (2001a) developed Hg PBTK model, close to the multi-compartment model, describing the respective kinetics of MeHg and iHg following exposure to MeHg. The compartments of the model represent MeHg (left of Figure 3-1B) or iHg (right of Figure 3-1B) in an organ or a group of organs or excreta, such as the GI tract, liver, kidney, blood, brain, whole body burden of MeHg. Biotransformation of MeHg into iHg is also represented by the model. The kinetic and physiological parameters were taken from the original models except for BW (Table S3-3). The implemented model was evaluated using available human data (Figure S3-4).

iAs PBTK model

El-Masri and Kenyon (2008) developed the iAs PBTK model which can simulate the oxidation and reduction of arsenite (As(III)) and arsenate (As (V)), and metabolism into the monomethylarsonic acid (MMA) and dimethylarsinic acid

(DMA). The model consists of four submodels for As(III), As(V), MMA, and DMA. Each submodel includes the GI tract, liver, kidney, blood, muscle, brain, skin, heart, lung compartments, and urine as an excretion route (Figure 3-1D) and represents the intake of As species separately. The kinetic and physiological parameters were taken from the original models except for BW (Table S3-4). The implemented model was evaluated using available human data (Figure S3-5).

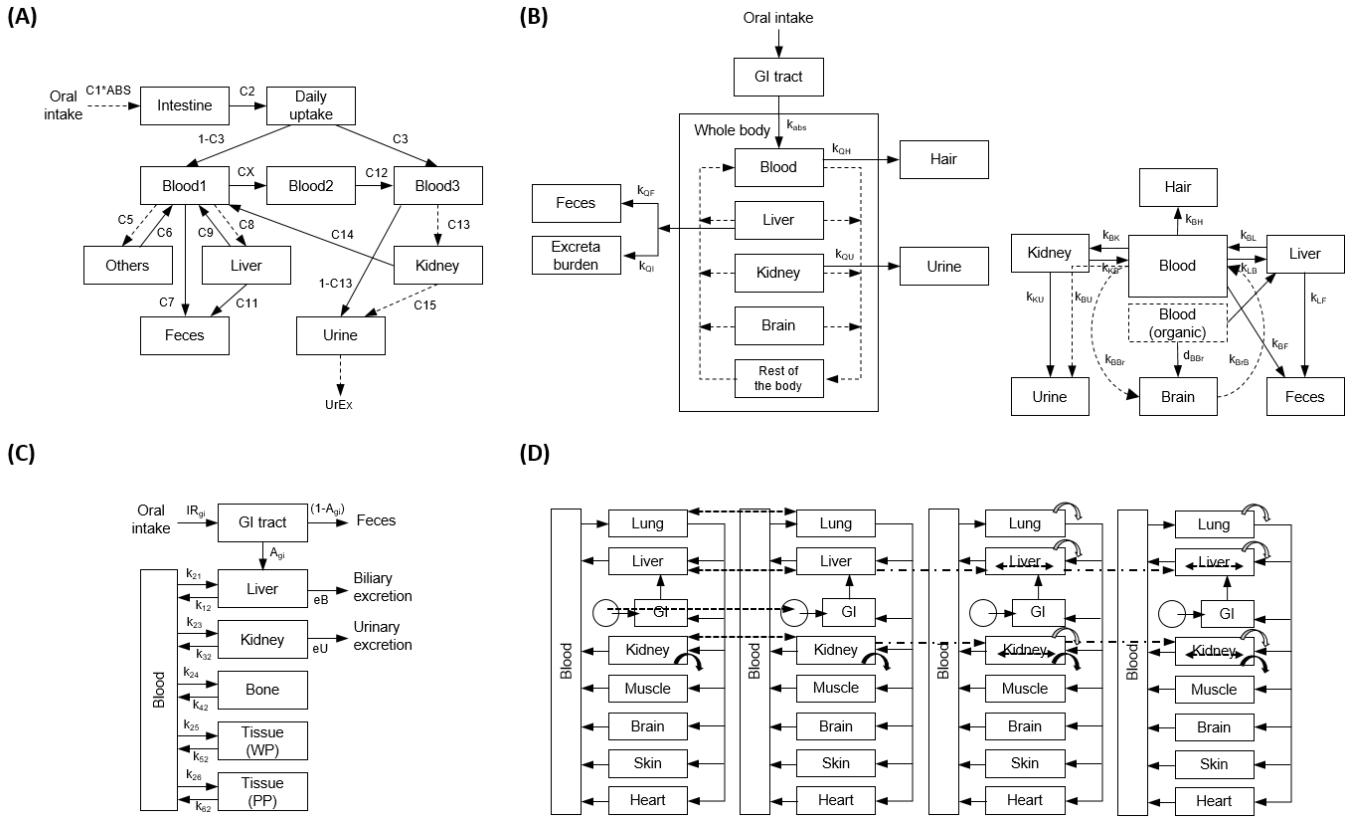

Figure 3-1. PBTK model structures of (A) Cd (MFDS, 2011), (B) MeHg (Carrier et al., 2001a), (C) Pb (Dede et al., 2018), and (D) iAs (El-Masri and Kenyon, 2008). Descriptions of model parameters are shown in Table S3-1 to S3-4.

3.2.4. Biomonitoring-based exposure assessment

Biomonitoring data

UCd, UiAs, BPb, and BHg concentrations were used as biomarkers of exposure that apply to the models. The concentrations below the limit of detection (LOD) were imputed as LOD/√2 for the calculation (Hornung and Reed, 1990b). UCd concentration was adjusted by urinary creatinine concentration as simulated in the model. For UiAs, both unadjusted and urinary creatinine-adjusted concentrations were considered to estimate the exposure amount.

For Hg, we estimated blood MeHg (BMeHg) concentrations from BHg concentrations to perform reverse dosimetry following the method described elsewhere (Lee et al., 2017b). The reason for this estimation is that the model reflects the demethylation of MeHg in the body (Carrier et al., 2001a) and BHg contains not only MeHg but also other Hg compounds. The estimation was performed using SAS 9.4 program and the results are shown in Table S3-5. For iAs, it was assumed that only As(III) or As(V) was ingested to perform reverse-dosimetry because the PBTK model simulates the intake of iAs by dividing it into As(III) and As(V) (El-Masri and Kenyon, 2008).

Reverse dosimetry

To convert the biomonitoring data into exposure amount, “exposure-concentration relationships (ECR)” of each metal for different age groups were obtained. The concept of ECR is similar to the reciprocal of the exposure conversion factor (ECF) proposed in previous studies (Tan et al., 2006). ECRs were computed as the

relationship between model input (exposure amount, μg/kg/day) and simulated biomarker concentration in urine or blood (μg/L) at steady-state (more than 5 times half-life). Only Cd could not apply this assumption because the half-life of UCd is extremely longer (> 10 years) than others. Therefore, the average of the UCd concentration by age belonging to each age group was used for the simulated biomarker concentration. We assumed that the exposures occurred through oral ingestion because the diet was the most contributing source of Cd, Pb, Hg, and As exposure (MFDS, 2020, 2021), and PBTK models used in this study can only reflect the oral route. Theoretical doses per exposure were applied to the model for each dosing interval which was set to 3 times per day or once a day or once a week. The exposure amount was calculated by dividing the biomarker concentration by the slope of ECR.

3.2.5. Scenario-based exposure assessment

Scenario-based exposure assessment was conducted to assess the aggregate exposure of different age groups (0-2 yrs, 3-6 yrs, 7-12 yrs, 13-18 yrs, 19-64 yrs, and 65+ yrs). Exposure assessment was conducted with a probabilistic approach. The median (p50) and 95th percentile (p95) were estimated from the distribution of the calculated data.

A series of exposure scenarios were considered to reflect different exposure sources and lifestyles of each age group. The exposure routes were ingestion (diet and non-diet (soil/dust)), inhalation (environment), and dermal contact (environment and consumer product). For the toddlers, we included oral exposure via hand-to-mouth and dermal exposure via toys in the exposure scenario. Metal concentrations were collected from the literature and reports of Korea (data not shown) (Choi et al., 2014; Ha et al., 2003; Hong et al., 2002; Jeong et al., 2008; Jeong et al., 2017; KFDA, 2002; Lee et al., 2013; Min, 2018; MoE, 2019; Oh et al., 2006; Son et al., 2008). For dietary exposure, a list of food items that compose more than 90% of food consumed by Koreans based on the 24-h dietary recall data of the Korea National Health and Nutrition Examination Survey (KNHANES) 2015-2016 and with a high content of heavy metals suggested by the Korea Ministry of Food and Drug Safety (MFDS) (MFDS, 2016b) were drawn up. Metal concentrations in the food items were obtained from MFDS (2016b).

Distributions of dietary ingestion rates were obtained from the KNHANES 2015-2016 24-h dietary recall data by age group. The distributions of exposure factors including body weight (BW) and inhalation rate were obtained from the data presented in the Korean exposure factors handbook (NIER, 2019) and the Korean

exposure factors handbook for children (NIER, 2016). Other factors, including exposure time, exposure frequency, ingestion rate (dust/soil, drinking water), skin adhesion amount (dust/soil), body surface area, etc. were collected from the literature and reports of Korea (data not shown) (MoE, 2019; NIER, 2009, 2016, 2019; Park et al., 2015; Park et al., 2017). Dermal absorption fraction and skin retention factor were obtained from foreign data (EPA, 2004; Health Canada, 2010; SCCS, 2016; Xu et al., 2017). If there were no distribution data for the exposure factors and concentrations, a fixed value was applied to the equation as in deterministic scenario-based exposure assessment.

The exposure amount was calculated using the exposure algorithm by exposure pathways and media (Table S3-6). The Monte Carlo (MC) simulation was performed to incorporate the variability and uncertainty of the model parameters. The MC simulation was set to 10,000 iterations using R (version 4.1.3). The estimated values were presented as EDI by body weight (μg/kg/day).

3.3. Results

3.3.1. Biomonitoring-based exposure assessment

The biomonitoring data used to perform the biomonitoring-based exposure assessment is summarized in Table 3-1. The concentrations of UCd adjusted to urinary creatinine concentration were found to be decreased from toddlers (0-2 yrs) to adolescents (13-18 yrs) and gradually increased in the elderly (65+ yrs), the highest among all age groups. The concentration of BPb was found to be the highest among the elderly while BHg was found to be the highest among adult (19-64 yrs) males. The concentrations of UiAs were the highest among toddlers.

The ECR charts of each metal by age group are shown in Figure S3-6 to S3-9. The slope of ECR was very similar for each dosing interval (i.e., three times per day, once a day, once a week) so the exposure was assumed to occur once a day for each metal (data not shown). The PBTK-based estimated daily intakes (EDI_{PBTK}) of each metal by age group are shown in Table 3-2. The estimated Cd exposure was the highest in toddlers (0.72 μg/kg/day for males and 0.66 μg/kg/day for females) and gradually decreased in adults, with an opposite trend of UCd concentrations. Males were more exposed to Cd than females in toddlers and young children (3-6 yrs), but the opposite trend was observed in adults and the elderly. The estimated Pb and MeHg exposure gradually increased with age. Meanwhile, for Pb, the p95 of the exposed amount which refers to the exposure level of a highly exposed population was the highest in children (7-12 yrs; 3.69 μg/kg/day). The estimated iAs exposure amounts were similar between values using UiAs concentration with different

dilution adjustment methods (i.e., unadjusted and urinary creatinine-adjusted concentrations; data not shown) so the estimates with unadjusted concentrations were presented in Table 3-2. The values were described as a range of values obtained by assuming that only As(III) or As(V) was ingested, gradually decreasing with age until adults, followed by a slight increase in the elderly.

To assess the potential health risk, the EDIs were compared with the tolerable daily intakes (TDI) proposed by the Korea Ministry of Food and Drug Safety (MFDS) (Table 3-2). For Cd exposure, all age groups did not exceed the TDI (0.83 μg/kg/day) based on p50 but p95 of the age groups including toddlers (male/female: 1.52/1.20 μg/kg/day), young children males (0.85 μg/kg/day), children (1.03/0.96 μg/kg/day), adolescents (1.02/0.86 μg/kg/day), and elderly (0.99/1.28 μg/kg/day) exceeded them. For Pb exposure, all age groups were found to exceed the BMDLs (benchmark dose lower confidence limit) (0.5 μg/kg/day for children and 0.63 μg/kg/day for adults). None of the age groups exceeded TDI of MFDS for MeHg (0.29 μg/kg/day) based on p50 and p95. For iAs, toddlers (0.44-1.75 μg/kg/day), young children (0.61-1.70 μg/kg/day), and p95 of children (0.74-1.35 μg/kg/day) were likely to exceed TDI of MFDS (1.29 μg/kg/day).

Table 3-1. Population characteristics and distribution of biomarker concentrations by age group [median (p95)]

	Dilution adjustment method	1-2 yrs	3-6 yrs	7-12 yrs	13-18 yrs	19-64 yrs (male)	19-64 yrs (female)	65+ yrs
N[a]		18 (-)	245 (-)	230 (100)	267 (100)	525 (149)	483 (142)	118 (109)
Body weight (kg, AM ± SD)		13.3 ± 1.9	20.5 ± 5.0	39.4 ± 11.8	61.3 ± 14.0	74.2 ± 10.8	58.9 ± 9.6	64.0 ± 10.4
Biomarker conc. (μg/L)								
UCd	Unadjusted	0.17 (0.43)	0.21 (0.57)	0.27 (0.61)	0.30 (0.70)	0.43 (1.44)	0.55 (1.91)	0.71 (2.77)
	Creatinine[b]	0.34 (0.70)	0.28 (0.69)	0.23 (0.43)	0.20 (0.38)	0.41 (1.11)	0.64 (1.97)	1.01 (2.95)
UiAs	Unadjusted	0.96 (3.24)	1.54 (3.95)	1.39 (3.74)	1.89 (5.50)	1.47 (4.19)	1.13 (3.75)	1.40 (6.71)
	Creatinine[b]	2.21 (5.00)	1.97 (4.88)	1.22 (2.95)	1.21 (3.11)	1.30 (3.61)	1.28 (4.24)	1.83 (5.72)
BPb[c]	-	-	-	1.84 (12.1)	1.71 (6.58)	3.15 (6.51)	2.71 (5.09)	3.34 (6.66)
BHg	-	-	-	2.55 (16.2)	2.75 (13.5)	6.28 (19.3)	4.49 (21.7)	4.86 (30.6)

* CAS, Covariate adjusted standardized method
[a] Urine data (blood data)
[b] Creatinine adjusted concentration ($\mu g/g_{crea}$)
[c] Unit: μg/dL

Table 3-2. Exposure estimates of metals by age groups using biomonitoring data (EDI_{PBTK}, μg/kg/day)

Metal	Statistics	1-2 yrs	3-6 yrs	7-12 yrs	13-18 yrs	19-64 yrs (male)	19-64 yrs (female)	65+ yrs	TDI of MFDS
Cd[a]	p50	0.72/0.66	0.32/0.32	0.40/0.40	0.47/0.34	0.21	0.23	0.28/0.41	0.83
	p95	1.52/1.20	0.85/0.75	1.03/0.96	1.02/0.86	0.75	0.83	0.99/1.28	
Pb	p50	-	-	0.56	0.50	0.85	0.81	0.97	0.50 (children)[b], 0.63 (adults)[c]
	p95	-	-	3.69	1.91	1.77	1.51	1.93	
MeHg	p50	-	-	0.023	0.029	0.053	0.048	0.051	0.29
	p95	-	-	0.113	0.174	0.240	0.197	0.197	
iAs	p50	0.44-1.75	0.61-1.70	0.28-0.50	0.29-0.44	0.10-0.14	0.09-0.14	0.11-0.17	1.29
	p95	1.48-5.90	1.57-4.35	0.74-1.35	0.85-1.28	0.29-0.41	0.30-0.45	0.53-0.81	

* TDI, tolerable daily intake; p50, median; p95, 95th percentile

[a] Male/female

[b] $BMDL_{01}$ (EFSA, 2010)

[c] $BMDL_{10}$ (EFSA, 2010)

3.2. Scenario-based exposure assessment

The results of the scenario-based exposure assessment are summarized in Table 3-3. The estimated Cd exposure was the highest in toddlers (0.332 μg/kg/day) and gradually decreased with age until adolescents (0.160 μg/kg/day), followed by increases in adults (0.185 μg/kg/day for males and 0.157 μg/kg/day for females) and elderly (0.144 μg/kg/day). The estimated Pb exposure was the highest in toddlers (0.451 μg/kg/day) and gradually decrease as age increased. The estimated Hg exposure was also the highest in toddlers (0.137 μg/kg/day) and the decreasing trend was observed as age increased. The estimated iAs exposure was the highest in young children (3.102 μg/kg/day) and the lowest in the elderly (1.187 μg/kg/day). The results of the deterministic approach were shown similar trends for Pb, Hg, and As, whereas the estimates of Cd exposure were found to be the highest in young children (0.463 μg/kg/day).

Dietary ingestion was the dominant source of all metals, followed by consumer products > environment > non-dietary ingestion for Cd, non-dietary ingestion > environment > consumer products for Pb, and environment > consumer products > non-dietary ingestion for As. For Hg, the contributions of other sources were negligible.

Table 3-3. Exposure estimates of metals using scenario-based exposure assessment approach (EDI_{SCN}, μg/kg/day)

Metal	Category	0-2 yrs		3-6 yrs		7-12 yrs		13-18 yrs		19-64 yrs (male)		19-64 yrs (female)		65+ yrs	
		p50	p95	p50	p95	p50	p95	p50	p95	p50	p95	p50	p95	p50	p95
Cd	Diet	0.276	0.946	0.281	1.469	0.237	1.972	0.157	0.979	0.179	1.261	0.151	1.021	0.143	0.733
	Non-diet[a]	0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Environment[b]	0.001	0.003	0.002	0.002	0.003	0.001	0.001	0.002	0.001	0.002	0.001	0.001	0.001	0.001
	Consumer products[b]	0.054	0.073	0.035	0.058	0.002	0.005	0.002	0.003	0.005	0.007	0.005	0.007	<0.001	<0.001
	EDI_{SCN}	**0.332**	**1.023**	**0.318**	**1.529**	**0.242**	**1.978**	**0.160**	**0.984**	**0.185**	**1.270**	**0.157**	**1.029**	**0.144**	**0.734**
Pb	Diet	0.268	0.870	0.258	0.797	0.189	0.786	0.122	0.385	0.156	0.521	0.151	0.496	0.131	0.466
	Non-diet[a]	0.136	0.183	0.087	0.146	0.038	0.098	0.054	0.087	0.009	0.014	0.012	0.018	0.011	0.018
	Environment[b]	0.046	0.061	0.031	0.051	0.017	0.046	0.014	0.022	0.026	0.038	0.022	0.033	0.016	0.023
	Consumer products[b]	0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	EDI_{SCN}	**0.451**	**1.115**	**0.376**	**0.994**	**0.244**	**0.930**	**0.190**	**0.494**	**0.191**	**0.573**	**0.185**	**0.547**	**0.158**	**0.507**
Hg	Diet	0.137	0.383	0.113	0.329	0.073	0.285	0.045	0.129	0.054	0.227	0.045	0.168	0.040	0.155
	Non-diet[a]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Environment[b]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	Consumer products[b]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	EDI_{SCN}	**0.137**	**0.383**	**0.113**	**0.329**	**0.073**	**0.285**	**0.045**	**0.129**	**0.054**	**0.227**	**0.045**	**0.168**	**0.040**	**0.155**
As	Diet	2.675	14.151	3.068	17.125	2.765	19.528	2.018	11.854	2.452	13.407	1.641	9.989	1.177	8.599
	Non-diet[a]	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Environment[b]	0.042	0.057	0.030	0.050	0.017	0.045	0.014	0.023	0.008	0.012	0.009	0.014	0.010	0.015
	Consumer products[b]	0.006	0.008	0.004	0.007	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.002	<0.001	<0.001
	EDI_{SCN}	**2.723**	**14.216**	**3.102**	**17.182**	**2.782**	**19.574**	**2.032**	**11.877**	**2.460**	**13.419**	**1.651**	**10.005**	**1.187**	**8.614**

* NA: Not available, [a] Dust/soil ingestion, drinking water, and hand-to-mouth exposure of toddlers (0-2 yrs); [b] Inhalation and dermal contact

3.3. Comparison of biomonitoring-based and scenario-based exposure assessment

In the case of Cd, $EDIs_{PBTK}$ were similar to $EDIs_{SCN}$ in young children and adults, whereas $EDIs_{PBTK}$ were 1.7-2.8 times higher than $EDIs_{SCN}$ in other age groups. Both approaches identified the toddlers as being the most highly exposed to Cd (EDI_{PBTK}: 0.72 and 0.66 μg/kg/day, EDI_{SCN}: 0.332 μg/kg/day). For Pb, $EDIs_{PBTK}$ were 2.3-6.1 times higher than $EDIs_{SCN}$ in all age groups and the difference was greater in adults and the elderly. For Hg and As, since reverse dosimetry PBTK modeling targeted specific compounds whereas the scenario-based exposure model was for total Hg and As, it was difficult to compare the two approaches directly.

3.4. Discussion

Biomonitoring is known as an indicator of internal dose and can provide directly related exposure levels of the target site or organ of environmental pollutants (Hays et al., 2007). In terms of risk assessment, exposure amounts are utilized by comparison to reference values (e.g., RfD, TDI, PTWI, etc.). However, biomarker concentration is difficult to compare with existing guidance values and needs to be converted into external exposure. The PBTK model can be used to reconstruct the exposure. The two approaches (i.e., reverse dosimetry PBTK modeling and scenario-based exposure modeling) were used to determine human exposure levels to four metals. The comparison among exposure estimates revealed several similarities and differences, which suggests the importance of considering the strengths and limitations of each approach.

The concentrations of biomarkers (i.e, UCd, BPb, BHg, and UiAs) used in this study (Table 3-1) were comparable to or higher than that of other Koreans. In detail, UCd concentrations (p50: 0.20-1.01 μg/g_{crea}) were similar to other Koreans (p50: 0.231-0.422 μg/L) but approximately two times higher than young children (p50: 0.091 μg/L) (Jung et al., 2022). BPb concentrations (p50: 1.71-3.34 μg/dL) were approximately two times higher than those of other Koreans (p50: 0.834-1.60 μg/dL; GM: 1.95 μg/dL for adults) (Jung et al., 2022; Park and Kim, 2021) but lower than the occupationally exposed populations (GM: 4.35 μg/dL) (Kim et al., 2014). BHg concentrations (p50: 2.55-6.28 μg/L) were higher than those of other Koreans (p50: 1.35-2.71 μg/L). The estimated BMeHg concentrations (GM: 3.61-3.98 μg/L, Table S3-5) were slightly lower than the measured concentrations of Korean adults

(GM: 4.44 μg/L) (Jung et al., 2013a) and higher than the estimated concentrations (GM: 2.16 μg/L) using the Korean National Environmental Health Survey 2009-2011 (KoNEHS I) data (Lee et al., 2017b). Considering the levels of our BHg measurements were also higher than those of Lee et al. (2017b) (GM: 3.08 μg/L), it is not surprising that these differences occur. UiAs concentrations (p50: 1.20-1.56 μg/L) were lower than Korean adults in a previous study (p50: 0.30 μg/L) (Lee et al., 2022).

The subjects of this study had higher levels of Pb and Hg (or MeHg) exposure than other Koreans, which can make higher estimates. Therefore, it needs to be taken into account when comparing EDI_{PBTK} with EDI_{SCN}. For Pb, $EDIs_{PBTK}$ were 2.3-6.1 times higher than EDI_{SCN} and exceeded the BMDL of MFDS in all age groups. Considering the higher BPb levels of this study, the exposure levels of the two approaches might be comparable in children and adolescents and EDI_{PBTK} does not exceed BMDL in all age groups based on p50.

For MeHg, a previous study estimated the exposure to MeHg using reverse dosimetry PBTK modeling in the general population of Korea (Lee et al., 2017b). EDI_{PBTK} of adults in this study (0.048-0.053 μg/kg/day) was higher than that of Lee et al. (2017b) (0.036 μg/kg/day), using KoNEHS I data, and it might be due to higher BHg levels of this study subjects. Meanwhile, several studies estimated exposure to MeHg in Koreans by considering only seafood intake. Similar intake amounts with this study (ranging from 0.048-0.053 μg/kg/day (3.27-3.86 μg/day)) were estimated in Choi et al. (2017) (0.048 μg/kg/day) and Kim et al. (2016) (3.72 μg/day), using KNHANES 2012-2014 data. Kim et al. (2016) also estimated total Hg intake by fish/shellfish intake and the values were slightly higher than MeHg intake,

suggesting that exposure to MeHg is mainly through fish/shellfish consumption.

