A Dissertation for the Degree of Doctor of Philosophy

Mercury exposure and associated health effects among the general populations of Korea

한국의 일반 인구집단 대상 수은 노출 및 건강영향

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이 승 호

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위 논문을 이승호 박사학위 논문으로 제출함.

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ABSTRACT

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Mercury (Hg) is a naturally occurring heavy metal compound and a ubiquitous contaminant of soil, air, foods, and other media. There are several forms of Hg in the environment; inorganic Hg such as amalgam, and organic Hg, especially methylmercury (MeHg), which is known for the most toxic form of Hg. According to two Korean national surveys, the geometric mean (GM) for Hg in blood was higher than that in similar national surveys done in Canada and the USA, and a substantial people in South Korea have the high level of blood Hg. Regarding to the biomonitoring level, the German Federal Environmental Agency set the guideline values for 5 μg/L (The HBM-I) as a control value for which no action is needed, and 15 μg/L (The HBM-II) as an action level for which medical care or advice is recommended. Meanwhile, the main exposure source for the general population is diet, especially fish

consumption. It is absorbed and condensed in the human body via fish intake. In fact, about 80 % of blood Hg consists of MeHg. In order to regulate the exposure amount, the United States Environmental Protection Agency (US. EPA) recommended 0.1 μg/Kg/day for MeHg as the reference dose (RfD). There have been several studies conducted investigating Hg exposure, however, there has been no study for the exposure amount of Hg based on the individual internal dose among the South Korean population. And the extent of Hg exposure should be investigated, especially for the related health effects as a suspected obesogen. In addition, Hg can be transferred from mother to fetus via placenta. Though women in South Korea have the high blood Hg compared to those in other countries, there has been no study for fetal body burden during pregnancy. Thus, exposure assessment for sensitive population is required.

Therefore, this study was conducted for investigating the exceedances above the guidance values among South Korean. And the corresponding exposure dose for MeHg was calculated, which can be compared to the RfD. Next, the associations between Hg and health outcomes were investigated using clinical chemistry markers. Lastly, fetal body burden was derived by using the PBPK model and investigated the growth effects of exposure to Hg among infants.

In Chapter II, the Korean National Environmental Health Survey (KoNEHS 2009-2011) were used to derive the exposure amount for Hg. Gender, age, and frequency of fish consumption were first identified as important predictors of KoNEHS blood Hg levels using generalized linear models. Stratified distributions of total blood Hg were then converted into distributions of blood MeHg using fractions of MeHg to total Hg from the literature. Next, a

published physiologically-based pharmacokinetic (PBPK) model was used to predict distributions of blood MeHg as a function of MeHg intake; ratios of MeHg intake to model-predicted blood MeHg were then combined with KoNEHS-based blood MeHg values to produce MeHg intake estimates. These intake estimates were ultimately compared with the Reference Dose (RfD) for MeHg (0.1 μg/kg/day) and reported as hazard quotient (HQ) for specific KoNEHS subgroups. The GM of blood Hg was 3.08 μg/L and about 25 % of total population exceeded the HBM-I. Though the exceedances were derived from single measurement per individual, however those were decreasing with the lower ICC and the more repeated data. The predicted exposure dose was 35.8 ng/kg/day for total population. And the corresponding HQ value was 0.36 for total (range: 0.24 ~ 0.63). The GM was only used for deriving the HQ because using the tail of simulation data requires careful interpretation. Considering the results were derived using only the GM, the potential at-risk population could be existed for each population.

In Chapter III, the perturbation of clinical chemistry markers including ALT, γ-GTP, total cholesterol, triglyceride, total lipid, and IgE were investigated as the effects associated with exposure to Hg. GMs of blood Hg and clinical chemistry markers were determined and odds ratios of out-of-reference range for each marker were estimated by gender and quantile of blood Hg adjusted for age, BMI, smoking and alcohol consumption. The blood Hg level was categorized into quantile groups - low: < 25th (2.05 μg/L), medium: 25th ~ 75th (4.75 μg/L), high: > 75th – to diagnose the effect of Hg on the reference ranges. The levels of ALT, γ-GTP and total lipid were increased with blood Hg group for both sexes, and the GMs for total cholesterol and TG were

significantly increased in men across the blood Hg. Regarding to γ-GTP, the high blood Hg group was associated with a 2.8-fold increase for being out-of-reference range in the male group (OR=2.77; 95% CI: 1.72-4.46), while 2.1-fold increase in the female group (OR=2.11; 95% CI: 1.35-3.30). High Hg group in men was associated with about 1.5-fold risk for total cholesterol. Especially, the significant contribution of blood Hg to high γ-GTP were retained after adjustment for other co-exposing chemicals in the multivariate linear model. Our results suggest that Hg exposure even as low as environmental levels among general population could perturbate lipid metabolism although further mechanistic researches needs to be confirmed.

In Chapter IV, the association between environmental exposures and health outcomes in children were investigated using the Children's Health and Environmental Chemicals in Korea (CHECK). The fetal body burden for Hg was derived by using PBPK model, and the growth effects from Hg exposure at the developmental period were analyzed using several statistical approaches. Among 106 paired data out of total 334 pairs, the GM for Hg in maternal blood and cord blood was 4.47, 7.35 μg/L, and that of placenta and meconium was 9.0, 36.9 ng/g, respectively. Though the sample size of infant's hair was too small (n=25) for the following analysis, however, the GM was as high as 443 ng/g. The derived fetal body burden was ranged between 26.3 and 86.9 mg based on the 106 cord blood. Cord blood Hg was positively associated with length at birth (*p*-value: 0.0132), while weight at birth (*p*-value: 0.1764) and head circumference (*p*-value: 0.4579) were not associated with cord blood Hg. According to the logistic regression model, the cord blood Hg were not significant for the standardized height (OR: 1.30 (0.55, 3.07)), weight (OR:

0.76 (0.36, 1.62)), and PI (OR: 0.817 (0.377, 1.77)). However, the LS mean estimates for the follow up weights represent the more rapid increasing slopes in the high cord blood Hg group compared to the low Hg group for both sexes. These results indicated that fetus would have high body burden, and exposure to Hg associated with the taller height at birth and the rapid weight increasing.

This study revealed the highly exposed population for Hg and conducted exposure assessment across the population based on the estimated MeHg. All HQs were below 1, but the upper 5 percentiles of HQ were above 1 except for those who consumed fish rarely. These results indicate that the potential at-risk population could be existed for each population, not only for those in consuming fish frequently. Also, Hg exposure even as low as environmental levels among general population could perturbate lipid metabolism although further mechanistic researches confirm. Furthermore, fetus would be exposed to certain amount of Hg (26.3 ~ 86.9 μg/kg/day), and that could be influenced on the growth at birth and weight inceasing in later life. Therefore, more researches for fetal body burden are required, and growth effects should be investigated adjusting for other confounders including Hg level in diet and co-exposure from other chemicals.

Keywords: mercury, methylmercury, biomonitoring, dose reconstruction, physiologically based pharmacokinetic model, hazard quotient, obesogen, body burden

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LIST OF ABBREVIATIONS

ALT: alanine aminotransferase

BPA: bisphenol A

CHECK: Children's Health and Environmental Chemicals in Korea

Cd: cadmium

CI: confidence interval

Coti: cotinine

ECF: exposure conversion factor

EDC: endocrine disrupting chemicals

EDI: estimated daily intake

US EPA: U.S. Environmental Protection Agency

GerES: German Environmental Survey

GM: geometric mean

γ-GTP: gamma-glutamyl transferase

HBM: human biomonitoring

Hg: mercury

HippA: hippuric acid

HQ: hazard quotient

ICC: intra-class correlation coefficient

IgE: immunoglobulin E

KoNEHS: Korean National Environmental Health Survey

LOD: limit of detection

MandA: mandelic acid

MBzP: mono-benzyl phthalate

MC: monte carlo

MECPP: mono-(2-ethyl-5-carboxypentyl) phthalate

MeHg: methylmercury

MEHHP: mono-(2-ethyl-5-hydroxyhexyl) phthalate

MEOHP: mono-(2-ethyl-5-oxohexyl) phthalate

MFDS: Korea Ministry of Food and Drug Safety

MHA: methylhippuric acid

MnBP: mono-n-butyl phthalate

MucA: t,t-muconic acid

Napt: 2-naphthol

NHANES: U.S. National Health and Nutrition Examination Survey

OHFLU: 2-hydroxyfluorene

OHP: 1-hydroxypyrene

OHPHE: 1-hydroxyphenanthrene

OR: odds ratio

PAH: polycyclic aromatic hydrocarbons

Pb: lead

PBA: 3-phenoxybenzoic acid

PBPK: physiologically-based pharmacokinetic

PheA: phenylglyoxylic acid

PI: ponderal index (g/Cm^3)

RfD: Reference Dose

RL: Reporting limit

TCS: triclosan

TG: triglyceride

VOC: volatile organic compounds

CHAPTER I.

BACKGROUNDS

Mercury (Hg) exposure in general population

Mercury is a natural compound and a ubiquitous contaminant of soil, air, foods, etc. It is found with several forms in environment such as inorganic compound including metallic or element form and organic compound as in methylmercury. Due to its readily absorption, accumulation and toxic effects (ATSDR, 1999), organic compound (mainly methylmercury) has been focused in toxicology and public health area. Besides, methylmercury is a main component of blood mercury (Jung et al., 2013; You et al., 2012b). The major exposure source of methylmercury is ingestion of pelagic organisms. Since microorganisms methylated inorganic mercury to methylmercury, this methylmercury disperses to pelagic organisms and be condensed in it (IPCS, 1990). Exposure to methylmercury is associated with neurotoxicity causing disorders of memory and speech capabilities, cardiovascular effects, reproductive effects, carcinogenicity including kidney cancer, etc. (Hong et al., 2012). Some of the recent reports suggest that high blood mercury might be associated with metabolic syndrome (Eom et al., 2014) and other sub-clinical health outcomes in Korean populations (Kim and Lee, 2012; Lee et al., 2012).

Due to the high level of concern surrounding Hg exposure, many regulatory agencies in the world set the health related reference values or allowable exposure limits of mercury. One of the world-widely reference values for blood mercury is German human biomonitoring (HBM) values, set by Commission on Human Biological Monitoring of the German Federal Environmental Agency. HBM-I (5 μg/L) is a control value below which no action is needed, while HBM-II (15 μg/L) implies intervention level over which medical care or

advice are needed (Drasch et al., 2002; Schulz et al., 2007; Schulz et al., 2011). These values were derived based on the two large studies of the Faroe islands and the Seychelles. 5 mg/kg was taken as a threshold, which was the concentration in mother's hair when the adverse effects had occurred in the children. Then, by applying the ratio of mercury between hair and blood, HBM-II of 15 μg/L was calculated (Drasch et al., 2002; Schulz et al., 2011). These values can be compared to the measurement of chemical substances in human, with considering the integrative uptake variability through different exposure pathways (Smolders et al., 2008).

Hg has been included in several national biomontoring surveys including the U.S. National Health and Nutrition Examination Survey (NHANES), German Environmental Survey (GerES) (Schulz et al., 2011), Canadian Health Measures Survey (CHMS) (Canada, 2013), and E.U. Consortium to Perform Human Biomonitoring on a European Scale (COPHES). Beginning in 2009, the Korean Ministry of Environment also implemented a nationwide biomonitoring project, entitled the "Korean National Environmental Health Survey" (KoNEHS) (Lim et al., 2012; Park et al., 2014). This survey includes measurements of blood Hg along with 18 other environmental chemicals. According to the first KoNEHS report (2009-2011), the geometric mean for total blood Hg was 3.09 μg/L, which is considerably higher than the results from the U.S. NHANES (0.703 μg/L from 2011-2012 (CDC, 2015)) and GerES III (0.58 μg/L in 1998 (Becker et al., 2002; Mahaffey et al., 2009)). Although there's a decreasing trend by year, considerable population had the higher blood Hg level than HBM-I among South Korean (Park et al., 2014).

Reconstruction of exposure dose

According to the WHO Guidance for identifying population at risk from mercury exposure, the intake amounts of methylmercury can be estimated with i) aggregation of food consumption information on fish species, serving size and frequency; ii) calculation with total mercury concentrations by the fish species ingested; iii) body weight of fish consumer (WHO and UNEP, 2008). Accordingly, many surveys estimated the intake amounts of methylmercury by multiplying consumption frequencies of dietary sources (*e.g.* fish, seafood), concentrations of methylmercury in the corresponding species and the consumption amounts (Cho et al., 2014; Dellatte et al., 2014; You et al., 2012b). The approach is relatively easier because it needs individual food consumption and the general information for mercury contents in the corresponding food. But it is indirect and has substantial uncertainty in order to apply for individual subject.

To the contrary, dose conversion of biological samples via pharmacokinetic model seems to reflect the internal dose for individual. Two approaches are available for MeHg: simple one-compartment PK modeling and PBPK modeling. One-compartment modeling has assumptions – kinetic profiles could be explained with a volume of box (body) with an absorption and an excretion rate constants; given that absorption is close to zero-order and elimination follows first-order kinetics, there is no net change in blood concentration of methylmercury at steady state; Then, $dC/dt = k_0/V_d - K_e*C = 0$, where k_0 is corresponding to daily intake amounts of methylmercury, K_e is the elimination rate constant and V_d is the volume of distribution.

In case of PBPK modeling, mathematical equations in physiological organs

describe absorption, distribution, metabolism and excretion. The PBPK model for MeHg consists of adult model having twelve compartments and fetal portion having four compartments (Figure 1.1). Oral absorption was modeled as zero-order uptake followed by intestinal absorption. The transport of MeHg and its conversion to inorganic mercury were described by linear processes. Distribution in the blood was assumed to be plasma-flow–limited, with the exception of flow across the placenta, across the blood-brain barrier, and across the red cell membranes, which were assumed to be diffusion-limited. Excretion of MeHg was assumed to occur via transport to hair, with subsequent loss of hair, conversion to inorganic mercury by gut flora and in the liver, and by urinary excretion. The fetal compartments were assumed to grow during the time of gestation. And maternal plasma, RBCs, richly perfused tissues (representing changes in the uterus and mammary glands), and fat compartment are describe to increase over the course of pregnancy (Clewell et al., 1999; Gearhart et al., 1995).

This approach can provide physiologically plausible estimations and conducts simulations at various exposure conditions (Clewell et al., 1999; EPA, 2006). Recently, more and more regulatory agencies and scientists in the world are trying to develop and apply PBPK model in risk assessment coupled with exposure-reconstruction (Allen et al., 2007; Brown et al., 2015; Georgopoulos et al., 2009; Loccisano et al., 2013). In order to control daily intake amounts of MeHg, U.S. Environmental Protection Agency (EPA) established a reference dose (RfD) as 0.1 μg/kg/day, which is “an estimate of a daily exposure to the human population that is likely to be without appreciable risk of deleterious effects during a lifetime”. It was derived from the study in Iraq in which

developmental neurotoxicity was observed following ingestion of methylmercury-treated grain (Rice, 2004; Stern, 1997). Several studies conducted for investigating Hg exposure, however, there were no study of MeHg exposure for Korean population.

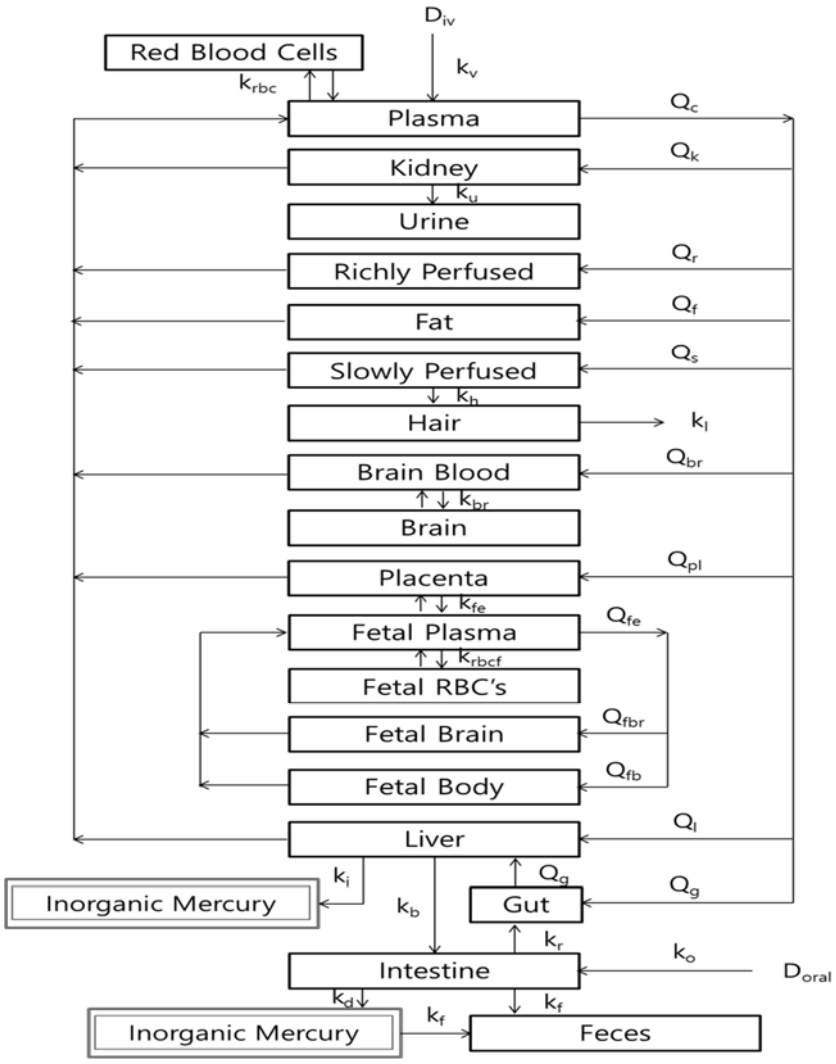

Figure 1.1. Physiologically based pharmacokinetic model for MeHg

Association between Hg and health outcomes

Different forms of Hg have different effects on human body, because they do not all move through the body in the same way. The general population is most commonly exposed to mercury from eating fish and marine mammals that contain MeHg. It is absorbed through the gastrointestinal tract easily, and it enters bloodstream before moving to other parts. Especially, it easily crosses the blood-brain-barrier and placenta so that accumulated in the brain of fetuses (ATSDR, 1999; Hong et al., 2012).

Exposure to Hg cause toxic effect on kidney, cardiovascular system and developing fetus along with nervous system (WHO and UNEP, 2008) via oxidative stress, lipid peroxidation, and mitochondria dysfunction, etc., which lead to deleterious effects on enzymes, cell membrane function, and neuron delivery materials (Hong et al., 2012; Mergler et al., 2007). In addition, several studies reported significant associations between high blood Hg and metabolic syndrome (Eom et al., 2014; Geier et al., 2016; Poursafa et al., 2014). Metabolic syndrome, which is defined as a cluster of disorders including central obesity, glucose intolerance, hypertension, low high-density lipoprotein cholesterol, and high triglycerides, has been suggested to be related to some environmental pollutants as well life style and diet (Chung et al., 2015; Moon, 2014). However, there were also some reports which showed no significant association with metabolic syndrome (Lee and Kim, 2013; Moon, 2014; Rothenberg et al., 2015; Rotter et al., 2015). It might indicate that Hg impacts on metabolic syndrome were not strong to lead the specific symptoms. Therefore, the health outcome following Hg exposure needs to be investigated using clinical markers.

Health outcomes after early life exposure

Mercury (Hg) can be transferred from mother to the fetus via placenta. Furthermore, the Hg level in cord blood was much higher than that in maternal blood (Butler Walker et al., 2006; Morrissette et al., 2004; Ong et al., 1993; Sakamoto et al., 2013). As a result, the maternal exposure for Hg could have influences on fetal development (ATSDR, 1999; Grandjean et al., 1997; Kim et al., 2016; Sakamoto et al., 2013).

There were several epidemiological studies after poisoning in Iraq and Japan. At first, the outbreak in Japan showed the occurrence of fetal poisoning and suggested that the fetus is more sensitive than the mother to toxic effects of MeHg (Harada, 1995). And then, community poisoning in Iraq reported symptoms such as gross impairment of motor, cerebral palsy, deafness and blindness (Amin-Zaki et al., 1974). The Faroe study also presented the association of prenatal Hg exposure and several adverse effects including tests of memory, attention, language, motor function, and visual spatial perception (Grandjean et al., 1997).

Since women in South Korea have the high blood Hg compared to other countries, however, there was no study for fetal exposure during pregnancy. In addition, exposure to Hg in early stage can interfere with the body's adipose tissue homeostasis and the ability to regulate weight control, thus leading to metabolic syndrome and obesity later in life (Hennig et al., 2012; Janesick and Blumberg, 2016). In this reason, the fetal body burden and the following health effects should be investigated.

Objectives

The objectives of this thesis were to evaluate Hg and MeHg exposures among South Korean population.

In the chapter 2, blood Hg measurements were compared to the HBM guidelines and MeHg intake estimates were compared to the RfD. HQ estimates were derived for different population subgroups to identify those with higher exposure potential and to inform risk management and mitigation strategies.

In the chapter 3, health outcomes associated blood Hg were determined using clinical chemistry markers. Especially, the risks of being out-of-reference ranges for hepatic and metabolic markers were analyzed. Furthermore, the effect of blood Hg was also evaluated after adjustment for co-exposure to environmental chemicals as confounders.

In the chapter 4, the fetal body burden for Hg during pregnancy was estimated using the PBPK gestation model, and investigated the growth effects of exposure to Hg among infants.

