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Exposures to Heavy Metals among Pregnant Women of Thailand: Pooled Urine Sample Analysis

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Abstract

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By Thanawadee Chantian

Background Pregnant women are at risk from heavy metals exposure due to physiologic changes, resulting in adverse pregnancy outcomes. Pooling approach is an approach which is beneficial in reducing financial and laboratory burden in limited-resource settings. The study aimed to assess background exposure of heavy metals among pregnant women and to evaluate the differences of urinary cadmium and lead level across geographic regions and across trimesters of pregnancy by using pooled sample analysis.

Methods We collected urine samples from all nationalities pregnant women who attended the ANC clinics during two weeks in six hospitals representing six different regions of Thailand. We asked clinical staff to split 1-ml of excess urine from routine ANC care into 50-ml tubes by trimesters. The samples were analyzed for cadmium and lead level as well as cotinine as biomarkers of tobacco smoking which might be a potential source of exposure.

Results Fifty-two pools samples of 2,112 individual pregnant women were collected by trimesters and by hospitals. Geometric mean for urinary cadmium and lead were 0.63±1.14 ng/mL and 0.82±1.07 ng/mL respectively. Log-transformed cadmium and lead levels were found statistical differences across the hospitals (p-value <0.0001 and 0.0014). The log-transformed urinary cadmium and lead showed the decreasing trends as trimester decreases, but they did not show statistically significant (p-value 0.66 and 0.28 respectively). Furthermore, there was a positive linear relationship between log-transformed urine lead and cotinine (p-value <0.001, r=0.38).

Conclusions Our study is the first study demonstrating the pattern of cadmium, lead and cotinine exposure by using pooled urine approach among Thai pregnant women. The results indicate that environmental tobacco exposure might be a potential source of lead exposure whereas diet might be a primary source of cadmium exposure among pregnant women in Thailand. Large-Scale human biomonitoring should be developed by using pooling approach to integrate this fruitful data into national public health surveillance.

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Exposures to Heavy Metals among Pregnant Women of Thailand: Pooled Urine Sample Analysis

I. Background and significance

Source of exposure to heavy metal in pregnancy

Pregnant women have a potential risk of exposure to heavy metals from contamination of occupational and non-occupational sources. Most occupational exposures occur during the manufacturing process in which heavy metals are employed. For instance, lead is commonly used in the manufacture of batteries, glazed pottery, fishing sinkers, water pipes, and paint. Cadmium is used as plastic stabilizers as well as battery-manufacturing and painting. Arsenic is involved in the production of certain pesticides and other agricultural products. Mercury is used in metal mining, smelting, incineration, and some industries manufacturing scientific instruments and medical devices (Klaassen, 2007). Non-occupational exposures primarily result from environmental contamination. For instance, cadmium is found as a contaminant in the food-chain (e.g., rice, seafood) and cigarette smoking (Agency for Toxic Substance and Disease Registry, 2008; Benson, Anake, Adedapo, Fred-Ahmadu, & Ayejuyo, 2017). Lead is found in lead-based paint, folk remedies, food containers such as pottery or porcelain, past use of leaded-gasoline as well as contamination in soil, outdoor air, groundwater and surface water surrounding industrial or lead-smelting areas (President's Task Force on Environmental Health Risks and Safety Risks to Children, 2016). Mercury is found in the food chain especially predator fish, dental amalgam and skin lightening products (Al-Saleh, 2016; Solan & Lindow, 2014). Arsenic is found in contaminated drinking water and food (Chung, Yu, & Hong, 2014). Moreover, cigarette smoking is also an important source of heavy metal exposure, especially

for arsenic, cadmium, chromium, nickel, and lead (Benson et al., 2017; Chung et al., 2014; Li et al., 2017).

In Thailand, heavy metal contamination has remained a significant issue for several decades especially contamination from natural and anthropogenic sources. Notably, food and water contamination are substantial concerns. For instance, arsenic and cadmium contamination was detected in jasmine rice, brown rice, and glutinous rice (Hensawang & Chanpiwat, 2017). One of the well-known cadmium contamination sources is from rice grain in Mae Sot, Tak province, in the northwestern region where paddy fields received irrigation from a creek and canal passing through a zinc (Zn) mine. The area enriches not only zinc but also cadmium naturally. The contamination has not been clearly proven whether it was contaminated from natural weathering of cadmium in soil or during the mining process (Khaokaew & Landrot, 2015; Padungtod, 2007). In addition, contamination in seafood has become a significant source of heavy metal exposure. Methylmercury contamination is found in fish from a canal in the upper Gulf of Thailand as well as high-level mercury contamination among fish and shellfish including shrimps, mussels, and oysters from Chaopraya river delta (Thongra-ar & Parkpian, 2003). Arsenic contamination in groundwater was found in the southern region of Thailand as a result of tin smelting (Jones et al., 2008) and northeastern region (Wongsasuluk, Chotpantarat, Siriwong, & Robson, 2018). Even though Thailand completely banned leaded gasoline in 1996, lead contamination from various sources have remained a problem. For instance, battery-acid used in solar power generating system (Swaddiwudhipong, Tontiwattanasap, Khunyotying, & Sanreun, 2013), electronic waste recycling facilities, and lead dust from boatyard industries (Untimanon, Geater, Chongsuvivatwong, Saetia, & Verkasalo, 2012). Moreover, a novel source of lead contamination of water and plants was identified as lead smelting into the creek named 'Clity' in the western region that has been going on until

present ("Clity: The novel story making new pages with environmental community justice," Sep 11, 2017).

Physiologic changes in pregnancy

Pregnant women are considered a vulnerable population for the effects of heavy metal exposure due to the physiological and anatomical changes during pregnancy. For instance, physiologic changes, such as an increase in respiratory rate, increase plasma and total body water volume, increase renal blood flow as well as altered gastrointestinal movement and gastric pH. These changes may increase cadmium distribution. Also, metallothionein (MT), which plays a role as a cadmium transporter, is different in each trimester of pregnancy. For instance, cadmium absorption is independent of MT in early pregnancy; in contrast, MT redistribution in late pregnancy contributes to cadmium redistribution (Committee on Human Biomonitoring for Environmental Toxicants, 2006). An increase in bone resorption during pregnancy also contributes to elevated blood lead level. Also, alterations in the methylation process during pregnancy affects inorganic arsenic levels which consequently can have an impact on adverse pregnancy outcome (Centers for Disease Control and Prevention, 2005). The placenta, one of the anatomical changes, is formed during pregnancy to serve as a barrier to protect any harm to the fetus. However, it serves as an active transport system for heavy metals. Lead and arsenic easily cross the placenta by a diffusion mechanism. Mercury vapor and methyl mercury pass through the placenta to the fetus through passive diffusion and amino acid transporter. On the other hand, inorganic mercury is accumulated in the placenta. Furthermore, the placenta accumulates cadmium using MT which binds to cadmium and is retained in the placenta (Choi, Knudsen, Mizrak, & Joas, 2017).

Effects of heavy metals in pregnancy and fetal outcomes

Several studies have identified associations between heavy metal exposure and adverse pregnancy outcomes such as low birth weight and preterm birth. According to a meta-analysis of 888 articles, arsenic is associated with increased risk of spontaneous abortion, the moderate risk for stillbirth as well as moderate risk for neonatal mortality and infant mortality (Ganzleben et al., 2017). Prenatal exposure to mercury may have an impact on adverse pregnancy outcomes including pregnancy-induced hypertension, low birth weight, and birth defects. However, four studies conducted in Korea, China, and Japan showed inconclusive results of mercury exposure and birth outcomes such as infant weight, length and head circumference (Chang et al., 2017). Cadmium induces oxidative stress, which damages cell structures and function and eventually leads to cell injuries and cell death (Committee on Human Biomonitoring for Environmental Toxicants, 2006). It accumulates in lungs and the gastrointestinal tract, then targets the organs- primarily liver, kidney, and placenta. Oxidative stress causes kidney injury and contributes to pre-eclampsia in pregnancy. Early life lead exposure is found to have an impact on neurocognitive development and behavior in childhood. Needleman et al., demonstrated that cognitive damage occurred even at blood lead levels below 10 μg/dL and a negative association between umbilical cord blood lead and neurobehavioral score persisted for at least 57 months of age. Additionally, elevated bone lead levels also increased the risk of juvenile offending behaviors and antisocial tendency (Leem et al., 2015; Marano et al., 2012).

Human biomonitoring

Biomonitoring is a fundamental approach to assess exposures to toxic chemicals and provides a great value in assessing and monitoring changes in the distribution of exposure and identifying vulnerable populations. When employed with epidemiologic investigations,

biomonitoring can provide evidence of exposure, and therefore allow the exploration of causal links to health outcomes (Barr, Wang, & Needham, 2005). Additionally, biomonitoring data can be used to estimate the dose or uptake of a chemical when used in models along with pharmacokinetic data (Committee on Human Biomonitoring for Environmental Toxicants, 2006). Nonetheless, some limitations exist regarding utilization and interpretation biomarker data (Committee on Human Biomonitoring for Environmental Toxicants, 2006). The biomarker data alone do not necessarily indicate health risks, but can be used in conjunction with health outcome data in epidemiologic studies to try to understand any relationship between the biomarker data and health risk (Centers for Disease Control and Prevention, 2005). Given the uncertainties present in exposure and health assessments, it is challenging to understand health implications from biomonitoring data alone, and, consequently, to inform risk assessments and translation into appropriate public health policies (Committee on Human Biomonitoring for Environmental Toxicants, 2006).

Even though human biomonitoring is useful in understanding individual and population-based exposures, biological samples must be collected strategically from a sufficient number of subjects. Personnel and laboratory costs in biomonitoring studies can be quite substantial. Thus biomonitoring studies are implemented typically only in developed countries such as the United States, France, Canada, and Germany (Choi et al., 2017; Ganzleben et al., 2017) that tend to have more funds to invest in such studies.