$EDIs_{PBTK}$ for Cd (0.21-0.72 μg/kg/day, p50-based) did not exceed TDI in all age groups, which indicated that Cd exposure is safe in our study populations although p95 of most age groups exceeded it. In addition, $EDIs_{PBTK}$ declined with age, in contrast to UCd concentration, which is thought to be because UCd concentrations represent cumulative exposure to Cd over a long time. Compared with the scenario-based approach, $EDIs_{PBTK}$ were comparable to $EDIs_{SCN}$ in young children and adults, but $EDIs_{PBTK}$ were two times higher than $EDIs_{SCN}$ in other age groups. Moon (2022) estimated the average dietary intake amount of Korean adults aged 20-59 years old (15.25 μg/day), which was higher than our results (0.157 (male) and 0.185 (female) μg/kg/day; 11.6 and 10.9 μg/day assuming average BW). It might be because they also used the KNHANES 24-h recall data from 2005 to 2017, including older data than ours, and Cd exposure have been continuously decreasing based on BCd levels (Moon, 2022). There are limited studies estimating Cd exposure using PBTK model by reverse-dosimetry approach. Satarug et al. (2013) estimated Cd exposure amounts from diets in the Thai population (50-56 (male) and 21-27 (female) μg/day), which were much higher than our results (15.6 (male) and 13.5 (female) μg/day). They used the model of Kjellstrom and Nordberg (1978) modified by Choudhury et al. (2001) and Diamond et al. (2003) but they used the model parameters optimized using data from the U.S. population.

EDI_{PBTK} for iAs in toddlers, young children, and p95 of children were likely to exceed TDI of MFDS (1.29 μg/kg/day), which indicates a potential risk of the age group. For As, a direct comparison of EDI_{PBTK} and EDI_{SCN} was also impossible because EDI_{PBTK} represents the exposure to iAs whereas EDI_{SCN} reflects the

exposure to total As. The difference in the estimates between the two approaches might come from the organic As such as arsenobetaine, arsenocholine, and arsenosugars. Arsenobetaine is the predominant As species of urine in the Korean population and the major As species present in seafood (Choi et al., 2022; Taylor et al., 2017). It is also supported by the fact that the food item with the largest contribution of As exposure is seafood according to the scenario-based exposure assessment and previous literature (MFDS, 2016a; Seo et al., 2016). Intake of iAs is known to be mainly provided by grains in Korean adults (Seo et al., 2016) and the consumption of multi-grain rice was associated with UiAs increase in our previous study (Choi et al., 2022). Several studies estimated the iAs intake amounts through grains or rice consumption in Korean adults and values were comparable to this study (0.09-0.17 μg/kg/day, 5.3-10.6 μg/day). The estimated iAs intake from grains was 9.6 μg/day (Seo et al., 2016) and those from white rice and husked rice were 0.17 and 0.02 μg/kg/day, respectively (Lee et al., 2018). To our knowledge, there was one study of Taiwanese adults living in an industrial area using reverse dosimetry PBTK modeling(Lin et al., 2020), and their estimate (2.00 μg/kg/day) was much higher than ours.

There are several uncertainties inherent in biomonitoring-based exposure estimates that need to be considered when using reverse dosimetry PBTK modeling. First, for reverse-dosimetry, chronic daily exposure was assumed, implying a kind of steady-state in the body and short half-lives of some chemicals may cause uncertainty in estimated exposure (Moreau et al., 2017). However, Cd, Pb, and Hg (including MeHg) are known to have long or moderate half-lives in the body, and their biomarkers (i.e., UCd, BPb, BHg) represent long or moderate terms of exposure

(Table 1-1). Meanwhile, iAs and its biomarker (UiAs) have a short half-life of several days (Table 1-1), however, previous studies investigated that biomarkers used in this study including UiAs had high intra-class correlation coefficients (ICCs), indicating the measurement of spot sample is a good predictor of exposure. For UCd, ICC ranged from 0.46-0.75 in Belgium participants (Smolders et al., 2014) and 0.76-0.78 in U.S. cohorts (Vacchi-Suzzi et al., 2017). For BPb and BHg, ICCs were 0.81 and 0.71, respectively, in the Korean elderly (Lee et al., 2017a). For UiAs, ICCs were 0.81 in Italians (Lovreglio et al., 2012) and 0.88 in U.S. participants (Rivera-Núñez et al., 2010).

Another uncertainty might arise from the assumption of reverse dosimetry that the exposures have occurred through oral ingestion. One reason for this is that the PBTK models used in this study can only represent the oral route. Given that food intake is the most contributing source of exposure to Cd, Pb, Hg, and As (MFDS, 2020, 2021), this assumption might be justified. However, Pb can also be exposed not only through ingestion but also through inhalation and/or dermal contact. In scenario-based exposure assessment, exposure contribution levels through inhalation and/or dermal contact for Pb (ranging from 7.0% to 13.6%) were higher than for other metals (approximately 1% or less) (Table S3-7). This may lead to an overestimation of EDI_{PBTK} when exposures via other routes are calculated as the oral equivalent doses because the absorption fraction could be varied depending on exposure routes. For example, Pb in submicron size can be almost completely absorbed through the respiratory tract, whereas only 3-50% of an oral dose of water-soluble Pb is absorbed in the human body (ATSDR, 2020). Accordingly, the forward-dosimetry approach needs to be performed, converting exposure amount into

biomonitoring concentration, using PBTK model with multiple routes to quantify the contribution level of unrecognized exposure routes or sources in internal dose.

Other uncertainty might occur to the characteristics of PBTK models. The models used in this study were for adults, except for Cd model. In addition, only body weight and some physiological parameters (e.g, renal function and fraction of hemoglobin) were considered for age- and sex-dependent variability although many parameters of the model can vary with age and sex (Clewell et al., 2004; Clewell et al., 2002). Besides, the used Pb model (Dede et al., 2018) could not reflect the detailed kinetics of Pb in bone. Pb is known to accumulate in mineralized tissues (e.g., bone and teeth) and can be eliminated from bone to blood long after exposure (Rădulescu and Lundgren, 2019). Therefore, the decrease in bone density due to aging and menopause in females also affects the BPb concentration (O'Flaherty, 2000; Symanski and Hertz-Picciotto, 1995). Therefore, future studies are needed by using a more elaborated model.

For scenario-based exposure estimates, lacking exposure algorithms, factors, and media concentrations can cause uncertainties in estimation. Scenario-based exposure assessment is an indirect estimation method that relies on an exposure scenario of receptors to target chemicals. Based on the exposure scenario, exposure algorithms, factors, and media concentrations are required to quantify exposure amounts. Metals including Cd, Pb, Hg, and As ubiquitously exist in the built and natural environment and can be found in building materials, household products, consumer products, and children's products. Therefore, it is difficult to estimate the exposure in all possible sources due to the lack of exposure algorithms, factors, and media concentrations. In this study, there were differences between

estimated Cd exposure amounts of the two approaches in infants, children, adolescents, and the elderly, implying the uncertainty of the scenario-based approach. Some previous studies also compared biomonitoring-based and scenario-based approaches and the estimates of the two methods were similar or the biomonitoring-based estimates were higher than scenario-based estimates. A study for Swedish adults has shown that estimate based on BHg levels was slightly higher (0.050 μg/kg/day) than those based on the food frequency questionnaire (FFQ) data (0.043 μg/kg/day) (Abass et al., 2018). Since there was a lack of Hg speciation data in food and blood, Abass et al. (2018) assumed MeHg/iHg ratio in food for model input and estimated Hg intake using multi-compartment two different MeHg and iHg TK models. To our knowledge, more studies comparing the exposure levels using both approaches have been performed on some phthalates. Some of them found that estimates using the biomonitoring-based approach were higher than the scenario-based approach (Lee et al., 2014; Moreau et al., 2017). Others found that the estimates of the two approaches were comparable (Cao et al., 2016; Yoon et al., 2022) but biomonitoring-based estimates were higher than the scenario-based estimates in children under 6 years old (Yoon et al., 2022). Yoon et al. (2022) mentioned that the reason for the difference in the exposure amount using the two approaches is a lack of information on exposure, especially in the young aged population.

In the scenario-based approach, aggregated exposure amounts were calculated to compare with the biomonitoring-based estimates because biomarker concentration reflects an integration of exposure from all routes and sources. However, the estimates of Hg and As were hard to be compared directly because the biomonitoring-based estimates were for MeHg and iAs, whereas scenario-based

estimates were for total Hg and total As. Hg and As are a group of compounds, and each species has different toxicity, but it is not easy to obtain speciated concentrations of all possible sources of exposure for the scenario-based exposure assessment. Therefore, previous studies estimated the exposure amount of MeHg and iAs through specific exposure sources such as fish and rice (Choi et al., 2017; Kim et al., 2016; Lee et al., 2018; Seo et al., 2016). In biomonitoring, we could predict the BMeHg concentration because there was data on the BMeHg/BHg fraction (Jung et al., 2013a) and could measure the concentrations of urinary As species in Koreans. In terms of risk assessment, it is necessary to distinguish highly toxic forms, MeHg and iAs. It implies that the biomonitoring-based approach might be more useful when the scenario-based approach is concerned with high uncertainty due to a lack of information.

In summary, integrated exposures to Cd, Pb, Hg, and As were estimated by biomonitoring-based exposure assessment with PBTK modeling and scenario-based exposure assessment with probabilistic exposure model for the general population of Korea, including toddlers to the elderly. By comparing the two different estimation approaches, their applicability and utility were evaluated. Biomonitoring-based approach can reflect internal exposure and biomonitoring data of metals (UCd, BPb, BHg, and UiAs) seem to reflect exposure well given their high ICC; however, the characteristics of the PBTK model and exposure via exposure routes other than oral ingestion might increase the uncertainty of the estimates. Meanwhile, scenario-based approach can be useful in risk management because the contribution level of each exposure source can be compared; however, the uncertainty of the estimates may increase as the information (exposure scenarios, algorithms, factors, and source

concentrations) is incomplete. Therefore, it is necessary to understand the inherent limitations of each method concerning its purposes of use, and areas of uncertainty discussed in this study need to be improved to perform a more elaborate exposure assessment. In future studies, our method can be applied to other chemicals. Furthermore, the relationship between external dose and internal dose can be explored by simulating the contribution level of various exposure scenarios to internal doses.

Chapter 4. Association between co-exposure to metals and blood pressure among the general population of Korea

4.1. Introduction

High blood pressure (BP) is the most important modifiable risk factor and the biggest contributor to cardiovascular diseases (CVDs). As systolic and diastolic blood pressure (SBP, DBP) increases, the risk of death from heart disease and cerebrovascular disease also increases. CVDs are the leading cause of death worldwide. In Korea, the mortality of CVDs has increased over the past decade and is the second leading cause of death (Lee et al., 2021; Shin et al., 2019). Moreover, the absolute number of people with hypertension increased steadily and exceeded 12.0 million although the average BP has modestly decreased (Kim et al., 2022).

Environmental exposure to metals has been known as one of the contributors to elevated BP. Several epidemiological studies have reported the association between BP and metals like As, Cd, Pb, and Hg, respectively (Zhao et al., 2021). These toxic metals can affect BP through biological mechanisms, such as oxidative stress, inflammation, vasoconstriction, renal tubular dysfunction, and so on (da Cunha Martins et al., 2018; Houston, 2011; Paithankar et al., 2021; Pinheiro Junior et al., 2020; Tinkov et al., 2018; Vaziri, 2008). Since the exposure sources to those metals ubiquitously exist in our daily life, including air, water, soil, dust, food, and consumer product, co-exposure to more than one metal is common (Yim et al., 2022). Once those metals are absorbed into the body, they may interact with each

other and not function independently. Therefore, it is important to investigate the interaction and joint effect of the metal mixture on common health outcomes. Several studies have reported the potential effect of the metal mixture on elevated BP (Bulka et al., 2019; Castiello et al., 2020; Howe et al., 2021; Kim and Park, 2022; Kupsco et al., 2019; Liu et al., 2021; Qu et al., 2022; Shih et al., 2021; Wang et al., 2021).

Nevertheless, studies on the association between metal exposure in non-adult populations and their BP are still limited. Elevated BP during childhood and adolescence is a precursor of hypertension and CVDs in adulthood (Yang et al., 2020). However, most of the studies have focused on adults or prenatal exposure of children in mother-children pairs (Howe et al., 2021; Kupsco et al., 2019). Few studies have examined the effect of metal exposure in childhood and adolescence on BP (Castiello et al., 2020; Desai et al., 2021; Yao et al., 2020).

Furthermore, many studies frequently use total As concentration as an exposure biomarker of As (Desai et al., 2021) although it largely reflects the exposure to organic arsenic species (e.g., arsenobetaine) via seafood consumption (Choi et al., 2022). Therefore, the speciation of inorganic arsenic (iAs), a toxicant form of the arsenic compound, is critical for linking As exposure and health outcomes.

The objectives of this study are (i) to investigate the association between metal (Cd, Pb, Hg, and iAs) exposure and BP, and (ii) to explore the joint effect and interaction of the metal mixture on BP in different age groups. For the analysis, we measured all metals in both urine and blood and study subjects included children and adolescents.

4.2. Methods

4.2.1. Study population and data collection

The Korean Ministry of Food and Drug Safety (MFDS) collected biological samples for measuring hazardous chemicals in the general Korean population (aged 1-98 years) from 2017 to 2018. Almost 2,500 subjects were recruited considering their residential distribution of geographical area. The present analysis included a total of 2,100 available spot urine and/or whole blood samples. The details of the project and sample collection method have been described previously (Choi et al., 2022). The survey was conducted in such a way that the participants filled out the questionnaire by themselves and the researcher inspected it. For children under the age of 10, their mother or primary caretaker responded to the questionnaire on their behalf. Information on demographic characteristics, socioeconomic status, lifestyle, and medical history were collected. For the analysis, we excluded participants with missing data on blood pressure and taking hypertension medication. We also excluded those with missing data on covariates and urinary creatinine concentration. Finally, 1,502 participants (450 urine and 186 blood for children and adolescents, 902 urine and 280 blood for adults) were left for the analysis (Figure 1).

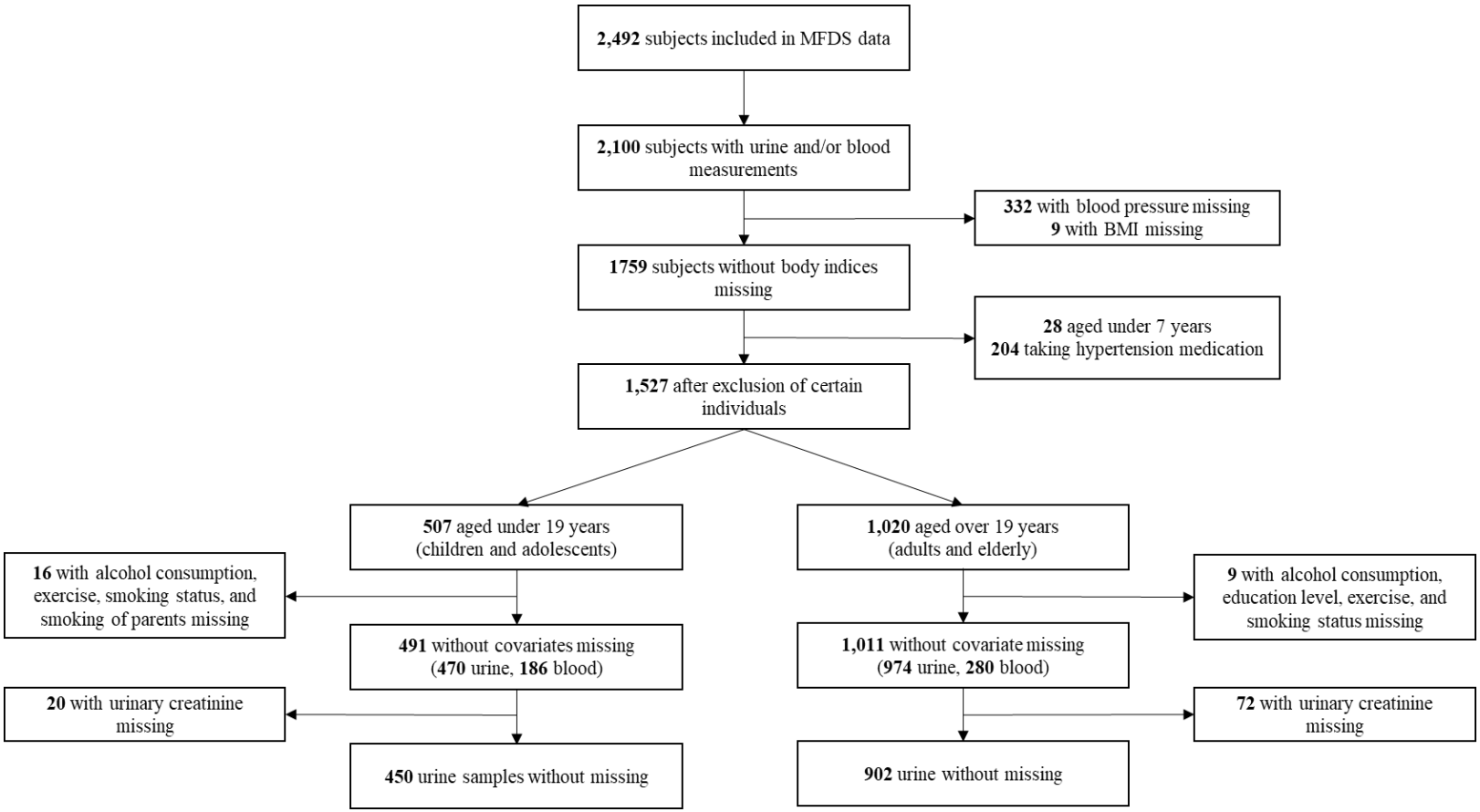

Figure 4-1. Flowchart of subjects included in our final analysis.

4.2.2. Blood pressure

Participants over 6 years old measured their BP according to the following procedures. Before the measurement, they were asked to rest for at least 5 minutes and checked whether they were taking blood pressure medication. If the SBP/DBP values were 120/80 mmHg or higher, they were measured again after 2-3 minutes. In this case, the remeasured data were used for the analysis.

4.2.3. Measurement of metals in urine and blood

All target metals were measured in both urine and blood. For the exposure biomarker of iAs, both iAs and the sum of iAs and monomethylarsonic acid (MMA) concentrations (iAs+MMA) were used for the analysis as in previous studies (Hata et al., 2016; Yoshinaga and Narukawa, 2020; Yusa et al., 2018). The sum of iAs, MMA, and dimethylarsinic acid (DMA) concentrations is generally used as a biomarker of iAs exposure. However, DMA concentration is sometimes excluded considering exposure characteristics of the populations because it is associated with seafood consumption.

Cd, Pb, and Hg

Sample preparation was performed as previously described with some modifications (NIER, 2008), which is resented in section 3.2.2. In brief, 100 μL of the blood samples were transferred to polytetrafluoroethylene (PTFE) microwave tube. Then, 100 μL of nitric acid (HNO_3, 65%, ultrapure grade) and 100 μL of hydrogen peroxide (H_2O_2, 30%, ultrapure grade) were added and the samples were digested using a microwave digester ultraWAVE (Milestone, Sorisole, BG, Italy). The digested samples were diluted to 10 mL with 1% HNO_3 (including 500 μg/L Yttrium (Y)) and filtered through a syringe filter (Phenex-RC membrane, 0.2 μm, Phenomenex, Torrance, CA, USA) before injection. For urine, the thawed samples were centrifuged (6,000 ×*g*, 20 min), and 1000 μL of the supernatants were transferred into a PP tube. Then, the transferred sample was diluted to a range of 20-50 mL with

a 1% HNO_3 (including 500 μg/L Y) and filtered through a syringe filter before injection.

All analytes were determined using a NexION® 2000 inductively coupled plasma mass spectrometer (PerkinElmer, Waltham, MA, USA). A radio frequency (RF) of 1.6 kW power was selected and the plasma gas flow was optimized at 15 L/min. The signal *m/z* 111 (Cd), 208 (Pb), 202 (Hg), and 89 (Y) was monitored. Analytical quality assurance was performed based on the protocol of United States Pharmacopeia (USP) chapters <232> with some modifications. Analytical quality assurance and quality control (QA/QC) was performed by analyzing the six replicates at the LOD level, triplicates, and blanks. Six replicates at LOD concentrations and triplicates of four different concentrations of the analytes were evaluated for every batch by spiking analytical standards into urine and blood. The accuracy and precision (%RSD) of the validated method met the criteria within the range of 70-150% and within 20%, respectively. Background contamination was monitored using procedural, solvent, and matrix blanks for urine and blood analyses. The coefficients of determination (R^2) were > 0.999. The limits of detection (LOD) were 0.01, 0.05, and 0.01 μg/L for urinary metals (Pb, Cd, Hg), blood Pb, and other blood metals (Cd, Hg), respectively.

Inorganic As

We analyzed As species rather than total As. Due to the high seafood consumption rate, the total As in our population mainly reflects organic As compounds, which are not toxic (Choi et al., 2022). The As species in urine (UiAs and UMMA) and blood (BiAs and BMMA) samples were determined using an ACQUITY UPLC H-Class

PLUS (Waters, Milford, MA, USA) coupled to a NexION® 2000 inductively coupled plasma mass spectrometer (PerkinElmer, Waltham, MA, USA) equipped with a dynamic reaction cell (DRC). The detailed method of sample preparation, instrumental analysis, and QA/QC are described in section 2.2.2. and Choi et al. (2022). In brief, 200 μL of blood samples were diluted to a range of 10-25 mL with a mobile phase. Then, the samples were centrifuged at 6,000 ×g for 20 min and filtered through a syringe filter before injection. For urine, the thawed samples were centrifuged (6,000 ×g, 20 min), and 1000 μL of the supernatants were transferred into a PTFE tube. Then, the transferred sample was diluted to a range of 10-25 mL with a mobile phase and filtered through a syringe filter before injection. Oxygen (O_2, ≥ 99.99%) was used for DRC gas and the signal at m/z 91 (AsO) was monitored. Chromatographic separation was performed using a CAPCELL PAK® C18 MG II (4.6 mm i.d. × 250 mm, 5 μm, Shiseido Ltd., Tokyo, Japan), and the mobile phase was composed of 10 mM sodium 1-butane sulfonate, 4 mM malonic acid, 4 mM tetramethylammonium hydroxide pentahydrate, and 0.05% methanol and adjusted to pH 2.7 with 10% nitric acid. The limits of detection (LOD) of all analytes were 0.3 μg/L in urine and 0.17 μg/L in blood.

The creatinine in the urine was determined using an Enzymatic Roche Coba 8000 analyzer (Roche Diagnostics, Indianapolis, IN, USA).

4.2.4. Covariates

Covariates were selected from the available variables, based on the previous studies, by using a directed acyclic graph (DAG). We used DAGitty (version 3.0, www.dagitty.net, (Textor et al., 2011)) to illustrate DAG and identify the minimally sufficient adjustment set of variables. As a result, the following covariates were included to adjust for confounding in all models: age (continuous), alcohol consumption (never or seldom vs. < 2/month vs. 2-4/month vs. ≥ 2/week, only for adults (≥ 19 yrs)), body mass index (BMI, continuous), exercise status (yes/no), sex, smoking status (non-smoker vs. former smoker vs. smoker, only for ≥ 19 yrs), and smoking status of parents (non-smoker vs. smoker, only for non-adults (< 19 yrs)). In non-adults, alcohol consumption and smoking status were excluded from the model because most of the subjects responded as never drinking and non-smoker. Pearson correlation analysis was conducted to assess the correlation among log-transformed metal concentrations in blood and urine.

4.2.5. Statistical analysis

The measurements below LOD were replaced with a value equal to the LOD divided by a square root of 2 (Hornung and Reed, 1990a). Due to the right skewness of the distribution, all measurements were log-transformed and centered. Then, these measurements were mean-centered for further analysis. For urinary metals, we used the covariate-adjusted standardized (CAS) method to handle the urinary creatinine may operate as a collider on the causal pathway between the exposure and outcome (O'Brien et al., 2016), of which the detailed method is described in section 2.2.3. In addition, a conventional creatinine-adjustment method was used to compare the results. We stratified the subjects by age group [non-adults (< 19 yrs) and adults (≥ 19 yrs)] and the analysis was performed separately by urinary and blood metal groups. All analyses were performed with R (version 4.1.3) and the statistical significance was set at $p < 0.05$.