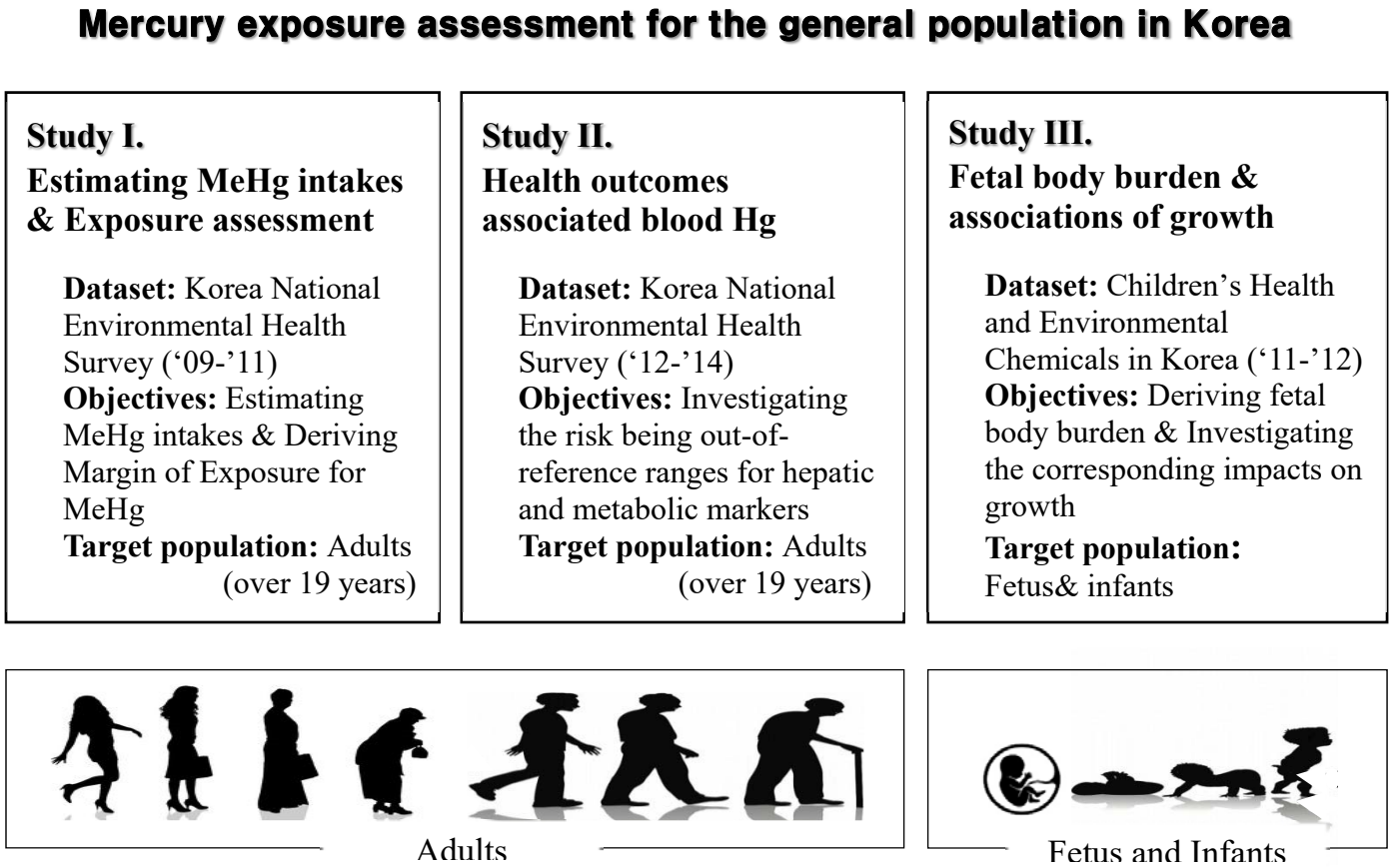

Figure 1.2. Summary of the research design

CHAPTER II.

Estimating methylmercury intake for the general population of South Korea using PBPK modeling

Introduction

Mercury (Hg) is a naturally occurring element and a ubiquitous contaminant of soil, air, foods, and other media. It exists in the environment in several forms, including elemental, inorganic, and organic forms. Methylmercury (MeHg), an organic form, has been extensively studied due to its ability to be highly absorbed, bioaccumulate, and cause neurotoxic, cardiovascular, reproductive, and carcinogenic effects (ATSDR, 1999; Hong et al., 2012). Exposure to MeHg is often associated with the consumption of fish (particularly large, fatty fish, such as tuna (Minnesota Department of Health), and can be monitored via measurement of MeHg in blood, or total Hg in blood, with MeHg generally comprising the majority of total blood Hg (Jung et al., 2013; You et al., 2012a). A number of recent studies have reported significant positive associations between total blood Hg levels and metabolic syndrome (Eom et al., 2014; Geier et al., 2016; Poursafa et al., 2014). Given the high level of concern surrounding MeHg exposure and toxicity, regulatory agencies have promulgated health-related reference values and allowable exposure limits. In addition, Hg has been included in several national biomonitoring surveys (either as total Hg or MeHg), including the U.S. National Health and Nutrition Examination Survey (NHANES), German Environmental Survey (GerES), Canadian Health Measures Survey (Health Canada), and E.U. Consortium to Perform Human Biomonitoring on a European Scale (COPHES). These biomonitoring programs are useful for investigating population exposures to specific chemicals, with thousands of measurements generally reported for each sampled population (typically one measurement per person). Beginning in

2009, the Korean Ministry of Environment also implemented a nationwide biomonitoring project, entitled the Korean National Environmental Health Survey (KoNEHS) (Lim et al., 2012; Park et al., 2014). This survey includes measurements of total blood Hg along with 18 other environmental chemicals. According to the first KoNEHS report (2009-2011), the geometric mean (GM) total blood Hg level was 3.08 μg/L, which is considerably higher than GM levels reported from the U.S. NHANES (0.703 μg/L from 2011-2012, N=7920 (CDC, 2015)) and GerES III (0.58 μg/L in 1998, N=4646 (Becker et al., 2002)).

To aid in the interpretation of biomarker data, biomonitoring equivalents (BEs) and other health-based guidance values have been developed for a growing number of environmental chemicals (Hays and Aylward, 2012). German human biomonitoring (HBM) values (set by the Commission on Human Biological Monitoring of the German Federal Environmental Agency) exist for blood Hg, and are based on two epidemiological studies conducted in the Faroe islands and the Seychelles (Drasch et al., 2002). The HBM-I value for blood Hg is 5 μg/L, below which no action is needed. The HBM-II value for blood Hg is 15 μg/L, above which medical care or advice is recommended (Schulz et al., 2007; Schulz et al., 2011). Despite the need for further study of the intermediate range between HBM-I and -II (Schulz et al., 2007), comparison of blood Hg levels (including those measured in the KoNEHS) to these guidance values can provide useful estimates of relative risk.

While blood Hg levels from KoNEHS may inform potential MeHg exposures, more direct assessment of health risks for this compound are based on actual MeHg measurements (or model predictions) and MeHg-specific

reference values. The U.S. Environmental Protection Agency (EPA) has established a Reference Dose (RfD) of 0.1 μg/kg/day for MeHg, (US.EPA, 2001), which is "an estimate of a daily exposure to the human population that is likely to be without appreciable risk of deleterious effects during a lifetime." In order to evaluate KoNEHS biomarker data in the context of the RfD, blood Hg data must be converted into MeHg intake estimates. In the present study, a previously validated physiologically based pharmacokinetic (PBPK) model for MeHg (Clewell et al., 1999) was used to facilitate this conversion. The predicted daily MeHg intake estimates were compared against the RfD to obtain a hazard quotient (HQ). The HQ, which could be a useful index for risk characterization, is the ratio of the potential exposure to a substance and the level at which no adverse effects are expected (in this case, an RfD)(Solomon et al., 2000). For the present study, an HQ > 1 indicates MeHg intake estimates that are above the RfD.

The objectives of the current study are to evaluate Hg and MeHg exposures amongst subgroups of the South Korean population via comparisons of: (1) blood Hg measurements to HBM guidelines; and (2) MeHg intake estimates to the EPA RfD. These analyses are intended to place the total blood Hg levels measured in South Koreans in a health risk context using a weight-of-evidence approach. HQ estimates are reported for different population subgroups to identify those with higher exposure potential and to inform risk management and mitigation strategies.

Materials and Methods

General population survey data

The Korean National Institute of Environmental Research (NIER) performed the first KoNEHS project from 2009 to 2011. Approximately 2000 adult subjects >19 years old were recruited each year of the study using stratified sampling units representing the residential distributions of geographical area, gender and age. A total of 6311 participants each provided one blood and urine sample along with questionnaire responses detailing their demographic information and lifestyle. Nineteen chemicals were measured in the biological samples, including total Hg in blood, which was measured using the gold-amalgam collection method and the Direct Mercury Analyzer 80 (DMA 80, milestone, Bergamo, Italy). The limit of detection (LOD) for blood Hg in KoNEHS was 0.04 μg/L. Values below the LOD (n=4) were included in the current analysis as LOD divided by the square root of 2. More information about the study design, methods of measurement and validation can be found elsewhere (NIER, 2011; Park et al., 2014).

Data Analysis procedures

The overall data analysis workflow is illustrated in Figure 2.1 – specific details about each step of the workflow are described in the subsequent sections. Briefly, variables that were statistically associated with blood Hg were first identified. Blood Hg levels for population subgroups were then compared with HBM guidance values. Next, blood Hg levels were converted to blood MeHg

levels using fractions of blood MeHg to total Hg from the literature (Jung et al., 2013). PBPK modeling was then performed to reconstruct MeHg intake based upon estimated blood MeHg concentrations. Finally, HQ values were calculated using estimated daily intake amounts and the U.S. EPA RfD for MeHg. All statistical analyses were performed using SAS® version 9.3 (SAS Institute Inc., Cary, NC, USA), and PBPK modeling was performed using MATLAB® (R2013b version8.2.0.701, Math Works, Natick, MA).

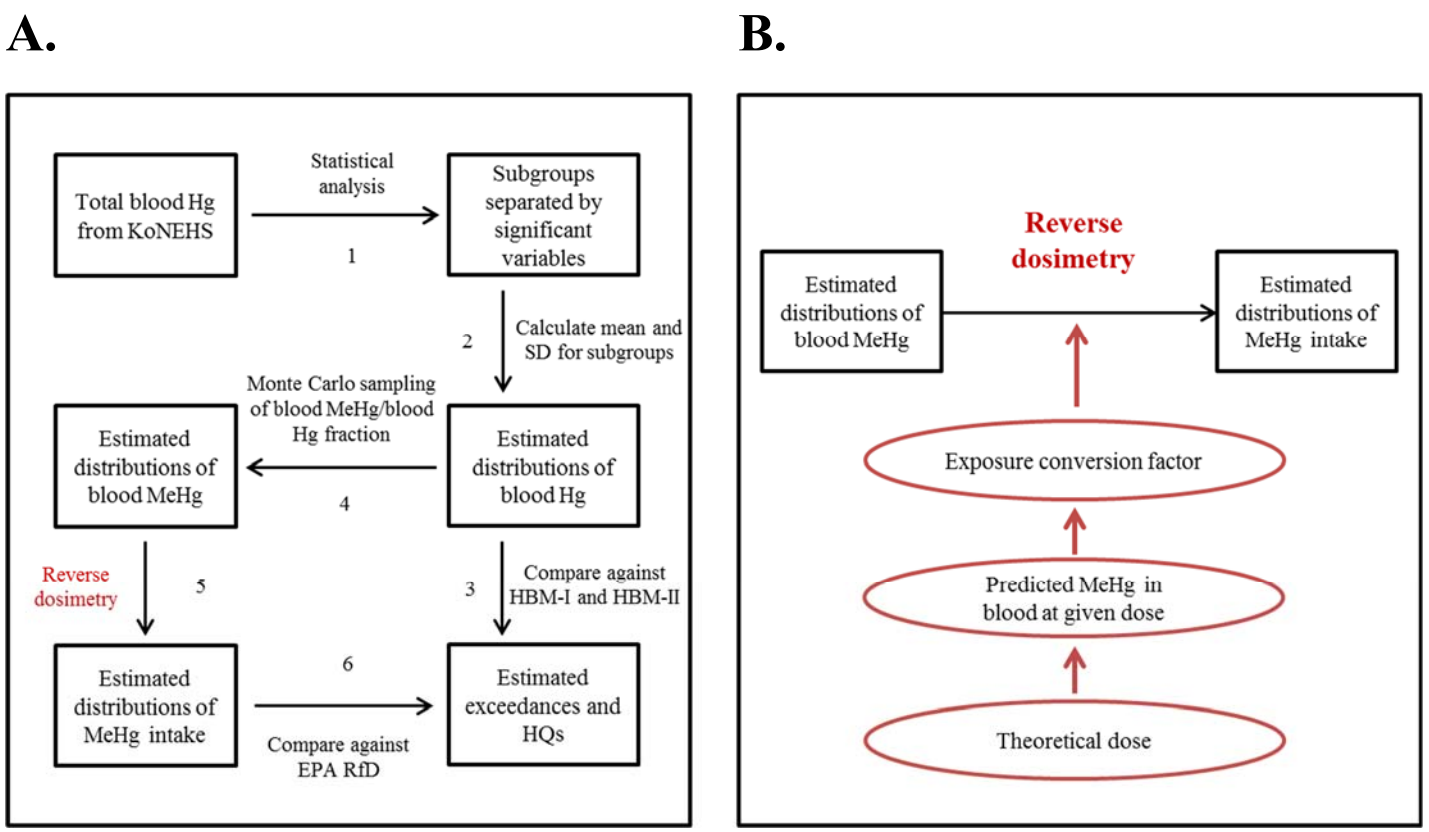

Figure 2.1 Overall data analysis workflow. [A] Procedures for comparing blood Hg measures against HBM-I and HBM-II values and estimated MeHg intake against the EPA RfD for MeHg. [B] More detailed schematic of the procedure for reverse dosimetry (step 5 in [A]).

Step #1: Identification of significant predictors of blood Hg levels

Across a total of 6311 subjects, biomarker results for 29 pregnant women and 13 subjects with missing records were excluded from analyses (*n*=6269). Results for pregnant women were excluded since blood Hg levels may be particularly transient during pregnancy due to rapid changes in body weight and diet. Since the distribution of blood Hg was right skewed, log-transformation was performed prior to all statistical analyses. This transformation satisfied assumptions of normality according to visual inspection of histograms and QQ-plots. Geometric means of total blood Hg were calculated (SAS Proc SURVEYMEANS) using sampling weights and survey strata. Bivariate analyses (SAS Proc SURVEYREG) were initially performed to identify independent variables that were correlated with blood Hg levels; evaluated demographic variables include age (19-29, 30-39, 40-49, 50-59, 60-69, >70), gender (male, female), BMI category (underweight: <18.5, normal: 18.5-23, overweight: 23-25, obese: >25), residential area (urban, rural, coast, monitoring station of heavy metals), smoking status (current smoker, nonsmoker), alcohol consumption status (current drinker, does not drink), and frequency of physical exercise (does not exercise, exercises regularly [30 minutes at least 3 times per week], exercises irregularly). Several variables related to seafood consumption were also investigated, including frequency of consumption (rarely, 1-3 times/month, 1-2 times/week, 3-4 times/week, 5-6 times/week, every day) and type of fish consumed. Categories of fish include large fish (e.g. whale, tuna), fish (e.g. mackerel, cod, salmon), crustaceans (e.g. shrimp, lobster, crab), seaweeds, shellfish, and other seafood (e.g. squid, sea cucumber).

Step #2: Model selection for defining population subgroups

Multiple regression models for blood Hg were constructed to include the candidate explanatory variables described above, as well as interaction terms (i.e., two-way interactions between age, gender, BMI, and seafood consumption). Backward stepwise elimination was used to select the best model (SAS Proc GLM) at a significance level of $p \leq 0.1$. Competing models were compared using Akaike information criteria (AIC), Bayesian information criteria (BIC), and the coefficient of determination. Main effects only of age, gender, and fish consumption frequency (but not type of fish consumed) were ultimately included in the final models.

Step #3: Calculating exceedance of blood Hg above HBM-I and HBM-II

Estimated means and standard deviations (in log space) were used to determine the percentage of each population subgroup (categorized by the aforementioned independent variables) with blood Hg levels exceeding HBM-I and HBM-II. These calculations were performed according to the method of Pleil and Sobus (Pleil and Sobus, 2013), and require knowledge of the intra-class correlation coefficient (ICC) for blood Hg. The ICC represents the ratio of between subject measurement variance to total variance and is calculated using repeat measures for individuals. The two most extreme cases are when ICC=0 or when ICC=1. When ICC=0, average measures across individuals are expected to be very similar, and repeated measures for any given individual are expected to be variable. When ICC=1, average measures across individuals are expected to be variable, and repeated measures for any given individual are

expected to be very similar. Since KoNEHS did not include repeat measures, we considered values of ICC ranging from 0 to 1 (representing the full range of possible ICC values) assuming either 3, 5, or 10 measurements per subject.

Step #4: Estimating MeHg concentrations in blood

Only total blood Hg was available in KoNEHS. Therefore, blood MeHg levels had to be estimated prior to reconstructing MeHg intake. Monte Carlo (MC) simulation was first used to generate distributions of blood Hg by gender, age, and fish consumption frequency based on observed distributions of Hg in KoNEHS; this step was performed to ensure a sufficient sample size for each subgroup (further information regarding the MC simulation is provided in table 4,5 section 3, Appendices). Next, blood Hg values were multiplied by a fraction of blood MeHg to blood Hg from the published literature. Specifically, stratified distributions of the MeHg/Hg fraction (assumed normal) were adopted from a previous Korean study, which collected paired measurements of MeHg and Hg in blood across 400 participants (Jung et al., 2013). For each subgroup (by age, gender, and fish consumption frequency), ten thousand random samples were selected (with replacement) based on theoretical normal distributions using SAS/IML. Due to limited data for older age groups, the distribution of MeHg/Hg for age group 60-69 was applied to age group 70+. Furthermore, due to a lack of data on fish consumption frequency, the total distribution of MeHg/Hg from participants in the Jung *et al.* study (n=354 participants age 20 or over) was applied across all KoNEHS subgroups for fish consumption frequency. The estimated blood MeHg levels based on KoNEHS data were compared to measurements of blood MeHg from the US NHANES

2011-2012 (n = 9,756). Geometric means were estimated for specific NHANES subgroups (SAS Proc SURVEYMEANS) after adjusting for sampling weights and survey strata. A total of 5,165 NHANES measures were considered after removing missing values and measures for participants below age 20.

Step #5: PBPK modeling and reconstruction of MeHg intake

1) PBPK modeling

Absorption, distribution, metabolism, and elimination of MeHg were simulated using a published human PBPK model (Clewell et al., 1999). Since pregnant women were not included in this analysis, the gestation-related compartments (e.g., uterus, fetus) were excluded from the model. All kinetic and physiological parameters were adopted from the original models, with the exception of body weight – these values were sampled from the measurements of the KoNEHS participants. We assumed that the primary source of MeHg exposure was via ingestion for the general Korean population (Eom et al., 2014; Kim and Lee, 2010). A local sensitivity analysis was performed on all 48 model parameters to identify parameters with the greatest impact on the predicted blood MeHg (Clewell et al., 1994). Five sensitive parameters were identified, including body weight, volume of slowly-perfused tissues, partition of gut-to-blood, partition of hair-to-blood, and MeHg excretion into hair. Further information regarding sensitive parameters of the PBPK model are provided in table 6, section 3, Appendices.

2) Prediction of daily intake amounts of MeHg

In order to convert estimated MeHg in blood to MeHg intake, the PBPK model was used to implement a reverse dosimetry method known as the "Exposure Conversion Factor" (ECF) approach (Clewell et al., 2008; Tan et al., 2006). ECFs were computed as the ratio of the model input (daily exposure amount, μg/kg/d) to simulated MeHg concentrations in blood (μg/L) at steady state (between 7000-9000 hours). MC simulations were used to randomly sample (10,000 iterations per scenario) sensitive parameters. The distribution for body weight was derived directly from the KoNEHS records (Appendices Table 6). Distributions for the remaining sensitive parameters were obtained from the literature (Allen et al., 2007; Clewell et al., 1999). All other model parameters were to set to their mean values. To consider variation in diets among the Korean population, three exposure scenarios were considered in the MC analysis; exposure frequency (i.e., the number of eating events during which MeHg exposure occurred) was set to once a day, once a week, or once a month with the same dose per exposure (1 μg/kg/exposure). All simulations were used to predicted steady-state blood MeHg concentrations, which were the basis for ECF estimates (i.e., ECF=intake dose/steady-state MeHg concentrations in blood). Central tendency values of ECF distributions were multiplied by estimated blood MeHg (calculated in Step #4) to predict estimated daily intake (EDI) of MeHg (ng/kg/day) for each group.

Step #6: Calculation of hazard quotient

HQ estimates were derived based upon the distributions of estimated MeHg intake. Specifically, HQ estimates were calculated as the ratio of the GM of the estimated intake to the EPA RfD (0.1 μg/kg/d) for each population subgroup.

Results

Total blood Hg in the KoNEHS

Descriptive statistics for blood Hg levels and exceedance estimates for each subgroup are given in Table 2.1. The GM and the 95th percentile estimates of total blood Hg for the entire population were 3.08 and 9.91 μg/L, respectively [range 0.028-49.52 μg/L]. Results from multiple regression models showed that blood Hg levels varied by gender ($p < 0.0001$), age group ($p < 0.0001$), and frequency of fish consumption ($p < 0.0001$). Levels of blood Hg were significantly higher in males (GM=3.65 μg/L; 95% CI: 3.49-3.83) compared to females (GM= 2.62 μg/L; 95% CI: 2.51-2.73). Total blood Hg levels increased with age until the subjects reached their 60's, then decreased with age (see Table 1). Regarding consumption of fish, those who eat fish every day had about twice the blood Hg levels [GM=4.86 μg/L; 95% CI: 4.24-5.56] as those who eat fish rarely [GM=2.16 μg/L; 95% CI: 2.00-2.32].

Exceedance of blood Hg levels above HBM-I and HBM-II

Comparisons of blood Hg levels to HBM-I (5 μg/L) and HBM-II (15 μg/L) showed exceedance rates of 25% and <2% across all subjects, respectively (Table 2.1). The largest exceedance rates were observed for those who eat fish every day, with estimates of 48% (using HBM-I) and 5% (using HBM-I). These results were derived from single (spot) measurements of KoNEHS participants assuming no within-person variation – that is, assuming ICC=1. Since the true ICC is unknown in this population, exceedance values were estimated for additional cases where ICC was set to 0, 0.25, 0.5 and 0.75. The

impact of ICC, as well as the number of repeats (*m*) used in calculating ICC, is shown in Figure 2.2 (with additional results provided in tables 1-3, Appendices). Here, a gradual decrease in exceedance is observed given deceasing ICC and increasing *m*.

Table 2.1 Descriptive statistics for total blood Hg and exceedances above HBM-I and HBM-II

		Total Hg in blood (μg/L)				Exceedance (%)	
		GM	**GSD**	**Range**	**p95**	**HBM-I**	**HBM-II**
All (n = 6298)		3.08	2.20	(0.028, 49.52)	9.91	24.9	1.32
Gender	Male (*n* = 2924)	3.65	2.67	(0.028, 49.52)	12.1	33.4	2.66
	Female (*n* = 3374)	2.62	1.71	(0.028, 46.76)	7.39	16.1	0.38
Age	19-29 (*n* = 747)	2.34	1.49	(0.070, 46.34)	6.53	11.6	0.17
	30-39 (*n* = 1180)	3.23	2.18	(0.028, 30.21)	10.5	25.9	1.14
	40-49 (*n* = 1333)	3.45	2.41	(0.028, 49.52)	10.2	29.8	1.76
	50-59 (*n* = 1495)	3.75	2.74	(0.028, 46.76)	12.5	34.7	2.88
	60-69 (*n* = 1110)	3.05	2.22	(0.066, 44.40)	10.2	24.9	1.42
	70+ (*n* = 433)	2.58	1.86	(0.040, 37.42)	7.58	17.9	0.72
Fish consumption	Rarely (*n* = 533)	2.16	1.41	(0.309, 37.42)	6.50	10.0	0.16
	1-3/Month (*n* = 2024)	2.82	2.03	(0.028, 46.76)	8.85	21.4	1.01
	1-2/Week (*n* = 2653)	3.25	2.20	(0.028, 49.52)	10.1	26.2	1.20
	3-4/Week (*n* = 790)	3.92	2.73	(0.028, 46.34)	12.1	36.4	2.69
	5-6/Week (*n* = 110)	4.05	2.75	(0.950, 19.36)	12.4	37.9	2.71
	Everyday (*n* = 188)	4.86	3.41	(0.860, 44.40)	15.1	48.3	5.40

[Note] GM: geometric mean, Range: (minimum, maximum), p95: the 95th percentile, Exceedance: percent of subjects in each group who exceed the German human biomonitoring (HBM) guidance values for total Hg in blood; 5 μg/L for HBM-I and 15 μg/L for HBM-II. The GSD was computed based on the method suggested by SAS (Estimating the Standard Deviation of a Variable in a Finite Population, available at: https://support.sas.com/rnd/app/stat/examples/SurveyStdDev/new_example/index.html)

Estimates of MeHg in blood

The estimated global GM of MeHg in blood was 2.16 μg/L (see Table 2.2). The trends observed with the total blood measurement data (shown in Table 2.1) were also evident with the estimated MeHg data. Levels of blood MeHg were higher in males than females and generally increased with age and fish consumption frequency. Compared to other surveys, blood MeHg estimates in Korean adults were about three times higher than blood MeHg measurements in US adults (NHANES 2011-2012, GM=0.619 μg/L; Table 2.2), but about half that previously reported in a separate Korean study (GM=4.44; Table 2.2).