Biomarkers of exposure to heavy metals

In assessing heavy metal exposure using biomonitoring, the biological half-life of each heavy metal must be considered; they vary by metal and area of distribution (e.g., soft and hard tissues may have different half-lives for a given metal). Cadmium has a biological half-life in

humans of approximately 10-30 years (Klaassen, 2007). Since cadmium accumulates in the kidney, urinary cadmium is used to measure cadmium exposure. Both single, spot urine cadmium or first-morning void urine cadmium is an accurate measurement of the low-level exposures experienced by the general population (Thai et al., 2016). Even though mercury has many forms, its half-life is approximately 1-2 months. Ten percent of mercury vapor will be excreted by exhalation, the rest of it will be converted into inorganic mercury, which is mainly excreted in urine and feces. Also, ninety percent of methylmercury is excreted in feces, and the rest is excreted in urine (Klaassen, 2007). Lead binding to hemoglobin in red blood cells has a half-life in the blood is around 30 days while it is distributed to bone and hair. In trabecular bone where lead accumulates, it has a half-life of 20 years (Klaassen, 2007). Even though approximately 70% of lead is excreted through urine and reflects recent lead absorption, blood lead is more commonly used as a biomarker than urine lead due to less individual variation and contamination (Heffernan et al., 2013). Urinary arsenic can represent current exposure due to its short half-life. Arsenic is well-absorbed in the gastrointestinal tract, and its half-life is only 10 hours before excretion within three days.

Tobacco smoking and heavy metal exposure

Tobacco smoking is considered a significant pathway of heavy metal exposure especially to lead and cadmium due to absorption from the soil to tobacco plants(Chang et al., 2017). According to data collected from the National Health and Nutrition Examination Survey (NHANES), blood and urine cadmium are higher among cigarette smokers compared to smokeless tobacco and non-tobacco users. Also, there is a positive relationship between serum cotinine and blood cadmium, and urine cadmium among smokers (Marano et al., 2012). A study from Korean National Health Examination Survey (KNHANES) found the statistically

significantly lower mean levels of blood cadmium and lead among former smokers and non-smokers compared to current smokers (Leem et al., 2015). Recently, tobacco is not considered a significant source of arsenic exposure because of elimination of the use of arsenical pesticides in tobacco cultivation (Chang et al., 2017). To assess tobacco exposure, nicotine can be measured in serum or urine although its metabolites cotinine and 3-hydroxycotinine are more commonly measured. Urine cotinine and 3-hydroxycotinine are commonly used biomarkers of active smoking and second-hand tobacco smoke exposure (Mattes, Yang, Orr, Richter, & Mendrick, 2014).

Pooled urine samples

Pooled biological sampling is an approach which combines multiple individual specimens into a group by using well-defined criteria (Amy L. Heffernan et al., 2014). The pooling approach can be used for assessing trends of biomarkers in human exposure level over time in the population as well as patterns of exposure by demographic and geographic data. This approach may contribute to identifying susceptible population and some insight in environmental exposure in different locations. Additionally, it is a cost-effective way to detect emerging or unexpected exposures during an investigation (Amy L. Heffernan et al., 2014). Heffernan mentioned three factors that have an impact on pooling approaches including measurement error and variation in pooled sample measurements, pooling error, and shape of the underlying population's distribution (A. L. Heffernan et al., 2014).

Error and validity issues

Although the measured value in pooled samples is comparable to the arithmetic mean of individual levels making up the pools, the primary statistical concerns in using pooled analysis are bias in the estimation of central of tendency and limitations in assessing inter-individual

variation. Biases in the estimation of central of tendency may occur if the data distribution is lognormal (S. P. Caudill, 2010; A. L. Heffernan et al., 2014). Also, this approach has limitations in estimating the geometric mean, median, and variance in the population. Caudill proposed statistical approaches to estimate population variance and percentile estimates in biomonitoring data collected from the NHANES by using log-normality assumptions, analysis of multiple pools to estimate population variance and repeat more analytic batches of each pool to estimate measurement error (S. P. Caudill, 2010). Moreover, pooling error is probably caused by physical errors in extracting and transferring precise volumes by staff during pooling. The larger the number of individual specimens being pooled, the greater error may be. Notably, using weight sampling by volume of individual specimens may contribute to more error (A. L. Heffernan et al., 2014). On the other hand, the larger number of individual specimens will provide a more accurate estimation the actual mean and reduce the variance in that estimate. Therefore, balancing between an adequate number of specimens in each pool to describe the variance and to avoid an error due to pooling is taken into account (A. L. Heffernan et al., 2014).

Pooling approach in practice

Since the pooling approach provides benefit in reducing laboratory burden, it has been applied to biomonitoring programs, for example in the Australian population by the National Research Centre for Environmental Toxicology (Entox) at the University of Queensland and in the US NHANES. Also, many environment toxicants have been tested in pooled samples. For instance, PCB-153 (Samuel P. Caudill, 2012), polycyclic aromatic hydrocarbons (PAH) (Thai et al., 2016), bisphenol A (Heffernan et al., 2013), phthalates (Gomez Ramos et al., 2016), and

pesticides (Heffernan et al., 2016). All of the studies mentioned above collected individual samples from either routine laboratory testing for pooling or specimen banking.

In Thailand, an extensive biomonitoring program has not been integrated into public health surveillance leading to a lack of national data on environmental toxicant exposure. Financial constraints and a lack of laboratory support are the main hindrances in launching a biomonitoring surveillance program. To provide support for a national biomonitoring program and to assess exposures to emerging environmental toxicants among pregnant women in Thailand, we undertook a pooled biomonitoring study to evaluate exposures in pregnant Thai women.

The primary aim of our study is to assess background exposure of heavy metals among pregnant women in multiple regions of Thailand using cross-sectionally collected pooled urine samples collected from women in different trimesters of pregnancy. Also, specific objectives are to evaluate differences in urinary heavy metal levels among trimesters of pregnancy and to evaluate differences in urinary heavy metal levels among hospitals in different geographic regions in Thailand.

Hypotheses

1. There are differences in levels of urinary heavy metals across trimesters of pregnancy
2. There are differences in levels of urinary heavy metals across different geographic regions in Thailand

II. Methods

Our study protocol received an exemption of IRB approval from Emory University since used residual sample used for routine health screening and no identifiers were retained. Additionally, we received ethical approval from the Thai Department of Disease Control and

the six hospitals including Samutprakan (SAM), Saraburi (SAR), Pichit (PIC), Maharat Nakhon Ratchasima (MAH), Rayong (RAY) and Surat Thani (SUR) hospitals.

Participants recruitment

We conducted the study in six hospitals from six provinces to represent different regions throughout Thailand. SAM, SAR, PIC, MAH, RAY and SUR hospitals were selected by purposive sampling based on occupational medicine clinic networks; these hospitals represent a vicinity of Bangkok, central, north, northeast, east and south regions of Thailand. The study population is pregnant women who attended antenatal clinics (ANC) for routine antenatal care from six hospitals during the two weeks that were sampled for each hospital. Our inclusion criteria were all nationalities pregnant women who attended the ANC clinics, physically lived in that province during the study period and received urine testing at the clinics. Samples were collected from August to October 2017.

According to the government's protocol, a urine test for albumin and sugar is usually performed at every visit to screen risk of preeclampsia and gestational diabetes. After the routine clinical test is done, the residual urine is typically discarded. SUR, PIC, and MAH have tests performed the ANC clinic. Once pregnant women visit the ANC clinic, each pregnant woman will receive a plastic cup with a lid to collect her urine as usual and will be tested for urine albumin and sugar by using a dipstick at the clinic by nurses. Afterward, the tested urine is discarded at the clinic. In SAR, RAY and SAM, the testing occurs at both the ANC clinic and pathological laboratory. An aliquot of urine from each pregnant woman is sent to a pathological laboratory for testing.

Exposure assessment

We asked the ANC clinic staff including nurses, nurse assistants or laboratory staff to record maternal age and gestational age of pregnancy and to split 1-mL of the excess urine (from routine testing) without identifiers to pool into a 50-mL polypropylene tube by trimester of pregnancy. The trimesters of pregnancy were defined as (1) first trimester was less than 14 weeks, (2) second trimester was 14 to 28 weeks, and (3) third trimester was more than 28 weeks. This process was repeated until each 50-mL tube was full. Then a new tube was used to continue pooling. During aliquoting the urine, three pools were kept cold with ice/ice packs, and we also asked the hospitals to prepare field blanks for quality control purpose by using an empty 50-mL polypropylene tube with lids opened. All pools were kept cold at 4°C in the refrigerator until the two weeks of the collection was finished.

After the collection was completed, all samples were shipped to the Reference Center of Toxicology Laboratory of Bureau of Occupational and Environmental Diseases, Thailand for storage at -20°C before shipping to the Laboratory of Exposure Assessment and Development for Environmental Research (LEADER) of Rollins School of Public Health of Emory University. The samples were then homogenized, separated into smaller aliquots, and then kept frozen at -20° C until analysis.

A total of 52 pools comprised of residual urine of 2,112 individual pregnant women was collected representing each of the three trimesters of pregnancy and each of the six hospitals.

Outcome assessment

Since there was a laboratory technical problem with analyses of mercury and arsenic, the only heavy metals that were measured were urinary lead and cadmium. Also, urinary cotinine

analysis was performed to evaluate environmental tobacco smoke exposure which is a potential source of heavy metal exposure.

Ten mL of urine from each pool was aliquoted into 15-mL tubes before analysis. We prepared a laboratory blank and field blanks to account for background levels in the LEADER lab and hospital. Fifty-mL Milli-Q water was added to all 50-mL field blank tubes and a Milli-Q water-rinsed tube. Similar to the urine samples, we then aliquoted 10 mL into 15-mL tubes.