Multiple linear regression (MLR) model was conducted to assess the associations between log-transformed urinary and blood metal concentrations and BP (SBP and DBP). Firstly, the effects of individual metals on BP were assessed by including one metal concentration in the model, adjusted by the covariates mentioned above. Next, multiple metal concentrations were included in the covariate-adjusted models to investigate the associations between concentrations of four metals (Cd, Pb, Hg, iAs) and BP measurements. For non-adults, to consider the exposure and physiological differences, interactions by age were assessed by including interaction terms between each log-transformed and centered metal concentration and age into the model because age is an important factor of internal

exposure to metal and blood pressure increase. These interaction models were also adjusted for the same covariates as the main analysis and the statistical significance was set at $p < 0.05$. Then, to investigate potential heterogeneities in the age group, stratified linear regression analysis was also performed by dividing non-adults into children (7-12 yrs) and adolescents (13-18 yrs). The analysis was conducted only for urine data due to the small sample size of blood data (n = 84 for children and n = 79 for adolescents).

Environmental mixtures are known to have potential non-linear and non-additive relationships with health outcomes (Bobb et al., 2015), although the aforementioned regression models assume linearity and additive effects. Therefore, we conducted Bayesian kernel machine regression (BKMR) analysis to investigate the non-linear association, allowing for interactions among metals and the joint effect of metal mixture. Models were run at 10,000 Monte Carlo iterations with a Markov chain. The R package "bkmr" was used for the analysis.

To confirm the joint effect of the metal mixture and identify the contribution of each metal in two different media, we analyzed weighted quantile sum (WQS) regression. The WQS index, weighted sums of each metal concentration, was calculated to represent the relative importance of the whole burden of metals in each media. The models were adjusted for the same set of covariates as MLR and BKMR models. The R package "gWQS" was used for WQS analysis.

4.3. Results

4.3.1. Population characteristics

Characteristics of the study population are shown in Table 4-1. A total of 1,502 subjects participated in this study and were comprised of 1,011 adults and 491 non-adults. For adults, the average age was 42.5 years, BMI was 24.0 kg/m^2, and blood pressure (SBP/DBP) was 125.2/75.0 mmHg. Most of them had a college degree or above (71.8%) and were non-smokers (69.6%). The average age of the non-adults was 12.7 years, BMI was 20.8 kg/m^2, and blood pressure was 115.6/66.9 mmHg. Most of them responded to never or seldom drinking (83.1%). The characteristics and BP of the study population according to the biological media are presented in Appendix Table S4-1 and S4-2.

Table 4-1. Characteristics of the study population (n = 1,502)

Characteristics	Adults (≥ 19 yrs) N = 1,011	Non-adults (< 19 yrs) N = 491	*p*-value
Continuous variables (AM ± SD)			
Age (year)	42.5 ± 14.3	12.7 ± 2.9	-
Body mass index (BMI, kg/m^2)	24.0 ± 3.4	20.8 ± 4.4	< 0.001
Systolic blood pressure (mmHg)	125.2 ± 17.5	115.6 ± 14.8	< 0.001
Diastolic blood pressure (mmHg)	75.0 ± 11.6	66.9 ± 10.3	< 0.001
Categorical variables (n (%))			
Sex			0.874
Male	496 (49.1)	238 (48.5)	
Female	515 (50.9)	253 (51.5)	
Alcohol consumption			< 0.001
Never or seldom	197 (19.5)	408 (83.1)	
< 2/month	277 (27.4)	51 (10.4)	
2-4/month	300 (29.7)	27 (5.5)	
≥ 2/week	237 (23.4)	5 (1.0)	
Exercise			0.002
Yes	434 (42.9)	253 (51.5)	
No	577 (57.1)	238 (48.5)	
Smoking status			-
Non-smoker	704 (69.6)	-	
Former smoker	149 (14.7)	-	
Smoker	158 (15.6)	-	
Smoking status of parents			-
Non-smoker	-	268 (54.6)	
Smoker	-	223 (45.4)	

4.3.2. Metal concentrations in urine and blood

Distributions of urinary and blood metal concentrations are shown in Table 4-2. For urine, adults had higher UCd and UPb than non-adults (Unadjusted GM: 0.46 vs. 0.27 and 1.62 vs. 1.23 μg/L for UCd and UPb, respectively), whereas UiAs concentration was higher in the younger group (1.23 vs. 1.56 μg/L). UHg concentration was higher in non-adults than in adults (0.50 vs. 0.58 μg/L) but the differences were not statistically significant (p = 0.728). For blood, all metal concentrations of adults were higher than that of non-adults but the differences were not statistically significant for BiAs (p = 0.729). Detection rates of all metals were greater than 85% in both urine and blood.

The correlations between urinary and blood metals were shown in Figure 4-2. Most of the metals had positive correlations with each other, although the correlations were weak to moderate – the correlation coefficients (|r|) ranged from 0.01 to 0.65. BPb and BCd had the strongest correlation (r = 0.65, p < 0.001) in non-adults.

Table 4-2. Distribution of metals in urine and blood (μg/L)

Biomarkers	Dilution adjustment	Adults			Non-adults			*p*-value[a]
		N	GM	Median (Q1-Q3)	N	GM	Median (Q1-Q3)	
UCd	Unadjusted	974	0.46	0.48 (0.27-0.86)	470	0.27	0.29 (0.20-0.42)	< 0.001
	Creatinine[b]	902	0.48	0.49 (0.29-0.83)	450	0.21	0.22 (0.16-0.29)	
	CAS	902	0.48	0.49 (0.31-0.77)	450	0.27	0.27 (0.20-0.36)	
UPb	Unadjusted	974	1.62	1.69 (1.07-2.64)	470	1.23	1.38 (0.84-2.12)	< 0.001
	Creatinine[b]	902	1.64	1.58 (0.95-2.69)	450	0.95	0.96 (0.57-1.75)	
	CAS	902	1.65	1.55 (1.01-2.68)	450	1.19	1.25 (0.73-2.24)	
UHg	Unadjusted	974	0.50	0.92 (0.24-1.86)	470	0.58	1.02 (0.35-1.94)	0.728
	Creatinine[b]	902	0.50	0.83 (0.23-1.88)	450	0.43	0.77 (0.21-1.66)	
	CAS	902	0.50	0.81 (0.22-2.02)	450	0.54	0.96 (0.30-1.97)	
UiAs	Unadjusted	974	1.23	1.32 (0.76-2.04)	470	1.56	1.32 (0.76-2.04)	< 0.001
	Creatinine[b]	902	1.27	1.29 (0.80-2.03)	450	1.20	1.24 (0.83-1.85)	
	CAS	902	1.28	1.31 (2.02-3.95)	450	1.51	1.53 (1.04-2.26)	
BCd	-	280	1.04	1.02 (0.71-1.49)	186	0.51	0.48 (0.34-0.64)	< 0.001
BPb[c]	-		2.95	2.98 (2.10-4.05)		2.23	1.81 (1.40-2.95)	< 0.001
BHg	-		4.02	5.37 (2.96-9.19)		1.41	2.70 (1.09-4.62)	< 0.001
BiAs	-		1.10	1.19 (1.64-2.29)		1.08	1.11 (1.46-2.24)	0.729

* CAS, covariate-adjusted standardized; GM, geometric mean; Q1, quartile 1 (25^{th} percentile); Q3, quartile 3 (75^{th} percentile)
[a] *p*-values were obtained from the Student's t-test, [b] Unit: $\mu g/g_{crea}$, [c] Unit: μg/dL

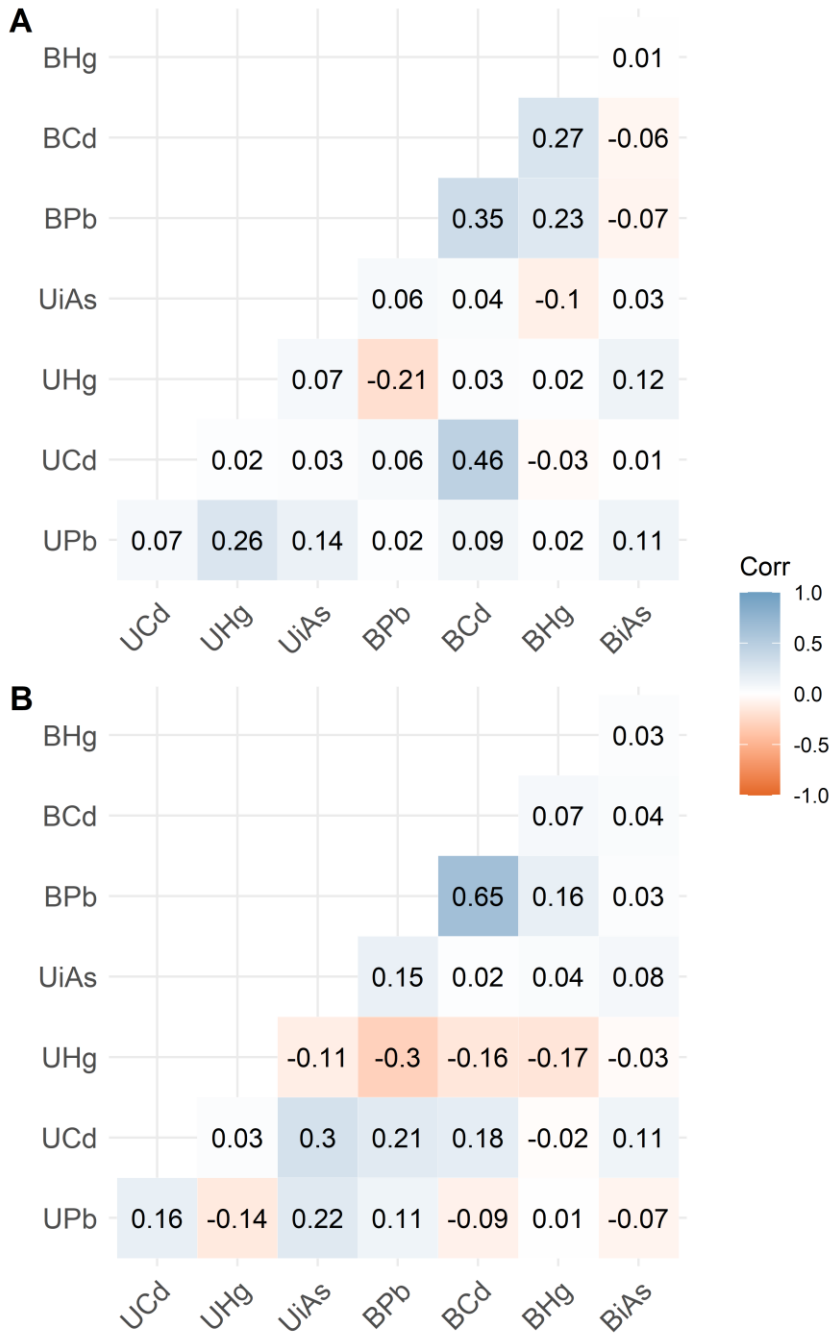

Figure 4-2. Pearson correlations among urinary and blood concentrations of four metals in (A) adults (aged ≥ 19 years old) and (B) non-adults (aged < 19 years old).

4.3.3. Associations between metal exposure and blood pressure from multiple linear models

The conventional MLR model was used to estimate the associations between individual metals and BP measurements (Table 4-3). UPb concentration was associated with increased BP in all age groups. In detail, the adults had an association of UPb concentration and SBP increase [β (95% CI): 1.18 (0.02, 2.34) mmHg] while non-adults had associations of UPb and SBP [β (95% CI): 1.14 (0.09, 2.20) mmHg] and DBP [β (95% CI): 1.06 (0.20, 1.91) mmHg]. In non-adults, UCd and BHg concentrations were also associated with SBP [β (95% CI): 2.56 (0.17, 4.95) mmHg for UCd and 0.92 (0.04, 1.80) mmHg for BHg] and/or DBP [β (95% CI): 2.17 (0.23, 4.11) mmHg for UCd].

Table 4-3. Differences in blood pressure for the increase in levels of each metal of adults and non-adults from covariate-adjusted multiple metal models

Biomarker	SBP (mmHg) β (95% CI)	*p*-value	DBP (mmHg) β (95% CI)	*p*-value
Adults (≥ 19 yrs)				
Log UCd	0.77 (-0.86, 2.40)	0.355	0.35 (-0.78, 1.49)	0.544
Log UPb	**1.18 (0.02, 2.34)**	**0.046**	-0.03 (-0.84, 0.78)	0.942
Log UHg	0.27 (-0.21, 0.74)	0.740	0.23 (-0.10, 0.56)	0.167
Log UiAs	0.69 (-0.66, 2.05)	0.315	0.16 (-0.79, 1.10)	0.745
Log BCd	2.67 (-1.85, 7.18)	0.246	0.11 (-2.68, 2.91)	0.936
Log BPb	-0.26 (-5.05, 4.54)	0.916	-0.34 (-3.30, 2.62)	0.822
Log BHg	0.17 (-1.18, 1.51)	0.810	0.20 (-0.63, 1.03)	0.640
Log BiAs	1.66 (-1.87, 5.19)	0.355	-0.36 (-2.54, 1.83)	0.748
Non-adults (< 19 yrs)				
Log UCd	**2.56 (0.17, 4.95)**	**0.036**	**2.17 (0.23, 4.11)**	**0.029**
Log UPb	**1.14 (0.09, 2.20)**	**0.034**	**1.06 (0.20, 1.91)**	**0.016**
Log UHg	0.05 (-0.42, 0.52)	0.842	0.35 (0.03, 0.73)	0.074
Log UiAs	1.35 (-0.50, 3.19)	0.153	0.64 (-0.86, 2.15)	0.401
Log BCd	0.47 (-2.20, 3.14)	0.729	0.33 (-1.68, 2.33)	0.748
Log BPb	1.08 (-1.54, 3.70)	0.418	1.12 (-0.85, 3.08)	0.264
Log BHg	**0.92 (0.04, 1.80)**	**0.040**	0.51 (-0.15, 1.17)	0.132
Log BiAs	0.20 (-2.95, 3.35)	0.900	-2.29 (-4.64, 0.06)	0.557

Note: Models were adjusted to age, sex, alcohol consumption, BMI, exercise, and smoking status for adults and age, sex, BMI, exercise, and smoking of parents for non-adults. "Log" refers to natural log-transformation of biomarker concentration. Each metal predicts each blood pressure measurement (SBP and DBP) separately. Bolded values represent statistical significance ($p < 0.05$).

To investigate the associations between multiple metals with BP, we analyzed MLR by including all metals in the model (Table 4-4). The results were similar to the model of individual metals (Table 4-3), in which UPb concentration was marginally or significantly associated with increased BP in all age groups. In detail, UPb concentration had marginal associations with SBP in all age groups and a significant association with DBP in non-adults [β (95% CI): 0.99 (0.12, 1.85) mmHg]. In non-adults, UCd concentration was positively associated with DBP [β (95% CI): 2.05 (0.03, 4.05)] and BHg concentration was marginally significant with SBP increase. For urinary metals, the results were comparable regardless of the urinary dilution adjustment method (i.e., CAS and urinary creatinine adjustment) (Table S4-3).

In addition, the results showed generally similar patterns between the models that used iAs (Table 4-3 and 4-4) and iAs+MMA (Table S4-4) concentrations, except for the multiple metal model of blood metal group in non-adults. BiAs+MMA showed an association between iAs exposure and DBP decrease [β (95% CI): -2.47 (-4.81, -0.13) mmHg] (Table S4-4), whereas BiAs resulted in null associations (Table 4-4).

Table 4-4. Differences in blood pressure for the increase in levels of metals of adults and non-adults from the covariate-adjusted multiple metal models

Biomarker	SBP (mmHg) β (95% CI)	*p*-value	DBP (mmHg) β (95% CI)	*p*-value
Adults (≥ 19 yrs)				
Urinary metal group				
Log UCd	0.55 (-1.09, 2.19)	0.510	0.29 (-0.85, 1.44)	0.616
Log UPb	1.03 (-0.13, 2.23)	0.088	-0.16 (-0.99, 0.67)	0.702
Log UHg	0.17 (-0.31, 0.65)	0.483	0.23 (-0.10, 0.57)	0.171
Log UiAs	0.56 (-0.45, 2.64)	0.423	0.14 (-0.81, 1.09)	0.775
Blood metal group				
Log BCd	3.37 (-1.68, 8.43)	0.190	0.12 (-3.02, 3.26)	0.941
Log BPb	-1.45 (-6.72, 3.82)	0.589	-0.55 (-3.83, 2.72)	0.740
Log BHg	-0.05 (-1.48, 1.37)	0.942	0.22 (-0.66, 1.11)	0.622
Log BiAs	0.54 (-3.06, 5.89)	0.223	0.29 (-1.96, 2.55)	0.799
Non-adults (< 19 yrs)				
Urinary metal group				
Log UCd	2.25 (-0.23, 4.73)	0.075	**2.04 (0.03, 4.05)**	**0.047**
Log UPb	1.01 (-0.05, 2.08)	0.063	**0.99 (0.12, 1.85)**	**0.026**
Log UHg	-0.20 (-0.76, 0.36)	0.482	-0.11 (-0.56, 0.35)	0.649
Log UiAs	0.60 (-1.33, 2.53)	0.541	-0.03 (-1.60, 1.53)	0.969
Blood metal group				
Log BCd	-0.37 (-3.92, 3.19)	0.840	-0.59 (-3.25, 2.06)	0.660
Log BPb	0.91 (-2.62, 4.45)	0.610	1.27 (-1.37, 3.91)	0.343
Log BHg	0.88 (-0.02, 1.77)	0.054	0.53 (-0.15, 1.20)	0.165
Log BiAs	0.16 (-2.99, 3.30)	0.922	-2.29 (-4.64, 0.06)	0.056

Note: Models were adjusted to age, sex, alcohol consumption, BMI, exercise, and smoking status for adults and age, sex, BMI, exercise, and smoking of parents for non-adults. All metals were included together in the models and analyzed for urine and blood respectively. "Log" refers to natural log-transformation of biomarker concentration. Each metal predicts each blood pressure measurement (SBP and DBP) separately. Bolded values represent statistical significance ($p < 0.05$).

Interactive effects between metal exposure and age are shown in Table S4-5. Only UPb had a marginally significant interaction with age for SBP (p = 0.089). In the stratified linear regression model, UPb showed a marginally significant association with SBP for adolescents (β = 1.44, p = 0.085), with approximately 2 times higher estimates than children (β = 0.66, p = 0.350). Both UPb and UCd concentrations had significant positive associations with DBP in non-adults overall (Table 4-3 and 4-4); however, the association between UPb and DBP consisted only in children [β (95% CI): 1.87 (0.52, 3.22)] (Table 4-5). UCd was associated with both SBP [β (95% CI): 4.31 (0.51, 8.11)] and DBP [β (95% CI): 3.52 (0.89, 6.15)] only in adolescents (Table 4-5).

Table 4-5. Differences in blood pressure for the increase in levels of metals in children (n = 211) and adolescents (n = 239) from covariate-adjusted multiple metal models

Biomarker (μg/L)	Children (7-12 yrs)				Adolescents (13-18 yrs)			
	SBP (mmHg) β (95% CI)	*p*-value	DBP (mmHg) β (95% CI)	*p*-value	SBP (mmHg) β (95% CI)	*p*-value	DBP (mmHg) β (95% CI)	*p*-value
Log UCd	0.59 (-2.96, 4.14)	0.745	2.27 (-1.20, 5.74)	0.198	**4.31 (0.51, 8.11)**	**0.026**	**3.52 (0.89, 6.15)**	**0.009**
Log UPb	0.66 (-0.72, 2.04)	0.350	**1.87 (0.52, 3.22)**	**0.007**	1.44 (-0.20, 3.09)	0.085	0.59 (-0.55, 1.73)	0.310
Log UHg	-0.04 (-0.79, 0.71)	0.922	-0.22 (-0.95, 0.51)	0.558	-0.36 (-1.20, 0.48)	0.402	-0.14 (-0.72, 0.44)	0.640
Log UiAs	1.70 (-1.26, 4.65)	0.259	-1.27 (-4.16, 1.62)	0.387	0.30 (-3.15, 3.76)	0.862	1.10 (-1.29, 3.49)	0.365

Note: Models were adjusted to age, sex, BMI, exercise, and smoking of parents and all metals are included together in the models. "Log" refers to natural log-transformation of biomarker concentration. Each metal predicts each blood pressure measurement (SBP and DBP) separately. Bolded values represent statistical significance ($p < 0.05$).

4.3.4. Associations between metal exposure and blood pressure from mixture models

We analyzed BKMR to assess the joint effect of the metals on BP and the interactions between metals in the mixture. For urinary metals, there was significant or suggestive evidence of BP increase in all age groups (Figure 4-3). In adults, UPb had marginal effects on SBP increase when other metals were at the 75^{th} percentile (Figure 4-4A). In non-adults, UPb showed significant effects on SBP and DBP increase when other metals were at the 50^{th} percentile and 75^{th} percentile (Figure 4-4C and 4-4D). Those results imply the possible interaction among metals, however, interactions were not confirmed in the bivariate exposure-response function (Figure S4-5 and S4-6). Additionally, UPb had significant dose-response relationships with BP and indicated non-linear associations with SBP in all age groups (Figure 4-5). For blood metals, there were no significant associations between BP and metals mixture (Figure S4-2 to S4-4).

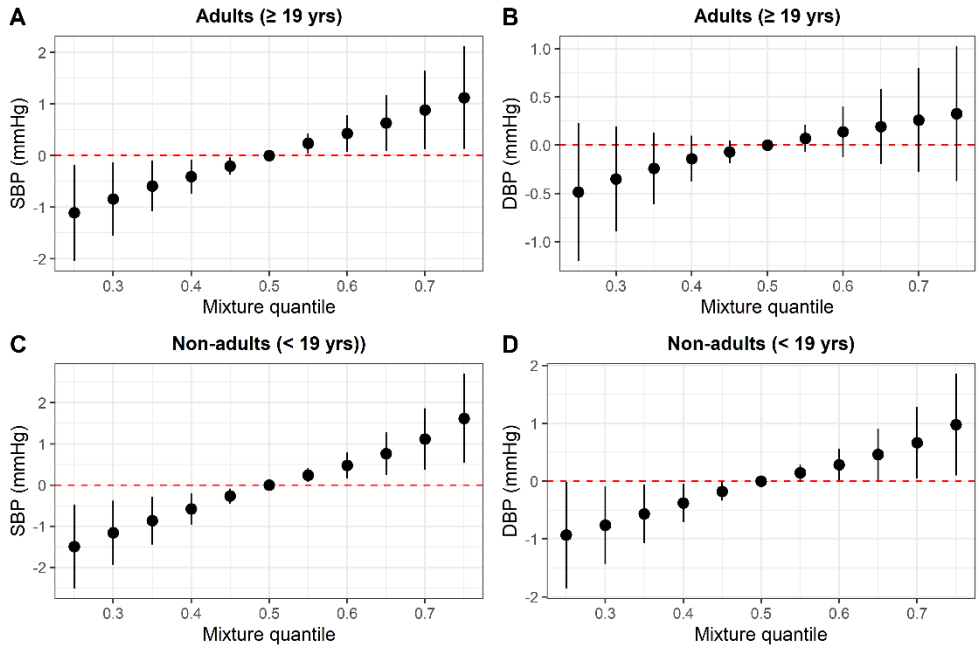

Figure 4-3. Overall effect (95% CI) of the metal mixture on BP when all urinary metals at a particular percentile were compared to all metals at the 50th percentile. The results were obtained by BKMR analysis stratified by age group [adults (A, B) and non-adults (C, D)] and adjusted to age, sex, alcohol consumption, BMI, exercise, and smoking status for adults and age, sex, BMI, exercise, and smoking of parents for non-adults.