Table 2.2 Comparison of estimated blood MeHg with measurements from other studies (μg/L)

		Present study		NHANES (2011-2012)*			Jung et al. (2013)		
Variables	**Class**	**GM**	**p95**	***N***	**GM**	**p95**	***N***	**GM**	**p95**
All		2.16	7.26	5165	0.619	5.83	400	4.44	14.0
Gender	Male	2.47	8.45	2553	0.646	6.52	189	5.13	16.6
	Female	1.89	5.83	2612	0.594	5.19	211	3.91	11.6
Age	19-29	1.63	4.82	1038	0.477	4.5	36	3.12	10.3
	30-39	2.21	7.06	880	0.533	5.05	88	4.20	12.5
	40-49	2.35	7.73	834	0.643	7.2	76	4.75	15.5
	50-59	2.68	9.27	831	0.727	6.94	88	5.69	14.4
	60-69	2.28	7.72	822	0.829	7.53	66#	5.93#	17.8#
	70+	1.93	6.45	760	0.621	5.42	-	-	-
Fish	Rarely	1.50	4.65	-	-	-	-	-	-
Consump-	1-3/month	1.98	6.71	-	-	-	-	-	-
tion	1-2/week	2.27	7.25	-	-	-	-	-	-
	3-4/week	2.74	9.00	-	-	-	-	-	-
	5-6/week	2.83	9.07	-	-	-	-	-	-
	Everyday	3.39	11.25	-	-	-	-	-	-

[Note] GM: geometric mean, p95: the 95th percentile; * over 19 years; #: over 60 years and older

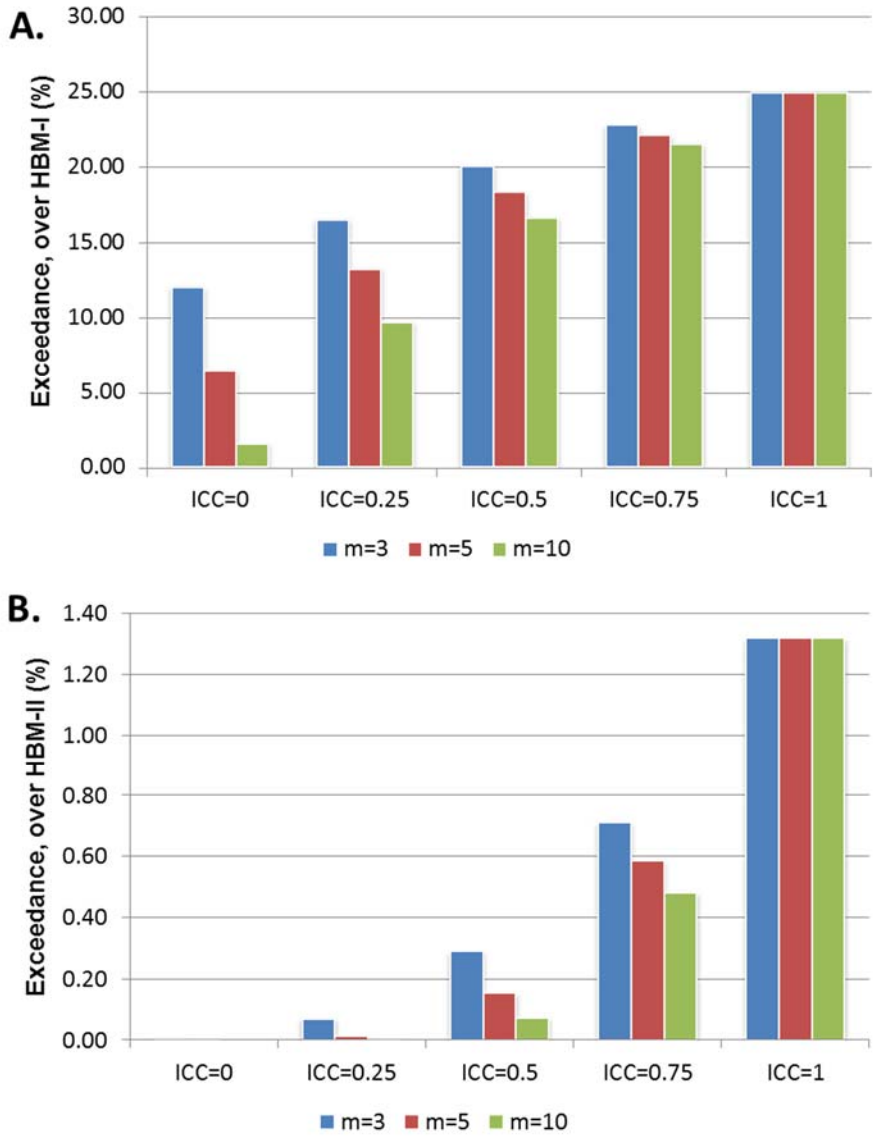

Figure 2.2 Exceedance estimates over HBM-I and HBM-II at various intra-class correlation coefficients (ICCs). [A] Exceedance over HBM-I (5 µg/L) equals 24.9% at ICC=1. [B] Exceedance over HBM-II (15 µg/L) equals 1.32% at ICC=1. 'm' indicates number of theoretical repeated measurements per subject.

Estimates of MeHg intake

The time profiles of estimated blood MeHg across the three different dosing scenarios (i.e., daily, weekly, and monthly) are displayed in Figure 2.3. Simulations were run until steady state blood concentrations were reached for each scenario. Average blood MeHg concentrations were 51.7 μg/L, 7.5 μg/L, and 1.9 μg/L for daily, weekly, and monthly exposures, respectively, at a unit dose of 1 mg/kg/day (Figure 2.3 [A]). The central tendency ECF estimate under each of the three dosing scenarios was very similar (Figure 2.3 [B]), confirming both a linear relationship between exposure and biomarker levels, and independence between ECF values at steady state and exposure frequency. A global ECF value of 0.017 was ultimately used to estimate values of MeHg intake that correspond to blood MeHg levels. The GM EDI of MeHg was 35.8 ng/kg/day for the whole population (see Table 2.3). The EDI for those who eat fish every day (56.1 ng/kg/day) was more than twice that of people who eat fish rarely (25.1 ng/kg/day). The central tendency EDI for each group was lower than the EPA RfD (100 ng/kg/day) for MeHg.

Estimates of hazard quotient

The GM HQ estimates across all population subgroups were between 0.24 and 0.63 (Table 2.3). The largest HQ (0.63) was observed for men who consume fish every day. The smallest HQ (0.24) was observed for women who consume fish rarely. Regarding age, HQ estimates increased until subjects reached their 60's in both males and females.

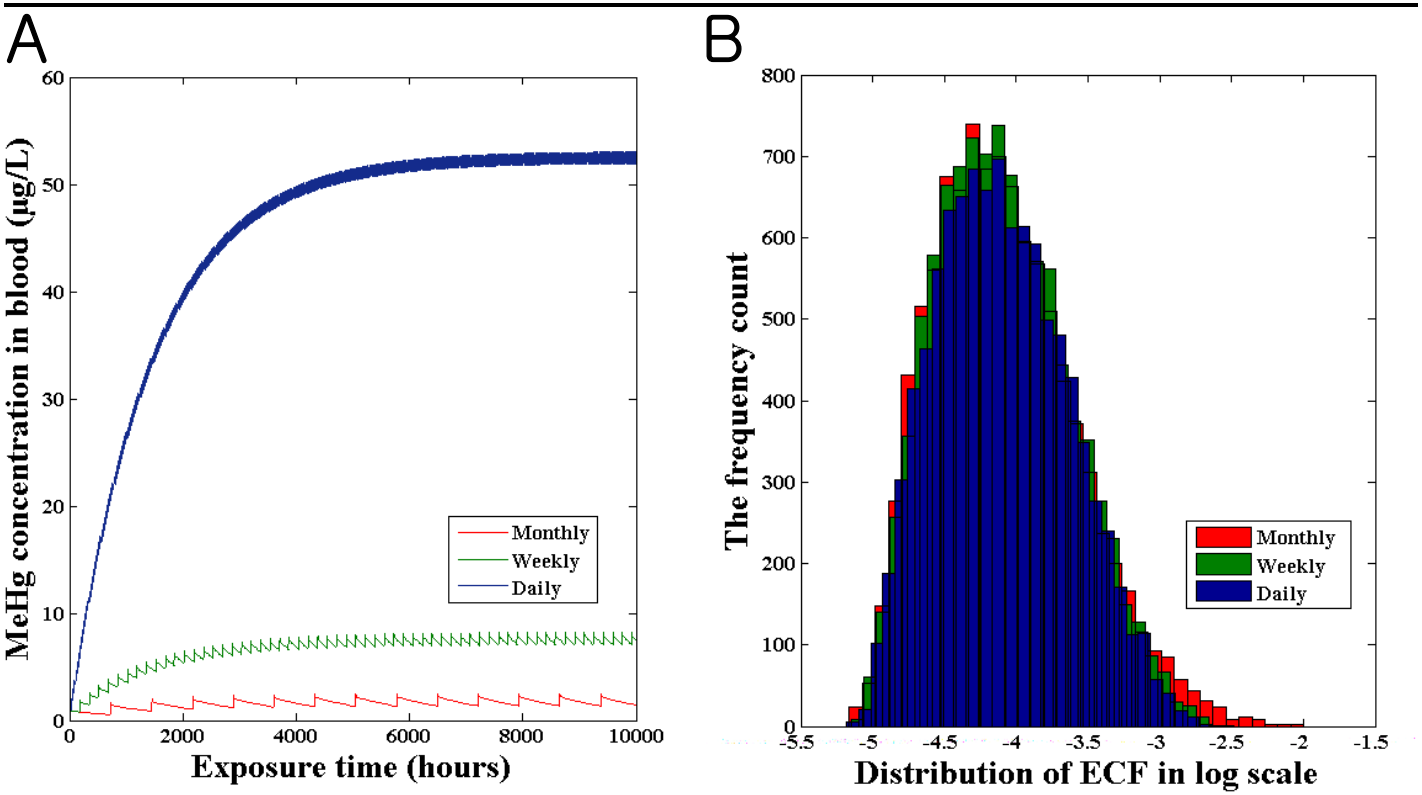

Figure 2.3 Simulated time profiles of MeHg in blood [A] and ECF distributions [B] by exposure scenario. The exposure in each scenario was set at 1 µg MeHg/kg BW/exposure event, but with different exposure event frequencies (i.e., daily, weekly, and monthly).

Table 2.3 The estimated daily intake (EDI) of MeHg (ng/kg/day) and hazard quotient (HQ) for MeHg in the adult Korean population (2009-2011)

		Male		Female		Total	
		EDI	**HQ**	**EDI**	**HQ**	**EDI**	**HQ**
All		41.1	0.41	31.3	0.31	35.8	0.36
Age	19-29	29.3	0.29	25.1	0.25	27.4	0.27
	30-39	44.3	0.44	31.8	0.32	37.0	0.37
	40-49	47.2	0.47	34.4	0.34	39.1	0.39
	50-59	50.0	0.50	38.2	0.38	44.9	0.45
	60-69	41.0	0.41	31.1	0.31	38.1	0.38
	70+	34.6	0.35	27.9	0.28	32.1	0.32
Fish Consump-tion	Rarely	25.8	0.26	24.4	0.24	25.1	0.25
	1-3/month	37.8	0.38	28.8	0.29	32.8	0.33
	1-2/week	43.9	0.44	33.3	0.33	37.6	0.38
	3-4/week	53.2	0.53	39.0	0.39	45.6	0.46
	5-6/week	56.8	0.57	36.5	0.36	47.3	0.47
	Everyday	62.6	0.63	50.9	0.51	56.1	0.56

[Note] EDI indicates the GM of the estimated daily intake of MeHg.
HQ= GM of the predicted intake (μg/kg/day) / RfD (0.1 μg/kg/day)

Discussion

Many studies have indicated that levels of blood Hg in the Korean population are among the highest in the world (Cho et al., 2014; Kim and Lee, 2010; NIER, 2011; Park et al., 2014; You et al., 2012a). Based on our analysis of the KoNEHS data, assuming a limiting case where ICC=1 (i.e., negligible within-person variance in blood Hg levels), we would predict about 2% of the Korean population to have blood Hg levels higher than the HBM-II (15 μg/L), and 25% to have levels that exceed the HBM-I (5 μg/L) (Schulz et al., 2011).

Although confirmation is needed, recent studies suggest that these high levels of blood Hg might be associated with metabolic syndrome and obesity among the general population in Korea (Bae et al., 2016; Chung et al., 2015; Eom et al., 2014). To further inform the level of risk, the current study presents a weight-of-evidence approach for interpreting blood Hg data from KoNEHS.

Exceedances of blood Hg above HBM considering ICC

Single spot measures of blood Hg may be associated with exposures occurring over previous days, weeks, and even months (Yaginuma-Sakurai et al., 2012). Some within-person variance is expected in blood Hg levels, which has implications for exposure reconstruction and risk assessment efforts. Specifically, a single spot measure of blood Hg may not adequately reflect an individual's true average Hg body burden. To address this uncertainty, we estimated exceedance levels for population subgroups across a full range of ICC values (i.e., from 0 to 1). While this approach suggests a range of potential

exceedances for each subgroup, utilization of an observed ICC is preferable, and should yield more accurate results. In KoNEHS, there were no repeated measurements of analytes, so ICCs were not able to be calculated. In the absence of data, using an ICC of 1 is considered a health-protective approach - that is, erring towards maximum caution. However, an ICC of 0.71 was reported based on maximum 6 measures of blood Hg per subject among 1429 elderly population aged over 60 who lived in urban in South Korea (Lee *et al.*, 2017). Using this ICC, the adjusted exceedance over HBM-I for KoNEHS 60's and over 70's would be 21 %, and 13.5%, respectively. When comparing these estimates to the unadjusted exceedances of 25 % (60's) and 18 % (70's), it becomes clear that risk estimates can be prone to over inflation when based on single-measure studies, and a default assumption of ICC=1 (Table 1). As such, exceedance estimates provided in Figure 2.2 should be carefully interpreted if new data on ICC levels in the Korean population become available.

The approaches of estimating MeHg exposure

Although blood Hg is a good biomarker of exposure, its use in traditional risk assessment is still under discussion. In terms of risk assessment, intake amounts are typically utilized as the basis of comparison to health-based reference or guidance levels. According to the WHO guidance for identifying populations at risk of Hg exposure, intake amounts of MeHg can be estimated using: i) food consumption information on fish species, serving size, and frequency; ii) measured total Hg concentrations in the fish species ingested; and iii) the body weight of the consumer (WHO and UNEP, 2008). Based on this guidance, intake amounts of MeHg have been previously estimated using

information on typical Hg content in foods (Dellatte et al., 2014; Mahaffey et al., 2004; Shang et al., 2010; Zhang et al., 2009). Additionally, stochastic exposure and dose simulations have been used to estimate dietary MeHg intake for comparison with measured blood levels (US EPA, 2012). The probabilistic approach of Xue et al. (2012) (Xue et al., 2012) was recently used to generate US population percentile estimates of dietary MeHg exposure by source, age, gender, and eating occasion. Using data from NHANES 1999-2006, Xue et al. reported high MeHg blood concentrations for Asian, Pacific Islander, Native American, and multiracial groups (A/P/N/M) relative to other racial/ethnic groups, and estimated MeHg intake at 0.04 μg/kg/day for A/P/N/M (for ages 21 to 49 years). Interestingly, the predicted intake from Xue et al. is very similar to our predicted intake of 0.036 μg/kg/day (Table 2.3) for the KoNEHS participants. This suggests that populations with similar genetic backgrounds and culturally-based eating patterns have comparable MeHg intakes.

While dietary data and forward prediction models are commonly used to evaluate MeHg intake, biomarkers are increasingly finding use as a means of reconstructing exposures and assessing risk (Sobus et al., 2015). Several studies have used biomarkers of MeHg to reconstruct intake amounts for comparison with reference values (e.g., RfDs) (Allen et al., 2007; Clewell et al., 1999; Legrand et al., 2010; Rice et al., 2000; Xue et al., 2012). In the KoNEHS, total blood Hg was measured rather than MeHg. Since the RfD is available for MeHg (based on its neurotoxic and developmental effects (US EPA, 2001b)), but not for total Hg, the current study utilized a multi-step workflow (Figure 2.1) to reconstruct and evaluate MeHg intake.

Estimates of blood MeHg for South Korean

An early step in our workflow was predicting blood MeHg levels based on KoNEHS reported blood Hg levels and literature reported fractions of blood MeHg to total Hg. Predicted levels of blood MeHg in the present study (GM=2.16 μg/L) were about half as large as those directly measured as part of another Korean study (the same study from which blood MeHg/blood Hg values were obtained). The study (i.e., (Jung et al., 2013)) reported a total GM level of 4.44 μg/L across 400 participants (Table 2.2). Importantly, sample measures observed below the 25th percentile (1.5 μg/L) in this study were excluded from final summary statistics. Given this data censoring, it is not surprising that values reported by (Jung et al., 2013) exceeded those estimated for the KoNEHS participants.

A comparison of blood MeHg estimates from KoNEHS against measurements from the US NHANES 2011-2012 (GM=0.619 μg/L for adults over 19 years) clearly indicates different exposures across populations. Interestingly, whereas the global GM of US NHANES measures was four times lower than that of KoNEHS, blood MeHg levels for Asian Americans in the NHANES (GM=1.58 μg/L) were close to KoNEHS estimates. In fact, NHANES blood MeHg measures for Asian Americans over 50 years of age (GM=2.64 μg/L) were higher than our estimates for age-matched participants in KoNEHS (GM=2.28 μg/L). As an explanation for these similarities, we posit that immigrants from Asian countries retained their dining habits, such as consuming fish frequently (Gordon et al., 2000). This conjecture assumes comparable Hg levels in fish consumed in both countries - further investigation is required to explore this hypothesis as the basis for similar blood MeHg

levels across populations.

Interpretation of HQs using the predicted MeHg intakes

PBPK modeling was used as a later step in our workflow (Figure 2.1) to enable the conversion from estimated blood MeHg to estimated MeHg intake. One-compartment PK models have previously been used to predict daily MeHg intake (Albert et al., 2010; Ginsberg and Toal, 2000; Rice, 2004; Stern, 2005). In the present study, a PBPK model for MeHg was used, allowing incorporation of variability in physiological and pharmacokinetic properties (Allen et al., 2007; Georgopoulos et al., 2009; Loccisano et al., 2013; Shipp et al., 2000). Intake amounts of MeHg were estimated following MC sampling of sensitive model parameters. The estimated HQs for the KoNEHS participants based on reconstructed MeHg intake ranged from 0.24 to 0.63. Regarding age, HQ estimates increased until subjects reached their 60's in both males and females. This result is somewhat expected, since the frequency of fish consumption decreased among the elderly population (Shin et al., 2012). The largest HQ was observed for frequent fish consumers (Table 2.3), which suggests the highest exposure for this subgroup. However, HQ estimates across all subgroups were below 1, indicating MeHg intake estimates are below the RfD for all subgroup. We note that HQ estimates reported here were derived from central tendency estimates of reconstructed MeHg intake distributions. In fact, HQ estimates based on the upper 5 percentile EDI were above 1 in most subgroups except those who consumed fish rarely and aged 20’s. However, using the tail of the simulated distribution could lead overestimation, central tendency values were utilized to yield the most accurate HQ for each sampled

subgroup. Future work should explicitly consider HQ estimates based on additional percentiles of the MeHg intake distributions. This would allow refined estimates of potential risks for subgroups of the Korean population. Further investigation is also needed to determine the real-life impacts of these findings for the general Korean population. In particular, further studies are needed to set health guidance values for fish consumption and to make quantitative determinations of the likelihood of deleterious health outcomes among susceptible subgroups resulting from high MeHg intake.

CHAPTER III.

Mercury impacts on out-of-reference range for clinical chemistry including hepatic and metabolic markers

Introduction

Increasingly, endocrine disrupting chemicals (EDCs) have been issued for their obesogenic property. Prenatal or early life exposure to the obesogenic chemicals can interfere with the lipid metabolism, its homeostasis and the ability to regulate weight control, which leads to metabolic syndrome and obesity later in life (Hennig et al., 2012; Janesick and Blumberg, 2016). Among EDC metals, mercury (Hg) is strongly suspected as obesogen (Geier et al., 2016; Hyman, 2010).

The main exposure sources of Hg for the general population are diet (Kim et al., 2013; Xue et al., 2012; Zhang et al., 2009). Especially, fish consumption is the major exposure pathway of methylmercury (MeHg) which is the most toxic form among Hg, and MeHg consists of total mercury in human blood as a main component (Jung et al., 2013; You et al., 2012a). Exposure to Hg cause toxic effect on kidney, cardiovascular system and developing fetus along with nervous system (WHO and UNEP, 2008) via oxidative stress, lipid peroxidation, and mitochondria dysfunction, etc., which lead to deleterious effects on enzymes, cell membrane function, and neuron delivery materials (Hong et al., 2012; Mergler et al., 2007).

The Commission on Human Biological Monitoring of the German Federal Environmental Agency established two human biomonitoring (**HBM**) values which are guided the measurement of chemicals or their metabolites in human tissues or specimens (Schulz et al., 2007). For blood Hg, HBM-I is 5 μg/L, which is a control value, and HMB-II is 15 μg/L, which is an intervention value required medical care. Although there's a decreasing trend by year, about

25 % of Korean population had the higher blood Hg level than HBM-I (Park et al., 2014). Several studies have focused on the relationship between metal exposure including Hg and metabolic syndrome among general population (Eom et al., 2014; Lee and Kim, 2013; Moon, 2014), which needs confirmation, which could be confounded with co-exposure chemicals.