Urinary cotinine was measured in urine samples by liquid chromatography tandem mass spectrometry (LC-MS/MS). To perform the sample extraction, 200 uL of urine was spiked with 50 uL of labeled internal standard solution (prepared in acidified water at a concentration of 10 ug/mL) and enzymatically digested using 100 uL of β-glucuronidase enzyme solution [prepared in 0.5 M ammonium acetate (pH 5.1) at a concentration of 20,000 unit per mL] for more than 12 hours. After enzymatic digestion, a liquid-liquid extraction was performed using 2 mL of dichloromethane. The extract was evaporated to dryness and the dried residue was reconstituted using 100 uL of water prior to injection onto a Kinetex EVO C18 column (4.6 mm x 100 mm, 5 um particle size) for chromatographic separation and analysis using LC-MS/MS. The mobile phase was (A) 6.5 mM ammonium acetate (pH 5.1) and (B) acetonitrile. The injection volume was 2 uL. The target compound was monitored using multiple reaction monitoring (MRM) mode. One quantitative ion (m/z 177.1->98) and one confirmative ion (m/z 177.1->80.1) were monitored for the native compound while one quantitative ion (m/z 180.1->101.1) was monitored for the labeled analog. The concentration of the target compound was determined from the ratio of the native to labeled standard in the sample, by comparison to an 8-point calibration curve. The calibration curve was prepared using acidified water as a matrix, covering the quantification range of 0.25 ng to 1,000 ng. A factor of five

was used to calculate the final concentration in ng/mL sample. In each analytical run, one sample blank and two quality control materials were included. The quality control materials were prepared by spiking the cotinine standard into 200 uL of 1:1 diluted non-smoker urine to yield 20 ng (QCL) and 500 ng (QCH) samples, respectively. The blank sample was prepared using 1:1 diluted non-smoker urine in a similar manner as the QCs and unknown samples. The method was validated by successful participation in the German External Quality Assessment Scheme (G-EQUAS).

For heavy metals analysis, 1000 μL urine samples were spiked with 50 μL 4 ppm indium, mixed well and digested with 2% nitric acid then vortex mixed. The prepared samples were analyzed for lead and cadmium using inductively coupled plasma-mass spectrometry (ICP-MS). Calibration standards, field blanks, lab blank and quality control samples were analyzed in tandem with samples. Then, all data were integrated to calculate concentration by using linear regression of standard curves.

Statistical analysis

Point estimates of the geometric mean, median and percentiles were evaluated for of urinary cadmium, lead, and cotinine. A urine value below the lower limit of detection (LOD) was substituted with the imputed value of LOD value divided by square root of two (Hornung & Reed, 1990). Since the data was not normally-distributed, natural log transformation was performed. Comparing the urinary level of these heavy metals, mean of natural log and 95% confident intervals were illustrated by graphical comparison and using ANOVA to compare mean among geographic regions and trimester. Further analysis was conducted by using Tukey's Studentized Range Test to see which pairs would display significant differences.

Pearson's correlations between natural log of these heavy metals and cotinine were assessed to point out characteristics of a possible source of exposure.

III. Results

Basic demographic data

A total of 52 pools from 2,112 individual pregnant women were collected. The number and percentage of pools, as well as the individuals making up the pools by hospitals and trimesters, are described in **Table 1**. The largest proportion of the pools were from SUR (south: 23.08%), followed by MAH (northeast: 21.15%), SAR (central) and RAY (east) accounting for 15.38% from each hospital, SAM (Bangkok vicinity: 13.46%) and PIC hospitals (north:11.54%). More than 50% of the pools were from pregnant women in the third trimester (51.92%), followed by the second trimester (32.69%) and the first trimester (15.38%). The average age of pregnant women contributing to the pools was 27.08 ± 1.95 years.

Exposure, confounder and outcome summary

Since the distributions of urinary cadmium, lead, and cotinine were right-skewed, geometric means and geometric standard deviations of both metals and cotinine were calculated. Additionally, natural log-transformations of urinary cadmium, lead, and cotinine were used in the statistical comparison.

Cadmium

Of fifty-two pools, all urine samples had urinary cadmium greater than LOD (0.33 ng/mL). The calculated geometric mean (GM) and geometric standard deviation (GSD) of urinary cadmium was 0.63±1.14 ng/mL. The range of urinary cadmium concentration was between 0.02-2.93 ng/mL.

Descriptive statistics of urinary cadmium by hospitals is described in **Table 2**. Samples from PIC hospital revealed the highest urinary cadmium concentration (GM±GSD: 1.95 ± 1.14 ng/mL), followed by MAH (1.44±1.1 ng/mL), SUR (0.63± 1.14 ng/mL), SAR (0.54±1.06 ng/mL), SAM (0.42±1.16 ng/mL), and RAY (0.16 ±1.38 ng/mL).

The concentration of urinary cadmium by trimesters was displayed in **Table 5**. The level of urinary cadmium revealed a decreasing trend by trimesters. Pregnant women in the first trimester had the highest geometric urine cadmium concentration (0.84±1.26 ng/mL), followed by second trimester (0.62±1.33 ng/mL) and third trimester (0.60±1.17 ng/mL).

Lead

For urinary lead, the descriptive results are summarized in **Table 3**. Of fifty-two pools, fifty pools were available to analyze. Ninety-eight percent of the samples had urinary lead greater than LOD (0.27 ng/mL). Geometric mean and geometric standard deviation of urinary lead was 0.82±1.07 ng/mL. The range of urine lead concentration was between <0.27-1.78 ng/mL. Unlike urinary cadmium, the geometric mean of urinary lead showed the greatest in SAM (1.41±1.04 ng/mL), followed by SAR (1.07±1.14), RAY (0.82±1.28 ng/mL), SUR (0.77±1.12 ng/mL), MAH (0.61±1.14 ng/mL) and PIC hospitals (0.58±1.16 ng/mL).

Urinary lead by trimester is shown in **Table 6**. Pregnant women in the first trimester had the highest urinary lead level (1.06±1.11 ng/mL), followed by pregnant women in the second and third trimester (0.84±1.13 ng/mL and 0.75±1.1 ng/mL respectively).

Cotinine

We also explored urine cotinine level as a biomarker of tobacco smoke exposure, which is one of the crucial sources of heavy metals exposure. All samples had urine cotinine level higher

than LOD (1.25 ng/mL). The distribution of urinary cotinine was right-skewed with a bimodal peak **(Figure 4)**. Geometric mean and standard deviation of urinary cotinine were 24.95±1.27 ng/mL. The range varied between 1.53 – 386 ng/mL. The geometric mean and geometric standard deviation by the hospitals are shown in **Table 4**. The urinary cotinine revealed the highest in PIC (77.50±1.86 ng/mL), followed by SAM (61.34±1.84 ng/mL), SUR (28.26±1.70 ng/mL), RAY (1.06±1.11 ng/mL), SAR (16.02±1.78 ng/mL), and MAH hospital (9.12±1.66 ng/mL).

Moreover, the geometric mean and geometric standard deviation of urinary cotinine by trimesters of pregnancy are shown in **Table 7**. It can be seen that pregnant women in the second trimester had the highest urinary cotinine (47.54±1.52 ng/mL), followed by the first trimester (23.95±2.02 ng/mL) and the third trimester (16.82 ±1.36 ng/mL).

Primary outcome

ANOVA statistics were used for examining the difference of natural log-transformed urinary cadmium, lead and cotinine. Our results showed the evidence that natural log-transformed urinary cadmium levels were different among hospitals (p-value <0.0001). A graphical comparison is shown in **Figure 2**. Post hoc analysis was carried out to see which pairs of the hospitals revealed a statistical difference in **Table 8**. Of 15 pairs from the six hospitals, eleven pairs of hospitals had statistical difference among natural log-transformed urinary cadmium as following, MAH-RAY, MAH-SAM, MAH-SAR, MAH-SUR, PIC-RAY, PIC-SAM, PIC-SAR, PIC-SUR, RAY-SAM, RAY-SAR, RAY-SUR, and SAM-SUR hospitals. Similarly, it can be seen from **Figure 3** that natural log-transformed urinary lead revealed a statistical difference among hospitals (p-value 0.0014). Post hoc analysis showed the difference among SAM-MAH, SAM-PIC, SAM-SUR hospitals.

On the contrary, ANOVA test did not show the difference in an average of natural log-transformed urinary cotinine (p-value 0.11). A graphical comparison is shown in **Figure 5**.

Secondary outcome

Even though urinary cadmium and lead showed decreasing trends as trimesters increase, the average of natural log-transformed urinary cadmium and lead were not statistically different across trimesters (p-value 0.66 and 0.28 respectively). Graphical comparisons of log-transformed urinary cadmium, lead, and cotinine are shown in **Figure 6 to 8** respectively. However, the decreasing trend by trimesters was not detected in urine cotinine. Also, the urinary cotinine was not different across trimesters (p-value 0.15)

Correlation

To examine tobacco smoking as potential source of heavy metals, the correlations of those metals and cotinine were carried out. As we can see from **Table 10**, the results from Pearson correlation revealed that there was a positive linear relationship between natural log-transformed of urinary lead and cotinine with statistical significance (p-value <0.001, r=0.38). While the other pairs such as natural log-transformed urinary cotinine and cadmium (p-value 0.88, r=0.02) as well as natural log-transformed urinary cadmium and lead (p-value 0.69, r=-0.69) did not show a significantly linear relationship.

IV. Discussions

Feasibility

This study is the first study introducing pooled urine sample analysis to describe patterns of tobacco smoke and heavy metal exposure in Thailand. The results of this study showed that our pooling strategies could demonstrate the pattern of environmental exposure as well as identify a different level of cadmium and lead exposure by geographic regions. Additionally,

our pooling strategy is unique because we recruited participants based on routine healthcare service. Consequently, we were unable to control the number of pregnant women visiting the hospital across trimesters and hospitals.

The apparent advantages of the pooled sample approach are reducing cost and laboratory burden as well as avoiding an ethical issue associated with privacy of individual measurements. We tried to employ our protocol into routine healthcare service and found that the protocol is simple enough to provide the answer the research questions.