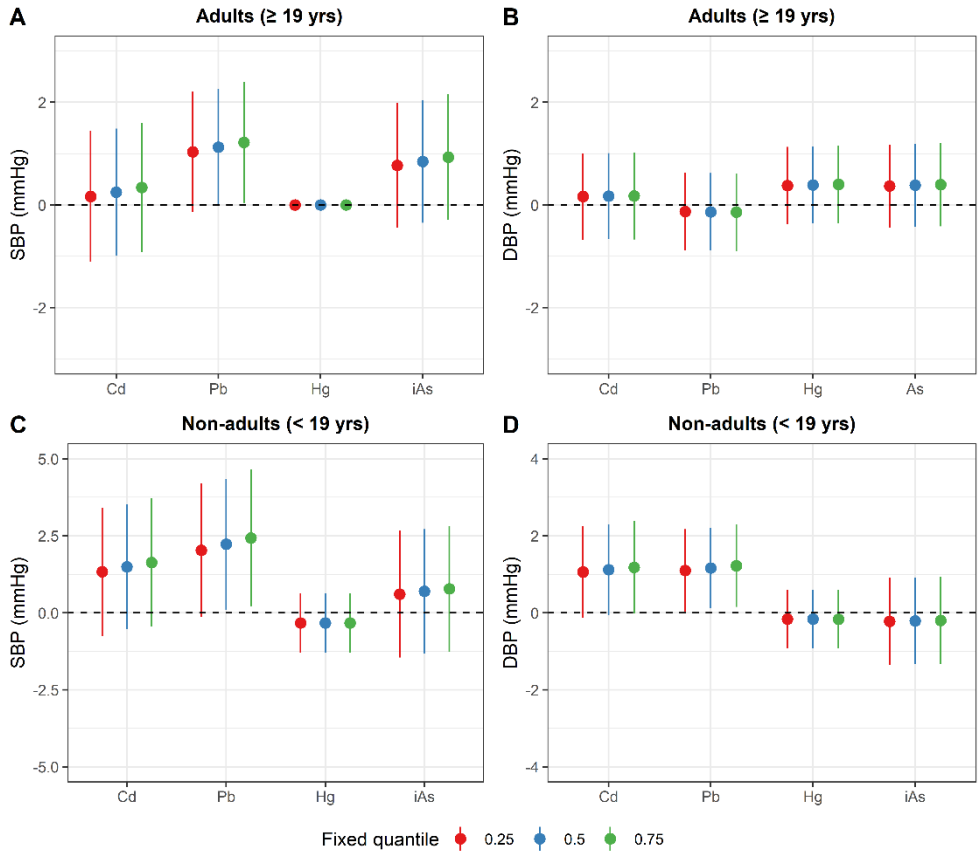

Figure 4-4. Associations of single urinary metals with BP were estimated while other urinary metals were fixed at their 25th (red), 50th (blue), and 75th (green) percentile, respectively. The results were obtained by BKMR analysis stratified by age group [adults (A, B) and non-adults (C, D)] and adjusted to age, sex, alcohol consumption, BMI, exercise, and smoking status for adults and age, sex, BMI, exercise, and smoking of parents for non-adults.

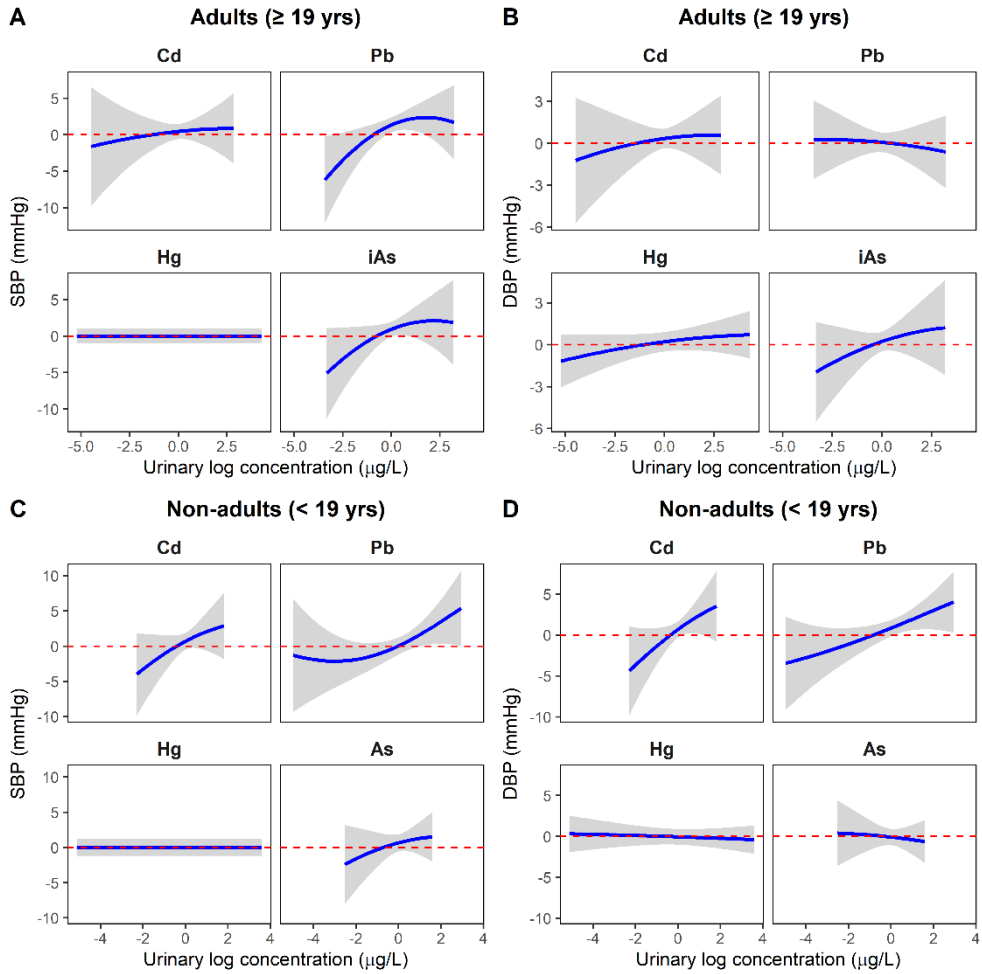

Figure 4-5. Single metal exposure-response relationship (95% CI) between each urinary metal and blood pressure while other urinary metals fixed at their 50th percentile. The results were obtained by BKMR analysis stratified by age group [adults (A, B) and non-adults (C, D)] and adjusted to age, sex, alcohol consumption, BMI, exercise, and smoking status for adults and age, sex, BMI, exercise, and smoking of parents for non-adults.

We adopted WQS to assess the association between the metal mixture and BP and identify the relative contribution of each metal (Figure 4-6). In adults, WQS indices were not significantly associated with BP in both urine and blood. In non-adults, WQS indices of urinary metals were significantly associated with increased SBP [β (95% CI): 1.10 (0.31, 1.90)]. The highest weighted urinary metal in the model was Pb (34.2%), followed by Cd (29.6%), iAs (28.2%), and Hg (8.0%).

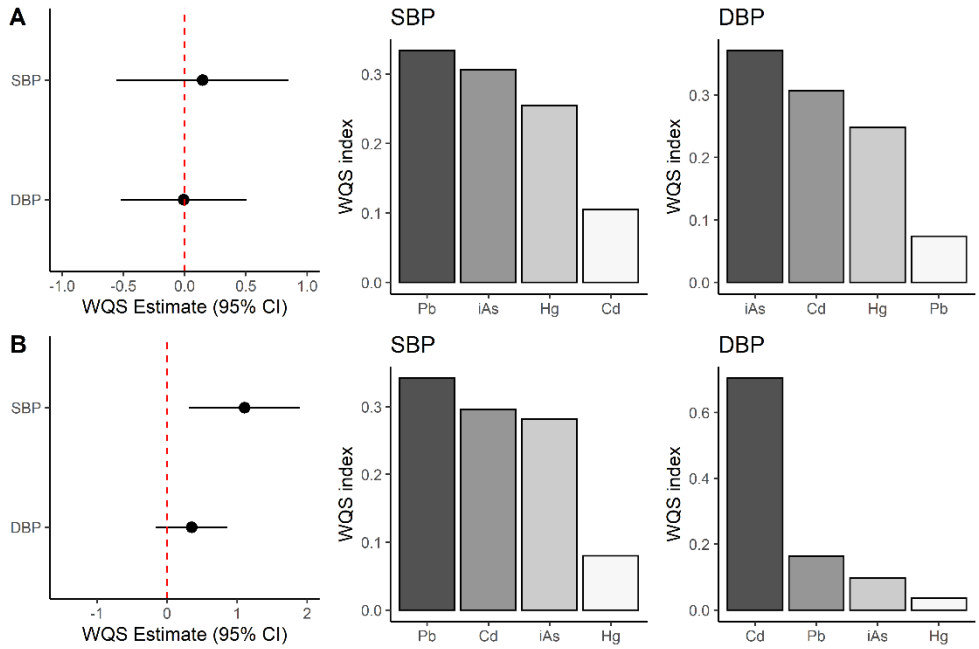

Figure 4-6. Mixture effect of urinary metals and contribution of each metal in (A) adults and (B) non-adults. In the forest plot, the point represents the estimate and the error bar represents the 95% CI of WQS estimates. Bar plot represents the WQS index of urinary metals for BP. Models were adjusted to age, sex, alcohol consumption, BMI, exercise, and smoking status for adults and age, sex, BMI, exercise, and smoking of parents for non-adults.

4.4. Discussion

In the real world, people are simultaneously exposed to various metals. Recently, several studies have focused on the joint effect of metal mixtures by using novel statistical methods to investigate the association with BP and hypertension (Yim et al., 2022). Most of them targeted adults whereas few studies focused on young populations. Therefore, the present study investigated the impact of metal exposure on BP in the general population of Korea including children and adolescents. We measured four toxic metals (Cd, Pb, Hg, and iAs) in both urine and blood, and analyzed a conventional multiple linear regression model and two mixture modeling approaches. A previous study has recommended using different methods and interpreting them together to get a more reliable conclusion (Zhang et al., 2019).

It was found that the metal mixture had a positive association with BP in all age groups and the result was more evident in non-adults. In adults, we could not find any significant effect of metals on BP in MLR analysis when adjusting for other metal concentrations. In mixture modeling approaches, the result of BKMR analysis showed a significant positive association between the metal mixture and SBP (Figure 4-3A), however, WQS regression was not significant (Figure 4-6A). In the case of DBP, there was an increasing trend as exposure increased but the result was not statistically significant (Figure 4-3B). Meanwhile, in non-adults, the positive associations between the metal mixture and BP were confirmed with the two mixture modeling approaches (Figure 4 and Figure 6) although the metal concentrations in this group were significantly lower than in adults (Table 2), indicating that non-adults may be more susceptible to metal exposure.

Several studies explore the association between co-exposure to metals (including Cd, Pb, Hg, or As) and BP and/or hypertension. Most of them found significant associations, but some did not (Everson et al., 2021; Kim and Park, 2022; Qu et al., 2022; Wang et al., 2021; Xu et al., 2021). Kim and Park (2022) evaluated the effects of three metals (BCd, BPb, BHg) on BP and hypertension among Korean adults using both logistic regression and WQS regression. They found all metals had associations with BP and the prevalence of hypertension, but the results were null for hypertension when adjusting the concentrations of other metals in the model. In addition, they showed a significant effect of metal mixture on BP increase, with Pb being the most predominant. Qu et al. (2022) explored the effects of mixtures of 13 metals (including Pb, As, Cd, and Hg) on BP. Using WQS regression, they found that metal mixture levels in blood were significantly associated with elevated SBP and DBP, with the largest contributor being Pb. BPb and BAs levels were associated with increased odds of pre-hypertension and/or hypertension, however, the results of other metals were null. Wang et al. (2021) investigated the association of UAs, UCd, UHg, and UPb with longitudinal changes in BP among midlife women using linear mixed-effect models. They found that all metals were associated with increased SBP and DBP, with comparing those with high concentrations to low concentrations. Meanwhile, Xu et al. (2021) evaluated the association between metal mixtures (including BCd and BPb) and BP using quantile-based g-computation but associations were not clear between metal levels and BP. Taken together, Pb was a common contributing metal among metal mixtures concerning the increase in BP.

For non-adults, previous studies investigated the association between the metal mixture and BP but the results were inconsistent (Castiello et al., 2020; Desai

et al., 2021; Yao et al., 2020). Desai et al. (2021) used BPb, BHg, UCd, and UAs (total As) concentrations and BP measurements of children and adolescents (aged 8-17 years) from the National Health and Nutrition Examination Survey (NHANES) data. There was a significant inverse association between the mixture of four metals in urine and DBP, but not other BP measures using BKMR analysis. Yao et al. (2020) also evaluated associations between metals (Cd, Pb, Hg, MeHg) and BP among children and adolescents (aged 8-17 years) using NHANES data. They performed multivariable linear and logistic regression analyses. There was no significant association between UPb and BP, whereas BPb was negatively associated with DBP among blacks, and positively associated with DBP among whites. BHg and UCd were inversely associated with DBP in all subjects and men. Castiello et al. (2020) used concentrations of seven metals (including As, Cd, Hg, and Pb) in urine and BP measurements from Spanish male adolescents (aged 15-17 years) and assessed the associations using linear regression. They found that both UAs and UCd concentrations were associated with increased SBP and the number of the detected metals was associated with increased BP.

Of the four toxic metals included, Pb was the most consistently associated with elevated BP in both adults and non-adults. The mechanisms linking Pb exposure and BP increase have been explored in the *in vitro* and animal *in vivo* studies, including oxidative stress, impaired nitric oxide (NO) system, inflammation, dysregulation of vasoactive hormones, alteration of cellular calcium ion (Ca^{2+}) transport and intercellular distribution of Ca^{2+}, and so on (Boskabady et al., 2018; Vaziri, 2008). In addition, various epidemiological studies have demonstrated that Pb has positive associations with BP (Navas-Acien et al., 2007; Prozialeck et al.,

2008). In linear regression models, UPb showed a statistically significant association with DBP in non-adults. Furthermore, the association between UPb and DBP differed between subgroups (children vs. adolescents), which was statistically significant in children. Additionally, according to the WQS index, Pb was the most or second largest contributor to metal mixtures (Figure 6), which was consistent with previous studies that suggested Pb was a significant contributor to BP increase in Koreans and Chinese (Kim et al., 2022; Qu et al., 2022). Our results were consistent with previous studies conducted in adults, which suggested positive associations between UPb and BP although these studies targeted adults (Mizuno et al., 2021; Wang et al., 2021). Moreover, UPb showed both consistent and inconsistent results in BKMR and conventional linear regressions. BKMR yielded significant associations with SBP in adults (Figure 4-5A) and both SBP and DBP in non-adults (Figure 4-5C and 4-5D), with consideration of metal co-exposure; however, only DBP had a statistically significant association with UPb in non-adults from linear regression models (Table 4-3 and 4-4). It might be because the association between UPb and DBP was relatively linear (Figure 4-5D) and no interaction was found, whereas UPb and SBP had a non-linear association (Figure 4-5A and 4-5C).

We found associations between Pb exposure and BP increase but the results were statistically significant when we assessed Pb exposure from urine. BPb is the most widely measured biomarker of exposure and reflects the recent exposure (half-life: ~ 1 month) and body burden. Korean national biomonitoring surveys, such as Korean National Health and Environmental Survey (KoNHES) and Korean National Health and Nutrition Examination Survey (KNHANES), have been measuring Pb in whole blood only. Nevertheless, UPb level has been often used as a biomarker of

exposure and showed positive associations with BP in some previous studies (Mizuno et al., 2021; Wang et al., 2021). The collection of urine for Pb measurement is noninvasive and is favored for long-term biomonitoring. Moreover, it is likely to reflect the filterable fraction of plasm Pb in the kidney (Barbosa et al., 2005; Sommar et al., 2014). However, the weakness of UPb is that its half-life and reliability are still unclear. Few studies have examined the intra-class correlation coefficients (ICCs) of UPb in the general adult populations of Sweden and China (Sallsten et al., 2022; Sommar et al., 2014; Wang et al., 2016) and the values were quite inconsistent (ranging from ≤ 0.1 to 0.53 (0.88 after dilution adjustment)). Sallsten et al. (2022) supposed those differences were attributed to the different exposure levels and sample collection intervals, however, the reason is still unclear. In further studies, it needs to be confirmed whether our findings are reproducible and investigate the efficiency of UPb as a biomarker of exposure in more diverse populations (e.g., children and adolescents, other countries/ethnicity).

Cd was also associated with elevated BP in our study subjects. From the stratified linear regression model, UCd level was positively associated with DBP in adolescents (Table 4-5). Cd is a well-known toxic element and has been known to have a positive association with BP in general populations (Gallagher and Meliker, 2010; Martins et al., 2021). The mechanisms linking Cd exposure and BP have been known to include oxidative stress, endothelial dysfunction related to impaired NO system, modification of catecholamine metabolism, and so on (da Cunha Martins et al., 2018; Prozialeck et al., 2008). Besides, Cd can affect the kidney whose role is in long-term BP control (Satarug et al., 2005), suggesting UCd levels are considered an indicator of long-term exposure to Cd (Vacchi-Suzzi et al., 2016). Previous studies

investigating the association between UCd concentration and elevated BP or risk of hypertension (Castiello et al., 2020; Franceschini et al., 2017; Huang et al., 2019; Oliver-Williams et al., 2018; Van Larebeke et al., 2015; Wu et al., 2019).

Hg has been known to affect BP increase and hypertension (da Cunha Martins et al., 2018; Houston, 2011) and we found limited evidence between Hg exposure and BP. In non-adults, BHg showed a statistically significant effect on DBP (Table 4-3) and the association became marginal after including other metals (Table 4-4), whereas UHg had no association with BP. It seems to be because the distributions of Hg species in blood and urine are different. BHg reflects the exposure to methyl Hg (MeHg), a highly toxic form because it composes approximately 70% of the total Hg in the whole blood (Jung et al., 2013b). In UHg concentration, it is difficult to distinguish the exposure to MeHg and inorganic Hg as MeHg slightly excretes in urine (Hong et al., 2012). To clarify our results, mercury speciation needs to be considered in future studies.

For iAs, our result showed an inverse association between iAs exposure and DBP in non-adults when BiAs+MMA was used as an exposure biomarker. A previous study found a positive association between blood total As and the odds of pre-hypertension (Qu et al., 2022), however, it was the result of a single metal model. Urine is the most widely used biological media for the biomonitoring of As compounds. But UiAs had no significant association with BP in all age groups which is in agreement with previous studies (Desai et al., 2021). Blood As concentrations are often considered indicators of short-term internal dose of As (Chen et al., 2009; Choi et al., 2022), however, evidence is still limited and future studies are needed to confirm our result.

In addition, the results of the stratified analysis in non-adults indicated different effects of metal exposure on BP between children and adolescents. Children had an association between DBP and UPb concentration and adolescents had associations between BP (SBP and DBP) and UCd concentration (Table 4-5). The difference might be due to the different exposure levels and/or physiological development between subgroups. According to the urinary concentrations, exposure level of Cd was slightly lower in children (0.26 μg/L) than in adolescents (median: 0.28 μg/L), whereas that of Pb was higher in children (1.29 μg/L) than in adolescents (1.21 μg/L). Similar patterns were also observed based on the blood data (children vs. adolescents: 0.45 vs. 0.51 μg/L for BCd and 1.84 vs. 1.76 μg/dL BPb), supporting the results as being due to different levels of exposure between subgroups. Moreover, both childhood and adolescence are critical periods of development when developing organ systems that are highly sensitive to exposure to environmental pollutants. In particular, adolescence is the onset of pubertal status, which might contribute to the age-related association between metal exposure and BP. Exposure to Cd was reported to be positively associated with adrenocorticotropic hormone (ACTH) levels in adolescents (Castiello et al., 2020).

Overall, the current study found that urinary metals produced more visible results than blood metals. Indirect cardiovascular effects may occur as a result of renal injury because the kidney is a primary target organ of some metals, like Pb and Cd. Unfortunately, we could not collect data on kidney conditions (e.g., serum creatinine concentration, albumin-creatinine-ratio, blood urea nitrogen, etc.) and our cross-sectional research design has limitations in identifying the causal relationships. In addition, it might be due to the longer half-life of urinary metals than blood metals.

Urinary metals are thought to reflect long-term exposure compared to blood metals (Table 1-1) and appear to be suitable biomarkers of long-term exposure under steady-state conditions (Castiello et al., 2020).

This study has several limitations. First, the present study could not consider other potential confounders or mediators due to the limited data from the questionnaire. Although we included key covariates such as age, sex, and smoking (Yim et al., 2022), it would have been better if other covariates such as dietary intake, nutrient intake, family medical history (e.g., hypertension, diabetes, hyperlipidemia), biochemical markers, and lipid profiles could be taken into account when developing the statistical model. In particular, as mentioned above, if biochemical markers on kidney function were available, the size of the direct and/or indirect effect between metal exposure and BP could have been investigated. Second, the small number of target metals without essential elements. Essential trace elements such as Zn, Se, and Mo are known to affect BP or have potential interactions with toxic metals (Bulka et al., 2019; Howe et al., 2021; Osorio-Yañez et al., 2016; Skröder et al., 2015; Zhong et al., 2021). In addition, exposures to other non-metals with metals need to be considered in future studies. Third, non-adults aged from 7 to 18 years old were put in one group for the analysis due to the sample size. Since there are some differences in the physiological and exposure characteristics between children and adolescents, future studies are needed to confirm our results. Finally, prenatal exposure to metals was not considered due to the cross-sectional study design. Recently, the potential effect of prenatal exposure to metals on BP has been suggested in several studies (Howe et al., 2021; Kupsco et al., 2019). Further research is warranted to prospectively explore these associations, especially in non-adults.

Chapter 5. Conclusions

This study consists of three studies about i) biomonitoring of As species, ii) exposure assessment for metals including Cd, Pb, Hg, and As using PBTK modeling, and iii) the epidemiological association study between combined exposure to those metals and health outcome. Before conducting the exposure assessment and epidemiological studies using biomonitoring data, I first aimed to address the current knowledge gap in metal biomonitoring (mentioned in section 1.5) by focusing on As species in the first study (Chapter 2). There were still limited data available for As species in biological media and exposure biomarkers of iAs in the general population of Korea, whereas biomarkers of Cd, Pb, and Hg have been well studied and continuously measured in the national biomonitoring survey. Then, I aimed to conduct the two case studies in the application of biomonitoring: i) exposure assessment of metals and ii) epidemiologic study of the association between combined metal exposure and blood pressure in the second (Chapter 3) and third studies (Chapter 4), respectively. For the metal biomonitoring data used in this study, both urine and blood samples from the general population of Korea, aged from 1 to 98 years, were collected by the Korean Ministry of Food and Drug Safety (MFDS), and Cd, Pb, Hg, and As species were measured in both media.

In the first study (Chapter 2), both urinary and blood As species (arsenate (As(V)), arsenite (As(III)), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenobetaine (AsB), arsenocholine (AsC)) were first measured in the Koreans. According to the Korean National Environmental Health Survey 2009-

2011 (KoNEHS I), total urinary As concentrations (p50: 41.7 μg/g_{crea}) of Koreans were comparable to those of populations living in highly contaminated regions of iAs (p50: 48.6-154 μg/g_{crea}) (Gao et al., 2019). However, this study found that AsB, a non-toxic As species, was the most common analyte in both urine and blood among the As species. Moreover, urinary DMA levels were higher than in highly exposed populations and both urinary DMA and AsB levels were associated with seafood consumption. Given those results, total urinary As concentration might overestimate the As exposure in Koreans due to organic As from seafood. In addition, exposure to known sources of iAs (e.g., multigrain rice, drinking water type, etc.) was associated with increased iAs levels in urine (UiAs), indicating specificity of UiAs as an exposure biomarker. Blood As species showed high detection rates (i.e., high sensitivity) as in the case of urinary As species, but no significant relationship existed between the concentration and exposure sources. Future research is needed to confirm whether blood is appropriate media for iAs biomonitoring in populations with low-level exposure to iAs. Furthermore, iAs methylation efficiency was linked to age, sex, and smoking status, indicating adolescents, smokers, and males might be more susceptible to iAs exposure in the study subjects.

In the second study (Chapter 3), biomonitoring-based exposure assessments were conducted for four metals (Cd, Pb, Hg, and As), and the results were compared to estimates using a conventional method, i.e., scenario-based exposure assessment. The comparison between exposure estimates based on reverse dosimetry using PBTK model and the probabilistic exposure model showed similarities and differences, indicating that it is important to consider the strengths and limitations of each approach for exposure assessment. In general, biomonitoring-based estimation

has inherent uncertainty that comes from biological half-lives of environmental pollutants. However, these uncertainties are relatively low for metals because biomarkers used in this study (urinary Cd, blood Pb, blood Hg, and urinary iAs) have long half-lives and/or high ICC. In addition, many PBTK models were developed for metals, of which complexity is depending on the purpose of modelers, and the characteristics of the selected model – model compartments, parameters, and exposure routes – can cause uncertainties. Other uncertainty may arise in the assumption that exposure to heavy metals in the general population mainly occurs through oral ingestion, and other routes such as inhalation less contribute, and further research is needed. For the probabilistic exposure model, a lack of exposure data (exposure algorithms, factors, and media concentration) can cause uncertainties although metals have more sufficient exposure data than other environmental pollutants. Therefore, continuous monitoring of media and exposure factors is needed to reduce the uncertainties. Each method needs to be performed together, for a more elaborated exposure assessment of Cd, Pb, Hg, and As considering its strength and inherent uncertainties.

In the last study (Chapter 4), the association between metal co-exposure and BP was investigated in the general population of Korea including adults and non-adults. Several statistical methods (e.g., multiple linear regression, BKMR regression, and WQS regression) have been applied to evaluate the associations. Urinary Pb and Cd levels had significant associations with diastolic BP (DBP) in non-adults although exposure levels were lower in non-adults. In addition, the joint effect of the mixture was found in both adults and non-adults using BKMR analysis but the results were more evident in non-adults. WQS regression analysis showed

that Pb was also the largest contributor as results from other previous studies. Taken together, this study showed that co-exposure to metals needs to be considered in the risk management of metals, and non-adults might be more susceptible to metal exposure than adults. In addition, there is a need for further research on biomarkers – UPb, BiAs, and urinary and blood Hg species – and associations between their concentrations and health outcomes to confirm our results.