The aim of this study is to determine the health effect of Hg with clinical chemistry markers using general biomonitoring surveys. Alanine aminotransferase (ALT) is a traditional marker of non-alcoholic fatty liver disease and γ-glutamyl transferase (γ-GTP) is a substrate enzyme which plays a central role in glutathione metabolism. Also, the two hepatic markers are suggested to have an association with metabolic syndrome (Oh et al., 2011). Cholesterol and triglycerides (TG) are the plasma lipids as well total lipid. Cholesterol is a vital structure component of cell membranes, and TG and total lipid are major source of energy for cells (Hilbert and Lifshitz, 2007). Immunoglobulin E (IgE) is the antibody isotype that contains the ε heavy chain and it is a monomer with five domains in the immunoglobulin structure (Amarasekera, 2011). According to a published article, there was association between serum IgE and obesity (Fitzpatrick et al., 2012). We examined that the levels of blood Hg could be explained by demographic and specific behaviors, and gauged the risk of being out-of-reference ranges for the clinical chemistry markers matched with the level of blood Hg. We also evaluated the effect of blood Hg after adjustment for co-exposure to environmental chemicals as confounders.

Materials and Methods

Study population

Since 2009, the Korean National Environmental Health Survey (KoNEHS) has been conducted periodically by the Korean National Institute of Environmental Research (NIER). About 2000 subjects (≥ 19 years) were recruited annually by using stratified multistage sampling units representing the residential distributions of geographical area, gender and age. The survey included an interview survey and examination survey which measured the concentration levels of 21 toxic pollutants including mercury in human blood and urine. In the second cycle of KoNEHS (2012-2014), blood chemistry markers were added to investigate the association of exposure with health effects. Among a total of 6478 participants, 21 subjects were excluded due to missing data of blood Hg. All participants had no medical illness and signed an informed consent (Choi et al., 2017).

Measurements of clinical chemistry and Hg level in samples

Fasting blood samples were collected from the participants and transported in cold storage to the Seoul Medical Science Institute in Seoul, S.Korea. All samples were stored at -20 °C until analyzed. Serum levels of ALT (reference range: 10-49 IU/L), γ-GTP (reference range: < 73 IU/L for men; < 38 IU/L for women), total cholesterol (reference range: < 200 mg/dL), TG (reference range: < 250 mg/dL) were measured using ADVIA1800 Autoanalyzer (Siemens Medical Solutions, USA). Serum levels of total lipid (reference range: 400-800 mg/dL) was measured using U-3010 Spectrophotometer (Hitachi, Tokyo, Japan)

and IgE (reference range: ≤ 158 IU/mL for adult) was measured by ADVIA Centaur auto analyzer (Siemens Medical Solutions, USA). The reference ranges of each blood chemistry markers were reported. Blood mercury levels were analyzed by the gold-amalgam collection method using Direct Mercury Analyzer 80 (DMA 80, milestone, Bergamo, Italy). Reporting limit (RL) for blood Hg was 0.1 μg/L and values below the RL were included in the current analysis as RL divided by the square root of 2.

Statistical analysis

Since the distribution of blood analytes (*n*=6457) was right skewed, log transformation was performed before all statistical analyses. Candidate explanatory variables of blood Hg were evaluated with univariate analysis with Proc SURVEYREG of SAS, where the (natural) log-transformed blood Hg regressed on: sex (male, female), age (19-29, 30-39, 40-49, 50-59, 60-69, >70), BMI category (underweight: < 18.5, normal: 18.5-23, overweight: 23-25, obese: > 25 kg/m^2), residence region (urban, rural, coast, others), household income class (< $1500, $1500-$3000, $3000-$5000, $5000-$10000, > $10000, in USD), smoking status (non-smoker, ex-smoker, current smoker), alcohol drinking status (non-drinker, ex-drinker, current drinker), consuming frequency (< once a month, 1~2 times/month, 1~2/week, 3/week, almost everyday) and amount, marital status (never married, married, divorce/widowed/separated), parity (no, yes), menopause (no, yes), education (no formal education, elementary school, middle-school, high school, college, university, graduate, masterdom, doctorate), and cooking recipe of fish (rarely, baked, steamed/boiled, fried, raw), taking herbal medicine for last one year (no, yes).

Seafood consumption frequency was grouped into 'rarely', '1~3/month', '1~3/week', 'over 4/week'. Categories of seefood are large fish (*e.g.* whale, tuna), fish (*e.g.* mackerel, cod, salmon), crustaceans (*e.g.* shrimp, lobster, crab), seaweeds, shellfish, and others (*e.g.* squid, sea cucumber). *P*-value ($p < 0.05$) and the coefficient of determination (R^2) were used to identify the significant explanatory variables. Geometric means (GMs) of blood Hg and clinical chemistry markers were calculated by the significant variables while the comparison of means was conducted by Kruscal-Wallis test. Regarding the clinical chemistry markers, the GMs were displayed with the reference ranges and frequency out-of-range. The odds ratios (ORs) of out-of-reference range for each clinical marker were calculated by gender and quantile of blood Hg (low: < 25th, medium: 25th to 75th, high: > 75th) using logistic regression models. The adjusted variables were selected among the significant variables in case the R^2 is over 0.05, and interaction terms were also investigated. Competing models were compared using Akaike information criteria (AIC), Bayesian information criteria (BIC), and the R^2. In the final model, the ORs were calculated adjusting for age, BMI category, smoking status, alcohol consuming frequency and the interaction between blood Hg and sex. In order to address the confounding effect of other chemicals on the clinical chemistry, correlations between the blood Hg and other chemicals – blood lead (**Pb**), urinary cadmium (**Cd**), the metabolites of phthalates in urine (**MEHHP**, mono-(2-ethyl-5-hydroxyhexyl) phthalate; **MEOHP**, mono-(2-ethyl-5-oxohexyl) phthalate; **MnBP**, mono-n-butyl phthalate; **MECPP**, mono-(2-ethyl-5-carboxypentyl) phthalate; **MBzP**, mono-benzyl phthalate), the metabolites of VOCs in urine (**HippA**, hippuric acid; **MucA**, t,t-muconic acid; **PheA**,

phenylglyoxylic acid; **MandA**, mandelic acid; **MHA**, methylhippuric acid), the metabolites of PAHs in urine (**OHP**, 1-hydroxypyrene; **Napt**, 2-naphthol; **OHPHE**, 1-hydroxyphenanthrene; **OHFLU**, 2-hydroxyfluorene), environmental phenols in urine (**BPA**, bisphenol A; **TCS**, triclosan), 3-phenoxybenzoic acid (**PBA**), and cotinine (**Coti**) - were investigated before developing models. Details of the other chemicals were described elsewhere (Choi et al., 2017). And then, we performed multivariate linear model where the levels of each clinical marker were regressed on the corresponding blood mercury after adjusting for the other chemicals. All statistical analyses were performed using SAS® version 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

The GM and the 95th percentile of blood Hg among all participants were 3.11 μg/L and 9.44 μg/L, respectively. The distribution of blood Hg is presented in Figure 3.1. The exceedance rate over HBM-I is 22.5 % and HBM-II is 1.3 %. Levels of blood Hg were significantly higher in males (GM=3.70 μg/L) compared to females (GM=2.63 μg/L) as shown in Table 3.1. Regarding age, blood Hg levels increased until 60's, then decreased. Also, blood Hg levels increased with BMI category and household income. Those who eat fish over 4 times per week had about twice the blood Hg levels (GM=4.04 μg/L) as those who eat fish rarely (GM=2.17 μg/L). In addition, smoking, drinking alcohol, alcohol consumption frequency and amount, parity, menopause, education, marriage status, and cooking recipe were also significant variables, but taking herbal medicine was not influenced on the blood Hg level.

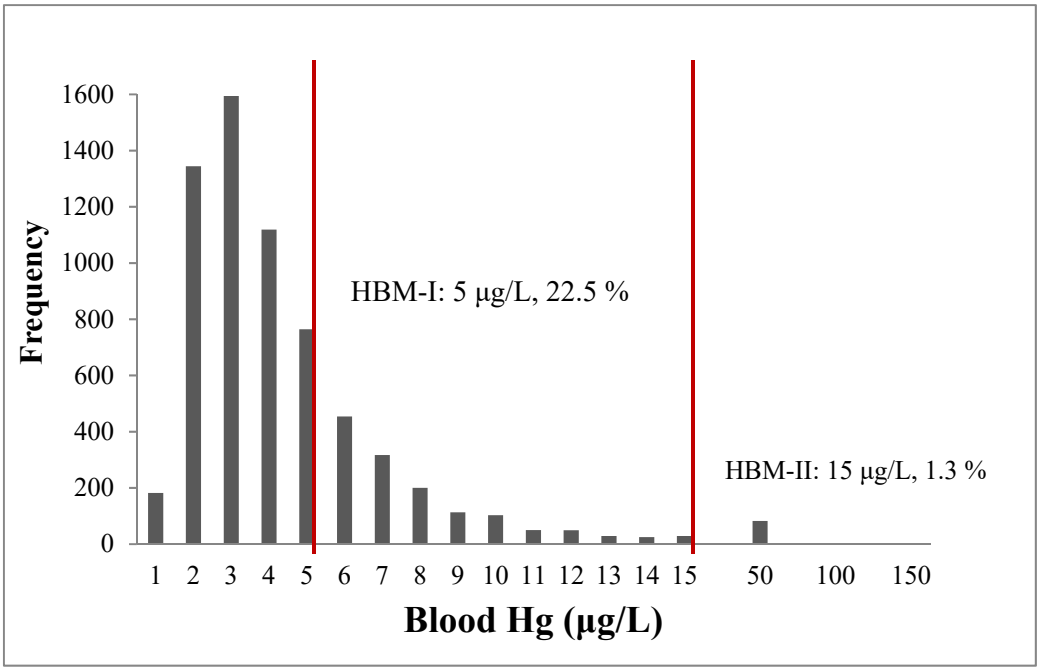

Figure 3.1 Histogram of blood Hg for total population

Table 3.1 Summary of blood Hg levels by demographic variables

	N	GM	95% CI for GM		P75	P95	*p*-value
All	6457	3.11	(3.02	3.21)	4.75	9.44	
Sex							
Male	2768	3.70	(3.57	3.84)	5.76	11.2	<.0001
Female	3689	2.63	(2.54	2.72)	4.04	7.73	
Age							
19-29	536	2.37	(2.23	2.52)	3.18	6.75	
30-39	1053	3.18	(3.04	3.33)	4.48	8.13	
40-49	1225	3.59	(3.44	3.75)	4.99	9.48	<.0001
50-59	1436	3.64	(3.47	3.82)	5.28	10.9	
60-69	1326	3.23	(3.05	3.43)	4.94	10.1	
70+	881	2.54	(2.38	2.71)	4.23	9.18	
BMI							
< 18.5	159	2.13	(1.85	2.45)	3.10	6.31	
18.5- <23.0	2209	2.74	(2.64	2.85)	4.10	7.74	<.0001
23.0- <25.0	1602	3.27	(3.13	3.41)	4.93	9.48	
25.0+	2487	3.55	(3.41	3.69)	5.31	10.4	
Residence region							
Urban	5081	3.11	(3.01	3.20)	4.73	9.22	
Rural	485	3.05	(2.69	3.46)	4.52	8.07	<.0001
Coastal area	227	4.29	(3.51	5.25)	6.75	15.00	
HM site	664	3.01	(2.79	3.25)	4.51	10.39	
Household Income (US $/Month)							
< 1500	1793	2.76	(2.62	2.92)	4.52	9.51	
1500 - 3000	1621	3.01	(2.84	3.18)	4.54	9.03	
3000 - 5000	1765	3.21	(3.08	3.35)	4.72	8.69	<.0001
5000 - 10000	1105	3.38	(3.20	3.57)	5.20	9.84	
> 10000	173	3.47	(3.06	3.92)	5.48	11.4	
Fish Consumption Frequency							
Rarely	622	2.17	(2.03	2.32)	3.28	7.76	
1-3/Month	2031	2.88	(2.77	2.99)	4.33	8.19	<.0001
1-3/Week	3378	3.42	(3.31	3.53)	5.04	9.57	
4-6/week	426	4.04	(3.68	4.43)	6.36	11.7	

The GMs and the number of samples which were out-of-reference range of blood chemistry markers are shown in the Table 3.2. About 15 % of men exceeded the reference range for γ-GTP, while 10 % of women exceeded. All GM values for each marker were significantly different by sex. Next, the blood Hg level was categorized into quantile groups - low: < 25th (2.05 μg/L), medium: 25th – 75th (4.75 μg/L), high: > 75^{th} - to diagnose the effect of Hg on the reference ranges. About 35.5 % of men and 17 % of women were included in the high blood Hg group. Figure 3.2 shows the GMs for each marker across the blood Hg by sex. The levels of ALT, γ-GTP and total lipid were increased with blood Hg group for both sexes, but the GMs for total cholesterol and TG were significantly increased in men across the blood Hg. The GMs for IgE showed an increasing trend only in women.

Table 3.2 Summary of clinical chemistry markers by sex

	Male			Female			Reference range
	Total N	GM (95% CL)	Out-of-range, N (%)	Total N	GM (95% CL)	Out-of-range, N (%)	
ALT (U/L)	2767	25.5 (24.8,26.2)	279 (10.1)	3689	17.7 (17.2,18.2)	229 (6.21)	10-49
γ-GTP (U/L)	2767	33.5 (32.3,34.7)	406 (14.7)	3689	17.3 (16.8,17.9)	382 (10.4)	M: <73, F: <38
T-Cholesterol (mg/dL)	2767	180 (178,182)	812 (29.4)	3689	181 (179,183)	1184 (32.1)	<200
T-Lipid (mg/dL)	2766	604 (593,615)	503 (18.2)	3689	570 (561,579)	538 (14.6)	400-800
TG (mg/dL)	2767	162 (157,167)	650 (23.5)	3689	125 (121,128)	493 (13.4)	<250
IgE (IU/mL)	2766	104 (96,113)	1180 (42.7)	3689	43.7 (41.0,46.7)	669 (18.1)	<158

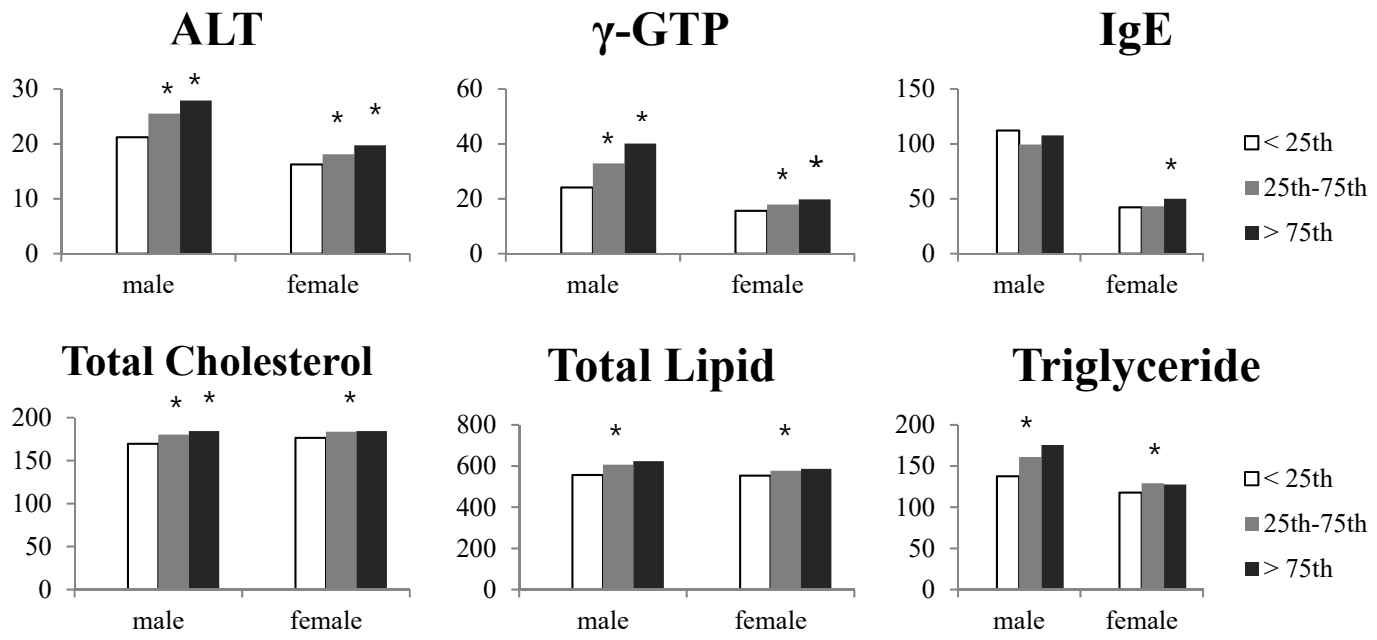

Figure 3.2 Geometric mean of blood chemistry markers across the blood Hg groups by sex

The percentages for exceeding the reference ranges were presented across the blood Hg groups by sex in Figure 3.3. The percentages for γ-GTP and total cholesterol are increased with blood Hg groups for both sexes. While over 40 % in men exceeded the reference range for IgE regardless of the blood Hg, the increasing trend was shown significantly in women. The trends of ALT and total lipid showed the increasing trend in the male population though statistical significance were not proved. Table 3.3 exhibits the ORs of the risk for being out-of-reference ranges. With regards to the γ-GTP, almost 2-fold increase was shown in the medium group, and 2.8-fold increase in the high group in men. Also, the adjusted ORs of the medium and the high Hg group among women were 1.53 and 2.11, respectively. High Hg group in men was associated with about 1.5-fold risk for total cholesterol. However, the other markers were not shown the significant ORs.

There were no significant correlations between blood Hg and the other chemical levels (see Table 7 in Appendices). The metabolites of VOCs showed the significant associations for ALT in men, but, the significance from those chemicals except HippA were removed in the final model. And blood Hg remains significant adjusting for the same covariates (age, BMI, smoking, drinking) including HippA. In women, blood Pb, the metabolites of phthalates (MEOHP, MnBP, MBzP), the metabolites of PAHs (Napt, OHFLU), and Coti had the significant association with ALT. And blood Hg remains significant with blood Pb and Coti in the final model (Table 8-a, 8-b in Appendices). In case of γ-GTP, blood Pb and the metabolites of VOCs showed the significant associations in men. And blood Hg was significant with blood Pb, and HippA

in men, while blood Hg and Coti were the only significant in women (Table 9-a, 9-b in Appendices). Blood Hg was not significant for total cholesterol in men, but 2 metabolites of VOCs (PheA, MandA), 4 metabolites of Phthalates (MEHHP, MEOHP, MnBP, MECPP), PBA, BPA were shown the significant association. In women, blood Hg and Coti were marginally significant and no other chemicals were significant. There were no associations between blood Hg with TG or with total lipid in both sexes. In addition, blood Hg showed not significant association with IgE in men, while it was the only significant chemical for IgE in women. (Table 10~13 in Appendices).

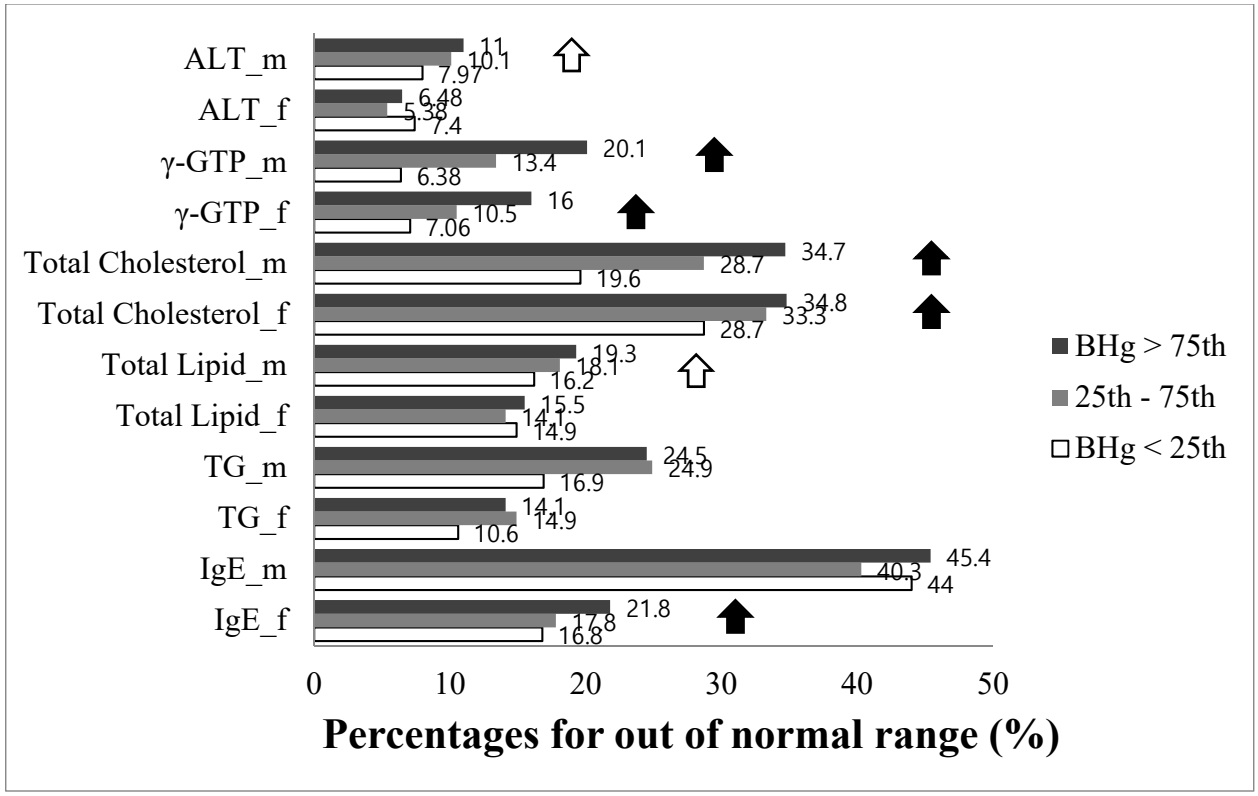

Figure 3.3 The percentages of the subjects whose blood chemistry was out-of-range across the blood Hg by sex - the bold arrow indicates the increasing trend significantly and the dotted arrow indicates the increasing trend but no statistical evidence.

Table 3.3 Odds Ratios for being out-of- the reference range across the blood Hg groups by sex

Markers	Group	Male			Female		
		Estimate	95% Confidence Interval		Estimate	95% Confidence Interval	
ALT	Medium	1.17	0.74	1.83	0.64	0.42	0.97
	High	1.23	0.77	1.97	0.71	0.41	1.24
γ-GTP	Medium	1.90	1.19	3.05	1.53	1.03	2.27
	High	2.77	1.72	4.46	2.11	1.35	3.30
Total-Cholesterol	Medium	1.26	0.93	1.71	1.12	0.88	1.42
	High	1.44	1.05	1.97	1.09	0.80	1.47
Total lipid	Medium	1.05	0.75	1.48	0.85	0.63	1.15
	High	1.05	0.74	1.50	1.02	0.70	1.49
Triglyceride	Medium	1.33	0.95	1.85	1.18	0.83	1.66
	High	1.12	0.79	1.59	0.79	0.50	1.24
IgE	Medium	0.81	0.63	1.05	0.86	0.65	1.12
	High	0.95	0.72	1.24	1.26	0.90	1.75

[Note] The logistic models were adjusted by age, BMI, smoking, alcohol consumption frequency. Group means the classification by the quantile levels of blood Hg - low: < 25th (2.05 μg/L), medium: 25th ~ 75th (4.75 μg/L), high : > 75th - and the ORs were calculated based on the low group as a reference.