Outcome interpretation

With regard to the results, the differences of the natural log-transformed urinary cadmium and urinary lead were detected across the regions with statistical significance. Nonetheless, the natural log-transformed urinary cotinine was not different across the regions. Additionally, the natural log-transformed urinary cadmium, lead, and cotinine were not statistically significant across trimesters of pregnancy. However, the natural log-transformed urinary cadmium and lead showed decreasing trends as trimesters of pregnancy increase. Moreover, a positive linear correlation was detected between natural log-transformed urinary lead and urine cotinine with statistical significance.

Ca*dmium*

As we can see from **Table 11**, the measured urinary cadmium levels from the pools are comparable with unadjusted urinary cadmium. Only a few studies reported unadjusted urinary cadmium including studies from Australia, China, Spain and the United States (NHANES) reported unadjusted urine cadmium. A median urine cadmium concentrations from 157 Australian non-smoker pregnant women was 0.66 μg/L which is also slightly higher than our results (median 0.62 ng/mL) (Hinwood et al., 2013). On the other hand, our results are higher

than the studies in Wuhan, China, and Spain. The stratified results by trimesters of pregnancy were reported. The Chinese study revealed geometric means of urine cadmium across the first, second and third trimesters as 0.48, 0.35, and 0.35 μg/L respectively (Cheng et al., 2017). Additionally, the study of 486 Spanish pregnant women of the first and third trimester showed median urine cadmium as 0.55 and 0.53 μg/L respectively (Forns et al., 2014). Comparing with NHANES 2013-2014 data (Centers for Disease Control and Prevention, 2017), the geometric mean of the urine cadmium was higher than the 95th percentile. All hospitals except PIC and MAH, their urine cadmium levels were between 75th-90th percentiles.

From the literature review, most studies reported a creatinine-adjusted level; however, our study was not adjusted for creatinine. The median and geometric mean of urine cadmium in our study are slightly lower than Bangladesh (Kippler et al., 2012), Australia (Hinwood et al., 2013a) and Japan (Shirai, Suzuki, Yoshinaga, & Mizumoto, 2010). These results are consistent with the study conducted in five cities in Asia; Nanning, Tainan, Manila, and Kuala Lumpur. The results showed that urine cadmium levels of Bangkok women, as well as dietary cadmium levels from rice, are either the lowest or the next lowest among those five cities (Zhang et al., 1999). These comparisons may indicate that cadmium burden among pregnant women in Thailand is higher than the U.S., China, and Spain. However, this burden is quite low among pregnant women in Thailand, compared to pregnant women in Asia and Australia.

In Thailand, there have been a few studies measuring urine cadmium shown in **Table 12**. We notice that our results are in the same range. Additionally, the statistical significance of the difference of urinary cadmium by geographic region could result from heterogeneity pattern of exposure. For instance, PIC hospital (north region) and MAH hospital (northeast region) where the geometric means of urine cadmium were highest, results are similar to the study

conducted in cadmium-contaminated areas. PIC hospital is located close to Tak province, a known cadmium contamination area (Swaddiwudhipong et al., 2015). Also, some water samples from the Nan River, which is passed by the province, was detected on cadmium and other metals in 2013 (Bureau of Water Quality Management of Thailand, 2013). Interestingly, we detected the lowest urine cadmium levels in RAY hospital, which is located in the high-density industrial area. This detection might support the notion that the primary cadmium exposure among pregnant women is diet.

Since tobacco smoking is one of the crucial sources of cadmium exposure, urinary cotinine is taken into account. Our data suggest that this urine sampling approach can demonstrate similar shape distribution of urinary cotinine level to nature of exposure which is bimodal between smokers and non-smoker in the population (Kim, 2016). In comparison to the findings of Jung et al. (2012), the results of our study demonstrate a lower median urinary cotinine level for female non-smokers with environmental tobacco exposure in South Korean. Only the study at Bhuddachinarat Hospital in the north of Thailand measured the cotinine to creatinine ratio (CCR) among 242 pregnant women visited ANC clinic. The average CCR among pregnant women who were exposed to ETS was 6.8±9.8 ng/mg creatinine. Also the CCR tended to decrease with each subsequent trimester of pregnancy (Srituee, 2012), which was unlike urine cadmium and lead. Additionally, we could see the difference of urine cotinine across neither geographic regions nor trimesters. Possible explanations might be a lack of statistical power due to the low number of the pools, and homogeneity of a pattern of environmental tobacco exposure by each region.

However, we did not detect the correlation among urinary cadmium and urine cotinine in our study. This inconsistency may be due to a primary source of cadmium exposure among this

population is diet rather than tobacco. Zhang et al. (1999) also found that rice contributed to 30% of cadmium burden. Another study in Bangkok suggested that cadmium intake were associated with Urine cadmium more than 1 μg/gram creatinine in 22.5% of women who never smoked (Satarug, Swaddiwudhipong, Ruangyuttikarn, Nishijo, & Ruiz, 2013). Additionally, another study conducted in the northwestern region of Thailand detected cadmium in vegetables (Wachirawongsakorn, 2015).

Lead

The results of urine lead in our study appeared to be much lower than the Spanish study, which is the only study reporting unadjusted creatinine urine lead. According to the adjusted creatinine level, our results are in the same range with the Australian study, but higher than pregnant women in China and Japan (**Table 13**) Additionally, the geometric mean in our studies was between the 75thand 90th percentile of female non-smokers in the U.S. from NHANES survey 2013-2014.

Only a few studies have measured urinary lead as a biomarker in Thailand. A study in Bangkok which was conducted among non-occupationally exposed adult women showed a higher geometric mean compared to our study (2.60±1.53 μg/g creatinine)(Zhang et al., 1999).

We also found the statistical difference among geographic regions, particularly SAM hospitals where the urinary lead was the highest. The highest urinary lead levels are relevant to the report from R506/2, the National Surveillance for Occupational and Environmental Diseases. In 2015, SAM was the province which reported the highest number of patients with lead poisoning. RAY, which had the third highest urine lead, was also ranked the fourth province which reported heavy metal poisoning (Chantian T., 2016). Both SAM and RAY hospital are

in known high-priority occupational health areas since there are abundant of the high-density industrial parks.

Trimesters

Our study revealed a decreasing trend of natural log-transformed urinary cadmium and lead levels, but the trends did not show a significant difference across trimesters. These results are consistent with the results observed in studies from China and NHANES that showed the decreasing trend of urinary cadmium as trimester increases (Cheng et al., 2017; Jain, 2013).

To demonstrate the difference of heavy metals levels across trimesters, Fort et al. (2014) found that urine lead levels among the first trimester were not statistically different from the third trimester. In contrast, urinary cadmium levels among the first trimester were higher than the third trimester with statistical significance. They also mentioned in their discussion that their results might be explained by physiological changes during pregnancy, such as an increase in plasma volume as well as glomerular infiltration rate (Fort et al., 2014). Being pregnant also has an impact on faster nicotine metabolism compared with what is observed during the postpartum period or before pregnancy (Bowker, Lewis, Coleman, & Cooper, 2015; Taghavi, Arger, Heil, Higgins, & Tyndale, 2018). We expected to see a decreasing trend of urinary cotinine as trimester increases due to behavioral changes by pregnant women and their family member. On the other hand, the non-differential exposure, as well as low statistical power, might be two main issues with this problem.

The positive relationship between the natural-log transformation of urine lead and cotinine suggests that environmental tobacco exposure may be one of the potential sources of exposure among pregnant women. Our finding is in agreement with NHANES data 1999-2004. The study found a dose-response relationship between the different exposure level of second-hand

smokers and urine lead. Only being a second-hand smoker without assessing other sources of lead exposure still showed the dose-dependent relationship (Richter, Bishop, Wang, & Swahn, 2009).

Limitations

In our study, there are two issues of limitations including the limitations of pooled sample approach by itself and study design. Firstly, since there was no available individual sample, we did not analyze for creatinine and adjusted with the measured values. This limitation results in difficulty in comparison with other studies in which they adjusted the heavy metal values. The purposes of adjusting with creatinine are to correct the variation from urine dilution and to determining if a spot urine sample is valid for laboratory analysis or not. Individually, the creatinine-adjusted analytes should be compared with a reference range in the same demographic group (Barr, Wilder, et al., 2005). Even though we did not include creatinine in the analysis for the population group, Heffernan has noted that without creatinine or specific gravity data of pooled samples, the variation in individuals' hydration can be assumed to be averaged out and that it will not introduce significant bias to an estimation of average concentration (Heffernan et al., 2013). Additionally, since a measured level from each pool is equal to the arithmetic mean, it can make the data look more normally-distributed. Therefore, it might dilute the observed effect of association or make bias from pooling towards the null. Moreover, we could not trace back if there are any outliers. Furthermore, the measured level of each pool equals to the arithmetic mean of individual samples combining into pools. It is difficult to use this method to quantify any biomarker which has a bimodal distribution such as cotinine. The distribution is representative of both nonsmokers and smokers. For instance, the survey in 2007-2009 and 2010 of Korea National Health and Nutrition Examination Survey (KNHANES) showed the markedly different urinary cotinine range from 0.009-7,817

ng/mL (Jung et al., 2012). Therefore, it is more complicated to interpret since the measured values depend on the individual levels and prevalence of tobacco exposure in each group. We would suggest further study to restrict smokers group to avoid an interference from an extremely high value, but uncertainty will remain among second-hand smokers which are more likely to underreport their exposure.