This study has several strengths in that all three studies targeted the same population data. The target population includes toddlers to the elderly (aged 1 to 98 yrs), and all metals (Cd, Pb, Hg, and As species) were measured in paired urine and blood samples. Finally, for the first time to our knowledge, As speciation was performed in both urine and blood (Chapter 2), and the results were used in exposure assessment (Chapter 3) and epidemiologic studies (Chapter 4). However, this study also includes limitations. First, this study is a cross-sectional study, and spot samples were used for the analysis. The blood Pb levels of our study populations were considerably higher than other general populations of Korea, although they were lower than occupational levels. Second, information on sociodemographics, exposure, and food consumption characteristics was limited. Third, most of the PBTK models used in this study were developed for adults and cannot distinguish between males and females although the kinetics of metals varies depending on age and sex. Additionally, children and adolescents (aged 7-18 years old) were put in one group for the analysis in Chapter 4 despite the differences in the physiological characteristics and exposure between children and adolescents. Finally, the valence between MMA and DMA compounds was not specified although trivalent MMA

(MMA(III)) and DMA (DMA(III)) are highly toxic and important in terms of risk assessment. All of those limitations need to be considered when interpreting our results.

Considering the overall results, the present study suggests insights on what / where to measure especially for As biomonitoring in low-contamination regions, and how to interpret the results in terms of reverse dosimetry and their associations with health outcomes. More research is required to confirm these findings and arguments; additionally, detailed studies on the early biologic effects of metal co-exposure should address the mechanisms of health effects after combined exposure to metals in humans at low doses.

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Appendix

Chapter 2.

Table S2-1. ICP-MS parameters

Parameter	Setting
Mass/dwell	90.9165/500 ms
Detector mode/scan mode	Dual/Peak Hopping
Readings/replicates	1,750
Measurement unit	Counts per second (cps)
Nebulizer	CT Q+ MEINHARD Plus Quartz Nebulizer
Spray chamber	Cyclonic
Nebulizer flow	0.96 - 1.04 mL/min
RF power	1,600 W
Rpq	0.45
DRC gas	Oxygen
DRC gas flow	0.5 mL/min
DRC rpq	0.5

Table S2-2. Results of quality assurance of urinary and blood arsenic species

Media	Species	Intra-day (n = 3)				Inter-day (3 days)			
		Spiked conc. (μg/L)	Obtained conc. (μg/L)[a]	Accuracy (%)	Precision (%RSD)	Spiked conc. (μg/L)	Obtained conc. (μg/L)[a]	Accuracy (%)	Precision (%RSD)
Urine	As(V)	1.0	1.0 ± 0.08	100.3	8.1	1.0	1.1 ± 0.14	93.6	12.7
		5.0	4.2 ± 0.13	83.1	3.1	5.0	5.0 ± 0.96	99.0	19.0
		20.0	16.3 ± 0.52	81.3	3.2	20.0	20.5 ± 2.9	97.6	14.3
	As(III)	1.0	0.9 ± 0.11	95.8	11.5	1.0	0.9 ± 0.10	108.1	11.0
		5.0	4.5 ± 0.08	89.9	1.8	5.0	5.3 ± 0.88	94.0	16.6
		20.0	17.2 ± 0.50	85.8	2.9	20.0	18.7 ± 1.99	106.8	10.6
	MMA	5.0	4.7 ± 0.08	93.4	1.7	5.0	5.3 ± 0.90	95.2	17.2
		50.0	46.4 ± 0.48	92.7	1.0	50.0	54.0 ± 6.87	92.5	12.7
		100.0	90.9 ± 0.89	90.9	1.0	100.0	103.4 ± 11.9	96.7	11.5
	DMA	5.0	4.9 ± 0.41	97.9	8.4	5.0	5.6 ± 0.57	88.7	10.1
		50.0	50.6 ± 0.64	101.1	0.7	50.0	47.3 ± 3.0	105.7	6.3
		100.0	97.7 ± 1.05	97.7	1.1	100.0	102.2 ± 4.2	97.9	4.1
	AsB	5.0	5.4 ± 0.33	108.7	6.1	5.0	5.2 ± 0.17	96.7	3.3
		50.0	54.9 ± 0.35	110.0	0.6	50.0	47.9 ± 6.5	104.5	13.5
		100.0	107.0 ± 0.48	107.1	0.4	100.0	102.5 ± 3.7	97.6	3.6
	AsC	5.0	5.4 ± 0.20	108.2	3.8	5.0	5.2 ± 0.63	96.7	12.3
		50.0	55.4 ± 0.52	110.7	0.9	50.0	51.0 ± 4.6	98.0	8.9
		100.0	107.1 ± 0.50	107.1	0.5	100.0	102.2 ± 4.9	97.9	4.8

Table S2-2. *(continued)*

Blood	As(V)	2.5	2.8 ± 0.14	112.3	5.0	1.0	2.7 ± 0.13	93.2	5.0
		10.0	10.8 ± 0.14	107.6	1.2	5.0	10.5 ± 0.27	95.1	2.6
		25.0	26.3 ± 0.35	105.0	1.4	20.0	25.7 ± 0.82	97.4	3.2
	As(III)	2.5	2.6 ± 0.13	102.9	5.2	1.0	2.4 ± 0.20	106.1	8.4
		10.0	10.4 ± 0.19	103.6	1.9	5.0	10.2 ± 0.50	98.4	5.0
		25.0	27.2 ± 0.20	108.6	0.7	20.0	25.2 ± 2.10	99.0	8.3
	MMA	2.5	2.4 ± 0.14	96.2	5.9	5.0	2.3 ± 0.12	108.9	5.3
		10.0	10.0 ± 0.22	100.4	2.2	50.0	10.1 ± 0.36	98.6	3.6
		25.0	25.8 ± 0.22	103.2	0.9	100.0	25.0 ± 1.05	100.0	4.2
	DMA	2.5	2.8 ± 0.26	112.9	9.1	5.0	2.4 ± 0.37	104.1	15.5
		10.0	10.2 ± 0.07	104.8	0.7	50.0	10.1 ± 0.37	99.5	3.7
		25.0	25.1 ± 0.89	100.5	3.6	100.0	25.5 ± 1.04	97.9	4.1
	AsB	2.5	2.8 ± 0.17	110.2	6.1	5.0	2.4 ± 0.35	104.7	14.6
		10.0	10.5 ± 0.72	104.8	6.9	50.0	10.5 ± 0.65	94.8	6.1
		25.0	27.5 ± 0.64	110.0	2.3	100.0	26.0 ± 1.28	96.1	4.9
	AsC	2.5	2.7 ± 0.03	90.6	1.6	5.0	2.4 ± 0.16	102.2	6.4
		10.0	9.7 ± 0.27	97.1	2.8	50.0	9.5 ± 0.34	105.1	3.6
		25.0	26.2 ± 0.67	104.7	2.6	100.0	24.2 ± 1.88	103.4	7.8

* As(V), arsenite; As(III), arsenate; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; AsB, arsenobetaine; AsC, arsenocholine; RSD, relative standard deviation
[a] Mean ± standard deviation.

Table S2-3. Distributions of calibrated[a] As species concentrations in urine and blood

Media	As species	Dilution adjustment method	*n*	Mean ± SD (μg/L)	Median (μg/L)	p95 (μg/L)	Range (μg/L)
Urine	iAs	Unadjusted	2,025	1.35 ± 1.44	1.06	3.30	< LOD – 30.4
		Covariate-adjusted standardized (CAS)	1,886	1.34 ± 1.49	1.06	3.11	< LOD – 41.7
	MMA	Unadjusted	2,025	1.35 ± 1.44	1.01	3.46	< LOD – 29.5
		CAS	1,886	1.30 ± 1.53	1.02	2.78	< LOD – 39.6
	DMA	Unadjusted	2,025	13.2 ± 14.0	9.34	36.4	< LOD – 168.1
		CAS	1,886	13.3 ± 13.5	9.37	36.7	< LOD – 138.9
Blood	iAs	-	598	1.04 ± 1.21	0.91	1.82	<LOD – 24.8
	MMA			0.35 ± 0.36	0.25	0.89	<LOD – 4.41
	DMA			0.55 ± 0.44	0.47	1.32	<LOD – 4.06

* ∑As, sum of As species (= As(V) + As(III) + MMA + DMA + AsB + AsC); iAs, inorganic As (= As(V) + As(III)); As(V), arsenite; As(III), arsenate; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; AsB, arsenobetaine; SD, standard deviation; p95, 95th percentile

[a] As metabolite concentrations were corrected for AsB concentrations.

Table S2-4. Proportions of iAs and their metabolites in urine and blood

Calculation method	As species	Urine			Blood		
		Mean ± SD	Median (IQR)	p95	Mean ± SD	Median (IQR)	p95
(i)	%iAs	3.34 ± 4.43	2.18 (1.10-4.15)	9.55	22.6 ± 14.1	20.2 (11.8-30.3)	45.9
	%MMA	3.53 ± 4.32	2.42 (1.25-4.38)	9.69	5.94 ± 6.59	4.33 (0.00[b]-8.16)	20.1
	%DMA	42.8 ± 22.6	40.5 (24.9-58.8)	83.9	14.4 ± 11.3	11.7 (7.12-18.5)	36.4
	%AsB	49.0 ± 25.9	51.1 (30.1-69.6)	87.8	50.8 ± 22.4	53.5 (35.5-68.1)	82.7
	%AsC	1.30 ± 3.75	0.00[a] (0.00[a]-0.97)	6.58	6.34 ± 8.21	4.53 (0.00[c]-7.85)	20.2
(ii)	%iAs	6.72 ± 6.49	5.31 (3.03-8.48)	16.0	52.7 ± 18.2	52.5 (41.9-64.1)	81.8
	%MMA	7.15 ± 6.84	5.90 (3.65-8.78)	15.2	13.2 ± 12.1	12.1 (0.00[b]-18.5)	36.2
	%DMA	86.1 ± 11.8	88.6 (82.8-93.0)	96.8	34.1 ± 18.5	33.0 (22.6-44.9)	64.8
	%iAs[d]	10.6 ± 8.08	9.16 (5.33-13.8)	23.7	54.8 ± 18.0	54.5 (43.8-66.6)	83.1
	%MMA[d]	10.1 ± 7.61	8.90 (5.74-12.7)	20.0	15.8 ± 13.8	15.1 (0.00[b]-22.1)	40.7
	%DMA[d]	79.3 ± 13.4	81.5 (73.5-88.2)	94.6	29.4 ± 17.3	27.4 (18.6-39.2)	58.3

* Calculation method (i) included all As species (∑As = As(V) + As(III) + MMA + DMA + AsB + AsC), (ii) included iAs and its metabolites (As(V) + As(III) + MMA + DMA) in denominator.

[a] Detection rate was 45.8%.

[b] Detection rate was 73.0%.

[c] Detection rate was 73.5%.

[d] Calibrated concentrations were used for the calculation.

Table S2-5. Determinants of covariates on proportions[a,b] of urinary As species

Covariates	Urinary As species					
	%iAs R^2 = 0.055		%MMA R^2 = 0.103		%DMA R^2 = 0.087	
	b (95% CI)	*p*-value	b (95% CI)	*p*-value	b (95% CI)	*p*-value
Blood As species						
%iAs	**0.05 (0.01, 0.09)**	**0.015**	-	-	-	-
%MMA	-	-	-0.03 (-0.06, 0.01)	0.149	-	-
%DMA	-	-	-	-	**0.06 (0.001, 0.12)**	**0.047**
Age group (years)						
13-18	**3.13 (0.64, 5.62)**	**0.014**	**3.17 (1.25, 5.09)**	**0.001**	**-6.51 (-10.4, -2.60)**	**0.001**
19-64	1.17 (-1.23, 3.57)	0.337	-0.05 (-1.90, 1.80)	0.958	-1.17 (-4.94, 2.59)	0.540
≥ 65	-0.12 (-2.97, 2.73)	0.936	-0.67 (-2.86, 1.52)	0.547	0.60 (-3.87, 5.07)	0.791
Sex						
Female	**-1.79 (-3.26, -0.31)**	**0.018**	**-1.15 (-2.29, -0.02)**	**0.047**	**2.99 (0.67, 5.30)**	**0.012**
BMI (kg/m^2)						
Overweight (23-24.9)	-0.18 (-2.02, 1.66)	0.849	0.46 (-0.96, 1.87)	0.525	-0.31 (-3.20, 2.57)	0.830
Obese (≥ 25)	-0.02 (-1.75, 1.72)	0.985	-0.34 (-1.68, 0.99)	0.611	0.28 (-2.43, 2.99)	0.840
Alcohol consumption						
≤ 1/month	-0.93 (-1.21, 3.07)	0.369	-1.07 (-2.64, 0.51)	0.184	2.03 (-1.18, 5.23)	0.214
2-4/month	0.93 (-1.21, 3.07)	0.394	0.57 (-1.08, 2.22)	0.495	-1.31 (-4.66, 2.05)	0.444
≥ 2/week	-0.58 (-2.91, 1.74)	0.622	-1.08 (-2.88, 0.71)	0.237	1.78 (-1.86, 5.43)	0.337
Electricity charge(KRW/month)						
≥ 30,000, < 50,000	0.34 (-1.37, 2.06)	0.693	-0.73 (-2.05, 0.59)	0.280	0.51 (-2.18. 3.20)	0.709
≥ 50,000	0.82 (-0.96, 2.61)	0.364	-0.34 (-1.72, 1.04)	0.630	-0.24 (-3.03, 2.56)	0.867
Unknown	2.66 (-0.95, 6.28)	0.149	2.39 (-0.40, 5.18)	0.093	-4.78 (-1.04, 0.89)	0.098
Smoking status						
Smoker	1.57 (-0.98, 4.13)	0.227	**3.16 (1.19, 5.13)**	**0.002**	**-4.62 (-8.63, -0.60)**	**0.024**

* %iAs: proportion of inorganic As (= As(V) + As(III)), As(V): arsenite, As(III): arsenate, %MMA: proportion of monomethyl arsenic, %DMA: proportion of dimethyl arsenic

b: regression coefficients after adjustment for other covariates (i.e., proportion of each As species in blood, age, sex, BMI, electricity charge, smoking status, alcohol consumption, type of drinking water, consumption of blue-backed fishes, other fishes, laver, other seaweeds, and other seafood),

Reference: 7-12 years (age group), male (sex), normal (BMI), never or seldom (alcohol consumption), < 30,000 KRW/month (electricity charge), non-smoker (smoking status)

[a] Each proportion of As measurements were calculated by dividing by the sum of concentrations of iAs and its metabolites (iAs + MMA + DMA), [b] Concentrations used for calculating %iAs, %MMA, and %DMA were corrected for AsB concentrations

Chapter 3.

Table S3-1. Parameter for human Cd PBPK model (MFDS, 2011)

Parameter	Value (unit)	Description
Wa	Male: 43, Female: 33 (kg)	Bodyweight in adulthood
Wb	3.2 (kg)	Bodyweight at birth
Wc	Male: 33, Female: 32 (kg)	Bodyweight with maximum growth
Q	1.865	Wa adjustment factor according to age change
Wa_S [a]	1.00018×10^{-7}	Wa adjustment factor according to age change
H	5 (year)	Age with maximum growth
L [b]	Male: 0.0095, Female: 0.017	Logistic constant
K	220.08	Logistic constant
WFbl	0.068	Blood volume (fraction of bodyweight)
WFki	0.0038	Kidney volume (fraction of bodyweight)
WFli	0.0206	Liver volume (fraction of bodyweight)
Scr	Male: (0, 0.2329) (14, 0.6109) (20, 0.9) (60, 0.9) (80, 0.9934) (age, mg/dL) Female: (0, 0.2329) (14, 0.6109) (20, 0.7) (62.5, 0.7) (80, 0.81) (age, mg/dL)	Changes in blood creatinine levels
F_CRcl	Male: 1, Female: 0.85	Sex adjustment factor of creatinine clearance
F_CRur	male: (0, 0.0149) (80, 0.0088) (age, unitless) female: (0, 0.0083) (80, 0.0082) (age, unitless)	Urinary creatinine adjustment factor
C1	0.052	Oral bioavailability
K_abs	(0, 4.0) (18, 1.5) (40, 1.0) (80, 0.7) (age, unitless)	Oral absorption fraction by age
C2	0.05 (1/day)	Absorption rate
C3	0.35 (1/day)	Daily uptake → blood 3 compartment
C4	1 (μg/day)	Maximum Cd amount into Blood 3 in a day
C5	(0, 0.462) (80, 0.452) (age, 1/day)	Blood 1 → other tissue compartment by age
C6	0.00028 (1/day)	Other tissue → Blood 1 compartment
C7	0.273 (1/day)	Fecal excretion rate from blood 1 compartment
C8	(0, 0.248) (80, 0.238) (age, 1/day)	Blood 1 →liver compartment by age

Table S3-1. *(continued)*

C9	3.0×10^{-5} (1/day)	Liver → blood 1 compartment
C10	0.00011 (1/day)	Liver →blood 3 compartment
C11	0.00015 (1/day)	Biliary excretion rate
C12	0.012 (1/day)	Blood 1 → blood 2 compartment
C13	(0, 0.946) (20, 0.946) (80, 0.683) (age, 1/day)	Blood 3 → kidney compartment by age
C14	0.000039 (1/day)	Kidney → Blood 1 compartment
C15	(0, 0.000065) (30, 0.00065) (80, 0.000765) (age, 1/day)	Kidney → urine compartment by age
C16	0.1	Fraction of blood 1 and blood 3 to whole blood
C17	2.0×10^{-7} (1/day)	Rate constant increases from age 30 for C15
CX	0.017	1-C5-C7-C8
UrEx	(0, 0.04) (1, 0.03) (3, 0.02) (6, 0.015) (20, 0.01) (age, 1/d/kg)	Urinary excretion rate constant by age and bodyweight

[a] Optimized using bodyweight of the study subject
[b] Obtained from O'Flaherty et al. (1998)

Table S3-2. Parameter for human Pb PBPK model (Dede et al., 2018)

Parameter	Value (unit)	Description
QCC	340 (L/day/kg)	Cardiac blood flow
HCT	0.45	Hematocrit ratio
BIND	0.437 (mg Pb/L cell)	Pb binding capacity of erythrocytes
KBIND	3.72×10^{-4} (mg Pb/L cell)	Binding constant of erythrocytes
G	1.2	Ratio of unbound erythrocyte Pb concentration to plasma Pb concentration
Fraction of blood flow		
QLC	0.25	Fraction of cardiac blood flow going to the liver
QKC	0.17	Fraction of cardiac blood flow going to the kidney
QBC	0.03	Fraction of cardiac blood flow going to bone
QWC	0.44	Fraction of cardiac blood flow going to tissues (well-perfused)
QPC	0.11	Fraction of cardiac blood flow going to tissues (poorly-perfused)
Constant for tissue volume(L/kg)		
VLC	0.025	Liver
VKC	0.0042	Kidney
VBC	0.14	Bone
VBLC	0.073	Blood
Partition coefficient		
Pliver	100	Liver/plasma
Pkidney	100	Kidney/plasma
Pwp	100	Well-perfused tissues/plasma
Ppp	20	Poorly-perfused tissues/plasma
Pbone	1000	Bone/plasma
Transfer rates(1/day)		
Agi	0.06-0.12	Absorption rate
eU	0.47	Urinary excretion rate
eB	0.2	Biliary excretion rate

Table S3-3. Parameter for human Hg PBPK model (Carrier et al., 2001a)

Parameter	Value	Description
Organic mercury (1/day)		
K	12.987	Constant ratio Whole body/Blood
kabs	5.544	Oral absorption rate constant
kQI	0.01347	Metabolism rate constant of organic mercury to inorganic mercury
kQF	9.0668×10^{-5}	Whole body to feces transfer coefficient of organic mercury
kQU	≈0	Whole body to urine transfer coefficient of organic mercury
kQH	2.3825×10^{-4}	Whole body to hair transfer coefficient of organic mercury
kelim	0.0138	Whole body elimination rate constant of organic mercury
Inorganic mercury (1/day)		
dBL	0.175	Blood-to-liver transfer coefficient combined with liver metabolism rate constant of organic mercury
dBBr	≪dBL	Blood-to-brain transfer coefficient combined with brain metabolism rate constant of organic mercury
kLB	0.894	Liver to blood transfer coefficient of inorganic mercury
kBK	17.1234	Blood to kidney transfer coefficient of inorganic mercury
kKB	0.001	Kidney to blood transfer coefficient of inorganic mercury
kKU	0.006949	Kidney to urine transfer coefficient of inorganic mercury
kBH	0.14	Blood to hair transfer coefficient of inorganic mercury
kBU	0.06994	Blood to urine transfer coefficient of inorganic mercury
kBF	3.9917	Blood to feces transfer coefficient of inorganic mercury
kLF	1.5476	Liver to feces transfer coefficient of inorganic mercury
kBBr	0.0028	Blood to brain transfer coefficient of inorganic mercury
kBrB	0.052	Brain to blood transfer coefficient of inorganic mercury
Constant for tissue volume(L/kg)		
VBLC	0.073	Blood

Table S3-4. Parameter for human iAs PBPK model (El-Masri and Kenyon, 2008)

Parameter	Value (unit)	Description
Tissue volume[a]		
VG	1.2 (L)	Tissue volume of GI tract
VS	2.6 (L)	Tissue volume of skin
VB	1.4 (L)	Tissue volume of brain
VH	0.35 (L)	Tissue volume of heart
VK	0.28 (L)	Tissue volume of kidney
VLi	1.82 (L)	Tissue volume of liver
VM	55.5 (L)	Tissue volume of muscle and remaining tissues
VLu	0.56 (L)	Tissue volume of lung
Blood flow		
Qpv	1 (1/min)	Blood flow of GI (blood flow of portal vein)
QS	0.26 (1/min)	Blood flow of skin
QB	0.63 (1/min)	Blood flow of brain
QH	0.2 (1/min)	Blood flow of heart
QK	1 (1/min)	Blood flow of kidney
Qhe	0.31 (1/min)	Blood flow of liver (blood flow of hepatic artery)
QM	1.8 (1/min)	Blood flow of muscle and remaining tissues
QC	5.2 (1/min)	Total cardiac output
Partition coefficient		
GI	2.7 (As(V)), 8.3 (As(III)), 2.2 (MMA), 2.1 (DMA)	Partition coefficients of GI tract
Skin	7.9 (As(V)), 7.4 (As(III)), 2.6 (MMA), 2.4 (DMA)	Partition coefficients of skin
Brain	2.4 (As(V)), 2.4 (As(III)), 2.2 (MMA), 3.3 (DMA)	Partition coefficients of brain
Muscle	7.9 (As(V)), 7.4 (As(III)), 2.6 (MMA), 2.4 (DMA)	Partition coefficients of muscle
Kidney	8.3 (As(V)), 11.7 (As(III)), 4.4 (MMA), 3.8 (DMA)	Partition coefficients of kidney
Liver	15.8 (As(V)), 16.5 (As(III)), 3.3 (MMA), 3.3 (DMA)	Partition coefficients of liver

Table S3-4. *(continued)*

Lung	2.1 (As(V)), 6.7 (As(III)), 1.3 (MMA), 1.3 (DMA)	Partition coefficients of lung
Heart	7.9 (As(V)), 7.4 (As(III)), 2.6 (MMA), 2.4 (DMA)	Partition coefficients of heart
DMA kinetics		
Ka	0.007(1/min)	Oral absorption rate
Kred	0.004(1/min)	Reduction of DMA
Kox	0.65	Oxidation of DMA(III)
Kurine/DMA	0.13(1/min)	Urine excretion constant
MMA kinetics		
Ka	0.007(1/min)	Oral absorption rate
Kred	0.008(1/min)	Reduction of MMA
Kox	0.63	Oxidation of MMA(III)
Vmax	6.6×10^{-7}(mole/min)	Methylation of MMA(III) (MMA(III)→DMA)
Km	3×10^{-6}(M)	Methylation of MMA(III) (MMA(III)→DMA)
Kinh	4×10^{-5}(M)	Methylation of MMA(III) (noncompetitive inhibition)
Kurine/MMA	0.3(1/min)	
Inorganic As kinetics		
Ka(As(V))	0.003(1/min)	Oral absorption rate
Ka(As(III))	0.004(1/min)	Oral absorption rate
Kred	0.003(1/min)	Reduction of As(V)
Kox	0.25	Oxidation of As(III)
Vmax	5.3×10^{-7}(mole/min)	Methylation of As (As(III)→MMA)
Km	3×10^{-6}(M)	Methylation of As (As(III)→MMA)
Vmax	2×10^{-6}(mole/min)	Methylation of As (As(III)→DMA)
Km	3×10^{-6}(M)	Methylation of As (As(III)→DMA)
Kinh	4×10^{-5}(M)	Methylation of As (noncompetitive inhibition)
Kurine/As	0.007(1/min)	Urine excretion constant