Discussion

Reportedly, the blood Hg in general Korean population has been high-ranked in the world (Cho et al., 2014; Lee et al., 2017; You et al., 2012a) despite moderate decreasing trends. The present measurements indicated about a quarter of population exceeded the HBM-I of Hg (5 μg/L) for 2012~2014, which were coupled with a significant increase of ALT and γ-GTP in serum. Especially, the latter showed a significant association with blood Hg out of its reference range (Table 3.3). The levels of total cholesterol, total lipid and triglyceride increased with the blood Hg in male population.

Hg compounds exist in four different forms with different toxicological effect. Among them, the alkyl Hg, such as MeHg, are more lipid soluble and passes readily through biological membranes. Because bile is the major route of excretion, MeHg can be reabsorbed into the blood, via enterohepatic system (ATSDR, 1999; Hong et al., 2012). Covalent bonding with protein sulfhydryl groups occur widespread and nonspecific enzyme dysfunction, inactivation, and denaturation (Pincus and Abraham Jr., 2007). Considering that blood Hg can easily bind to cystein residues (Clarkson et al., 2007) such as glutathione to allow penetration into cellular membrane (Aschner and Aschner, 1990), the MeHg-cysteine complex could be delivered down to the bile tract and then hydrolyzed by γ-GTP and dipeptidase (Clarkson et al., 2007; Kim et al., 2014b). γ-GTP regulates the transport of amino acids across cell membranes by catalyzing the transfer of a glutamyl group from glutathion to a free amino acid (Pincus et al., 2007). In addition, Hg enhances lipid peroxidation in the liver and kidney by inactivating superoxide dismutase and catalase which is two

important enzymes for scavenging H_2O_2 (Salonen et al., 2000). Hg also inactivates paraoxonase, an extracellular antioxidative enzyme (Gonzalvo et al., 1997). Our results manifested that blood Hg showed not only the increasing trend, but it was also related to the risk being out-of-reference range for γ-GTP for both sexes. Furthermore, the significant effect of blood Hg was retained after including the other environmental chemicals (e.g. lead and VOCs) in the statistical model. As an early biological effect marker of oxidative stress, the elevated γ-GTP could be a reflection of increased oxidative stress (Lim et al., 2004), which was supported by several animal and human studies reporting association between Hg exposure and γ-GTP (Schaefer et al., 2011; Singh et al., 2007; Wadaan, 2009).

Another interesting result was the difference for lipid profiles with blood Hg by sex. In the present study, the GM of the total cholesterol, TG and total lipid were shown increasing trend with blood Hg group among males, while there were no trends visually among female (Figure 3.2). Regarding the total cholesterol, the OR of being out-of-reference range was significant in the male population (OR=1.44; 95% CI: 1.05-1.97) while not in the female population. These results were comparable with a cross-sectional study, which reported that Iranian adolescents with metabolic syndrome showed relatively higher blood metals including Hg (Poursafa et al., 2014). Interestingly, there was also a difference of blood Hg levels between gender. Since males showed higher blood Hg than females, we investigated the interaction of sex with BMI or fish consumption frequency, and found a significant three-way interaction among sex, BMI and fish consumption ($p = 0.0433$). It suggested differential effects of blood Hg among BMI category in male (BMI*fish consumption, p-

value=0.047) while not significant of blood Hg in female (BMI*fish consumption, p-value=0.573).

Several epidemiological studies reported that blood mercury showed no significant association with metabolic syndrome (Lee and Kim, 2013; Moon, 2014; Rothenberg et al., 2015; Rotter et al., 2015) while other studies found the significant effects of the mercury (Eom et al., 2014; Geier et al., 2016; Poursafa et al., 2014). These inconsistent results might indicate that the impacts of Hg are not strong enough to lead obesity though their association is significant. Because majority of the studies which results were not significant focused on the specific health effect such as obesity, metabolic syndrome, and BMI, we investigated the influence on each clinical marker and the risk for being out-of-range in the present study. And our results demonstrated that there is the significant increasing trend in hepatic and metabolic markers with blood Hg level. The second reason of the inconsistency could be the gender-dependent effects. Most of the studies did not separate the study population by sex and a few study adjusted the sex effect for the statistical model. This could be made the impacts of Hg attenuate and incomparable. In fact, sex-related differences in oxidative stress and antioxidant defense mechanism were found in rats (Dimitrijevic et al., 2017; Jung and Metzger, 2016; Olivieri et al., 2002; Taskiran et al., 1997). Some studies also indicated that the higher catalase activity, the more antioxidant defense mechanism in female rats (Taskiran et al., 1997). Considering female hormone (e.g. 17β-estradiol) could reduce oxidative stress (Jung and Metzger, 2016), this could be explanatory although it needs more confirmation. Comparably, our results also showed that the GM of lipid

profiles were shown increasing trend across blood Hg group and the risk being out-of-reference range for total cholesterol was significant in men.

This study suggests the new evaluation method for Hg impacts by measuring the risk of being out-of-range. Despite its contribution, the results of this study should be interpreted carefully. Because the endpoint of our study is out-of-reference range, the results can be presented as an index of pre-obesity though it could not be connected to obesity directly. Evaluation of Hg effects with co-exposed chemicals is additional strength of the present study. We determined that there were no significant interactions between Hg and other environmental chemicals such as lead, cadmium, phthalates, VOCs and PAHs. Also, the effects of blood Hg for ALT and γ-GTP were significant after adjustment for co-exposure chemicals in both sexes. The results of this study indicate that Hg has the significant association with clinical chemistry markers though current exposure level might not lead the specific diseases. Therefore, efforts in reducing Hg exposure should be considered.

CHAPTER IV.

Fetal body burden for mercury exposure during pregnancy and the association of growth

Introduction

Due to growing concerns for environmental contaminants, exposome and early life exposure studies are increasing (Rappaport, 2012). Especially, exposed to endocrine disrupting chemicals (EDCs) in developmental stage have been issued for their obesogenic property (Hennig et al., 2012; Janesick and Blumberg, 2016). As a suspected obesogen (Geier et al., 2016; Hyman, 2010), Mercury (Hg) can be transferred from mother to the fetus via placenta. And it showed a significant positive association with metabolic syndrome among South Korean (Chung et al., 2015; Eom et al., 2014).

According to the published data, the geometric mean of blood Hg was about 4.5 μg/L for women in South Korea (Kim et al., 2016; You et al., 2012b). The level is considerably higher than the reported geometric mean (GM) for women of child bearing age in US (0.65~1.35 μg/L) (Birch et al., 2014; Cusack et al., 2017) while much lower than the reported GM for the pregnant women in Japan (9.1 ng/g) (Sakamoto et al., 2013). Blood Hg mainly consists of MeHg which is the most toxic form among Hg (Jung et al., 2013; You et al., 2012b), since the main exposure source of Hg is fish consumption for the general Korean population (Kim et al., 2013). Thus, in 2015, Korea Ministry of Food and Drug Safety (MFDS) have announced for the pregnant women to eat below 400 g of general fishes such as mackerel, cod, saury, and below 100 g of large fishes such as tuna, shark per week (MFDS, 2015).

Regarding to reconstruct exposure, the published physiologically-based pharmacokinetic (PBPK) model was developed based on the study of an acute poisoning incident in Iraq (Clewell et al., 1999). This model can be described

the kinetics of methylmercury (MeHg), and predict the amount in the body mathematically during pregnancy. The strength of this method is that it seems to reflect the internal dose for individual based on the actual measurement of biological samples.

Due to the high level of Hg in blood, several studies have been conducted for Hg among South Korean (Bae et al., 2016; Chung et al., 2015; Chung and Myong, 2016; Kim et al., 2014a; Lee et al., 2017). However, the exposed amount for fetus and the following effects were not fully investigated. Therefore, we estimated fetal body burden which is the total exposure amounts for Hg via placenta during pregnancy using the PBPK model, and investigated the influences on growth for infants in the present study.

Materials and Methods

Study population and sampling

From January 2011, the Children's Health and Environmental Chemicals in Korea (CHECK) has been conducted to investigate association between environmental exposures and health outcomes in children. A total of 334 pregnant women were recruited from several university hospitals in Seoul, Pyungchon, Ansan, Guro, and Jeju, South Korea until December 2012. Among those participants, 106 subjects were available to analyze maternal blood at delivery and umbilical cord blood both. Face to face interview was conducted to obtain personal information and pregnancy-related information, including age, weight, gestational period, caesarean section, past delivery experience. Especially, body weight, length and head circumference of newborns were measured at birth directly and then, weight and length were followed up by a telephone interview for maximum 44 month. Blood and urine samples of pregnant women were collected at the day before delivery, and placenta with umbilical cord blood samples were collected at the delivery. The first urine of newborns was collected within postpartum, and meconium was collected from diapers within 48 hours after birth. Breast milk by hand expression and about 5 cm of infant's hair was collected on the 30th day after delivery. All samples except meconium were stored at -80 ℃ and meconium samples were stored at -25 ℃ until analysis. The present study was approved by the institutional review board at the school of public health, Seoul National University, South Korea (IRB no. 8-2012-04-20), and all subjects provided written informed consent. All samples and data were processed blindly.

Measurement of mercury level in each matrix

Mercury levels in each matrix was measured according to the method 7473 outlined by EPA, USA (2007) with minor modifications. Briefly, 100 μL of whole blood, urine and breast milk samples were prepared after mixing in a roll-mixer. And 0.1 g of placenta and meconium samples were weighed using analytical balance (XB220A, Precisa Gravimetrics AG, Dietikon, Switzerland). Hair samples were washed with distilled water, acetone, and 3 % Triton X-100, in turn. After washing by distilled water again, the samples were dehydrated using dry oven (OF-22GW, JEIO Technology, Korea) at 105 ℃ for 30 min. Lastly, 0.01g of hair samples were prepared before analysis. Total mercury levels were measured by using an automatic mercury analyzer (SP-3D, Nippon Instruments Co., Japan) using heat vaporization, gold amalgamation, and cold-vapor atomic absorption technique. The limit of detection (LOD) for Hg in maternal blood, maternal urine, cord blood and breast milk was 0.01 μg/L. And LOD for Hg in placenta and fetus meconium was 0.1 ng/g. For the first urine of neonate and infant's hair, LOD was 0.04 μg/L, and 0.4 ng/g, respectively. In the quality assurance and quality control for mercury measurements, the accuracy and recovery ranges calculated with spiked blood and urine were 90–110% and 85–100%, respectively.

Exposure reconstruction of MeHg intake using PBPK modeling

1) Calculation of partition coefficient for Hg

Using the measured Hg level among the 106 paired data, partition coefficient of each matrix was calculated based on the Hg level in maternal level.

Therefore, distribution of the partition coefficient for maternal urine, cord blood, placenta, fetus' meconium, and infant's hair was calculated.

2) PBPK modeling

The measured Hg level in each matrix was applied to the published human PBPK model for MeHg to estimate the exposed amount for Hg (Clewell et al., 1999). This model explains absorption, distribution, metabolism, and elimination of MeHg. The fetal compartments were assumed to grow during the time of gestation. And maternal plasma, RBCs, richly perfused tissues (representing changes in the uterus and mammary glands), and fat compartment are describe to increase over the course of pregnancy (Clewell et al., 1999; Gearhart et al., 1995). All kinetic and physiological parameters were adopted from the original models, with the exception of body weight - body weight was set to 54 kg which was the GM of the body weights before pregnancy in our data.

3) Prediction of fetal body burden for MeHg

In order to predict fetal body burden for MeHg, we assumed that MeHg level reached the steady state before the conception and the subject eat fish continuously during pregnancy. At first, we derived the 5^{th}, 50^{th}, 95^{th} percentile values for Hg in maternal blood and took the cord blood Hg of the paired newborns. And the MeHg fraction was applied for the range of maternal blood Hg and cord blood Hg, respectively, to estimate range of MeHg in cord blood Hg (You et al., 2012b). The PBPK model was simulated to reconstruct the exposed dose corresponding to the estimated cord blood MeHg as well

maternal blood MeHg for 270 days of pregnancy. Fetal body burden, which is the total exposure amounts for MeHg via placenta during pregnancy, was calculated by accumulating the given amounts from the placenta to fetal plasma using the PBPK model. The simulations of PBPK model was performed by Berkeley Madonna 8.3.9 (University of California at Berkeley, Berkeley, CA).

Statistical analysis

Among a total of 334 pregnant women, geometric means (GMs) and the distributions of Hg in each matrix were calculated. And partition coefficients of each matrix were derived using paired data. Due to the right skewed distribution, Hg levels in cord blood were converted to the natural log scale. Before analyzing the follow-up data, logical and coding errors were taken out - same values for more than three months, decreased more than 1 cm for height or 1 kg for weight compare to the previous follow-up month. And the participants were categorized by quantile of cord blood Hg to investigate the growth effect by groups. Association between cord blood Hg and growth variables including height, weight, head circumference were investigated using several approaches. **First,** the association between cord blood Hg and the growth variables at birth were analyzed using general linear model. Also, we investigated the follow-up month which could be influenced by Hg exposure via placenta. Therefore, the growth variables at 1, 3, 6, 9, 12, and 24 month after birth were analyzed respectively adjusting for newborn's sex, living area, maternal age group, gestation day, delivery experience, maternal body mass index, drinking alcohol frequency and the exposed time of passive smoking as

well cord blood Hg. Backward stepwise elimination was used to select the best model (SAS Proc GLM) at a significance level of $p \leq 0.1$. Competing models were compared using Akaike information criteria (AIC), Bayesian information criteria (BIC), and the coefficient of determination. **Second,** the growth variables were standardized with the mean of each month and evaluated using random slope and intercept model after adjustment for covariates. Maximum likelihood method (ML) was used for estimating the covariance parameters, and the covariance structure were used as unstructured (UN). Also, logistic regression was used for analyzing the standardized growth data. The evaluation criterion for relative growth was the median values for the growth variables – height and weight – at each month. **Lastly,** additional mixed-effects models were developed for the individual follow-up height and weight to investigate the effets of exposure to Hg after adjusting for covariate effects. Following is the model structure.

$$Y_{ij} = \beta_0 + \beta_1 month_{ij} + \beta_2\sqrt{month_{ij}} + \beta_3 sex_i + \beta_4 cord\ \ blood\ \ Pb_i + \beta_5 cord\ \ blood\ \ Hg\ \ group_i + \left(\beta_6\sqrt{month_{ij}} * cord\ \ blood\ \ Hg\ \ group_i\right) + (\beta_7 sex_i * cord\ \ blood\ \ Hg\ \ group_i) + \varepsilon_{ij}$$

i indicated indivduals and j indicated measurements of the individual.

Follow-up more than three times only used to estimate least square mean (LS mean) derived using mixd model (n=103). According to the previous report, Pb is associated with growth significantly. Therefore, we also adjusted the cord blood Pb in the model. The Hg levels were higher than LOD for each matrix except missing. All statistical analyses were performed using SAS® version 9.3 (SAS Institute Inc., Cary, NC, USA).

Results

Basic characteristics of study participants

A total of 334 pregnant women were participated in the present study. The age of the pregnant women was between 23 and 46 years (mean: 33.5 ± 4.04 years), and gestation period was ranged from 259 to 293 days (mean: 275.5 ± 7.7 days). About 3 % and 14 % of the respondent were smoked and drunk alcohol during pregnancy, respectively. The GM of weight for boys was 3.33 Kg while girl's weight was 3.22 Kg. The length of boys was a little higher than the girl's though there was no statistical difference (Table 4.1).

Table 4.1 Demographics among all participants

	Boys	Girls	Total
All	167	167	334
Region			
Seoul	71	68	139
Middle city (PyeonChon, Jeju)	59	56	115
Industrial estate (Ansan)	37	43	80
Maternal age			
20's	29	18	47
30's	130	132	262
40's	8	17	25
BMI			
< 18.5	52	68	120
18.5-23	69	69	138
23-25	19	14	33
> 25	27	16	43
Smoking (during pregnancy) [a]			
No	129	110	239
Yes	2	6	8
Alcohol (during Pregnancy) [b]			
No	116	104	220
Yes	20	16	36
Passive smoking (during pregnancy) [c]			
0 hr	83	72	155
less than 1 hr (/day)	52	52	104
more than 1 hr (/day)	6	8	14
Newborn (at birth) [d]			
Weight (Kg)	3.33 (1.11)	3.22 (1.11)	3.28 (1.11)
Length (Cm) [e]	50.0 (1.06)	49.8 (1.06)	49.9 (1.06)
Head circumference (Cm) [f]	34.4 (1.06)	34.0 (1.04)	34.2 (1.05)
Ponderal Index (g/Cm^3) [g]	2.65 (1.19)	2.61 (1.18)	2.63 (1.18)

[Note] a. The number of total missing was 87; b. The number of total missing was 78; c. The number of total missing was 61; d. The numbers are presented GM and geometric standard deviation for continuous variables; e. The number of total missing was 14; f. The number of total missing was 31; g. The number of total missing was 14.

The distributions of Hg levels in each matrix and the partitioning among the paired subjects

The number of cord blood sample was 277 out of 334 participants. Among those participants, 106 subjects were available to analyze maternal blood at delivery and umbilical cord blood both. The GM of maternal blood Hg and cord blood Hg was 4.47, and 7.35 μg/L, respectively. The Hg levels of placenta and fetus meconium was as high as 9.0, 36.9 ng/g, respectively. Though the number of infant's hair sample was only 25, the GM was 443 ng/g (Table 4.2). The correlation coefficients for Hg in placenta, fetal meconium and hair with cord blood Hg were 0.57, 0.22, and 0.23, respectively. Based on the Hg level in maternal blood, the partition coefficient of cord blood ranged from 0.46 to 3.30 (median: 1.68). The median partitioning of placenta and meconium was 2.12 and 7.41, respectively. It showed much higher Hg levels of meconium and cord blood than of maternal blood. The partitioning of cord blood to placenta was between 0.28 and 1.95 (median: 0.83) (Table 4.3).

Table 4.2 Distributions of Hg levels by each matrix in paired data

Matrices	N	GM	LCL	UCL	P25	P50	P75	P95
Maternal blood (μg/L)	106	4.47	4.15	4.81	3.43	4.32	5.62	8.64
Umbilical cord blood (μg/L)	106	7.35	6.79	7.96	5.77	7.37	9.12	15.5
Maternal urine (μg/L)	105	1.41	1.21	1.64	0.82	1.4	2.24	4.92
Fetus urine (μg/L)	61	0.53	0.42	0.67	0.35	0.51	0.82	2.32
Breast milk* (μg/L)	61	0.55	0.46	0.65	0.37	0.53	0.84	1.84
Placenta (ng/g)	100	9.0	8.31	9.8	6.49	8.72	11.5	17.4
Fetus meconium (ng/g)	72	36.9	30.1	45.3	20.1	34.1	52.3	207
Infant's hair (ng/g)	25	443	352	558	240	506	623	974

* Breast milk was collected after 30 days from delivery.

Table 4.3 Hg partitions of each matrix in paired data

Partition coefficients	N	min	p25	p50	p75	p95	max
Cord blood vs. Maternal blood	106	0.46	1.48	1.68	1.99	2.43	3.30
Placenta vs. Maternal blood	100	0.85	1.66	2.12	2.52	3.05	4.06
Fetus meconium vs. Maternal blood	72	2.10	5.44	7.41	11.8	27.4	159
Infant hair vs. Maternal blood	25	19.4	63.0	133	163	173	174
Maternal urine vs. Maternal blood	105	0.04	0.20	0.31	0.49	1.18	5.00
Cord blood vs. Placenta	100	0.28	0.63	0.83	1.03	1.49	1.95

Fetal body burden

The 5th percentile, 50th percentile, 95th percentile of the distribution for maternal blood Hg was 2.62, 4.34, 8.64 μg/L, respectively. And the paired cord blood Hg was taken for considering the individual partitioning – 5th percentile: 5.2, 50th percentile: 7.79, 95th percentile: 17.18 μg/L. Applying the proportion of MeHg from total Hg to the cord blood Hg, the estimated MeHg level in cord blood was 4.06, 6.08, 13.4 for 5th, 50th, 95th percentile, respectively. As a result of the PBPK model, the corresponding fetal body burden for MeHg was ranged from 26.3 to 86.9 mg among our subjects (Figure 4.1).

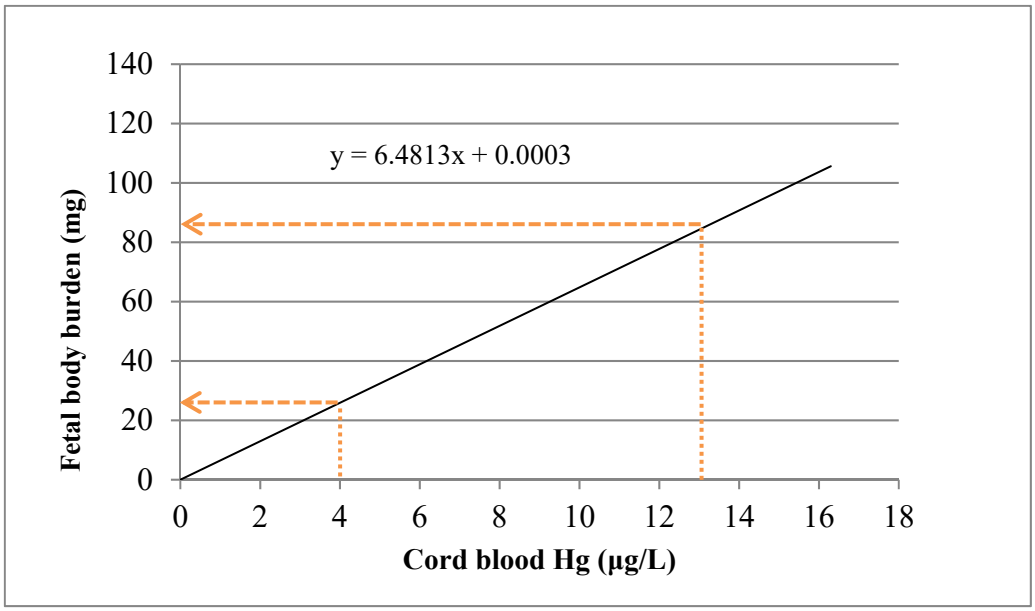

Figure 4.1 The relationship between cord blood Hg and fetal body burden

Growth effect from Hg exposure

On average, follow-up was conducted by 5.1 times until maximum 44 months after birth. Hg in cord blood categorized by the quartiles of cord blood Hg; 25th percentile=5.5 μg/L, 50th percentile=7.37 μg/L, 75th percentile=9.6 μg/L. The growth information of all subjects showed no differences across the cord blood Hg group (see figure 2 in Appendices). As it shows, no difference is existed by the cord blood group. Table 4.4 shows summary information of growth variables - height, weight, and PI by month among all participants.

The association between cord blood Hg and length at birth were evaluated using general linear model after adjustment for living area, normal delivery, maternal BMI, gestation day, delivery experience, drinking and the time of passive smoking (Table 4.5). Cord blood Hg was positively associated with length at birth (estimate: 1.204, *p*-value: 0.0132) and living area, and gestation day were also significant factors for the length at birth. However, the association between cord blood Hg and the height of the follow-up months were not significant. Weight at birth (estimate: 0.077, *p*-value: 0.1764) and head circumference (estimate: 0.189, *p*-value: 0.4579) was not associated with cord blood Hg. Also, the cord blood Hg was not significant for those weight and PI of the follow-up months.