Secondly, we had some constraints in study design related to representativeness, statistical issues, variation in the number of participants, and exposure assessment issues. In this study, we purposefully selected each hospital from the six different regions. These samples might not represent a whole country. Additionally, we have a limited number of the pools, which might not provide enough statistical power to detect the difference among those pools. Furthermore, the study design was cross-sectional. The samples from pregnant women from each trimester were not the same person. Therefore, the effects of inter-individual variation might occur. From this point, there was a variation in the number of samples across trimester especially fewer number in a first and second trimester. The variation might be explained by the lower rate of ANC among first trimester and increasing frequency of appointment by trimesters. Therefore, it might result in a lack of statistical power to detect the difference. For exposure assessment, we have limited demographic data such as smoking or occupational exposure to control this covariate. Environmental tobacco smoking is the covariate that we tried to identify by using urine cotinine to distinguish between smokers and non-smokers. Since the cotinine level among non-smokers and smokers vary from a single digit to a thousand ng/mL, the variation of measured level might be concealed after the samples were pooled. If the pool has a high percentage of smokers or has at least one smoker who has extremely high cotinine levels, we might see high urinary cotinine levels in that pool too. Since the samples were pools, we could only estimate the prevalence of exposure by pools. However, we considered a

balance between the number of exposure variables we wanted, the burden of hospital staff and ethical issue.

Although there are several limitations in our study, the pooled urine approach may be fruitful to provide some background data and encourage hospital staff as well as researchers to conduct further study to improve a practical and cost-efficient protocol of human biomonitoring.

Conclusions and Recommendations

Conclusions

Our study indicates that the pooled urine sample may be feasible to employ in large-scale human biomonitoring in a limited-resource setting. We showed evidence that the data from pooled sample provide the results that met the particular purposes including describing background exposure of heavy metals as well as identify susceptible population to inform policymaker. Also, the background level of urine cadmium and lead among pregnant women of Thailand did not show a high burden, compared to international studies. Therefore, the difference of urinary cadmium among hospitals implies the different types of exposure source by region. Even though declining trends of urinary cadmium and urinary lead across trimesters were detected, we could not see a statistical difference. To determine if environmental tobacco exposure is correlated with urinary lead and cadmium, the correlation between urinary cotinine and urinary lead was detected but not for urinary cadmium. This correlation indicates that environmental tobacco exposure might be a potential source of lead exposure whereas diet might be a primary source of cadmium exposure among pregnant women in Thailand.

Recommendations for future research

The results of this study primarily provide background data among pregnant women in Thailand as well as a brief overview of a pattern of lead, cadmium as well as cotinine. However, the low levels of these biomarkers do not mean the pregnant women are safe. We would suggest conducting further studies both epidemiological studies and methodology research. Since biomonitoring data by itself could not inform us about risk factors or health outcomes, further epidemiological studies will be required to identify risk factors including potential route and source of exposure as well as to explore the association with adverse pregnancy outcomes at individual levels. Moreover, we also need research focused on method development. For instance, varieties of pooling strategies such as pooling samples from specimen banking would be beneficial in allowing study design for newly emerging toxicants as well as a pooled-unpooled technique, which is an approach that individual specimens are randomly sampled and analyzed; then the analyzed samples will be pooled and reanalyzed. This approach may capture the strengths of statistical properties but it requires intensive resources (Amy L. Heffernan et al., 2014; Schisterman, Vexler, Mumford, & Perkins, 2010).

Policy recommendations

Recently, Thailand has not developed large-scale human biomonitoring programs due to financial constraint and laboratory burden. We demonstrated using pooled sample approach to provide background information among pregnant women and identify the susceptible population. Our data are fruitful for allowing stakeholders to have a better understanding of the pattern of exposure in the areas and to prioritize public health interventions. Also, the approach can be used as a tool to monitor and evaluate the effectiveness of the implementation of policies and interventions. In addition, generalizing the pooled sample approach into other populations is needed to consider if the sampling could represent the study population or not.

For instance, using samples from pathology laboratories might represent only sick people unless people visit ANC clinic and annual check-up clinic. The Thai Ministry of Public Health may employ this strategy in developing the national human biomonitoring program. This data will provide more information on the exposure regarding spatial and temporality. Furthermore, the Bureau of Occupational and Environmental Diseases and Bureau of Epidemiology, federal organizations which are responsible for public health surveillance, may integrate a cost-effective human biomonitoring program into national public health surveillance for support outbreak investigation as well as health monitoring in provinces which have environmental problems.

Even though heavy metal levels among pregnant women were not remarkably higher than other countries, there is no safe level for heavy metals such as lead. Pregnant women should not be exposed to these toxins. We would suggest that the Department of Health, which is responsible for maternal and child health promotion, include heavy metal and cotinine screening into first ANC visit. Health education on preventing themselves from heavy metal toxicants and tobacco exposure are encouraged to integrate with routine maternal care to prevent adverse pregnancy outcomes.

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Table and Figures

Figure 1 Location of the participating hospitals in the study

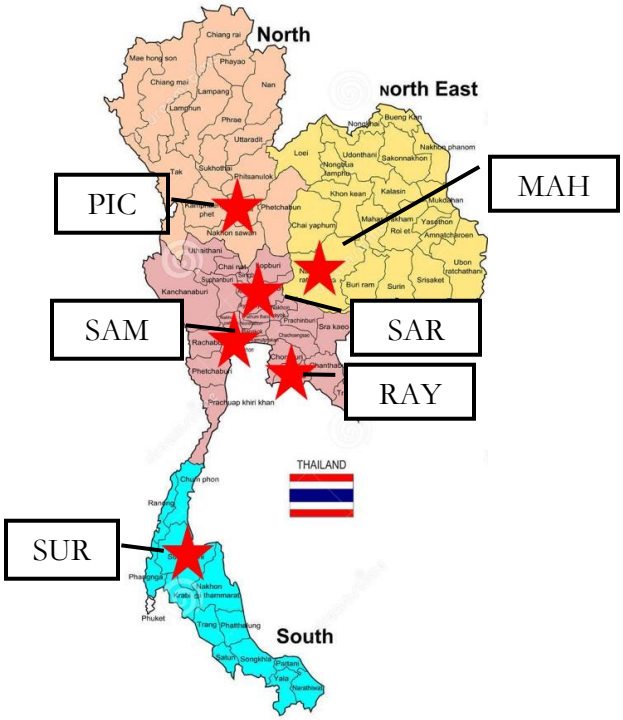


Table 1 Number and percentage of the pools and individual making up the pools

Characteristics	Number of individuals (n=2,112)	Number of pools (n=52)	Percent
Hospital (location)			
MAH (northeast)	427	11	21.15
PIC (north)	236	6	11.54
RAY (east)	301	8	15.38
SAM (Bangkok vicinity)	233	7	13.46
SAR (central)	343	8	15.38
SUR (south)	572	12	23.08
Trimester			
1	251	8	15.38
2	688	17	32.69
3	1173	27	51.92
Mean maternal age (Mean ± S.D.)		**27.08±1.95**	

Table 2 Arithmetic mean, standard deviation (SD), geometric mean, geometric standard deviation (GSD), median, 10th percentile, 90th percentile, minimum and maximum of urine cadmium by the hospitals

Hospital	Number of pools (n=52)	Arithmetic mean	SD	Geometric mean	GSD	10th percentile	Median	90th percentile	Min	Max
Total urine cadmium	**52**	**0.89**	**0.7**	**0.63**	**1.14**	**0.25**	**0.62**	**1.93**	**0.02**	**2.93**
MAH (northeast)	11	1.51	0.54	1.44	1.1	1.1	1.34	2.47	0.94	2.59
PIC (north)	6	2.03	0.61	1.95	1.14	1.09	2.06	2.93	1.09	2.93
RAY (east)	8	0.2	0.13	0.16	1.38	0.02	0.19	0.38	0.02	0.38
SAM (Bangkok vicinity)	7	0.45	0.19	0.42	1.16	0.26	0.38	0.85	0.26	0.86
SAR (central)	8	0.55	0.1	0.54	1.06	0.44	0.52	0.71	0.44	0.71
SUR (south)	12	0.69	0.29	0.63	1.14	0.41	0.62	1.11	0.25	1.17

Table 3 Arithmetic mean, standard deviation (SD), geometric mean, geometric standard deviation (GSD), median, 10th percentile, 90th percentile, minimum and maximum of urine lead by the hospitals

Hospital	Number of pools (n=50)	Arithmetic mean	SD	Geometric mean	GSD	10th percentile	Median	90th percentile	Min	Max
Total urine lead	**50**	**0.92**	**0.39**	**0.82**	**1.07**	**0.45**	**0.85**	**1.49**	**<0.27**	**1.78**
MAH (northeast)	11	0.66	0.22	0.61	1.14	0.43	0.74	0.88	0.21	0.94
PIC (north)	5	0.61	0.21	0.58	1.16	0.38	0.54	0.87	0.38	0.87
RAY (east)	7	0.94	0.39	0.82	1.28	<0.27	1.02	1.35	<0.27	1.35
SAM (Bangkok vicinity)	7	1.42	0.19	1.41	1.04	1.25	1.33	1.79	1.25	1.79
SAR (central)	8	1.13	0.4	1.07	1.14	0.62	1.13	1.64	0.62	1.64
SUR (south)	12	0.83	0.32	0.77	1.12	0.47	0.78	1.29	0.40	1.51

Table 4 Arithmetic mean, standard deviation (SD), geometric mean, geometric standard deviation (GSD), median, 10th percentile and 90th percentile, minimum and maximum of urine cotinine by the hospitals

Hospital	Number of pools (n=52)	Arithmetic mean	SD	Geometric mean	GSD	10th percentile	Median	90th percentile	Min	Max
Total urine cotinine	**52**	**76.92**	**96.42**	**24.95**	**1.27**	**2.54**	**27.66**	**221.38**	**1.53**	**386.00**
MAH	11	47.04	113.80	9.12	1.66	2.41	4.14	49.30	1.75	386.00
PIC	6	139.66	111.63	77.50	1.86	5.16	146.43	280.71	5.16	280.71
RAY	8	57.26	70.17	25.00	1.68	4.11	22.97	177.87	4.11	177.87
SAM	7	126.46	107.52	61.34	1.84	5.97	169.42	263.13	5.97	263.13
SAR	8	40.99	52.92	16.02	1.78	2.28	25.39	150.99	2.28	150.99
SUR	12	81.09	92.76	28.26	1.70	1.76	49.05	192.89	1.53	284.71