[a] Percent tissue volumes were obtained from Brown et al. (1997) and multiplied by 70 kg of body weight

Table S3-5. Estimated blood MeHg from the blood Hg measurements

Age group (yrs)	GM	p50	p95
All	3.06	3.08	16.4
7-12	1.70	1.72	8.57
13-18	2.16	2.17	13.3
19-64 (male)	3.98	4.00	18.2
19-64 (female)	3.61	3.65	15.0
65+	3.87	3.91	21.5

Table S3-6. Equations used for the scenario-based exposure assessment

Exposure route	Exposure source	Equation for exposure estimation
Oral ingestion	Diet, drinking water	$EDI = \frac{Cf \times FIR \times ABS}{BW}$ BW: Body weight (kg) ABS: Absorption fraction (unitless) Cf: Metal concentration in food/water (ng/g or ng/L) FIR: Food/water intake rate (g/day or L/day)
	Dust, soil	$EDI = \frac{Cs \times SIR \times UT \times ABS}{BW}$ BW: Body weight (kg) ABS: Absorption fraction (unitless) Cs: Metal concentration in dust/soil (ng/g) SIR: Intake rate of soil/dust by exposure places (mg/day) UT: Fraction of exposure time by exposure places (unitless)
	Consumer products	$EDI = \frac{Cp \times q \times f \times UR \times IF \times ABS}{BW}$ BW: Body weight (kg) ABS: Absorption fraction (unitless) Cp: Metal concentration in consumer products (ng/g) q: Use amount (mg/day) f: Use frequency (event/day) UR: Fraction of use (unitless) IF: Fraction of ingestion (unitless)
	Hand-to-mouth (toys)	$EDI = \frac{DH \times TH \times HA \times HM \times AD \times HC \times$ BW: Body weight (kg) ABS: Absorption fraction (unitless) DH: Transition rate (ng/cm^2/min) TH: Mouthing time (min/hr) HA: Skin contact area (cm^2) HM: Fraction of mouth-hand contact (unitless) AD: Activity time (hr/day) HC: Fraction of hand-sucking children (unitless) HR: Fraction of unabsorbed (unitless)

Table S3-6. *(continued)*

Dermal contact	Consumer products	$EDI = \frac{Cp \times q \times f \times UR \times RF \times ABS}{BW}$ BW: Body weight (kg) ABS: Absorption fraction (unitless) Cp: Metal concentration in consumer products (ng/g) q: Use amount (mg/day) f: Use frequency (event/day) UR: Fraction of use (unitless) RF: Skin retention factor (unitless)
	Dust, soil	$EDI = \frac{Cs \times AR \times BA \times ABS}{BW}$ BW: Body weight (kg) ABS: Absorption fraction (unitless) Cs: Metal concentration in dust/soil (ng/g) AR: Amount of adsorbed dust/soil BA: Body surface area (cm^2/day)
	Toys	$EDI = \frac{DH \times TH \times HA \times HM \times AD \times ABS}{BW}$ BW: Body weight (kg) ABS: Absorption fraction (unitless) DH: Transition rate (ng/cm^2/min) TH: Mouthing time (min/hr) HA: Skin contact area (cm^2) HM: Fraction of mouth-hand contact (unitless) AD: Activity time (hr/day)
Inhalation	Air	$EDI = \frac{Ca \times IR \times UT \times ABS}{BW}$ BW: Body weight (kg) ABS: Absorption fraction (unitless) Ca: Metal concentration in air (mg/m^3) IR: Inhalation rate (m^3/day) UT: Fraction of exposure time by exposure places (unitless)

Table S3-7. Exposure contribution (%) of each source for metals using scenario-based exposure assessment approach

Metal	Category	0-2 yrs		3-6 yrs		7-12 yrs		13-18 yrs		19-64 yrs (male)		19-64 yrs (female)		65+ yrs	
		p50	p95	p50	p95	p50	p95	p50	p95	p50	p95	p50	p95	p50	p95
Cd	Diet	83.1%	92.5%	88.4%	96.1%	97.9%	99.7%	98.1%	99.5%	96.8%	99.3%	96.2%	99.2%	99.3%	99.9%
	Non-diet	0.3%	0.1%	-	-	-	-	-	-	-	-	-	-	-	-
	Environment	**0.3%**	0.3%	**0.6%**	0.1%	**1.2%**	0.1%	**0.6%**	0.2%	**0.5%**	0.2%	**0.6%**	0.1%	**0.7%**	0.1%
	Consumer products	16.3%	7.1%	11.0%	3.8%	0.8%	0.3%	1.3%	0.3%	2.7%	0.6%	3.2%	0.7%	-	-
	EDI_{SCN}	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**
Pb	Diet	59.4%	78.0%	68.6%	80.2%	77.5%	84.5%	64.2%	77.9%	81.7%	90.9%	81.6%	90.7%	82.9%	91.9%
	Non-diet	30.2%	16.4%	23.1%	14.7%	15.6%	10.5%	28.4%	17.6%	4.7%	2.4%	6.5%	3.3%	7.0%	3.6%
	Environment	**10.2%**	5.5%	**8.2%**	5.1%	**7.0%**	4.9%	**7.4%**	4.5%	**13.6%**	6.6%	**11.9%**	6.0%	**10.1%**	4.5%
	Consumer products	0.2%	0.1%	-	-	-	-	-	-	-	-	-	-	-	-
	EDI_{SCN}	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**
Hg	Diet	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%
	Non-diet	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Environment	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	Consumer products	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	EDI_{SCN}	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**
As	Diet	98.2%	99.5%	98.9%	99.7%	99.4%	99.8%	99.3%	99.8%	99.7%	99.9%	99.4%	99.8%	99.2%	99.8%
	Non-diet	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Environment	**1.5%**	0.4%	**1.0%**	0.3%	**0.6%**	0.2%	**0.7%**	0.2%	**0.3%**	0.1%	**0.5%**	0.1%	**0.8%**	0.2%
	Consumer products	0.2%	0.1%	0.1%	0.04%	-	0.01%	-	-	-	-	0.1%	0.02%	-	-
	EDI_{SCN}	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**	**100%**

* NA: Not available, -: Intake below <0.001 μg/kg/day, [a] Dust/soil ingestion, drinking water, and hand-to-mouth exposure of toddlers (0-2 yrs); [b] Inhalation and dermal contact

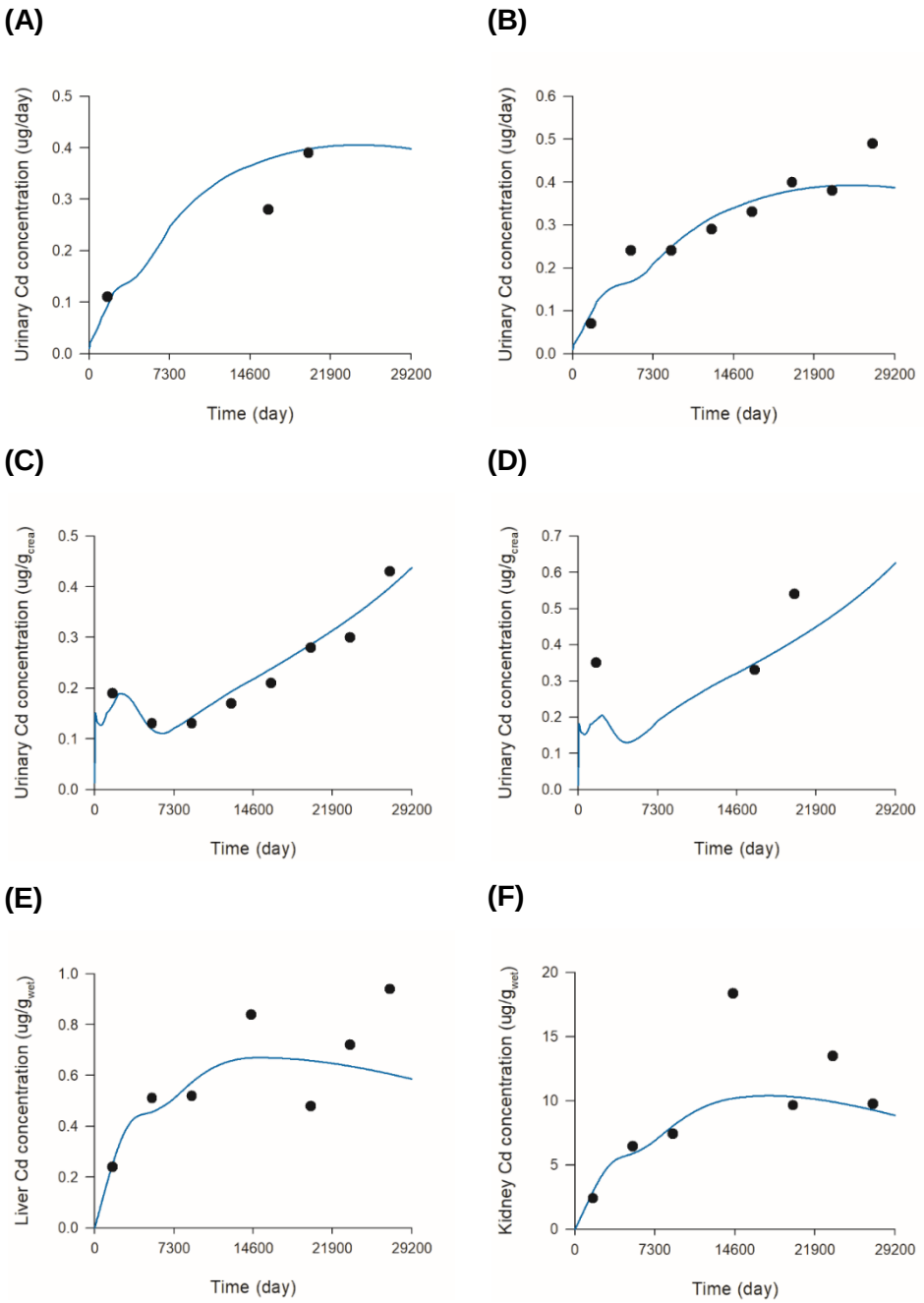

Figure S3-1. Solid lines represent Cd PBTK modeling-based estimates and symbols represent measurements. Each figure compares the model estimates and measurements of (A) urinary Cd concentration (male), (B) urinary Cd concentration (female), (C) creatinine adjusted urinary Cd concentration (male), (D) creatinine adjusted urinary Cd concentration (female), (E) liver Cd concentration, and (F) kidney Cd concentration in the Swedish population (Elinder et al., 1978).

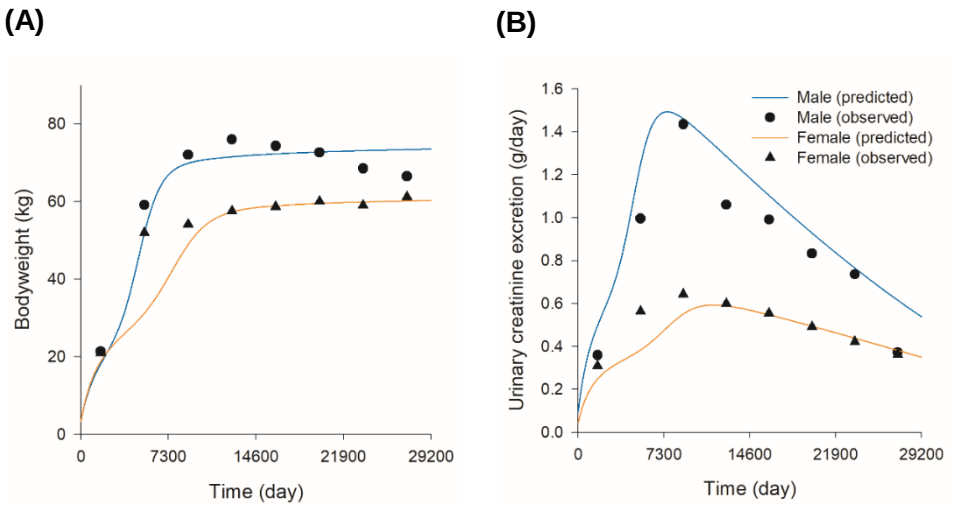

Figure S3-2. Solid lines represent Cd PBTK modeling-based estimates and symbols represent measurements. Each figure compares the model estimates and measurements of (A) bodyweight (our subjects) and (B) urinary creatinine excretion amount in Korean males and females (MFDS, 2011).

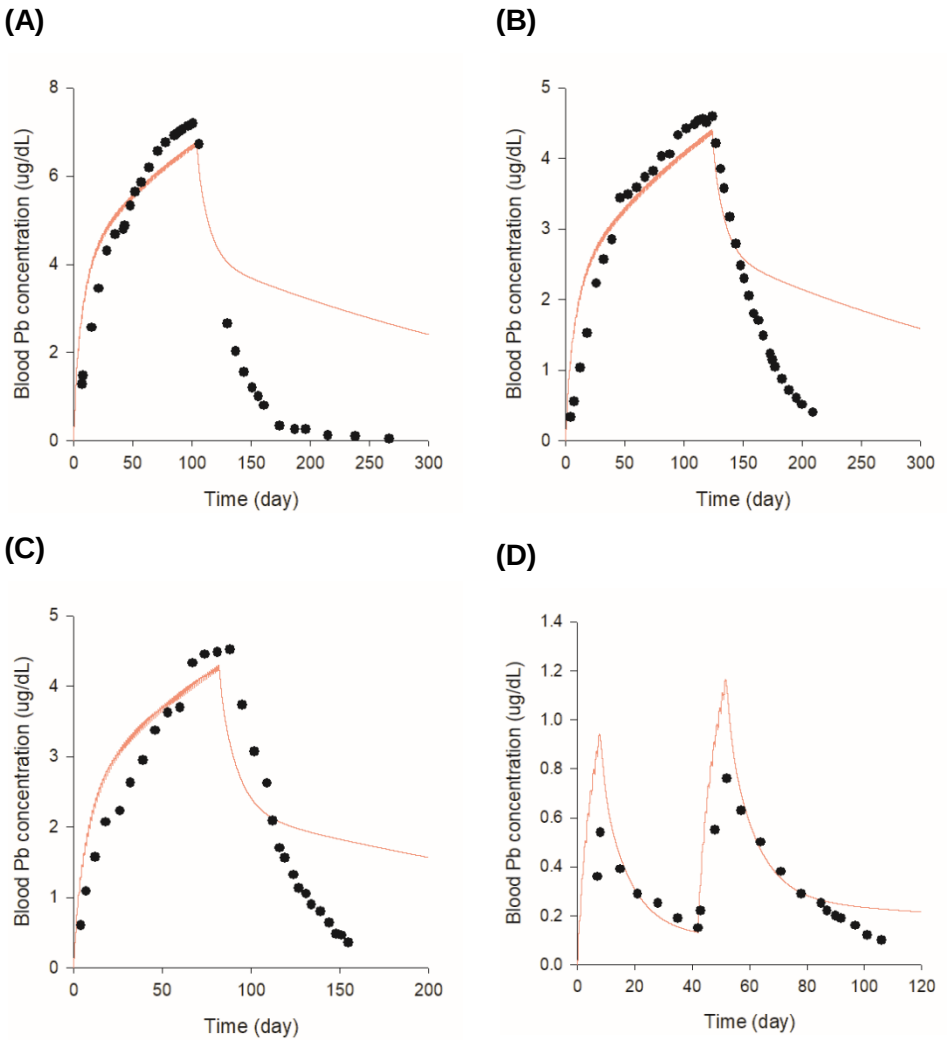

Figure S3-3. Solid lines represent Pb PBTK modeling-based estimates and symbols represent measurements after oral exposure to isotope-labeled Pb in humans (Rabinowitz et al., 1976). Exposure durations and amounts are (A) 204 µg/day for 104 days, (B) 185 µg/day for 124 days, (C) 105 µg/day for 82 days, and (D) 99 µg/day for the first 8 days and again from 42 to 52 days.

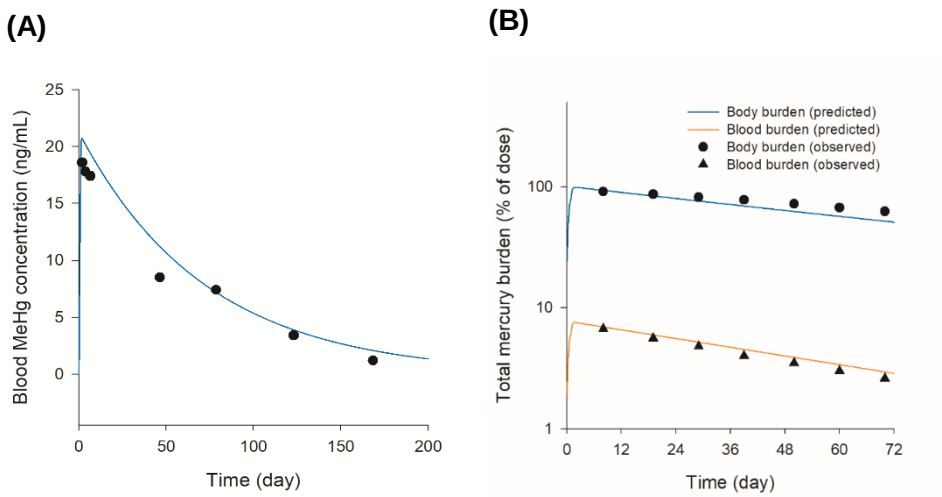

Figure S3-4. Solid lines represent Hg PBTK modeling-based estimates and symbols represent measurements. Each figure compares the model estimates and measurements of (A) blood MeHg concentration in human volunteer after ingestion of 1400 μg MeHg (20 μg/kg MeHg in a 70 kg man) (Kershaw et al., 1980) and (B) total mercury burden (% of dose) in human volunteers after intravenous dosing of 3.85 μg MeHg (Smith et al., 1980).

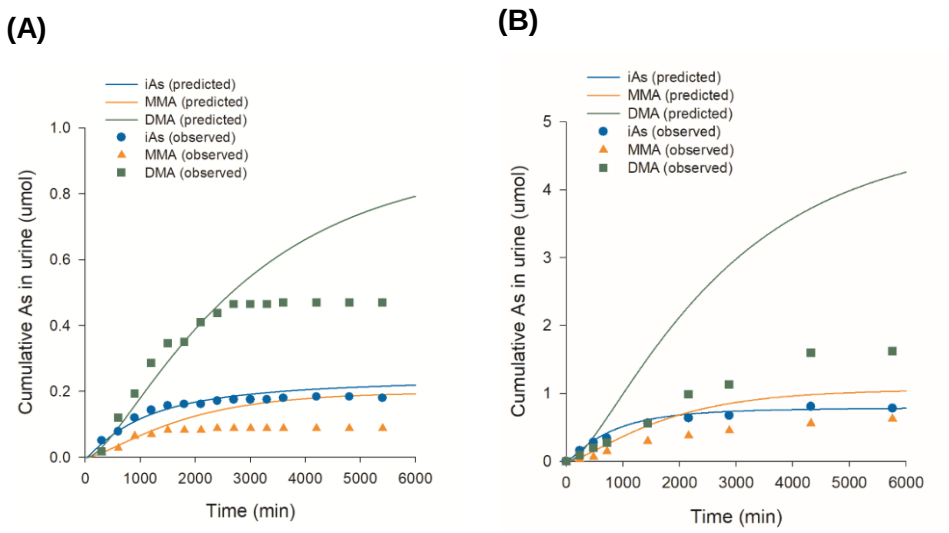

Figure S3-5. Solid lines represent iAs PBTK modeling-based estimates and symbols represent measurements. Each figure compares the model estimates and measurements of cumulative urinary iAs (blue), MMA (orange), and DMA (green) amounts in human volunteers after the ingestion of (A) 500 μg As in the form of sodium arsenite and (B) 100 μg As in the form of sodium arsenate (Buchet et al., 1981).

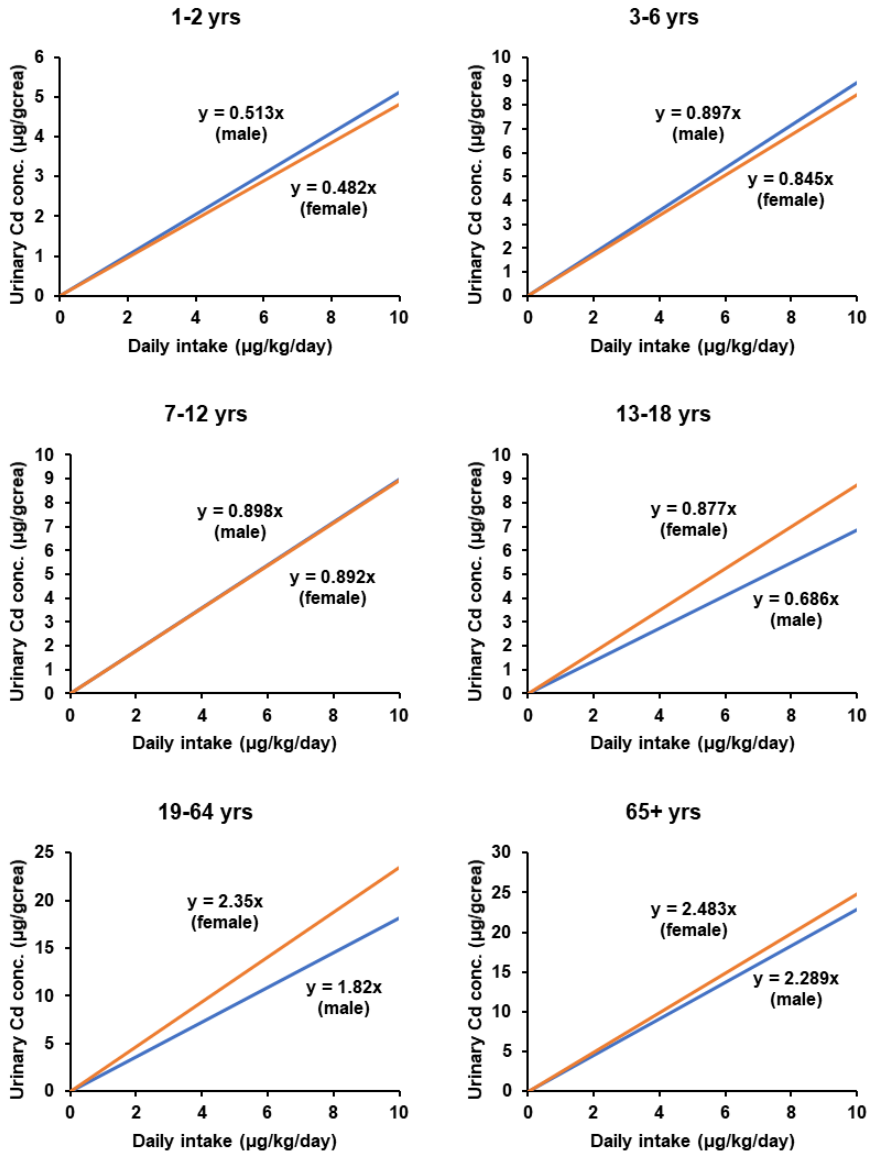

Figure S3-6. Exposure-concentration relationship (ECR) chart of Cd (x-axis: Cd daily intake (μg/kg/day), y-axis: urinary Cd concentration (μg/g_{crea})) by age and gender (male(blue line), female (orange line)) for reverse dosimetry.