Table 4.4. Summary information of growth variables by follow up months (among all participants)

	Height (cm)				Weight (Kg)				PI			
	N	mean	sd	p50	N	mean	sd	p50	N	mean	sd	p50
at birth	320	50.0	2.75	50.0	334	3.29	0.35	3.28	320	2.68	0.69	2.63
month=1	53	54.6	3.38	55.0	64	4.44	0.79	4.50	52	2.75	0.45	2.70
month=2	48	59.0	2.47	59.0	59	5.76	0.86	5.81	48	2.86	0.40	2.88
month=3	80	62.5	3.21	62.4	87	6.71	0.97	6.70	77	2.80	0.45	2.84
month=4	37	65.0	2.34	65.0	39	7.47	1.01	7.50	36	2.74	0.31	2.77
month=5	30	66.7	2.26	67.0	36	7.97	1.06	7.99	30	2.73	0.27	2.72
month=6	54	69.4	2.57	69.3	59	8.51	1.06	8.60	52	2.57	0.27	2.57
month=7	38	70.5	2.21	70.6	36	9.05	1.12	9.10	34	2.58	0.30	2.59
month=8	32	72.0	2.67	72.4	40	9.23	1.11	9.25	32	2.48	0.28	2.51
month=9	78	73.0	2.92	72.8	84	9.50	1.02	9.40	77	2.44	0.25	2.46
month=10	39	72.8	3.36	73.0	40	9.67	1.03	9.70	35	2.51	0.39	2.43
month=11	28	75.4	2.43	75.2	31	9.72	0.92	9.80	27	2.26	0.22	2.32
month=12	67	76.8	2.86	76.3	72	10.00	1.11	9.80	66	2.20	0.22	2.21
month=15	35	79.2	4.10	80.0	41	10.88	1.48	10.7	33	2.20	0.40	2.17
month=24	45	86.8	3.66	86.0	52	12.60	1.31	12.5	44	1.93	0.18	1.94
month=27	30	89.6	3.80	89.0	37	12.95	1.40	12.6	29	1.81	0.19	1.76

Table 4.5 Relationship between cord blood Hg and the growth variables - length, weight, head circumference

Parameter	Estimate	Standard Error	t Value	Pr > |t|
Length at birth (cm)				
Intercept	14.29	8.759	1.63	0.1044
Cord blood Hg	1.204	0.481	2.5	**0.013**
Region - Seoul vs Ansan	-1.185	0.514	-2.31	0.0222
Region - Pheongchone, Jeju vs Ansan	-1.622	0.516	-3.14	0.002
Normal delivery	-0.293	0.494	-0.59	0.5531
Maternal BMI	0.014	0.063	0.22	0.8248
Gestation day	0.121	0.031	3.89	0.0001
Delivery experience - no	-0.574	0.426	-1.35	0.1789
Drinking - no vs. yes	0.266	0.563	0.47	0.6365
Passive smoking – '0 hr' vs '> 1 hr'	1.442	0.940	1.53	0.1271
Passive smoking – '< 1 hr' vs '> 1 hr'	0.532	0.968	0.55	0.5835
Weight at birth (Kg)				
Intercept	-0.869	0.999	-0.87	0.3857
Cord blood Hg	0.077	0.056	1.36	**0.176**
Newborn's sex - female vs male	-0.119	0.047	-2.55	0.0116
Region - Seoul vs Ansan	-0.064	0.059	-1.1	0.2744
Region - Pheongchone, Jeju vs Ansan	-0.121	0.061	-1.97	0.0497
Maternal age group - 20's vs. 40's	-0.057	0.120	-0.47	0.6365
Maternal age group - 30's vs 40's	0.064	0.103	0.62	0.5341
Maternal BMI	0.016	0.007	2.18	0.0306
Gestation day	0.013	0.003	3.88	0.0001
Drinking - no vs. yes	0.104	0.068	1.53	0.1266
Head circumference (cm)				
Intercept	24.563	4.645	5.29	<.0001
Cord blood Hg	0.189	0.254	0.74	**0.458**
Region - Seoul vs Ansan	-0.247	0.263	-0.94	0.3505
Region - Pheongchone, Jeju vs Ansan	0.149	0.282	0.53	0.5986
Newborn's sex - female vs male	-0.368	0.210	-1.75	0.0815
Normal delivery	-0.677	0.261	-2.6	0.0102
Maternal age group - 20's vs. 40's	-1.475	0.540	-2.73	0.007
Maternal age group - 30's vs 40's	-0.929	0.478	-1.94	0.0537
Maternal BMI	0.101	0.032	3.11	0.0022
Gestation day	0.030	0.016	1.83	0.0696
Delivery experience - no	-0.135	0.226	-0.6	0.5511
Drinking - no vs. yes	0.524	0.308	1.7	0.0909

Next, the growth variables were standardized by follow-up month at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 24, and 27, respectively. The standardized data were regressed on the cord blood Hg after adjustment for covariates used in the previous mixed model. While the association between cord blood Hg and the standardized data for height (*p*-value: 0.4547) and weight (*p*-value: 0.4833) were not significant, the standardized PI were marginally significant (p-value: 0.0665). According to the logistic regression model, the cord blood Hg were not significant for the standardized height (OR: 1.30 (0.55, 3.07)), weight (OR: 0.76 (0.36, 1.62)), and PI (OR: 0.817 (0.377, 1.77)) (Table 4.6).

Table 4.7 represents each parameters for explaining the follow-up height and weight. Though cord blood Hg group was not significant in the model, especially month and $\sqrt{month}$ were contributed to describe the follow-up growth. Figure 4.2 shows the LS mean of follow-up growth by months. The lines represent LS mean estimates by sex, and the asterisk symbols represent mean values of each month. The high group and the low group of cord blood Hg were only expressed. LS mean for the heights were not shown any differences between the high and the low Hg group, however, those for the weights seemed to have different increasing rate by Hg group. For both sex, high Hg group showed more rapid increasing of weight.

Table 4.6 Relationship between cord blood Hg and the standardized data for height, weight, and PI and ORs for the relative growth

	Mixed model			Logistic model		
Parameter	**Estimate**	**Standard**	**Pr > |t|**	**Odds Ratio**	**95% Confidence Limits**	
Standardized height						
Cord blood Hg	0.205	0.273	**0.4547**	1.30	0.55	3.07
Follow-up month	-0.007	0.008	0.3714	0.95	0.91	0.99
Newborn's sex - female vs male	-0.286	0.204	0.1635	0.79	0.43	1.46
Delivery experience - no vs yes	-0.116	0.218	0.5950	1.23	0.65	2.30
Gestation day	0.027	0.014	0.0494	1.10	1.05	1.14
Maternal BMI	-0.036	0.035	0.3032	0.94	0.84	1.04
Drinking alcohol - no vs. yes	0.260	0.337	0.4407	2.71	0.97	7.53
Passive smoking – '0 hr' vs '> 1 hr'	0.174	0.431	0.6864	1.68	0.42	6.79
Passive smoking – '< 1 hr' vs '> 1 hr'	-0.341	0.455	0.4550	0.76	0.17	3.33
Standardized weight						
Cord blood Hg	-0.169	0.241	**0.4833**	0.76	0.36	1.62
Follow-up month	-0.009	0.004	0.0501	1.00	0.97	1.04
Newborn's sex - female vs male	-0.252	0.183	0.1716	0.30	0.17	0.52
Delivery experience - no vs yes	0.063	0.195	0.7467	1.29	0.75	2.23
Gestation day	0.020	0.012	0.1015	1.04	1.01	1.08
Maternal BMI	0.010	0.030	0.7473	0.96	0.88	1.06
Drinking alcohol - no vs. yes	0.629	0.287	0.0293	6.84	2.33	20.1
Passive smoking – '0 hr' vs '> 1 hr'	0.502	0.393	0.2028	2.46	0.74	8.15
Passive smoking – '< 1 hr' vs '> 1 hr'	0.321	0.411	0.4347	2.30	0.65	8.14
Standardized PI						
Cord blood Hg	-0.650	0.352	**0.0665**	0.82	0.38	1.77
Follow-up month	-0.001	0.009	0.8981	1.01	0.97	1.05
Newborn's sex - female vs male	0.254	0.265	0.3392	1.23	0.7	2.17
Delivery experience - no vs yes	0.460	0.284	0.1072	0.84	0.47	1.48
Gestation day	0.002	0.018	0.9298	0.96	0.92	0.99
Maternal BMI	0.041	0.044	0.3552	1.13	1.02	1.24
Drinking alcohol - no vs. yes	0.473	0.433	0.2761	2.74	0.95	7.93
Passive smoking – '0 hr' vs '> 1 hr'	0.292	0.555	0.5992	1.32	0.35	5.01
Passive smoking – '< 1 hr' vs '> 1 hr'	0.735	0.585	0.2106	1.98	0.48	8.12

Table 4.7. Relationship between cord blood Hg and the follow-up data for height and weight

Parameter	Estimate	Standard error	DF	t value	pr>|t|
Height					
Intercept	50.0945	0.9119	97	54.93	<.0001
Follow up month	0.07548	0.03173	101	2.38	0.0192
$\sqrt{\text{Follow up month}}$	7.162	0.2322	96	30.84	<.0001
Cord blood Hg group - Medium vs Low	-0.6881	0.8875	435	-0.78	0.4386
Cord blood Hg group - High vs Low	-0.3551	1.1368	435	-0.31	0.7549
Cord blood Pb	0.09602	0.2537	435	0.38	0.7053
Newborn's sex - Female vs Male	-1.0008	0.9807	435	-1.02	0.3081
$\sqrt{\text{Follow up month}}$ * cord blood Hg group - Medium vs Low	0.3825	0.22	435	1.74	0.0828
$\sqrt{\text{Follow up month}}$ * cord blood Hg group - High vs Low	0.1379	0.2515	435	0.55	0.5836
Cord blood Hg group * Newborn's sex - Medium&female	0.5766	1.1643	435	0.5	0.6207
Cord blood Hg group * Newborn's sex - High&female	0.8809	1.3732	435	0.64	0.5216
Weight					
Intercept	3.2629	0.2155	96	15.14	<.0001
Follow up month	-0.02707	0.009317	102	-2.9	0.0045
$\sqrt{\text{Follow up month}}$	1.985	0.07962	100	24.93	<.0001
Cord blood Hg group - Medium vs Low	-0.05124	0.2093	510	-0.24	0.8067
Cord blood Hg group - High vs Low	0.1305	0.27	510	0.48	0.6292
Cord blood Pb	0.003057	0.05991	510	0.05	0.9593
Newborn's sex - Female vs Male	-0.2844	0.2343	510	-1.21	0.2254
$\sqrt{\text{Follow up month}}$ * cordblood Hg group - Medium vs Low	0.1862	0.08541	510	2.18	0.0297
$\sqrt{\text{Follow up month}}$ * cordblood Hg group - High vs Low	0.213	0.09845	510	2.16	0.031
Cord blood Hg group * Newborn's sex - Medium&female	0.04771	0.2759	510	0.17	0.8628
Cord blood Hg group * Newborn's sex - High&female	-0.1331	0.3276	510	-0.41	0.6847

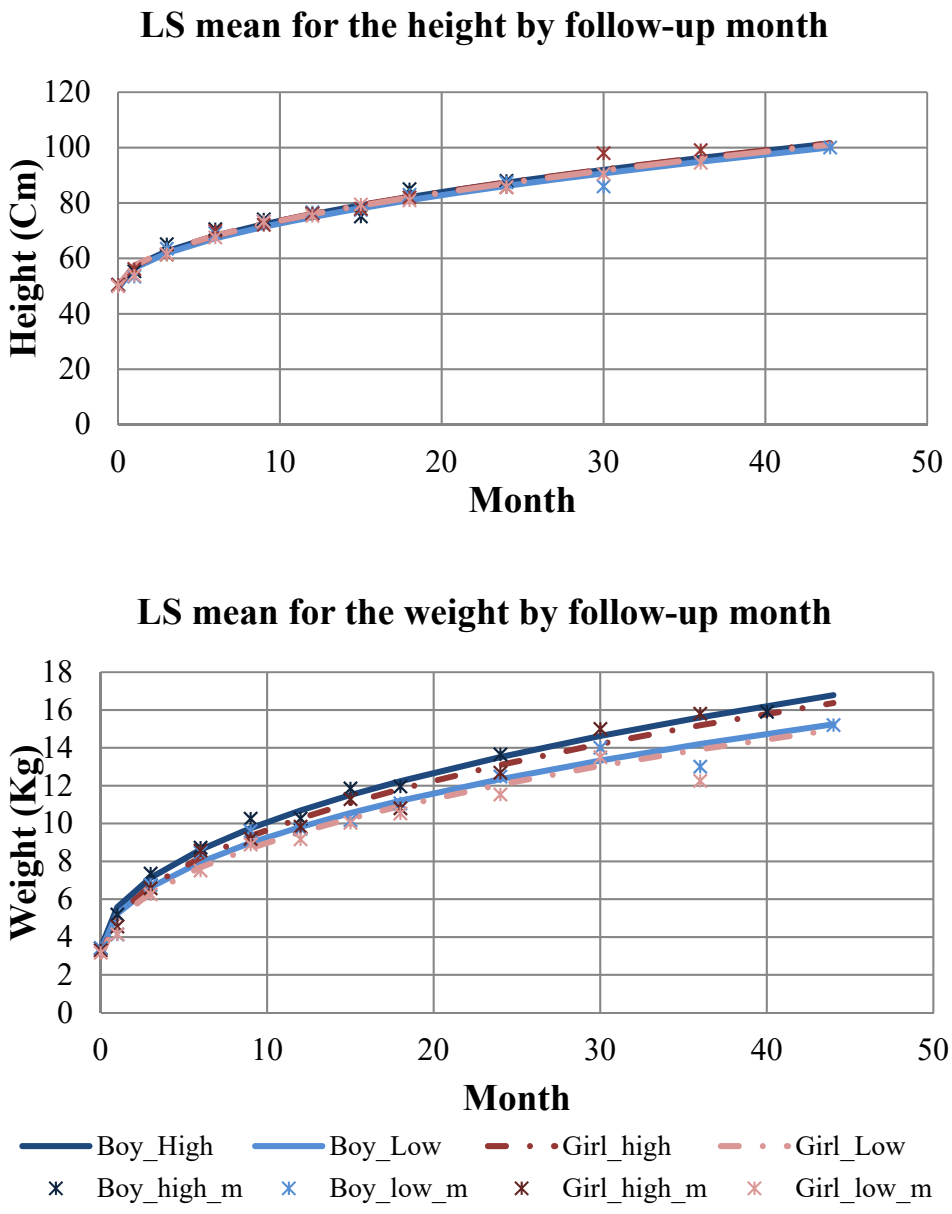

Figure 4.2 The LS mean of follow-up data by months – height (up), weight (down) [Note] The lines represent the LS mean estimates, and the asterisks represent the follow-up measurements.

Discussion

In the present study, we presented the distribution and the partitioning of Hg among the pregnant women and the newborns. And we derived the fetal body burden for MeHg during pregnancy, and evaluated the growth effect from the measured level of Hg for up to 44 month. Corresponding to the distribution of cord blood Hg level, the fetal body burden was about 26.3 to 86.9 mg. Though the cord blood Hg showed a significant association with length at birth, however, no statistical association with the follow-up data were found. Seemingly, LS mean estimates for the weight represents the different growth rates by cord blood Hg group.

The GM of maternal blood Hg in this study was 4.47 μg/L which is close to 5 μg/L of the human biomonitoring (HBM)-I value (Schulz et al., 2007). This level is much lower than those in Japan (9.1 μg/L) or Singapore (15.8 μg/L), while it is quite higher than those reported in US (0.48 μg/L) and Canada (1.66 μg/L) (Table 4.8). Though the magnitude of this level might not require medical care, but it needs effort to mitigate Hg exposure during pregnancy. Due to the maternal exposure for Hg, it could have influences on fetal development (ATSDR, 1999; Grandjean et al., 1997; Kim et al., 2016; Sakamoto et al., 2013). Furthermore, the Hg level in cord blood was much higher than that in maternal blood though the ratios of cord blood Hg and maternal blood Hg were different by the exposed level and the population.

Table 4.8 The proportions of MeHg in total Hg from several studies

	Sample	N	THg	MeHg	unit	MeHg/ THg (%)	References
Korea [a]							this study
	Maternal blood	106	4.47	-	μg/L	-	
	Cord blood	106	7.35	-	μg/L	-	
	Placenta	100	9	-	ng/g	-	
Japan [b]							(Sakamoto et al., 2013)
	Maternal blood	48	9.1		ng/g		
	cord blood	48	14		ng/g		
	placenta	48	51.2	43.1	ng/g	0.84	
	cord tissue	48	71.7	67.2	ng/g	0.94	
Singapore [c]							(Ong et al., 1993)
	Maternal blood	29	15.8	5.46	μg/L	0.35	
	Cord blood	29	18.8	8.82	μg/L	0.47	
Sweden [b]							(Vahter et al., 2000)
	Maternal blood	112	-	0.73	μg/L	-	
	Cord blood	98	-	1.4	μg/L	-	
US [a]							(Wells et al., 2017)
	Cord blood	263	1.4	0.95	μg/L	0.68	
US [a]							(Morrissette et al., 2004)
	Maternal blood	101	0.48	0.23	μg/L	0.48	
	Cord blood	92	0.52	0.39	μg/L	0.75	
Canada [a]							(Butler Walker et al., 2006)
All	Maternal blood	385	1.66	1.33	μg/L	0.80	
	Cord blood	402	2.7	2.5	μg/L	0.93	
Caucasian	Maternal blood	134	0.87	0.69	μg/L	0.79	
	Cord blood	134	1.22	1.14	μg/L	0.93	
Inuit	Maternal blood	146	3.51	2.87	μg/L	0.82	
	Cord blood	169	6.96	6.16	μg/L	0.89	

[Note] a. the numbers represent GM; b. the numbers represent median; c. the numbers were not specified

Table 4.3 presents the distributions of the partitioning for Hg from each matrix. Regarding to the fetal variables, such as placenta, cord blood, meconium, and infant's hair, Hg levels in those matrices were much higher than in maternal blood. Although the number of sample was small, the partitioning of the infant's hair to maternal blood was between 19.4 and 174 (median: 133). Based on the information, we investigated the proportion of MeHg from total Hg at each matrix to apply for partition coefficients in the PBPK model. Because we measured only total Hg and the PBPK model requires partition coefficients for MeHg (Berlin et al., 1975; Clewell et al., 1999; Sumino et al., 1975). Thus, we searched and compared the Hg levels at each tissue, and decided that the partition coefficients of Hg are differed to those of MeHg (Ou et al., 2014; Stern and Smith, 2003; Sumino et al., 1975; Yoo et al., 2002). The GMs of total Hg and MeHg in maternal blood and cord blood were summarized in the table 4.8. For the reasons, we decided not to use the partition coefficient derived from our data. In addition, we took the Hg level of the paired cord blood from the distribution of maternal blood, instead using the distribution of cord blood, to reflect the ratios from the measurements. In fact, the 5^{th}, 95^{th} percentiles of cord blood among the paired 106 subjects were 4.14, 15.5 μg/L, respectively, while the paired cord blood levels from the 5^{th}, 95^{th} percentiles of maternal blood Hg were 5.20, and 17.2 μg/L, respectively. The fetal body burden was predicted based on the latter range after applying the proportion of MeHg from total Hg (You et al., 2012b).

According to our results, the association between cord blood Hg and the length at birth were significant while the follow-up growth data were not

shown any statistical significant. The cord blood Hg showed positive estimates for height, weight and PI at birth in the general linear model (Table 4.5). It might indicate that newborn is taller and larger at birth as the exposure to Hg increased. In addition, the LS mean estimates sowed the different slopes for weight by the cord blood Hg group. High Hg group represented the rapid increasing rate of weight for both sexes (Figure 4.2). The difference between the high Hg and the low Hg group appeared from six months after birth for both sexes. These results would be the effects of Hg or the other environmental chemicals such as Pb, phthalates. Interestingly, cord blood Pb has significant linear relationship with height and weight (data not shown) though the significance was removed in the mixed model (Table 4.7). Likewise, a research from the same panel showed that the changes of child height, weight, and BMI z-scores at follow-up months were positively associated with lead level (Kim et al., 2017). In addition, diethylhexyl phthalate (DEHP) may affect body mass change in early life through obesity-related markers (Kim et al., 2016). The present study did not focus on mechanisms of Hg in newborns and infants, however, the detailed explanation including co-exposure to other environmental pollutants should be considered for describing the association between Hg and the growth.

As an important factor being affected on the growth directly, Hg in diet for the infants were required to investigate the association of following growth. However, it couldn't be considered in the present analysis due to the small number of available data. Another limitation is unknown information for fish consumption before pregnancy. Therefore, we assumed that maternal blood Hg reached to the steady state before conception, and women exposed at a certain

amount of MeHg, continuously.

The U.S. Environmental Protection Agency (EPA) has established a Reference Dose (RfD) of 0.1 μg/kg/day for MeHg, (Rice, 2004; Rice et al., 2000), which is "an estimate of a daily exposure to the human population that is likely to be without appreciable risk of deleterious effects during a lifetime". Although the Hg effect was not significant for the following growth based on our results, the measured Hg levels in fetal body including cord blood, meconium, hair were much higher than the maternal blood. In addition, the magnitude of the fetal body burden was ranged from 26.3 to 86.9 (median: 39.4 μg/kg/day) before birth. Therefore, pregnant women should consider and make efforts to mitigate the Hg exposure especially from diet. In addition, further research should be considered the fish consumption and MeHg level from diet to investigate the relationship between follow-up growth and the Hg level.

CHAPTER V.

CONCLUSIONS

Summary and Conclusions

The present study was conducted for investigating exceedances above the guidance values among South Korean. And the corresponding exposure dose for MeHg was calculated, which can be compared to the RfD. Next, the associations between Hg and health outcomes were investigated using clinical chemistry markers. Lastly, fetal body burden was derived by using PBPK model and investigated the growth effects of exposure to Hg among infants.

In the first study, the GM of blood Hg was 3.08 μg/L and about 25 % of total population exceeded the HBM-I according to the first cycle of KoNEHS (2009~2011). Because the exceedance was calculated based on the single measurement for individual, the exceedance was re-investigated by simulating with considering intra-class correlation (ICC). As a result, the exceedance rates were decreasing with the lower ICC and the more repeated data for individual. The most important explanatory variables were sex, age group and fish consumption frequency. The GM of blood MeHg in male population (2.47 μg/L) was higher than female's, and the population whose age is 50's (2.68 μg/L) was the highest among age group, as well the population who consumed fish everyday (3.39 μg/L). Next, the predicted exposure dose was 35.8 ng/kg/day for total population. Though the predicted values were lower than RfD, the HQ value was 0.36 for total population (range: 0.24 ~ 0.63). All the HQs were below than 1, however, the potential at-risk population could be existed for each population since the GM only used for deriving the HQ.