Table 5 Arithmetic mean, standard deviation (SD), geometric mean, geometric standard deviation (GSD), median, 10th percentile, 90th percentile, minimum and maximum of urine cadmium by trimesters

Trimester	Number of pools (n=52)	Arithmetic mean	SD	Geometric mean	GSD	10th percentile	Median	90th percentile	Min	Max
1	8	1.01	0.67	0.84	1.26	0.36	0.83	2.25	0.36	2.25
2	17	0.94	0.72	0.62	1.33	0.12	0.67	2.21	0.02	2.47
3	27	0.82	0.71	0.6	1.17	0.24	0.53	1.78	0.11	2.93

Table 6 Arithmetic mean, standard deviation (SD), geometric mean, geometric standard deviation (GSD), median, 10th percentile, 90th percentile, minimum and maximum of urine lead by trimesters

Trimester	Number of pools (n=50)	Arithmetic mean	SD	Geometric mean	GSD	10th percentile	Median	90th percentile	Min	Max
1	7	1.09	0.29	1.06	1.11	0.76	1.24	1.51	0.76	1.51
2	17	0.94	0.41	0.84	1.13	0.47	0.82	1.65	0.20	1.79
3	26	0.85	0.39	0.75	1.1	0.4	0.81	1.43	0.21	1.58

Table 7 Arithmetic mean, standard deviation (SD), geometric mean, geometric standard deviation (GSD), median, 10th percentile, 90th percentile, minimum and maximum of urine cotinine by trimesters

Trimester	Number of pools (n=52)	Arithmetic mean	SD	Geometric mean	GSD	10th percentile	Median	90th percentile	Min	Max
1	8	74.08	83.41	23.95	2.02	50.99	1.53	221.38	1.53	221.38
2	17	115.27	112.53	47.54	1.52	98.48	4.11	280.71	1.76	386.00
3	27	53.61	83.92	16.82	1.36	12.29	2.54	229.23	1.75	284.71

Figure 2 Comparison among natural log of urine cadmium by the hospitals

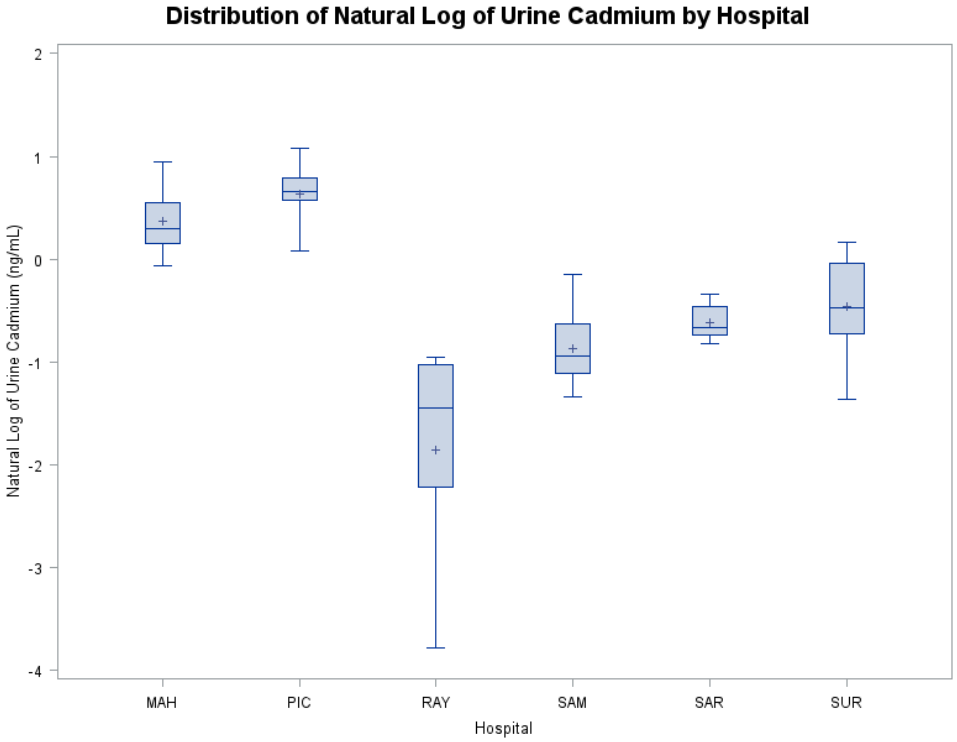


Table 8 Comparison the difference of mean natural log of urine cadmium by the hospitals

Hospital	MAH	PIC	RAY	SAM	SAR	SUR
MAH	0	0.3	2.23*	1.23*	0.98*	0.82*
PIC		0	2.53*	1.53*	1.28*	1.12*
RAY			0	0.99*	1.25*	1.41*
SAM				0	0.26*	0.41
SAR					0	0.16
SUR						0

*** Comparisons significant at the alpha=0.05 by Tukey's Studentized Ranged Test**

Figure 3 Comparison among natural log of urine lead by the hospitals

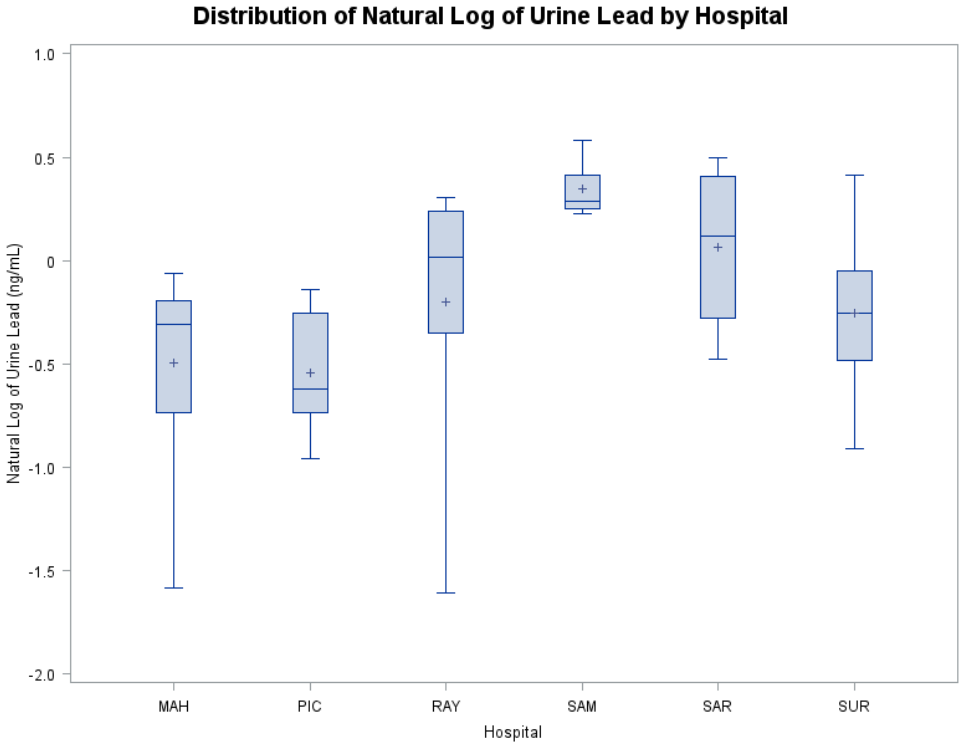


Table 9 Pairwise comparison of different of mean natural log of urine lead by the hospitals

Hospital	MAH	PIC	RAY	SAM	SAR	SUR
MAH	0	0.05	0.3	0.84*	0.56	0.24
PIC		0	0.34	0.89*	0.61	0.29
RAY			0	0.54	0.26	0.06
SAM				0	0.28	0.60*
SAR					0	0.31
SUR						0