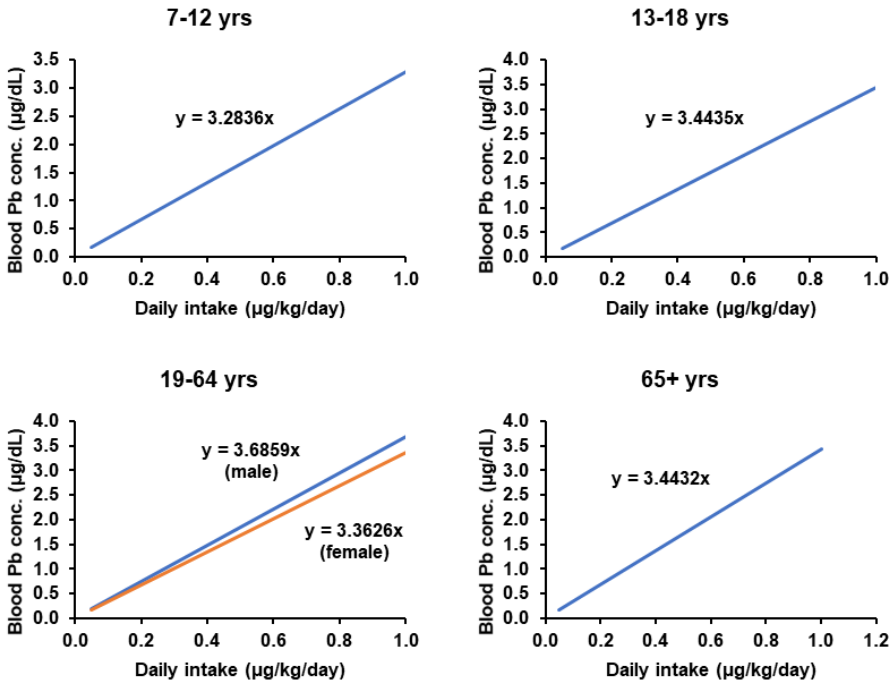

Figure S3-7. Exposure-concentration relationship (ECR) chart of Pb (x-axis: Pb daily intake (µg/kg/day), y-axis: blood Pb concentration (µg/dL)) by age and gender (for adults, male (blue line), female (orange line)) for reverse dosimetry.

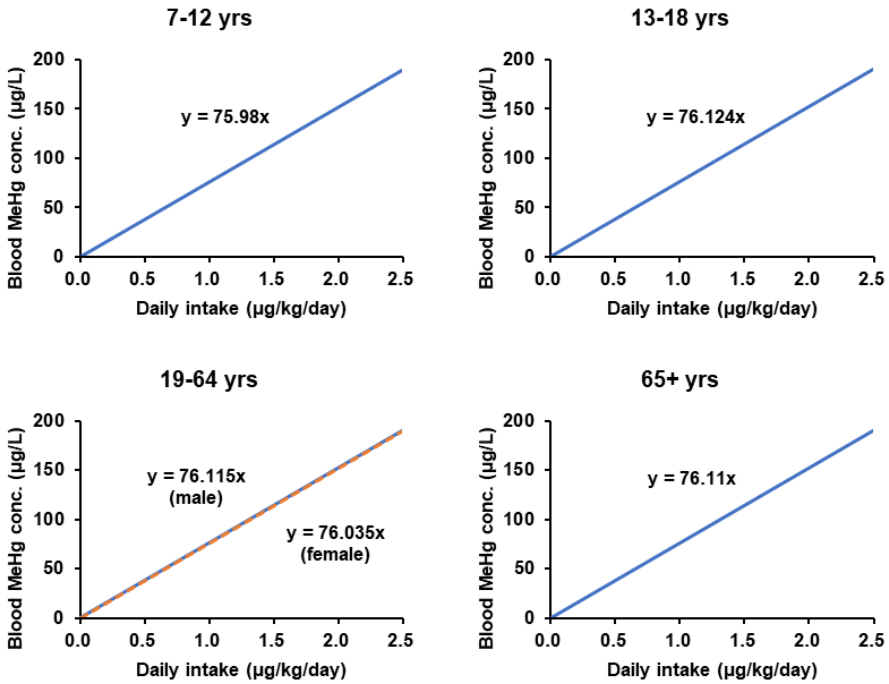

Figure S3-8. Exposure-concentration relationship (ECR) chart of MeHg (x-axis: MeHg daily intake (µg/kg/day), y-axis: blood MeHg concentration (µg/L)) by age and gender (for adults, male (blue solid line), female (orange dashed line)) for reverse dosimetry.

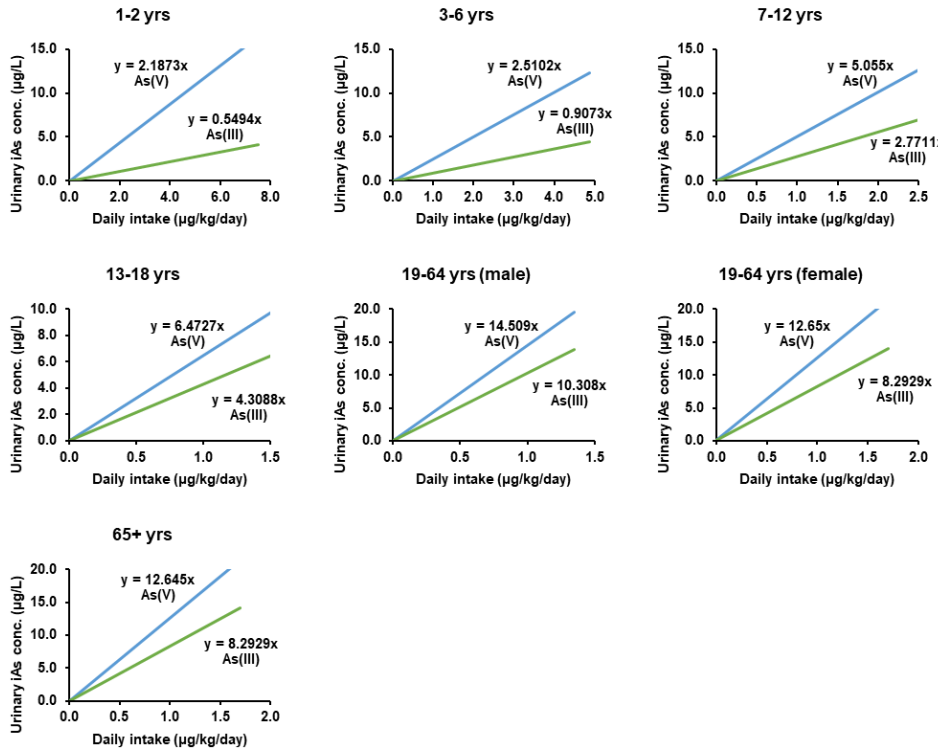

Figure S3-9. Exposure-concentration relationship (ECR) chart of iAs (x-axis: iAs daily intake (μg/kg/day), y-axis: urinary iAs concentration (μg/L)) by age and gender (for adults) for reverse dosimetry. The intake of iAs was assumed to be the case of ingesting only As(V) (blue line) or As(III) (green line), respectively.

Chapter 4.

Table S4-1. Demographic characteristics and blood pressure of the study population in adults

Characteristics	Urine data					Blood data				
	N (%)	SBP	*p*-value[a]	DBP	*p*-value[a]	N (%)	SBP	*p*-value[a]	DBP	*p*-value[a]
Total	902 (100)	124.9 ± 17.0	-	75.2 ± 11.8	-	280 (100)	128.4 ± 19.7	-	75.6 ± 11.4	-
Alcohol consumption			**< 0.001**		**< 0.001**			**0.020**		0.132
Never or seldom	164 (18.2)	125.9 ± 18.6		72.2 ± 10.8		80 (28.6)	130.8 ± 20.6		74.7 ± 10.6	
< 2/month	248 (27.5)	121.4 ± 15.9		73.2 ± 10.8		77 (27.5)	122.1 ± 15.8		73.5 ± 9.5	
2-4/month	272 (30.2)	124.0 ± 16.4		74.6 ± 11.7		67 (23.9)	129.2 ± 19.1		76.2 ± 12.1	
≥ 2/week	218 (24.2)	129.1 ± 16.9		79.3 ± 11.5		56 (20.0)	132.7 ± 21.9		79.1 ± 13.4	
BMI (kg/m^2)			**< 0.001**		**< 0.001**			**< 0.001**		**< 0.001**
Normal (< 23)	358 (39.7)	118.2 ± 15.2		70.4 ± 10.3		100 (35.7)	121.8 ± 20.1		71.4 ± 11.0	
Overweight (23-24.9)	223 (24.7)	125.7 ± 14.6		76.6 ± 10.3		81 (28.9)	129.7 ± 18.3		78.4 ± 11.5	
Obese (≥ 25)	321 (35.6)	131.9 ± 17.6		78.7 ± 12.0		99 (35.4)	133.9 ± 18.5		77.5 ± 10.6	
Exercise			**0.009**		0.391			**0.010**		0.180
No	524 (58.1)	123.7 ± 16.7		74.6 ± 11.5		139 (49.6)	125.7 ± 18.5		74.9 ± 11.8	
Yes	378 (41.9)	126.6 ± 17.3		75.3 ± 11.6		141 (50.4)	131.0 ± 20.4		76.3 ± 11.0	
Sex			**< 0.001**		**< 0.001**			**0.020**		**0.004**
Male	449 (49.8)	130.5 ± 15.0		78.6 ± 11.1		93 (50.0)	132.6 ± 18.1		77.7 ± 11.5	
Female	453 (50.2)	119.4 ± 17.1		71.2 ± 10.8		93 (50.0)	124.2 ± 20.3		73.5 ± 10.9	
Smoking status			**< 0.001**		**< 0.001**			**0.015**		**0.007**
Non-smoker	618 (68.5)	122.4 ± 17.0		72.6 ± 11.0		197 (70.4)	126.4 ± 19.4		74.1 ± 10.8	
Former smoker	137 (15.2)	129.8 ± 16.6		79.3 ± 11.4		52 (18.6)	133.6 ± 20.4		78.8 ± 11.8	
Smoker	147 (16.3)	130.8 ± 14.8		80.4 ± 10.9		31 (11.1)	132.6 ± 18.7		79.8 ± 12.5	

[a] *p*-values were obtained from the Kruskal-Wallis test.

Table S4-2. Demographic characteristics and blood pressure of the study population in non-adults

Characteristics	Urine data					Blood data				
	N (%)	SBP	*p*-value	DBP	*p*-value	N (%)	SBP	*p*-value	DBP	*p*-value
Total	450 (100)	115.7 ± 14.9	-	67.1 ± 10.4	-	186 (100)	116.4 ± 15.5	-	67.5 ± 10.3	-
Alcohol consumption			0.471		0.939			0.898		0.247
Never or seldom	374 (83.1)	115.3 ± 15.0		67.1 ± 10.8		153 (82.3)	116.5 ± 15.7		67.9 ± 10.5	
< 2/month	47 (10.4)	117.9 ± 13.9		66.7 ± 8.5		19 (10.2)	116.0 ± 12.3		67.1 ± 10.4	
≥ 2/month	29 (6.4)	116.4 ± 16.1		66.6 ± 7.6		14 (7.5)	115.9 ± 18.2		63.4 ± 5.3	
BMI (kg/m^2)			**< 0.001**		**< 0.001**			**0.005**		**0.046**
Normal (< 23)	324	112.6 ± 13.2		65.7 ± 10.4		138 (74.2)	114.5 ± 13.7		66.9 ± 10.5	
Overweight (23-24.9)	59	117.0 ± 11.4		68.3 ± 9.0		22 (11.8)	114.0 ± 12.4		66.0 ± 8.7	
Obese (≥ 25)	67	129.5 ± 17.3		72.5 ± 9.5		26 (14.0)	128.5 ± 21.2		71.7 ± 9.4	
Exercise			**0.004**		**< 0.001**			0.334		**0.011**
No	215	118.1 ± 16.1		69.2 ± 10.5		93 (50.0)	118.3 ± 17.4		69.6 ± 11.2	
Yes	235	113.4 ± 13.3		65.1 ± 9.9		93 (50.0)	114.5 ± 13.3		65.4 ± 8.7	
Sex			**< 0.001**		0.095			**0.016**		0.277
Male	220	118.8 ± 15.6		66.3 ± 11.2		93 (50.0)	119.3 ± 16.7		67.0 ± 10.9	
Female	230	112.7 ± 13.6		67.7 ± 9.6		93 (50.0)	113.5 ± 13.8		68.0 ± 9.6	
Smoking status of parents			0.082		0.631			**0.006**		0.792
Non-smoker	244	114.7 ± 14.8		67.0 ± 10.0		96 (51.6)	113.8 ± 15.7		67.0 ± 9.8	
Smoker	206	116.8 ± 15.0		67.1 ± 10.9		90 (48.4)	119.1 ± 15.0		68.0 ± 10.7	

[a] *p*-values were obtained from the Kruskal-Wallis test.

Table S4-3. Differences in blood pressure for the increase in concentrations of metals (conventional creatinine-adjusted) of adults and non-adults from the covariate adjusted models

Biomarker	**Adults (≥ 19 yrs)**				**Non-adults (< 19 yrs)**			
	SBP (mmHg) β (95% CI)	*p*-value	DBP (mmHg) β (95% CI)	*p*-value	SBP (mmHg) β (95% CI)	*p*-value	DBP (mmHg) β (95% CI)	*p*-value
Covariate adjusted individual metal model								
Log UCd	0.66 (-0.96, 2.27)	0.426	0.47 (-0.67, 1.59)	0.417	**2.89 (0.40, 5.39)**	**0.023**	**3.04 (1.02, 5.06)**	**0.033**
Log UPb	1.14 (-0.02, 2.30)	0.055	0.03 (-0.78, 0.84)	0.939	**1.20 (0.13, 2.27)**	**0.029**	**1.21 (0.34, 2.08)**	**0.006**
Log UHg	0.26 (-0.21, 0.73)	0.284	0.24 (-0.09, 0.57)	0.149	-0.18 (-0.74, 0.38)	0.534	-0.04 (-0.50, 0.41)	0.850
Log UiAs	0.63 (-0.73, 1.98)	0.365	0.24 (-0.70, 1.19)	0.616	1.41 (-0.45, 3.27)	0.136	1.02 (-0.49, 2.54)	0.185
Covariate adjusted multiple metal model								
Log UCd	0.45 (-1.18, 2.08)	0.590	0.39 (-0.74, 1.53)	0.496	**2.68 (0.11, 5.24)**	**0.041**	**2.90 (0.83, 4.98)**	**0.006**
Log UPb	1.00 (-0.19, 2.19)	0.098	-0.11 (-0.94, 0.72)	0.793	**1.11 (0.04, 2.19)**	**0.043**	**1.16 (0.29, 2.03)**	**0.009**
Log UHg	0.17 (-0.31, 0.65)	0.489	0.24 (-0.10, 0.57)	0.165	-0.19 (-0.75, 0.37)	0.499	-0.07 (-0.52, 0.38)	0.758
Log UiAs	0.50 (-0.86, 1.86)	0.471	0.21 (-0.74, 1.16)	0.661	0.70 (-1.22, 2.61)	0.479	0.28 (-1.27, 1.83)	0.719

Note: Models were adjusted to age, sex, alcohol consumption, BMI, exercise, and smoking status for adults and age, sex, BMI, exercise, and smoking of parents for non-adults. "Log" refers to natural log-transformation of biomarker concentration (conventional creatinine-adjusted). Each metal predicts each blood pressure measurement (SBP and DBP) separately. Bolded values represent statistical significance ($p < 0.05$).

Table S4-4. Differences in blood pressure for the increase in concentrations of metals of adults and non-adults from the covariate adjusted models

Biomarker	Adults (≥ 19 yrs)				Non-adults (< 19 yrs)			
	SBP (mmHg) β (95% CI)	*p*-value	DBP (mmHg) β (95% CI)	*p*-value	SBP (mmHg) β (95% CI)	*p*-value	DBP (mmHg) β (95% CI)	*p*-value
Covariate adjusted individual metal model								
Log UiAs+MMA	1.20 (-0.34, 2.74)	0.127	0.59 (-0.48, 1.67)	0.282	1.80 (-0.35, 3.94)	0.101	0.48 (-1.27, 2.23)	0.587
Log BiAs+MMA	2.21 (-1.41, 5.82)	0.23	0.33 (-1.91, 2.57)	0.773	0.41 (-2.73, 3.54)	0.798	-2.33 (-4.67, 0.00)	0.05
Covariate adjusted multiple metal model[a]								
Urinary metal group								
Log UCd	0.49 (-1.15, 2.14)	0.556	0.26 (-0.89, 1.40)	0.660	2.12 (-0.39, 4.64)	0.098	**2.15 (0.10, 4.19)**	**0.040**
Log UPb	1.05 (-0.13, 2.23)	0.082	-0.16 (-0.99, 0.66)	0.700	1.02 (-0.04, 2.08)	0.060	**1.00 (0.14, 1.86)**	**0.023**
Log UHg	0.17 (-0.31, 0.65)	0.495	0.23 (-0.10, 0.57)	0.177	-0.20 (-0.76, 0.37)	0.493	-0.11 (-0.57, 0.34)	0.628
Log UiAs+MMA	1.09 (-0.45, 2.64)	0.164	0.55 (-0.53, 1.63)	0.315	0.95 (-1.32, 3.21)	0.411	-0.35 (-2.19, 1.49)	0.710
Blood metal group								
Log BCd	3.37 (-1.68, 8.43)	0.190	0.12 (-3.02, 3.26)	0.941	-0.36 (-3.92, 3.20)	0.842	-0.66 (-3.31, 2.00)	0.625
Log BPb	-1.45 (-6.72, 3.82)	0.589	-0.55 (-3.83, 2.72)	0.740	0.92 (-2.62, 4.45)	0.610	1.24 (1.39, 3.87)	0.353
Log BHg	-0.05 (-1.48, 1.37)	0.942	0.22 (-0.66, 1.11)	0.622	0.88 (-0.02, 1.78)	0.056	0.53 (-0.15, 1.20)	0.124
Log BiAs+MMA	2.25 (-1.38, 5.89)	0.223	0.29 (-1.96, 2.55)	0.799	0.15 (-2.99, 3.29)	0.925	-2.47 (-4.81, -0.13)	0.039

Note: Models were adjusted to age, sex, alcohol consumption, BMI, exercise, and smoking status for adults and age, sex, BMI, exercise, and smoking of parents for non-adults. "Log" refers to natural log-transformation of biomarker concentration. Sum of iAs and MMA (iAs+MMA) was used as exposure biomarker of iAs in urine and blood. Each metal predicts each blood pressure measurement (SBP and DBP) separately. Bolded values represent statistical significance ($p < 0.05$).

[a] All metals are included together in the models and analyzed for urine and blood respectively.

Table S4-5. Interaction between age and metal exposure among non-adults (n = 450) from covariate adjusted individual metal models

Variable	SBP (mmHg) β (95% CI)	*p*-value	DBP (mmHg) β (95% CI)	*p*-value
Log UCd*Age	0.32 (-0.51, 1.16)	0.447	0.38 (-0.30, 1.06)	0.267
Log UPb*Age	0.28 (-0.04, 0.60)	0.089	-0.09 (-0.35, 0.17)	0.502
Log UHg*Age	0.04 (-0.16, 0.25)	0.683	0.10 (-0.07, 0.26)	0.249
Log UiAs*Age	-0.04 (-0.63, 0.54)	0.879	0.22 (-0.26, 0.70)	0.363

Note: Models were adjusted to age, sex, alcohol consumption, BMI, exercise, and smoking status for adults and age, sex, BMI, exercise, and smoking of parents for non-adults. "Log" refers to natural log-transformation of biomarker concentration. Each metal predicts each blood pressure measurement (SBP and DBP) separately.

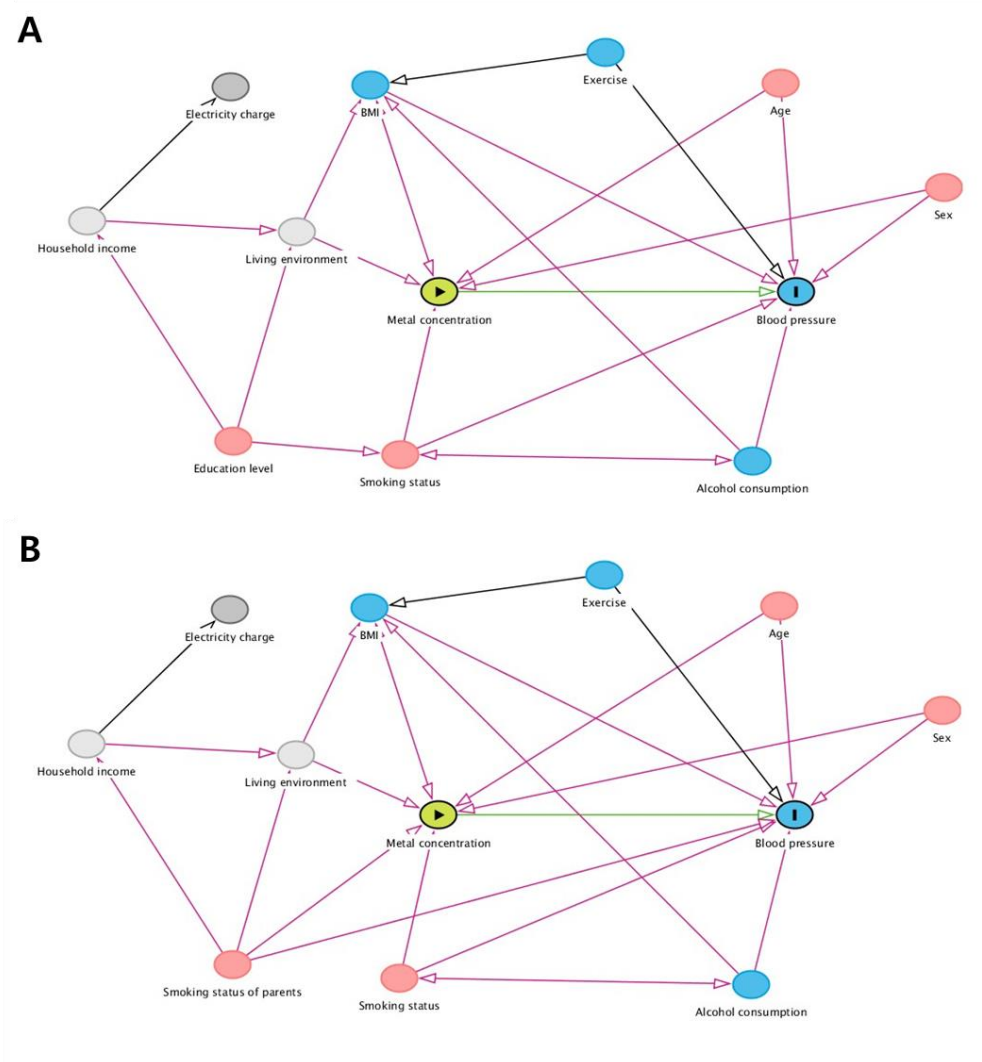

Figure S4-1. Directed acyclic graph (DAG) (produced using DAGitty, http://dagitty.net) of (A) adults and (B) non-adults.

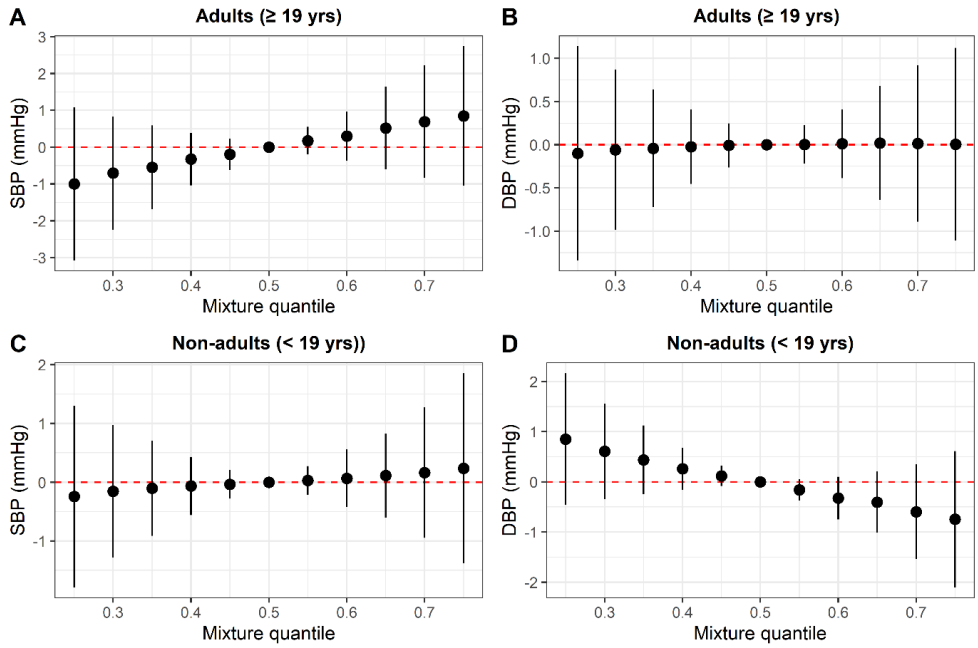

Figure S4-2. Overall effect (95% CI) of the metal mixture on BP when all blood metals at particular percentile were compared to all metals at 50th percentile. The results were obtained by BKMR analysis stratified by age group [adults (A, B) and non-adults (C, D)] and adjusted to age, sex, alcohol consumption, BMI, exercise, and smoking status for adults and age, sex, BMI, exercise, and smoking of parents for non-adults.