Second study demonstrated the association between blood Hg and clinical chemistry markers including hepatic, metabolic markers using the second cycle of KoNEHS (2012 ~ 2014). The GM for Hg in blood was 3.11 μg/L for all population. The blood Hg level was categorized into quantile groups - low: < 25th (2.05 μg/L), medium: 25th ~ 75th (4.75 μg/L), high: > 75th – to diagnose the effect of Hg on the reference ranges. The levels of ALT, γ-GTP and total lipid were increased with blood Hg group for both sexes, and the GMs for total cholesterol and TG were significantly increased in men across the blood Hg. In case of γ-GTP and total cholesterol, the exceedance out of the reference ranges were increased with blood Hg groups for both sexes. ALT and total lipid also showed the increasing trend in the male population though statistical significance were not proved. Regarding to γ-GTP, the high blood Hg group was associated with a 2.8-fold increase for being out-of-reference range in the male group (OR=2.77; 95% CI: 1.72-4.46), while 2.1-fold increase in the female group (OR=2.11; 95% CI: 1.35-3.30). High Hg group in men was associated with about 1.5-fold risk for total cholesterol. Especially, the significant contribution of blood Hg to high γ-GTP were retained after adjustment for other co-exposing chemicals in the multivariate linear model. Our results suggest that Hg exposure even as low as environmental levels among general population could perturbate lipid metabolism although further mechanistic researches confirm.

In the third study, Fetal body burden during pregnancy was derived using PBPK model based on the cord blood Hg, and the association between Hg in cord blood and growth variables - height and weight were analyzed for investigating health effects following Hg exposure at the developmental period.

The GM for Hg in maternal blood and cord blood was 4.47, 7.35 μg/L, and that of placenta and meconium was 9.0, 36.9 ng/g, respectively among 106 paired data out of total 334 pairs. Though the sample size of infant's hair was 25 which is too small for the following analysis, however, the GM was as high as 443 ng/g. The derived fetal body burden was ranged between 26.3 and 86.9 mg based on the 106 cord blood. Cord blood Hg was positively associated with length at birth (*p*-value: 0.0132), while weight at birth (*p*-value: 0.1764) and head circumference (*p*-value: 0.4579) were not associated with cord blood Hg. According to the logistic regression model, the cord blood Hg were not significant for the standardized height (OR: 1.30 (0.55, 3.07)), weight (OR: 0.76 (0.36, 1.62)), and PI (OR: 0.817 (0.377, 1.77)). However, the LS mean estimates for the follow up weights represent the more rapid increasing slopes in the high cord blood Hg group compared to the low Hg group for both sexes. These results indicated that fetus would have high body burden, and exposure to Hg associated with the taller height at birth and the rapid weight increasing.

The major limitation of this study was unavailable MeHg measurement data. For this reason, the blood Hg and the fraction of MeHg in total Hg were generated based on the reported distribution across the population in the first study. Because it was simulation data, only the central tendency was used for deriving the exposed dose and HQ though those values were generated based on the real measurements. Secondly, there was no information for fish consumption before pregnancy in the chapter IV. Therefore, fetal body burden was estimated under two assumptions; 1) the MeHg level in maternal blood reached the steady state before pregnancy, 2) pregnant woman was exposed at

a certain amount of MeHg via fish intakes during pregnancy continuously. The third limitation was small number of available data from diet. As an important factor being affected to the following growth, tis should be considered for further analyses. Though it was not considered in the present study, the associations of the current exposure level should be re-investigated adjusting for other confounders including Hg level in diet and co-exposure from other chemicals.

This thesis revealed the highly exposed population for Hg and conducted exposure assessment across the population based on the estimated MeHg. Though the HQs were lower than 1, however, most of the 95 percentiles were above 1 except those who consumed fish rarely. These results indicate that the potential at-risk population could be existed for each population, not only for those in consuming fish frequently. Thus, those people should be managed for their exposure not by regulating fish consumption itself, but by choosing fishes which have the less MeHg amount. Also, the current Hg exposure would not lead the specific diseases among the general South Korean, but it might be perturbed the lipid metabolism although further mechanistic researches require. Furthermore, fetus would be exposed to certain amount of Hg (26.3 ~ 86.9 μg/kg/day), and that could be influenced on the growth at birth and weight inceasing in later life. Since the present study did not focus on mechanisms of Hg in newborns and infants, the detailed explanation should be confirmed for future. Therefore, more researches for fetal body burden are required, and growth effects should be investigated adjusting for other confounders including Hg level in diet and co-exposure from other chemicals.

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Appendices

1. The histogram of blood mercury regarding HBM guidelines

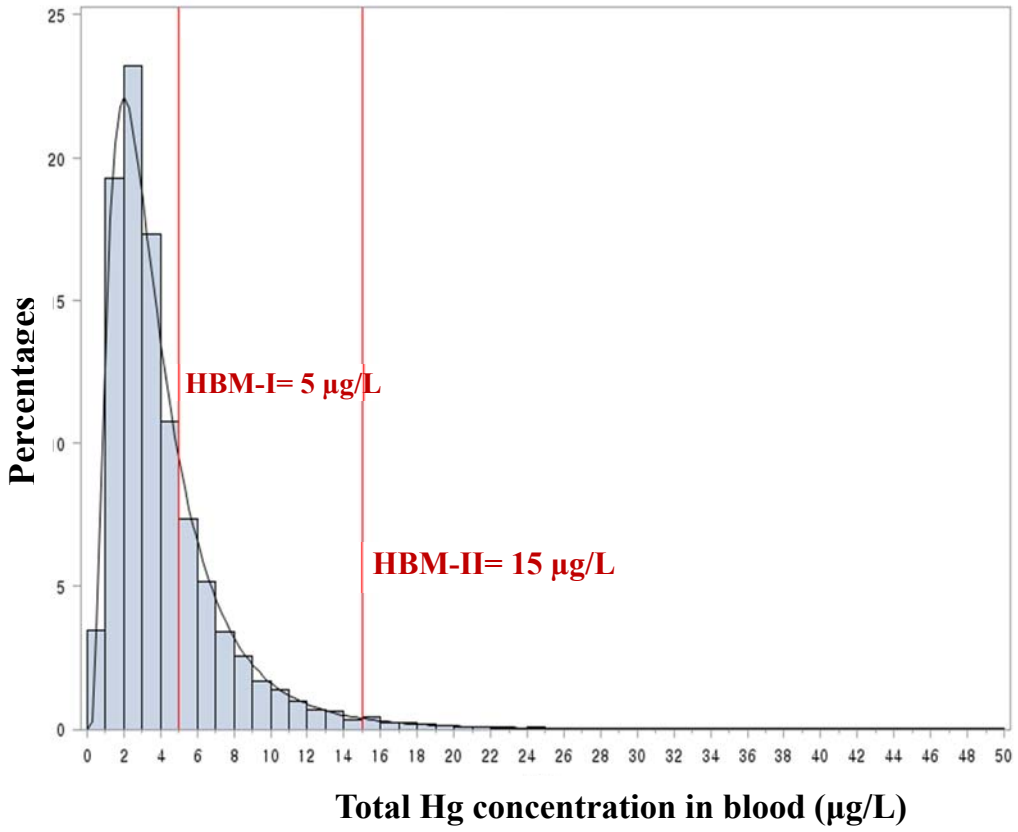

Figure 1. The distribution for total Hg in blood (µg/L) against HBM guidelines

2. Equation for calculating Exceedances beyond HBM-I, -II

The exceedances over HBM-I, -II were calculated using the equation suggested by Pleil & Sobus (2013). Since the KoNEHS conducted single measure per subject, so intra-class correlation coefficient could not be derived from KoNEHS. That is the reason of considering ICC equals 0, 0.25, 0.5, 0.75, 1. Below is the equation.

Exceedance (%) = 100 × [1 − lognorm.dist(BE, ln(GMg), ln(GSD), true)]

GSD = GMg × {ICC (m) × (Xmax − Xmin) + Xmin}

σ: standard deviation of logged data

μ: mean of logged data

GSDg = exp(σ): “global” geometric standard deviation of the initial data set

GMg = exp(μ): “global” geometric mean of the initial data set

Xmin = exp{ln[GSDg]/sqrt(m)}/GMg when ICC = 0

Xmax = GSDg/GMg when ICC = 1

m = number of measurements per person

The Exceedances above HBM-I, -II for each case are shown below.

1) The case of 3 repeated measures per individual (m=3)

Table 1. The exceedances above HBM-I, -II when the repeated measures per individual equal 3

Variables	class	ICC=0		ICC=0.25		ICC=0.5		ICC=0.75		ICC=1	
		Exceedance -I	Exceedance -II	Exceedance -I	Exceedance -II	Exceedance -I	Exceedance -II	Exceedance -I	Exceedance -II	Exceedance -I	Exceedance -II
Total		12.02	0.01	16.49	0.07	19.97	0.29	22.71	0.71	24.89	1.32
Gender	male	22.85	0.04	26.86	0.27	29.68	0.82	31.77	1.64	33.38	2.66
	female	4.33	0.0002	7.71	0.01	10.90	0.04	13.71	0.16	16.12	0.38
Age group	19-29	1.93	0.00002	4.26	0.001	6.84	0.01	9.33	0.06	11.61	0.17
	30-39	13.11	0.004	17.58	0.05	21.03	0.23	23.72	0.60	25.87	1.14
	40-49	17.91	0.01	22.27	0.12	25.47	0.44	27.89	1.00	29.79	1.76
	50-59	24.79	0.05	28.60	0.32	31.26	0.92	33.21	1.81	34.70	2.88
	60-69	11.99	0.01	16.47	0.08	19.96	0.32	22.69	0.78	24.87	1.42
	70+	5.60	0.001	9.35	0.02	12.70	0.12	15.55	0.35	17.94	0.72
Fish consumption	rarely	1.32	0.00002	3.24	0.001	5.52	0.01	7.82	0.05	9.98	0.16
	1~3/month	8.45	0.003	12.68	0.04	16.19	0.19	19.03	0.51	21.36	1.01
	1-2/week	13.50	0.005	17.97	0.06	21.41	0.25	24.09	0.63	26.21	1.20
	3-4/week	27.34	0.04	30.84	0.28	33.26	0.82	35.04	1.66	36.39	2.69
	5-6/week	29.65	0.04	32.85	0.28	35.05	0.83	36.66	1.67	37.88	2.71
	everyday	47.13	0.27	47.62	1.04	47.94	2.28	48.17	3.80	48.34	5.40

Unit: %, Exceedance-I: Exceedance over HBM-I, Exceedance-II: Exceedance over HBM-II

2) The case of 5 repeated measures per individual (m=5)

Table 2. The exceedances above HBM-I, -II when the repeated measures per individual equal 5

Variables	class	ICC=0		ICC=0.25		ICC=0.5		ICC=0.75		ICC=1	
		Exceedance-I	Exceedance-II	Exceedance-I	Exceedance-II	Exceedance-I	Exceedance-II	Exceedance-I	Exceedance-II	Exceedance-I	Exceedance-II
Total		6.48	0.00003	13.21	0.01	18.32	0.15	22.08	0.59	24.89	1.32
Gender	male	16.85	0.00077	23.99	0.07	28.38	0.51	31.30	1.42	33.38	2.66
	female	1.35	0.0000001	5.10	0.001	9.31	0.02	13.03	0.12	16.12	0.38
Age group	19-29	0.38	0.000000003	2.41	0.0001	5.51	0.005	8.70	0.04	11.61	0.17
	30-39	7.39	0.00002	14.27	0.01	19.38	0.12	23.10	0.49	25.87	1.14
	40-49	11.77	0.00012	19.09	0.03	23.97	0.25	27.34	0.84	29.79	1.76
	50-59	18.96	0.0011	25.87	0.09	30.03	0.58	32.77	1.56	34.70	2.88
	60-69	6.46	0.00005	13.20	0.02	18.32	0.18	22.06	0.65	24.87	1.42
	70+	2.01	0.000002	6.54	0.003	11.08	0.06	14.88	0.28	17.94	0.72
Fish consumption	rarely	0.21	0.000000002	1.71	0.0001	4.33	0.004	7.24	0.04	9.98	0.16
	1~3/month	3.79	0.00001	9.55	0.01	14.51	0.10	18.37	0.42	21.36	1.01
	1-2/week	7.72	0.00002	14.67	0.01	19.77	0.13	23.47	0.52	26.21	1.20
	3-4/week	21.83	0.00081	28.31	0.07	32.13	0.51	34.63	1.42	36.39	2.69
	5-6/week	24.51	0.00083	30.53	0.07	34.03	0.51	36.29	1.43	37.88	2.71
	everyday	46.30	0.02	47.27	0.40	47.79	1.61	48.12	3.40	48.34	5.40

Unit: %, Exceedance-I: Exceedance over HBM-I, Exceedance-II: Exceedance over HBM-II

3) The case of 10 repeated measures per individual (m=10)

Table 3. The exceedances above HBM-I, -II when the repeated measures per individual equal 10

		ICC=0		ICC=0.25		ICC=0.5		ICC=0.75		ICC=1	
Variables	class	Exceedance-I	Exceedance-II	Exceedance-I	Exceedance-II	Exceedance-I	Exceedance-II	Exceedance-I	Exceedance-II	Exceedance-I	Exceedance-II
Total		1.60	0.0000000001	9.65	0.001	16.62	0.07	21.47	0.48	24.89	1.32
Gender	male	8.72	0.0000000493	20.52	0.01	26.99	0.29	30.84	1.22	33.38	2.66
	female	0.09	0.0000000000	2.80	0.00001	7.76	0.01	12.37	0.09	16.12	0.38
Age group	19-29	0.01	0.0000000000	1.04	0.000001	4.28	0.001	8.11	0.03	11.61	0.17
	30-39	2.03	0.0000000000	10.61	0.001	17.66	0.05	22.48	0.39	25.87	1.14
	40-49	4.67	0.0000000014	15.38	0.003	22.38	0.13	26.80	0.70	29.79	1.76
	50-59	10.68	0.0000000969	22.55	0.01	28.72	0.34	32.34	1.35	34.70	2.88
	60-69	1.59	0.0000000002	9.66	0.001	16.62	0.09	21.46	0.54	24.87	1.42
	70+	0.19	0.0000000000	3.91	0.0001	9.48	0.02	14.24	0.22	17.94	0.72
Fish	rarely	0.00	0.0000000000	0.66	0.000001	3.27	0.001	6.70	0.03	9.98	0.16
consumption	1~3/month	0.60	0.0000000000	6.38	0.0004	12.82	0.04	17.73	0.34	21.36	1.01
	1-2/week	2.20	0.0000000000	11.00	0.001	18.06	0.06	22.86	0.42	26.21	1.20
	3-4/week	13.56	0.0000000536	25.17	0.01	30.92	0.29	34.24	1.22	36.39	2.69
	5-6/week	16.46	0.0000000564	27.61	0.01	32.91	0.29	35.93	1.23	37.88	2.71
	everyday	44.77	0.0000186638	46.82	0.10	47.63	1.07	48.07	3.04	48.34	5.40

Unit: %, Exceedance-I: Exceedance over HBM-I, Exceedance-II: Exceedance over HBM-II

3. Base information for the Monte Carlo simulations

1) In the Step #4,

the total mercury in blood was generated based on the normal distribution in natural log scale.

Table 4. Total mercury in blood for each sub-population

	Male		Female		Subtotal	
	Mean	SD	Mean	SD	Mean	SD
Total	1.296	0.731	0.963	0.654	1.127	0.712
Age1929	0.955	0.623	0.735	0.630	0.850	0.636
Age3039	1.365	0.699	0.972	0.585	1.173	0.675
Age4049	1.427	0.734	1.051	0.604	1.239	0.698
Age5059	1.496	0.750	1.153	0.668	1.322	0.730
Age6069	1.292	0.741	0.958	0.677	1.116	0.727
Age70+	1.125	0.679	0.838	0.722	0.950	0.719
Fish rarely	0.829	0.723	0.706	0.583	0.766	0.657
Fish 1-3/month	1.216	0.682	0.873	0.712	1.039	0.718
Fish 1-2/week	1.357	0.706	1.004	0.602	1.178	0.678
Fish 3-4/week	1.548	0.741	1.177	0.586	1.368	0.695
Fish 5-6/week	1.621	0.636	1.118	0.635	1.400	0.679
Fish Everyday	1.720	0.782	1.452	0.595	1.580	0.702

Unit: μg/L

2) In the Step #4
the fraction of methylmercury to total mercury was referred from the reported values of Jung's study. Simulations were performed with arithmetic mean, standard deviation, minimum, maximum, and each percentile value based on the normal distribution. Total, gender, and age-groups were generated by the corresponding factors for each class. For fish consumption group, those were generated based on the values for total population. And age groups by sex, fish groups by sex were generated based on the values for each sex because difference from sex was larger than the difference from age or fish consumption.

Table 5. Methylmercury fraction for each sub-population

	Male			**Female**			**Subtotal**		
	Mean	SD	Min	Mean	SD	Min	Mean	SD	Min
Total	0.689	0.137	0.416	0.746	0.179	0.307	0.719	0.163	0.307
Age 1929	0.689	0.137	0.416	0.746	0.179	0.307	0.707	0.162	0.495
Age 3039	0.689	0.137	0.416	0.746	0.179	0.307	0.707	0.168	0.307
Age 4049	0.689	0.137	0.416	0.746	0.179	0.307	0.698	0.161	0.371
Age 5059	0.689	0.137	0.416	0.746	0.179	0.307	0.735	0.170	0.421
Age 6069	0.689	0.137	0.416	0.746	0.179	0.307	0.761	0.142	0.440
Age 70+	0.689	0.137	0.416	0.746	0.179	0.307	0.761	0.142	0.440
Fish Rarely	0.689	0.137	0.416	0.746	0.179	0.307	0.719	0.163	0.307
Fish 1-3/month	0.689	0.137	0.416	0.746	0.179	0.307	0.719	0.163	0.307
Fish 1-2/week	0.689	0.137	0.416	0.746	0.179	0.307	0.719	0.163	0.307
Fish 3-4/week	0.689	0.137	0.416	0.746	0.179	0.307	0.719	0.163	0.307
Fish 5-6/week	0.689	0.137	0.416	0.746	0.179	0.307	0.719	0.163	0.307
Fish Everyday	0.689	0.137	0.416	0.746	0.179	0.307	0.719	0.163	0.307

Unit: %, Min: minimum, Maximum value for simulation was limited to 1.0.

3) In the step #5, the Monte Carlo simulations were performed for generating the 5 parameters in the PBPK model. Below is the distribution information for each sensitive parameter.

- Volume of slowly-perfused tissues
  : mean= 0.35, CV= 0.16, Normal distribution
- Partition of gut-to-blood
  : mean=1.0, CV=0.7, Log normal distribution
- Partition of hair-to-blood
  : mean=275, CV=0.7, Log normal distribution
- Excretion of methylmercury into hair
  : mean=7.5e-6, CV=0.25, Log normal distribution
- Body weight was retained from the sub-population using the Korean National Environmental Health Survey. Those were shown below. Because it was survey data, the mean was calculated with considering stratification and study weight information (PROC SURVEYMEANS in SAS 9.3). Also, the population SD was calculated based on the method suggested by SAS.
  (website:https://support.sas.com/rnd/app/stat/examples/SurveyStdDev/new_example/index.html)

Table 6. Body weight for each sub-population

	Male		Female		All	
	Mean	Population SD	Mean	Population SD	Mean	Population SD
Total	71.0	10.9	58.3	9.05	64.5	11.8
Age1929	72.3	12.4	56.8	10.0	64.9	13.7
Age3039	73.4	11.2	58.3	9.53	66.0	12.9
Age4049	72.1	10.6	58.5	8.44	65.4	11.7
Age5059	69.8	9.01	59.6	8.14	64.7	10.0
Age6069	67.5	8.82	59.4	8.69	63.3	9.64
Age70+	64.4	9.29	56.8	8.94	59.7	9.79
Fish rarely	69.3	12.1	57.0	8.56	63.0	12.1
Fish 1-3/month	70.8	10.9	58.0	9.49	64.2	12.0
Fish 1-2/week	71.2	10.7	58.4	8.71	64.7	11.7
Fish 3-4/week	71.6	10.4	59.1	9.19	65.5	11.7
Fish 5-6/week	70.6	10.2	60.6	9.00	66.2	10.9
Fish Everyday	72.4	11.1	60.5	9.14	66.2	11.7

Unit: Kg

4. Adjustment of other chemicals

Table 7. Correlation coefficient (ρ) between blood Hg and other chemicals

HMs		VOCs		Phthalates		PAHs		Phenols	
	ρ		ρ		ρ		ρ		ρ
BHg	1	HippA	-0.01087	MEHHP	0.00441	PBA	0.03234	BPA	-0.02873
BPb	0.08147	MucA	0.015	MEOHP	0.00298	Coti	0.06402	TCS	-0.02169
UHg	0.28922	PheA	0.05088	MnBP	0.03435	OHP	0.06837		
UCd	0.04758	MandA	0.05093	MECPP	0.00079	Napt	0.07148		
		MHA	0.03467	MBzP	-0.00649	OHPHE	0.06319		
						OHFLU	0.07023		

[Note] ρ indicates Pearson correlation coefficient, and the chemical levels were transformed by log scale.