***Comparisons significant at the alpha=0.05 by Tukey's Studentized Ranged Test**

Figure 4 Distribution of urine cotinine

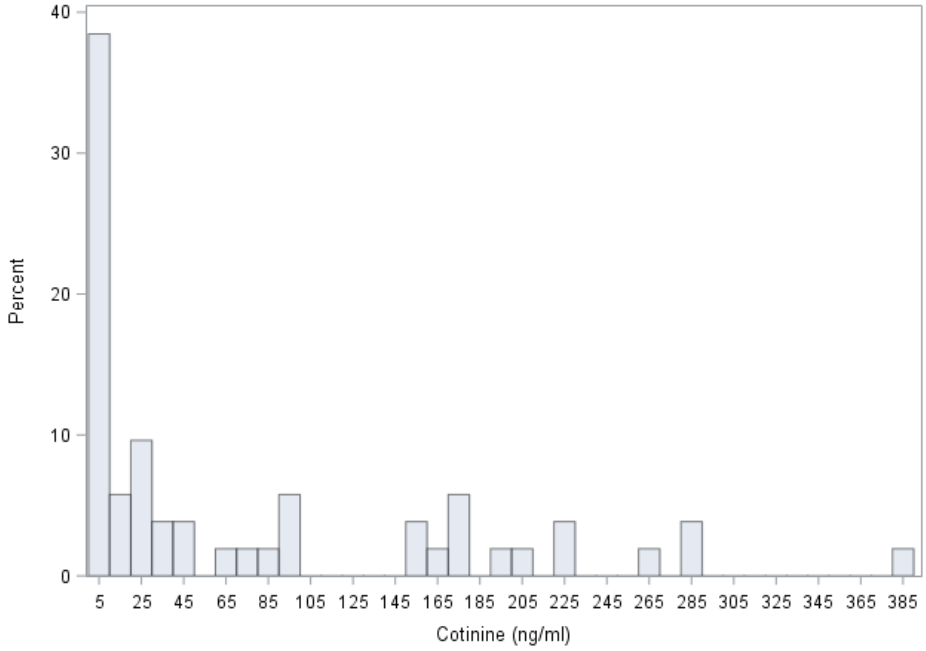


Figure 5 Comparison among natural log of urine cotinine by the hospitals

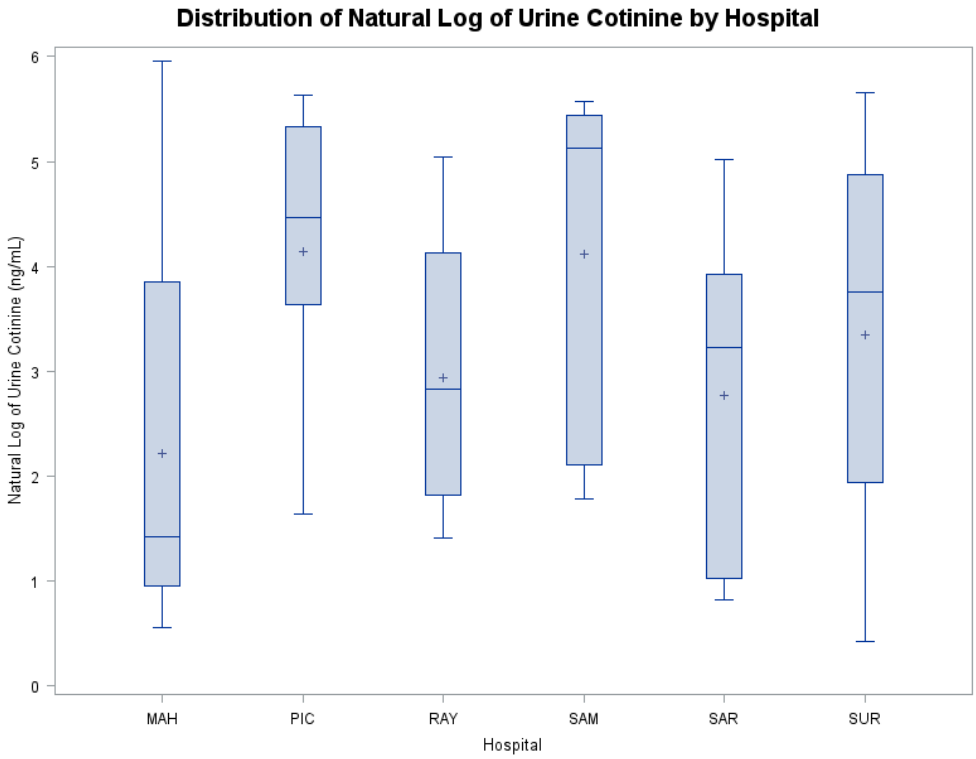


Figure 6 Comparison among natural log of urine cadmium by trimester

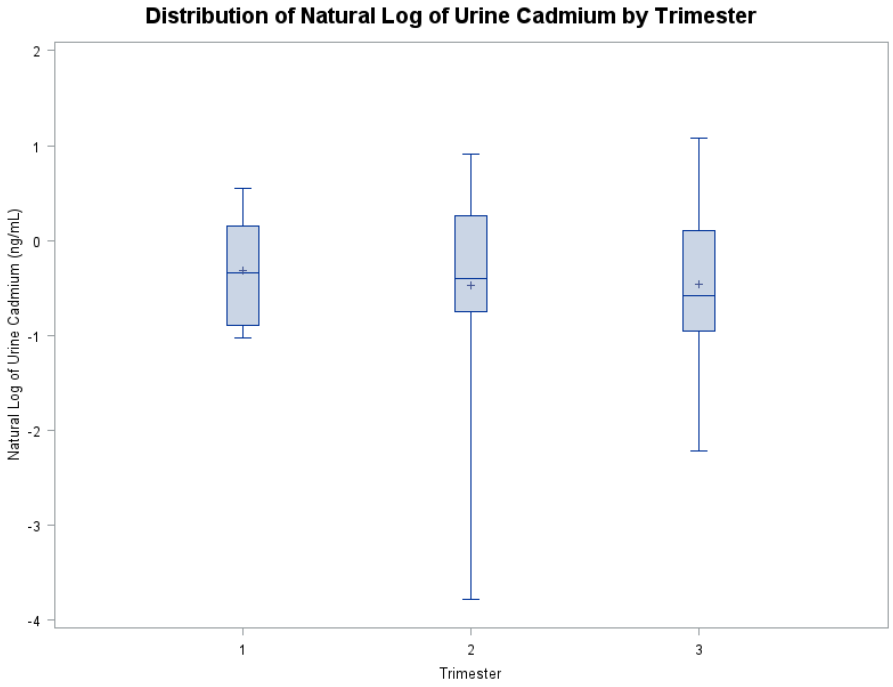


Figure 7 Comparison among natural log of urine lead by trimester

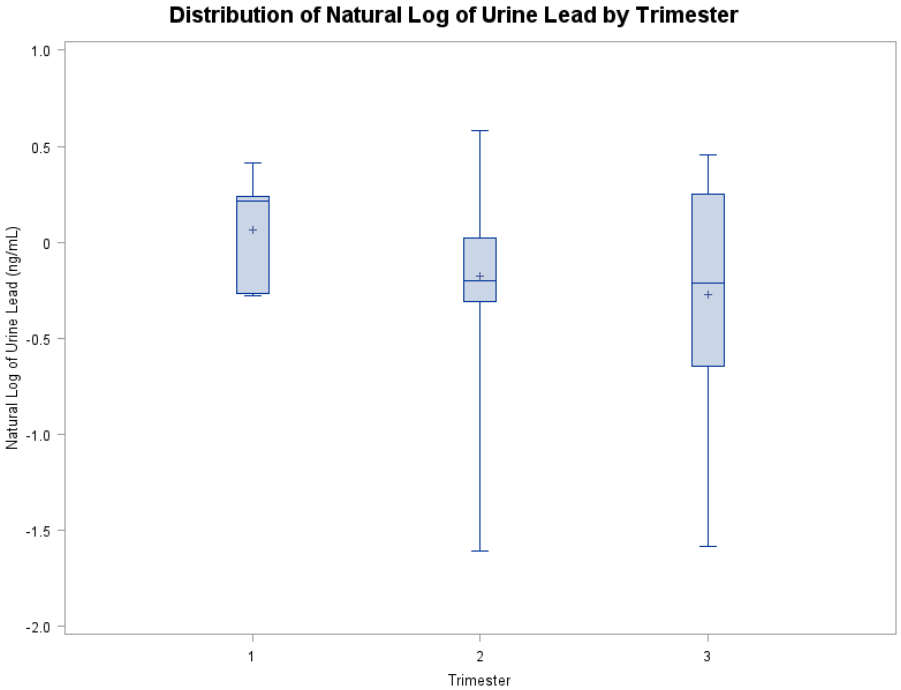


Figure 8 Comparison among natural log of urine cotinine by trimester

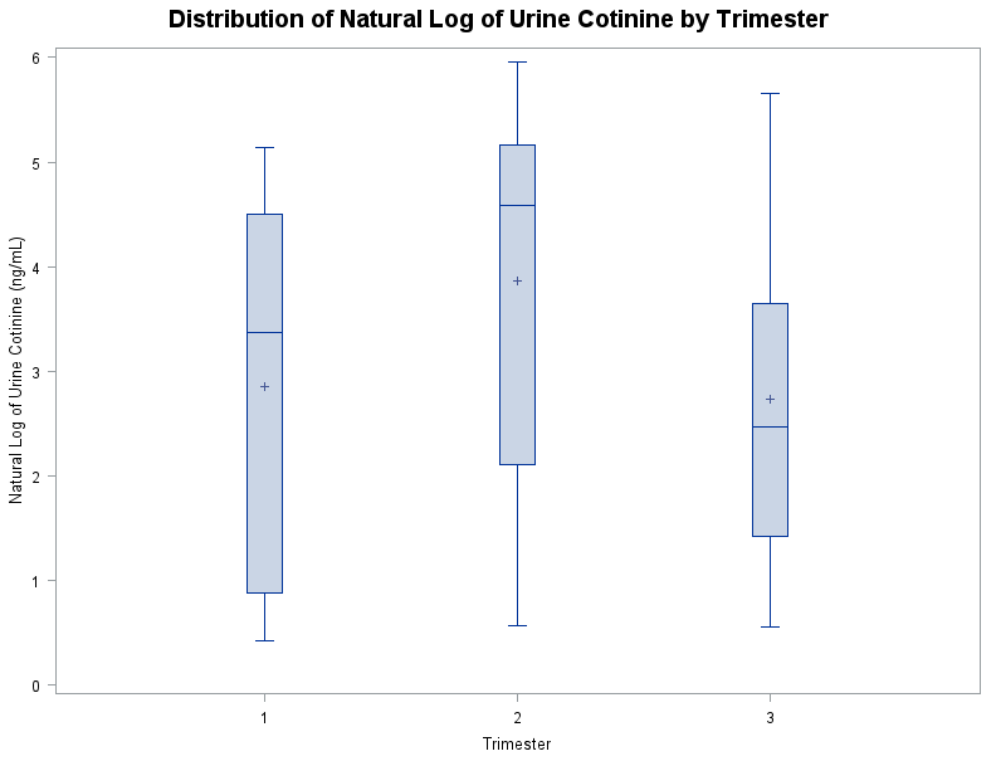


Table 10 Correlation among natural log of urine cadmium, lead and cotinine

Trace	Ln urine cotinine	Ln urine cadmium	Ln urine lead
Ln urine cotinine	1	0.02	0.33*
p-value		*0.88*	*0.02**
Ln urine cadmium		1	-0.06
p-value			*0.69*
Ln urine lead			1