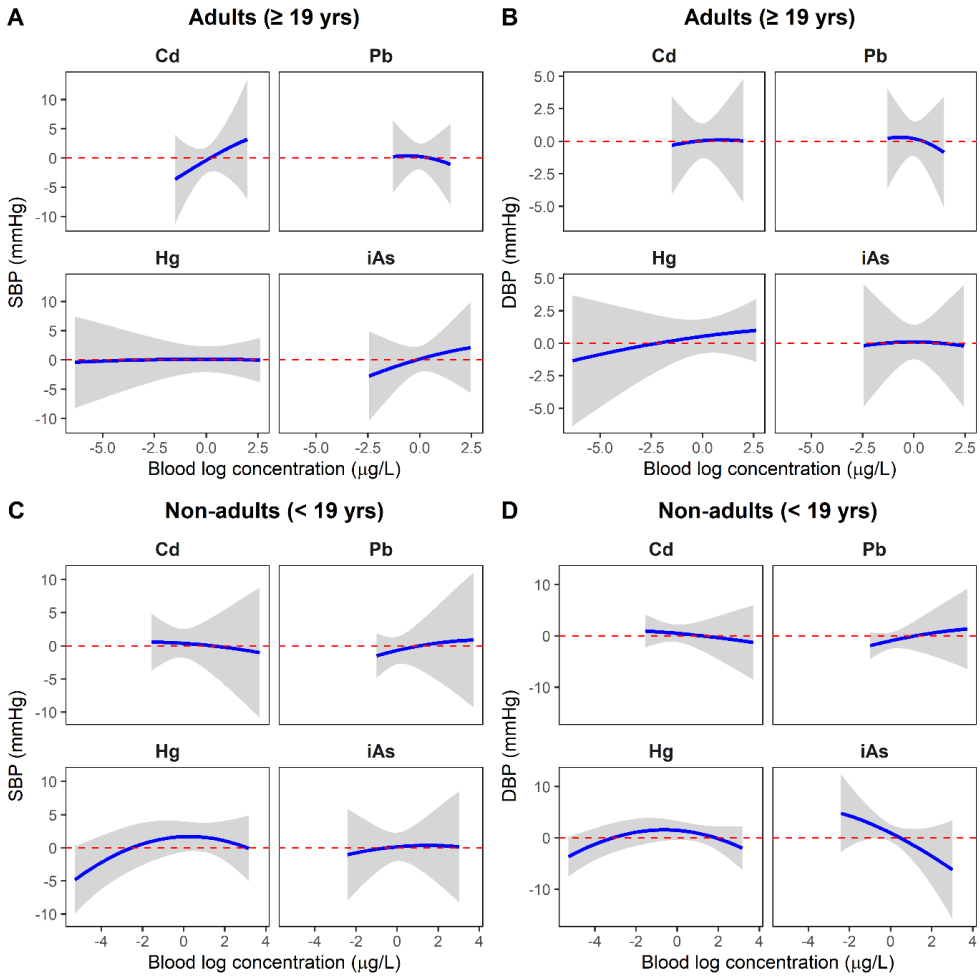

Figure S4-3. Single metal exposure-response relationship (95% CI) between each blood metal and blood pressure while other urinary metals fixed at their 50th percentile. The results were obtained by BKMR analysis stratified by age group [adults (A, B) and non-adults (C, D)] and adjusted to age, sex, alcohol consumption, BMI, exercise, and smoking status for adults and age, sex, BMI, exercise, and smoking of parents for non-adults.

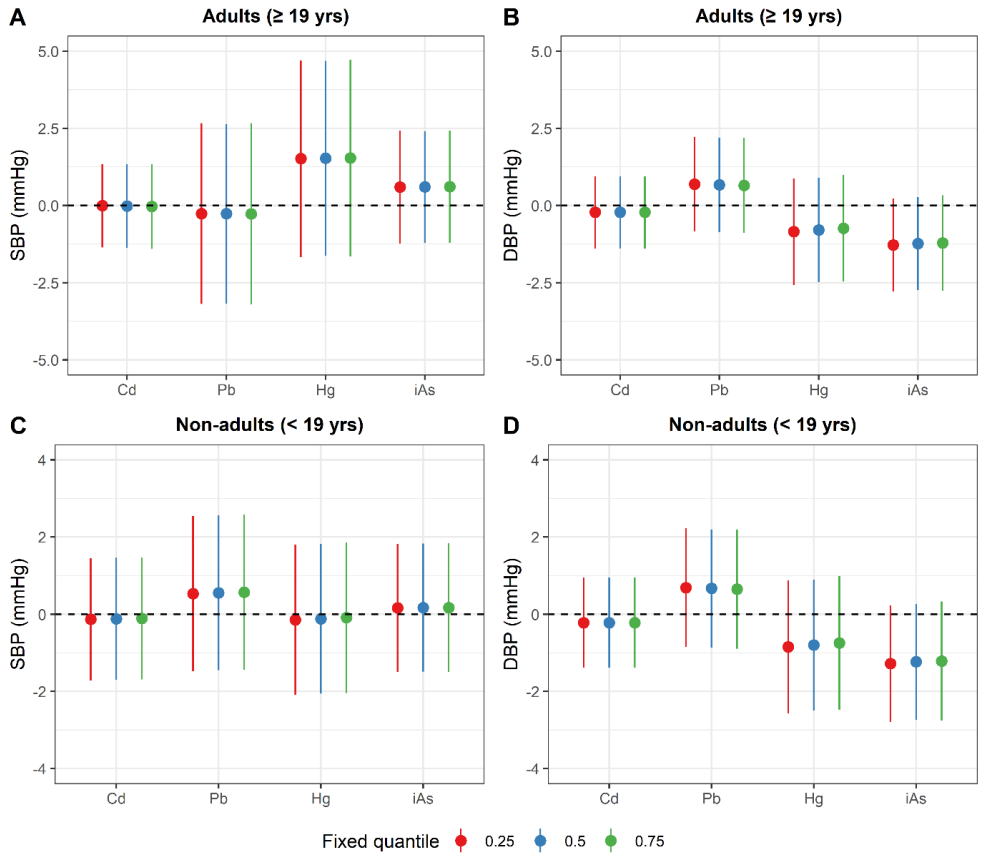

Figure S4-4. Associations of single blood metals with BP were estimated while other blood metals were fixed at their 25th (red), 50th (blue), and 75th (green) percentile, respectively. The results were obtained by BKMR analysis stratified by age group [adults (A, B) and non-adults (C, D)] and adjusted to age, sex, alcohol consumption, BMI, exercise, and smoking status for adults and age, sex, BMI, exercise, and smoking of parents for non-adults.

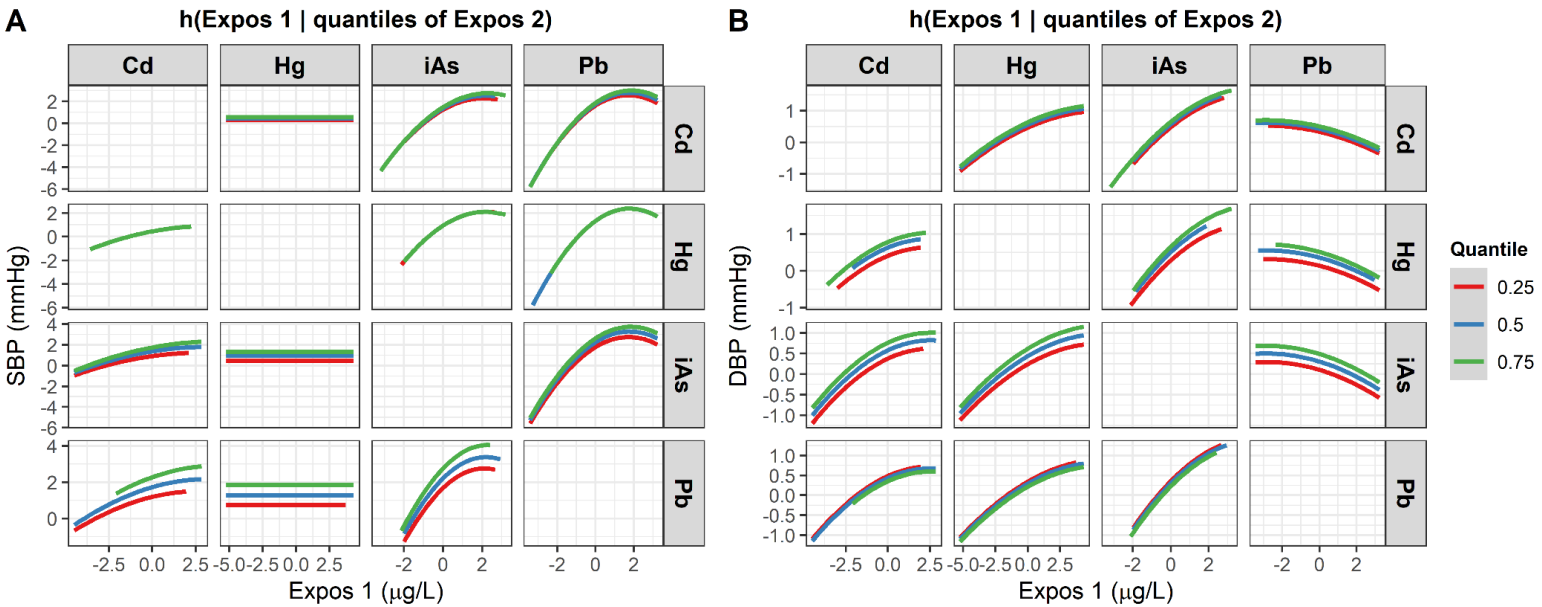

Figure S4-5. Bivariate exposure-response functions for Expos 1 when Expos 2 is fixed at their 25th, 50th, and 75th percentile and others are fixed at the median in blood metal mixture (A: SBP, B: DBP).

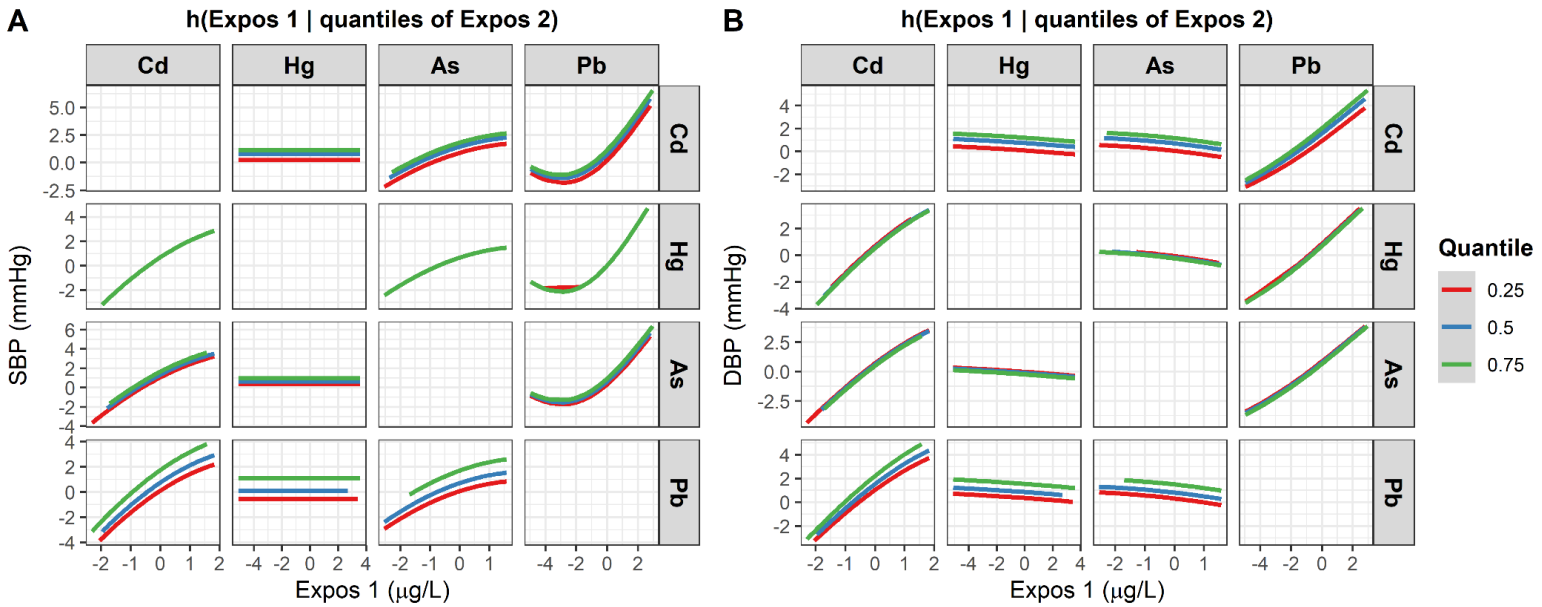

Figure S4-6. Bivariate exposure-response functions for Expos 1 when Expos 2 is fixed at their 25th, 50th, and 75th percentile and others are fixed at the median in the urinary metal mixture (A: SBP, B: DBP).

국문 초록(Abstract in Korean)

중금속 4종 인체노출평가에서의 노출 바이오마커 선택과 활용

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중금속은 자연적으로 존재하는 오염물질로 토양, 물, 공기 등 환경에 널리 분포한다. 이 중 셀레늄, 구리, 아연, 망간 등의 원소들은 체내 항상성을 유지하는데 필수적이지만, 특히 카드뮴, 납, 수은, 비소와 같은 물질들은 독성이 매우 높아 다양한 건강 문제를 야기할 수 있다. 또한 중금속은 자연계에 널리 존재하는 물질인만큼 노출원과 노출경로가 매우 다양하며, 일상생활에서 복합적으로 노출될 수 있다. 중금속의 실질적인 내적 노출 수준은 바이오모니터링을 통해 평가할 수 있으며, 노출평가를 비롯하여 건강영향과의 연관성을 분석하는데 활용할 수 있다. 이에 국내외 다양한 국가바이오모니터링 사업에서는 중금속, 특히 카드뮴, 납, 수은, 비소를 측정해오고 있다. 국내에서는 국민환경보건기초조사(이하 기초조사)와 같은 국가바이오모니터링 사업을 통해 중금속의 노출 수준을 확인하고 있으며, 대상물질은 소변 중 카드뮴, 혈중 납, 소변 중 수은, 혈중 수은이다. 비소 역시 노출 특성과 독성을 고려하였을 때 중요하게 고려되어야하는 물질이나, 제 1기 기초조사 (2009-2011) 이후로는 대상 물질에서 제외되었다. 국내 일반인구집단의 카드뮴, 납, 수은의 노출 수준은 점차 감소하고 있는 추세이나, 국외의 국가바이오모니터링 자료와 비교

하였을 때 상당히 높은 수준임을 확인할 수 있었다. 그러나 비소의 경우, 국내 인구집단의 노출 수준을 대표할 만한 자료가 부족하여 노출의 경시적인 변화나 국외와 비교하였을 때 어느 정도의 수준인지 확인이 어렵다. 또한 일반인구집단에서 카드뮴, 납, 수은은 혈중 및 소변 중 마커의 특성이 잘 알려져 있는 편이지만, 비소는 관련 연구가 상대적으로 부족한 실정이다. 따라서 본 학위논문에서는 비소를 중심으로 바이오모니터링 연구를 수행하여 기존 중금속 바이오모니터링에서 미흡했던 부분을 보완하고자 하였다. 또한, 4종 금속(카드뮴, 납, 수은, 비소)을 중심으로 바이오모니터링 자료를 해석 · 활용하는 방법을 보여주는 사례연구로써 노출평가와 역학 연구를 각각 수행하였다.

첫 번째 연구에서는 식약처에서 2017-2018년도에 전국민들을 대상으로 수집한 혈액과 소변(이하 식약처 표준인체시료) 총 2,025건으로부터 고성능액체크로마토그래프 및 유도결합플라즈마 질량분석기 (UPLC-ICP/MS)를 사용하여 비소종 6종(arsenate(As(V)), arsenite(As(III)), monomethylarsonic acid(MMA), dimethylarsinic acid(DMA), arsenobetaine(AsB), arsenocholine(AsC))의 분리 분석을 수행하고, 매체별 농도 대푯값과 비소종의 구성비를 확보하였다. 또한 소변 및 혈중 비소 농도 및 구성비와 관련있는 인구사회학적 및 노출 특성을 확인하였다. 우리나라 사람들의 비소종 농도의 합은 국외 고노출 지역과 비슷한 수준이었으나 소변과 혈액 내 비소 대부분은 독성이 낮은 유기비소인 AsB인 것으로 나타났다(각각 51.1%, 53.5%). 또한, 무기비소와 그 메틸화 대사체(MMA, DMA), AsC의 구성비는 소변과 혈액에서 유의미한 차이를 보였으며, 독성이 큰 무기비소가 차지하는 비율은 소변(2.18%)보다 혈액(20.2%)에서 더 큰 것으로 나타났다. 무기비소의 주요 노출원인 음용수 종류와 잡곡밥 섭취 여부는 소변 중 무기비소 농도 증가와 관련이 있었으며, 등푸른 생선류의 섭취는 소변과 혈액 중 AsB 농도 증가와 관련이 있는 것으로 나타났다. 해당 결과는 독성이 큰 무기비소의 노출평가를 위해서는 무기비소의 종 분리 분석이 필수적임을 시사한다. 무기비소와 MMA, DMA 농도로 계산한 무기비소의 메틸화 효율은 청소년과 흡연자에서 낮았으며, 여성의

경우 메틸화 수준이 남성보다 높았다. 종합하자면, 우리나라 사람들의 높은 총 비소 수준은 해산물 섭취로 인한 유기비소에 영향을 받은 것으로 이는 비소 바이오모니터링에서는 종분리분석이 필수적임을 의미한다. 또한, 혈중 비소종보다 요중 비소종이 노출원과 유의미한 관계가 있는 것으로 나타났는데 이는 요중 비소종이보다 적절한 노출바이오마커임을 시사한다. 그러나 혈액의 경우 무기비소의 구성비가 소변보다 높았다는 점에 있어 건강 영향과 연관한 추가적인 연구가 필요하다.

두 번째 연구에서는 식약처 표준인체시료로부터 측정한 생체시료중 카드뮴, 납, 수은, 무기비소의 농도를 이용하여 카드뮴, 납, 메틸수은, 무기비소의 연령 집단별 노출량을 산출하였다. 첫 번째 연구와 동일한 인구집단에서 비소종 외에 추가적으로 카드뮴, 납, 수은을 유도결합플라즈마 질량분석기 (ICP/MS)로 분석하였다. 노출량 추정에는 4종 금속의 인체 생리학적 독성동태(PBTK) 모델을 사용하였으며, 사용한 각 물질별 바이오마커는 소변 중 카드뮴, 혈중 납, 혈중 수은, 소변 중 무기비소였다. 수은의 경우에는 혈중 수은 중 메틸수은의 비를 이용하여 혈중 메틸수은 농도를 추정하였다. 다음으로, 확률론적 시나리오 기반 노출평가를 수행하여 다양한 매체와 노출경로로부터 카드뮴, 납, 수은, 비소의 노출량을 구한 뒤, 시나리오 기반 노출평가로 추정한 노출량과 비교하였다. 표준인체시료에서의 중금속 농도는 다른 국내 인구집단에서의 중금속 농도와 비슷하거나, 혈중 납의 경우 약 2배 정도 높은 수준이었다. PBTK 모델로 이용한 바이오모니터링 기반 노출량 추정치는 중위수 기준 카드뮴 0.21-0.72 μg/kg/day, 납 0.50-0.97 μg/kg/day, 메틸수은 0.023-0.053 μg/kg/day, 무기비소 0.09-3.51 μg/kg/day였다. 이는 식약처의 노출허용기준과 비교하였을 때 카드뮴과 메틸수은은 전 연령에서 기준치보다 낮은 수준이었으나, 납은 전 연령에서 기준치를 초과하였으며 무기비소는 영유아와 미취학아동 집단에서 기준치를 초과할 가능성이 있었다. 시나리오 기반 노출평가 결과와 비교하였을 때, 중위수 기준으로 카드뮴은 성인, 미취학아동 집단에서 두 방법의 추정치가 서로 비슷하였으나 다른 연령

집단에서는 바이오모니터링 기반 추정치가 1.7-2.8배 더 높았다. 납은 전 연령 집단에서 바이오모니터링 기반 추정치가 2.3-6.1배 더 높았다. 메틸수은과 무기비소의 경우 시나리오 기반 노출평가에서 총수은과 총비소를 대상으로 하였기에 두 방법 간 직접 비교는 불가능하였다. 서로 다른 두 가지 노출평가 방법의 추정치는 물질과 집단에 따라 비슷하거나 차이를 보였는데, 이는 노출평가 시 각 방법의 장단점을 고려할 필요성이 있음을 의미한다. 바이오모니터링 기반 노출평가의 경우, 내적 노출을 반영할 수 있지만 바이오마커 농도의 대표성, PBTK 모델의 특성(컴파트먼트 구성, 모델 파라미터, 노출 경로), 노출량 역산 시의 가정(경구 노출만 발생)이 예측치의 불확실성을 증가시킬 수 있다. 한편, 시나리오 기반 노출평가의 경우, 개별 노출원/노출경로의 기여 수준을 확인할 수 있지만 노출 관련 정보(시나리오, 노출계수, 오염도)가 부정확 할수록 예측값의 불확실성이 커질 수 있다. 각각의 방법을 사용할 때는 사용 목적에 따라 내재된 한계점을 고려할 필요가 있으며, 본 연구에서 검토한 한계점들은 이후 보다 정확한 노출평가를 수행하기 위해 개선될 필요가 있다.

세 번째 연구에서는 식약처 표준인체시료의 중금속 4종(카드뮴, 납, 수은, 무기비소)의 측정치와 참여자들의 혈압 자료를 이용하여 중금속의 복합노출과 혈압과의 연관성을 조사하였다. 이 때 전통적인 선형회귀분석과 베이지안 커널 머신 회귀(BKMR), 가중 분위수 합 회귀(WQS) 분석을 이용하였다. 인구집단은 미성년(만 19세 미만)과 성인으로 나누었으며, 중금속 측정치는 소변과 혈액으로 나누어 분석하였다. 선형회귀모형 분석결과, 다른 중금속들을 보정하였음에도 소변 중 납($p < 0.040$)과 카드뮴($p < 0.023$)이 미성년 집단에서 확장기 혈압(DBP) 증가와 유의미한 연관성이 있는 것으로 나타났으나, 성인에게서는 소변, 혈액에서 네 물질 모두 유의미한 결과를 보이지 않았다. 한편 성인의 경우, BKMR 분석 결과 소변 중 4종 금속의 복합 노출이 수축기 혈압(SBP)를 유의미하게 증가시키는 것으로 나타났으나, WQS 분석에서는 유의미하지 않았다($p = 0.684$). 미성년 집단의 경우 BKMR 분석에서 소변 중 금속의 복합 노출

과 SBP와 DBP 모두 유의미하게 증가하는 것으로 나타났으며, WQS 분석에서는 SBP가 유의미하게 증가하였다($p < 0.007$). 이 때, 소변에서 혈압 증가에 미치는 물질은 납(WQS index = 34.2%), 카드뮴(29.6%), 무기비소(28.2%), 수은(8.0%) 순이었다. 본 연구결과는 성인보다 미성년 집단이 노출 수준이 낮음에도 불구하고 중금속으로 인한 건강영향에 보다 민감할 수 있음을 시사한다. 또한, 여러 통계 분석 결과에서 공통적으로 소변 중 납이 혈압과 관련된 유의미한 마커인 것으로 보이며 이에 대한 추가적인 검증이 필요하다.

본 연구에서는 하나의 한국인 일반 인구집단으로부터 수집한 시료로부터 기존에 잘 알려지지 않았던 비소를 중심으로 바이오모니터링 연구를 수행하고, 4종 금속의 바이오모니터링 자료를 이용하여 노출평가와 역학 연구를 수행하였다. 그리고 이를 통해 바이오모니터링 시 어떤 마커 · 매체를 선택해야하는지, 수집한 자료를 어떻게 해석 · 활용할 것인지에 대한 답을 주고자 하였다. 본 연구는 개별 연구들이 모두 동일한 인구집단 자료를 이용하였다는 점, 전 연령을 포함한다는 점, 4종 중금속을 소변과 혈액에서 모두 측정했다는 점, 그리고 현재까지 잘 알려지지 않았던 한국인에서의 비소 노출 수준을 종분리 분석을 통하여 확인하고 이후 연구에 해당 자료를 활용하였다는 점에 의의가 있다. 다만 본 연구에서는 단면조사를 통해 얻어진 시료와 건강영향 자료를 분석하였다는 점에서 한계가 있으므로 이를 보완한 추가 연구가 필요할 것으로 사료된다.

주요어: 중금속, 바이오모니터링, 노출평가, 생리학적 독물동력학 (Physiologically-Based Toxicokinetic, PBTK) 모델, 복합노출, 혈압

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