[Abbreviation] **HMs**: BPb, blood lead; UHg, urinary mercury; UCd, urinary cadmium. **VOCs**: HippA, hippuric acid; MucA, t,t-muconic acid; PheA, phenylglyoxylic acid; MandA, mandelic acid; MHA, methylhippuric acid. **Phthalates**: MEHHP, mono-(2-ethyl-5-hydroxyhexyl) phthalate; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MnBP, mono-n-butyl phthalate; MECPP, mono-(2-ethyl-5-carboxypentyl) phthalate; MBzP, mono-benzyl phthalate. **PAHs**: PBA, 3-phenoxybenzoic acid; Coti, cotinine; OHP, 1-hydroxypyrene; Napt, 2-naphthol; OHPHE, 1-hydroxyphenanthrene; OHFLU, 2-hydroxyfluorene. **Phenols**: BPA, bisphenol A; TCS, triclosan

Table 8-a. The significance of blood Hg for ALT

		Male		Female	
Effect	DF	F Value	Pr > F	F Value	Pr > F
Model	13	25.7	<.0001	33.6	<.0001
Intercept	1	8134	<.0001	5351	<.0001
BHg	1	5.56	0.0189	25.1	<.0001
Age	5	11.7	<.0001	17.3	<.0001
BMI	3	53.9	<.0001	32.6	<.0001
Smoking	2	4.74	0.0093	1.14	0.3227
Drinking	2	0.74	0.477	4.82	0.0086

[Note] The chemical levels were transformed by log scale. Each F value and p-value was calculated using SurveyReg procedure by SAS 9.3.
Age: '19-29', '30-39', '40-49', '50-59', '60-69', '>70'; BMI: 'underweight' (< 18.5), 'normal' (18.5-23), 'overweight' (23-25), 'obese' (> 25 unit: kg/m2); Smoking: 'non-smoker', 'ex-smoker', 'current smoker'; Drinking: 'non-drinker', 'ex-drinker', 'current drinker'

Table 8-b. The significance of blood Hg for ALT adjusted for other chemicals

Male				Female			
Effect	DF	F Value	Pr > F	Effect	DF	F Value	Pr > F
Model	14	27.88	<.0001	Model	15	33.37	<.0001
Intercept	1	6146.24	<.0001	Intercept	1	4216.8	<.0001
Age	5	11.1	<.0001	Age	5	17.84	<.0001
BMI	3	54.83	<.0001	BMI	3	33.87	<.0001
Smoking	2	4.04	0.0184	Smoking	2	5.78	0.0034
Drinking	2	0.91	0.4024	Drinking	2	4.15	0.0166
BHg	1	4.16	0.0422	BHg	1	28.27	<.0001
HippA	1	16.3	<.0001	BPb	1	3.52	0.0616
				Coti	1	12.66	0.0004

[Abbreviations] BPb, blood lead; HippA, hippuric acid; Coti, cotinine;

Table 9-a. The significance of blood Hg for γ-GTP

		Male		Female	
Effect	DF	F Value	Pr > F	F Value	Pr > F
Model	13	37.0	<.0001	27.6	<.0001
Intercept	1	8391	<.0001	4907	<.0001
BHg	1	25.2	<.0001	20.1	<.0001
Age	5	7.00	<.0001	18.3	<.0001
BMI	3	31.8	<.0001	26.2	<.0001
Smoking	2	26.0	<.0001	10.5	<.0001
Drinking	2	20.9	<.0001	3.60	0.0284

[Note] The chemical levels were transformed by log scale. Each F value and p-value was calculated using SurveyReg procedure by SAS 9.3.
Age: '19-29', '30-39', '40-49', '50-59', '60-69', '>70'; BMI: 'underweight' (< 18.5), 'normal' (18.5-23), 'overweight' (23-25), 'obese' (> 25 unit: kg/m2); Smoking: 'non-smoker', 'ex-smoker', 'current smoker'; Drinking: 'non-drinker', 'ex-drinker', 'current drinker'

Table 9-b. The significance of blood Hg for γ-GTP adjusted for other chemicals

Male				Female			
Effect	DF	F Value	Pr > F	Effect	DF	F Value	Pr > F
Model	15	37.46	<.0001	Model	14	25.34	<.0001
Intercept	1	3416.41	<.0001	Intercept	1	3110.74	<.0001
Age	5	6.58	<.0001	Age	5	18.08	<.0001
BMI	3	31.57	<.0001	BMI	3	26.46	<.0001
Smoking	2	21.64	<.0001	Smoking	2	3.58	0.0291
Drinking	2	17.8	<.0001	Drinking	2	3.1	0.0464
BHg	1	16.4	<.0001	BHg	1	18.05	<.0001
BPb	1	11.17	0.0009	Coti	1	4.37	0.0374
HippA	1	41.81	<.0001				

[Abbreviations] BPb, blood lead; HippA, hippuric acid; Coti, cotinine;

Table 10. The significance of blood Hg for Total Cholesterol

		Male		Female	
Effect	DF	F Value	Pr > F	F Value	Pr > F
Model	13	19.1	<.0001	15.2	<.0001
Intercept	1	140646	<.0001	85171	<.0001
BHg	1	2.64	0.1048	2.93	0.0879
Age	5	18.9	<.0001	21.8	<.0001
BMI	3	15.6	<.0001	13.1	<.0001
Smoking	2	5.55	0.0042	3.05	0.0488
Drinking	2	5.03	0.007	1.62	0.1994

[Note] The chemical levels were transformed by log scale. Each F value and p-value was calculated using SurveyReg procedure by SAS 9.3.
Age: '19-29', '30-39', '40-49', '50-59', '60-69', '>70'; BMI: 'underweight' (< 18.5), 'normal' (18.5-23), 'overweight' (23-25), 'obese' (> 25 unit: kg/m2); Smoking: 'non-smoker', 'ex-smoker', 'current smoker'; Drinking: 'non-drinker', 'ex-drinker', 'current drinker'

Table 11. The significance of blood Hg for Triglyceride

		Male		Female	
Effect	DF	F Value	Pr > F	F Value	Pr > F
Model	13	20.0	<.0001	46.2	<.0001
Intercept	1	17176	<.0001	13074	<.0001
BHg	1	0.02	0.887	0.16	0.694
Age	5	8.82	<.0001	17.9	<.0001
BMI	3	43.9	<.0001	58.5	<.0001
Smoking	2	24.5	<.0001	3.39	0.0348
Drinking	2	6.61	0.0015	2.31	0.1005

[Note] The chemical levels were transformed by log scale. Each F value and p-value was calculated using SurveyReg procedure by SAS 9.3.
Age: '19-29', '30-39', '40-49', '50-59', '60-69', '>70'; BMI: 'underweight' (< 18.5), 'normal' (18.5-23), 'overweight' (23-25), 'obese' (> 25 unit: kg/m2); Smoking: 'non-smoker', 'ex-smoker', 'current smoker'; Drinking: 'non-drinker', 'ex-drinker', 'current drinker'

Table 12. The significance of blood Hg for Total Lipid

		Male		Female	
Effect	DF	F Value	Pr > F	F Value	Pr > F
Model	13	16.3	<.0001	21.1	<.0001
Intercept	1	107697	<.0001	80186	<.0001
BHg	1	0.70	0.4032	3.36	0.0676
Age	5	10.6	<.0001	15.0	<.0001
BMI	3	21.4	<.0001	25.5	<.0001
Smoking	2	14.7	<.0001	4.14	0.0167
Drinking	2	8.56	0.0002	1.33	0.2651

[Note] The chemical levels were transformed by log scale. Each F value and p-value was calculated using SurveyReg procedure by SAS 9.3.
Age: '19-29', '30-39', '40-49', '50-59', '60-69', '>70'; BMI: 'underweight' (< 18.5), 'normal' (18.5-23), 'overweight' (23-25), 'obese' (> 25 unit: kg/m2); Smoking: 'non-smoker', 'ex-smoker', 'current smoker'; Drinking: 'non-drinker', 'ex-drinker', 'current drinker'

Table 13. The significance of blood Hg for IgE

		Male		Female	
Effect	DF	F Value	Pr > F	F Value	Pr > F
Model	13	5.79	<.0001	3.55	<.0001
Intercept	1	1863	<.0001	777	<.0001
BHg	1	0.09	0.7639	5.85	0.0161
Age	5	8.59	<.0001	0.98	0.431
BMI	3	0.97	0.4058	8.06	<.0001
Smoking	2	15.9	<.0001	2.41	0.0914
Drinking	2	3.93	0.0205	0.96	0.3848

[Note] The chemical levels were transformed by log scale. Each F value and p-value was calculated using SurveyReg procedure by SAS 9.3.
Age: '19-29', '30-39', '40-49', '50-59', '60-69', '>70'; BMI: 'underweight' (< 18.5), 'normal' (18.5-23), 'overweight' (23-25), 'obese' (> 25 unit: kg/m2); Smoking: 'non-smoker', 'ex-smoker', 'current smoker'; Drinking: 'non-drinker', 'ex-drinker', 'current drinker'

5. Parameter values used in the PBPK model

Table 14. Parameter values for plasma flows and tissue volumes

Parameter	Description	value
BW	body weight (kg)	54.0
QCC	cardiac plasma output (L/hr/kg)	20.0
QPlm	plasma flow to placenta (L/hr)	58.5
QFeC	plasma flow to fetal tissue (L/hr)	54.0
Plasma flows (fraction of cardiac output)		
QBrBc	brain plasma	0.114
QFC	fat	0.052
QGC	gut	0.181
QKC	kidney	0.175
QLC	liver	0.046
QRC	richly perfused tissues	0.183
QSC	slowly perfused tissues	0.249
Tissue volumes (fraction of BW)		
VBrc	brain	0.020
VBrBc	brain plasma	0.007
VFc	fat	0.273
VGc	gut	0.017
VHc	hair	0.002
VIc	intestine	0.014
VKc	kidney	0.004
VLc	liver	0.026
VPc	plasma	0.041
VRBCc	red blood cell (RBC-hematocrit = 0.4)	0.024
VRc	richly perfused tissues	0.100
VSc	slowly perfused tissues	0.350
VRemain	remainder of body (non-perfused)	0.122

Table 15. Parameter values for partition coefficients and kinetic parameters

Parameter	Description	value
Partition coefficients for MeHg		
PBr	brain/blood	3.0
PBrB	brain blood/plasma	1.0
PF	fat/blood	0.15
Pfe	fetal plasma/placenta	2.0
PG	gut/blood	1.0
PHB	hair/blood	275
PK	kidney/blood	4.0
PL	liver/blood	5.0
PPl	placenta/blood	2.0
PRBC	red blood cell/plasma	12.0
PRBCFe	red blood cell/plasma for fetus	14.0
PR	richly perfused tissues/blood	1.0
PS	slowly perfused tissues/blood	2.0
PUr	urine/blood	0.025
Kinetic parameters (L/hr)		
kbrini	brain MeHg to inorganic Hg	1.2E-05
kbrili	loss of inorganic Hg from brain	0.001
kbrici	incorporation of inorganic Hg in brain	5.0E-05
kbi	biliary clearance of MeHg	0.0004
kbri	brain/brain plasma diffusion	0.01
kdi	MeHg to inorganic Hg in intestine	0.0001
kfe	placenta/embryo diffusion	1.0
kfi	fecal excretion of MeHg	0.0002
khi	excretion into hair	7.00E-06
kii	loss of MeHg to inorganic Hg in liver	1.00E-05
krbci	RBC to plasma diffusion	1.5
krbcfe	RBC to plasma diffusion in fetus	100
kri	intestinal reabsorption	0.005

6. The follow-up growth

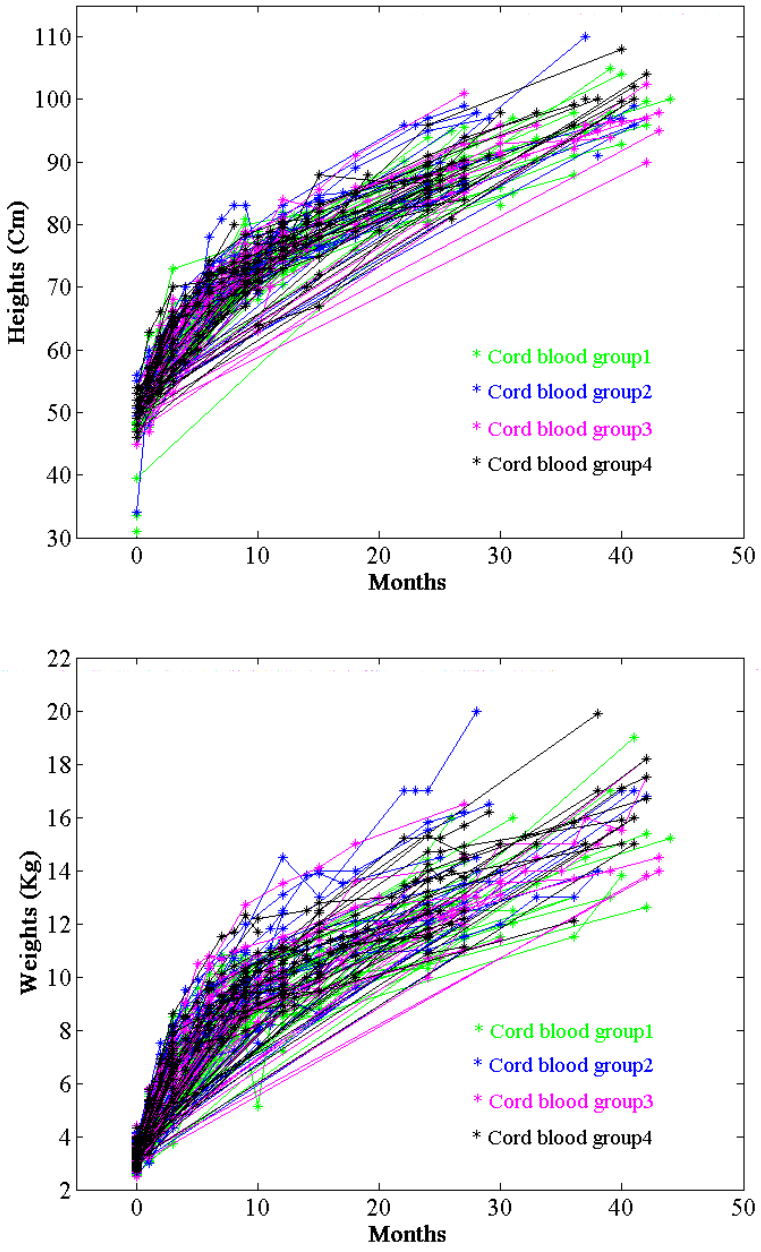

Figure 2. The growth information by follow-up month – height (up), weight (down)

국문 초록 (Abstract in Korean)

한국의 일반 인구집단 대상 수은 노출 및 관련된 건강영향

수은은 자연적으로 발생하는 중금속 물질로 대기나 토양, 그리고 해양 생태계에 존재한다. 형태에 따라 아말감 등의 무기수은과 메틸수은 같은 유기수은으로 구분되며 이중 메틸수은은 수은 중 가장 독성이 높은 형태로 알려져 있다. 국내 바이오모니터링 연구조사에 따르면 국내 평균 혈중 수은농도는 외국의 국가기반 바이오모니터링 자료에서 산출된 평균값보다 더 높은 수준이며 상당한 수의 한국인이 매우 높은 혈중 수은농도를 가지고 있음을 알 수 있었다. 이와 관련하여, 독일의 인체 바이오모니터링 위원회에서는 조처가 필요하지 않는 기준값으로 5 μg/L (HBM-I), 그리고 즉시 치료가 필요한 기준값으로 15 μg/L (HBM-II)를 제시하였다. 한편, 일반 인구집단에서 수은의 노출경로는 식이섭취이며, 특히 생선을 통해 내부에 축적되어 있던 메틸수은이 체내로 흡수되어 쌓이게 된다. 실제로, 체 내 혈 중 수은의 약 80 %는 메틸수은에 해당한다. 이에 따라 미국의 환경청 (US EPA)에서는 노출량을 규제하기 위해서 메틸수은의 일일섭취허용량 (RfD)을 0.1 μg/kg/day 로 제시하였다. 현재까지 국내에서는 개개인의 실제 내적노출량에 기반하여 수은 노출량을 산출한 연구는 없었다. 또한 수은이 비만유발 물질로 추정되고 있으므로 건강영향과 함께 연구함으로 관련된 건강영향과의 연관성이 확인할 필요가 있었다. 더욱이 한국의 여성들이 다른 나라 여성들에 비해 높은 혈중 수은 농도를 가졌고 수은이 태반을 통해 엄마로부

터 태아에게 노출이 된다는 점을 고려할 때 민감한 영향을 받을 가능성이 높은 태아 및 영유아에서의 수은 노출평가는 꼭 필요하다.

본 연구에서는 국내 인구집단 별 혈중 수은농도의 기준치 초과율을 연구하고 그에 상응하는 메틸수은 노출량을 산출하여 일일섭취허용량과 비교하였다. 또한 수은과 건강영향 간 연관성을 임상지표들을 사용해서 조사해보았다. 마지막으로, 태아의 노출 총 량을 산출하고 노출과 이후 성장과의 연관성을 살펴보았다.

첫 번째 연구에서는 국민환경보건기초조사 1기 자료 (2009-2011)를 이용하여 수은의 노출량을 산출하였다. 성별, 연령, 그리고 생선섭취량이 혈중 수은농도를 설명하는 가장 중요한 변수로 분석되었으므로 이 변수들에 따라 인구집단을 나누어 HBM 기준을 넘는 초과율을 산출하였다. 다음으로, 혈중 수은 중 메틸수은의 비를 이용하여 혈중 메틸수은의 농도를 얻은 뒤 PBPK 모델을 통해 인구 집단 별 노출량을 예측하였다. 이 섭취노출추정량은 일일섭취허용기준 (RfD)와 비교하여 각 집단 별로 위험지수 (HQ)를 산출하였다. 한국인의 평균 혈중 수은농도는 3.08 μg/L이며 전체 인구집단의 약 25 %가 HBM-I을 초과하는 것으로 드러났다. 이는 한 회 수집된 혈액에서 분석한 결과이므로 개인 내 변이를 고려하여 시뮬레이션 해보았을 때, 개인 내 상관계수(ICC) 가 작아질수록, 자료의 반복 개체수가 많아질수록 기준치 초과율이 작아지는 경향을 확인하였다. 전체 인구집단의 노출량은 35.8 ng/kg/day 이었고 이에 해당하는 HQ는 0.36 이었다. 집단 별로 산출된 노출량의 평균을 기반으로 계산된 HQ는 모든 인구 집단에서 1보다 낮은 수치를 보였으나 (range: 0.24 ~ 0.63) 상위 5퍼센타일의 HQ는 생선섭취를 거의 하지 않는 집단을 제외한 모든 집단이 1 이상의 값을 나타냈다. 본 연구는 시뮬레이션 자료를 이용한 노출평가이므로 극단 값의 사용은 주의

가 요구되지만, 그럼에도 해당 결과는 모든 집단에서 잠재적인 위험군이 일정수준 존재할 수 있음을 시사한다.

두 번째 연구에서는 국민환경보건기초조사 2기 자료 (2012-2014)를 가지고 수은 노출이 ALT, γ-GTP, total cholesterol, triglyceride, total lipid, and IgE 등의 임상화학지표의 교란에 미치는 영향을 조사하였다. 특히, 성별과 혈중 수은농도 그룹에 따라 각 지표 별 적합범위 (reference range)를 벗어나는 것에 대한 오즈비를 통해 혈중 수은과 각 지표들 간 연관성을 살펴보았다. 혈중 수은농도를 각각 4분위수에 따라 25^{th} 퍼센타일 미만(2.05 μg/L), 25^{th} - 75^{th} 퍼센타일(4.75 μg/L), 75^{th} 퍼센타일 이상으로 그룹을 나누고 임상학적 지표의 농도를 확인해본 결과 ALT, γ-GTP 그리고 총 지질은 남녀 모두에서 혈중 수은그룹에 따라 유의하게 증가하는 경향을 보였고, TG와 총 콜레스테롤은 남자에서만 유의하게 증가하였다. 특히, γ-GTP는 저농도 그룹에 비해 고농도그룹에서 남자는 약 2.8 배, 여자는 2.11 배 적합범위를 벗어날 오즈가 증가하는 것을 확인하였다. 또한 총 콜레스테롤 역시 남자의 경우 고농도 그룹이 저농도 그룹에 비해 약 1.5배 적합범위를 벗어날 오즈가 증가하였다. 특히, ALT와 γ-GTP의 경우, 다른 환경물질의 영향을 보정한 후에도 수은의 유의성이 계속 남아 있는 것을 확인하였다. 본 연구 결과는 환경 중 농도와 같이 낮은 수준의 수은 노출도 지질대사 등을 교란시킬만한 영향이 있을 수 있음을 알려주므로 관련된 메커니즘 연구를 통해 확인이 필요하다.

세 번째 연구에서는 한국 어린이의 건강 및 환경성 물질 연구자료를 이용하여 환경성 물질에의 노출과 건강영향의 연관성을 조사하였다. 태아의 수은 노출 총 량은 PBPK 모델을 이용하여 산출하였고, 발달기의 수은 노출

로 인한 성장 영향은 여러 통계적인 접근방법을 통해 분석하였다. 전체 산모태아 334 쌍 중 산모혈과 제대혈 자료가 모두 있는 106쌍을 대상으로 출산 전 산모의 혈중 수은 대비 출산 시 제대혈 및 태반, 태변, 한달 째 신생아의 머리카락 등의 수은농도를 비교해봤더니 산모와 제대혈의 평균 수은 농도는 각각 4.47, 7.35 μg/L 였으며 태반과 태변 중 수은 농도는 각각 9.0, 36.9 ng/g 이었다. 신생아 머리카락 시료는 분석에 사용하기에는 적은 수였으나 (n=25) 평균 수은농도는 무려 443 ng/g 이었다. PBPK 모델을 이용하여 106명의 제대혈 농도에 따른 태아의 체내 총 량의 범위는 약 26.3 ~ 86.9 mg으로 산출되었다. 제대혈 중 수은은 출산 시 신생아의 키와 유의한 양의 관계가 있었지만 (p-value: 0.0132) 출산 시 몸무게나 (p-value: 0.1764) 머리 둘레와는 (p-value: 0.4579) 연관성이 보이지 않았다. 표준화한 성장변수들을 가지고 중위수 이상인지 여부에 따른 로지스틱 분석을 수행한 결과 제대혈 중 수은은 키 (OR: 1.30 (0.55, 3.07)), 몸무게 (OR: 0.76 (0.36, 1.62)), 그리고 PI (OR: 0.817 (0.377, 1.77))와 유의한 영향을 보이지 않았다. 그러나 추적관찰된 키와 몸무게의 최소제곱평균 (LSmean) 추정치를 산출하여 그래프로 나타내보니 제대혈 중 수은의 고농도 그룹과 저농도 그룹에서 남녀 모두 몸무게 증가율이 다른 것을 확인할 수 있었다. 이 결과는 태아가 높은 수은 노출 총량을 가질 수 있으며, 또한 수은 노출이 출생 시에 더 큰 키와 이후 빠른 몸무게 증가와 연관이 있을 수 있음을 나타낸다.

본 연구를 통해 혈중 수은이 높은 인구집단을 확인하고 추정된 메틸수은 노출량을 기반으로 집단 별 노출평가를 수행하였다. 집단 별 노출추정량은 RfD 기준보다 낮았고 이에 따른 HQ 역시 모든 집단에서 1보다 낮은 값이 산출되었다. 시뮬레이션 자료를 이용한 평가이므로 평균을 이용하여 결과

를 산출하였으나, 상위 5퍼센타일 값의 경우 생선섭취를 거의 하지 않는 집단을 제외한 모든 집단의 HQ가 1을 초과하였다. 이는 생선섭취를 자주하는 집단이 아니더라도 각 집단 내 허용기준치 이상에 노출되는 사람들이 있음을 입증한다. 그러므로 이들에 대한 노출 관리가 필요하며 특히 생선섭취 자체를 줄이기보다는 수은함량이 적은 생선을 홍보하는 방법이 권장된다. 또한, 다른 환경성 물질의 노출을 보정한 뒤에도 혈 중 수은이 임상지표가 적합범위를 벗어나는 것과 유의한 연관성을 보였다는 점은 수은이 지질대사를 교란시키는 영향이 있을 수 있음을 나타내었다. 태아의 경우, 이미 발달기에 태반을 통해 상당한 양의 수은에 노출될 수 있음과 출생당시 키 그리고 이후 몸무게 증가에 수은이 영향을 줄 수 있음을 확인할 수 있었다. 본 연구는 수은의 영향을 단면연구와 추적관찰 연구 등을 통해 통계적으로 연관성을 분석, 제시하였으며 밝혀진 점들과 관련해서 추후 기전적인 연구를 통해 관계가 입증될 필요가 있다. 더욱이 수은 노출 총량으로부터 오는 영향은 식이 중 수은농도와 함께 다른 환경성 물질의 노출을 포함한 여러 교란인자들을 보정한 뒤 재분석되어야 할 것이다.

표제어: 수은, 메틸수은, 바이오모니터링, 생리학적 약동학 모델 (PBPK) 노출량 재구성, 비만유발물질, 노출 총 량(body burden), 위험지수(HQ)

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