***Statistical significance at level=0.05**

Table 11 Comparative studies of urine cadmium among pregnant women

Area	Study population	N	Measure of tendency	Value	Unit	Citation
Wuhan, China	Non-smoking pregnant women		GM	1.40±1.51	μg/g-Cr	Cheng, L., Zhang, B., Zheng, T., Hu, J., Zhou, A., Bassig, B. A., . . . Li, Y. (2017). Critical Windows of Prenatal Exposure to Cadmium and Size at Birth. Int J Environ Res Public Health, 14(1). doi:10.3390/ijerph14010058
	Trimester1	279	GM (AM)	0.48 (0.72)	μg/L	
	Trimester2	246	GM (AM)	0.35(0.50)	μg/L	
	Trimester3	276	GM (AM)	0.35 (0.46)	μg/L	
Spain	Non-smoking pregnant women	485				Forns, J., Fort, M., Casas, M., Caceres, A., Guxens, M., Gascon, M., . . . Sunyer, J. (2014). Exposure to metals during pregnancy and neuropsychological development at the age of 4 years. Neurotoxicology, 40, 16-22. doi:10.1016/j.neuro.2013.10.006
	1st trimester		Median	0.55	ng/mL	
	3rd trimester		Median	0.53	ng/mL	
Australia	Non-smoking pregnant women	157	Mean (Median)	0.74 (0.66)	μg/L	Hinwood, A. L., Callan, A. C., Ramalingam, M., Boyce, M., Heyworth, J., McCafferty, P., & Odland, J. O. (2013). Cadmium, lead and mercury exposure in non smoking pregnant women. Environ Res, 126, 118-124. doi:10.1016/j.envres.2013.07.005

GM: geometric mean AM: Arithmetic mean

Table 12 Comparative studies of urine cadmium in Thailand

Area	Study population	N	Measure of tendency	Value	Unit	Citation
Bangkok	Non-smoking Adult Thai women with non-occupational exposure	52	GM	1.40±1.51	μg/g	Zhang, Z. W., Shimbo, S., Watanabe, T., Srianujata, S., Banjong, O., Chitchumroonchokchai, C., . . . Ikeda, M. (1999). Non-occupational lead and cadmium exposure of adult women in Bangkok, Thailand. Sci Total Environ, 226(1), 65-74.
Ubon Ratchathani (northeastern)	General adult (non-exposure to groundwater drinking)	42	AM	2.38±4	μg/L	Wongsasuluk, P., Chotpantarat, S., Siriwong, W., & Robson, M. (2018). Using hair and fingernails in binary logistic regression for bio-monitoring of heavy metals/metalloid in groundwater in intensively agricultural areas, Thailand. Environmental Research, 162, 106-118. doi:https://doi.org/10.1016/j.envres.2017.11.024
	General adult (exposure to groundwater drinking)	58	AM	3.71±4	μg/L	
Northwestern	General adult (non-contaminated area)	279	Median (IQR)	0.476 (0.254-0.821)	μg/g	La-Up, A., Wiwatanadate, P., Uthaikhup, S., & Pruenglampoo, S. (2018). Association between urinary cadmium and chronic musculoskeletal pain in residents of cadmium-contaminated area in Northwest Thailand. Environ Sci Pollut Res Int. doi:10.1007/s11356-018-1665-3
	General adult (contaminated area)	280	Median (IQR)	1.457 (0.667-2.529)	μg/g	

Table 12 Comparative studies of urine cadmium in Thailand (Continue)

Area	Study population	N	Measure of tendency	Value	Unit	Citation
Pathum Thani (central)	General healthy adult 18-57 years old	50	AM	0.23±0.35	μg/g	Apinan, R., Satarug, S., Ruengweerayut, R., Tassaneeyakul, W., & Na-Bangchang, K. (2009). Cadmium exposure in Thai populations from central, northern and northeastern Thailand and the effects of food consumption on cadmium levels. Southeast Asian J Trop Med Public Health, 40(1), 177-186.
Khon Kaen (notheastern)		43	AM	0.51±0.76	μg/g	
Tak (Northwestern)		89	AM	0.63±1.41	μg/g	

GM: geometric mean AM: Arithmetic mean

Table 13 Comparative studies of urine cadmium and urine lead among pregnant women

Area	Study population	N	Measure of tendency	Value	Unit	Citation
Spain	Non-smoker	485				Forns, J., Fort, M., Casas, M., Caceres, A., Guxens, M., Gascon, M., . . . Sunyer, J. (2014). Exposure to metals during pregnancy and neuropsychological development at the age of 4 years. Neurotoxicology, 40, 16-22. doi:10.1016/j.neuro.2013.10.006
	1st trimester		Median	3.44	ng/mL	
	3rd trimester		Median	3.66	ng/mL	
Australia	Non-smoker	157	Mean (Median)	0.87 (0.7)	μg/g	Hinwood, A. L., Callan, A. C., Ramalingam, M., Boyce, M., Heyworth, J., McCafferty, P., & Odland, J. O. (2013). Cadmium, lead and mercury exposure in non smoking pregnant women. Environ Res, 126, 118-124. doi:10.1016/j.envres.2013.07.005
Jiang Su, China	Third trimester	205	GM (95%CI)	0.48 (0.38-0.60)	μg/g	Sun, H., Chen, W., Wang, D., Jin, Y., Chen, X., & Xu, Y. (2014). The effects of prenatal exposure to low-level cadmium, lead and selenium on birth outcomes. Chemosphere, 108, 33-39. doi:10.1016/j.chemosphere.2014.02.080
Japan	GA 9-40 wk	78	GM (GSD)	0.483 (4.00)	μg/g	Shirai, S., Suzuki, Y., Yoshinaga, J., & Mizumoto, Y. (2010). Maternal exposure to low-level heavy metals during pregnancy and birth size. Journal of Environmental Science and Health, Part A, 45(11), 1468-1474. doi:10.1080/10934529.2010.500942

GM: geometric mean AM: Arithmetic mean

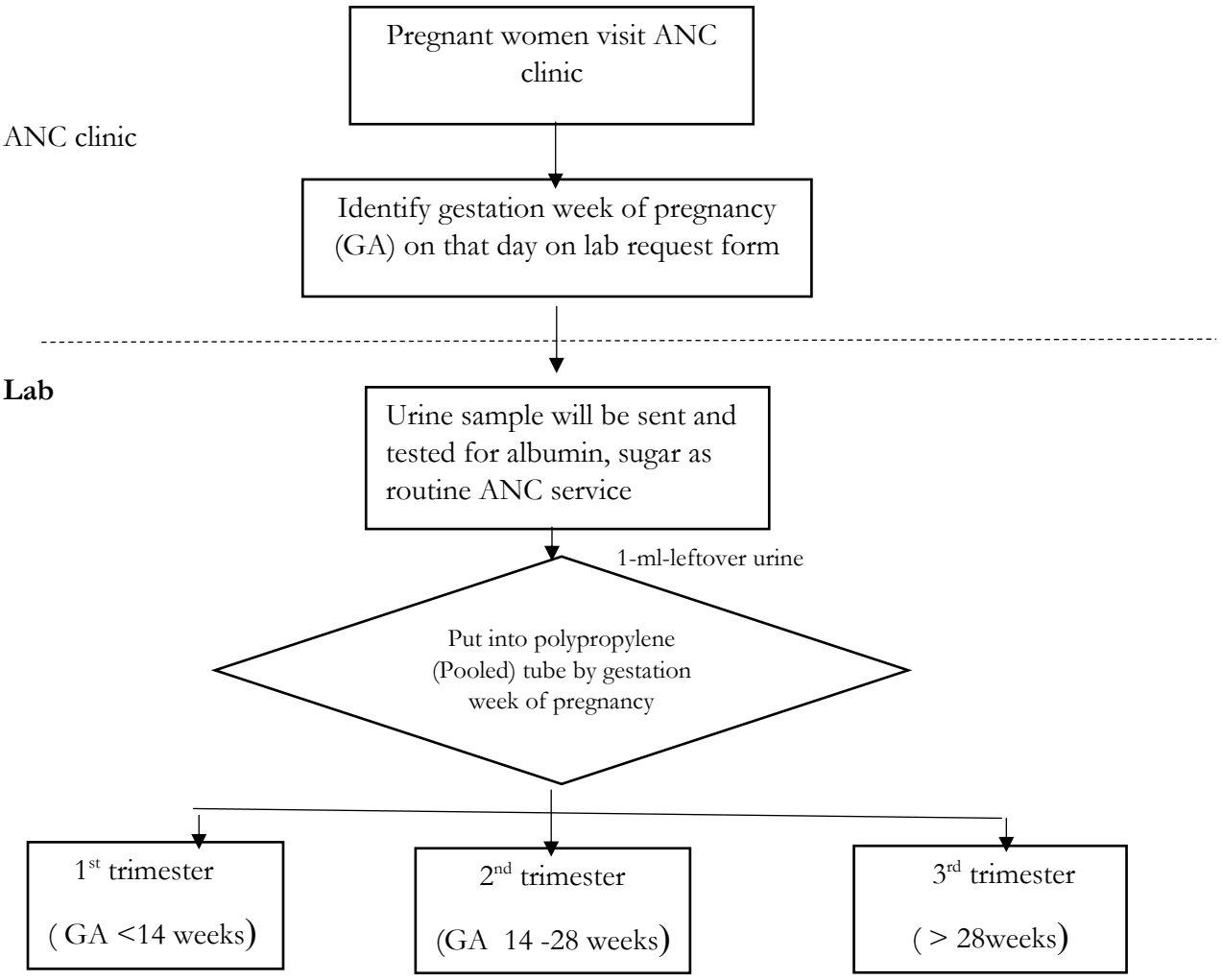
Table 14 Comparative studies of urine lead in Thailand

Area	Study population	N	Measure of tendency	Value	Unit	Citation
Bangkok	Non-smoking Adult Thai women with non-occupational exposure	52	GM	2.60±1.53	μg/g	Zhang, Z. W., Shimbo, S., Watanabe, T., Srianujata, S., Banjong, O., Chitchumroonchokchai, C., . . . Ikeda, M. (1999). Non-occupational lead and cadmium exposure of adult women in Bangkok, Thailand. Sci Total Environ, 226(1), 65-74.
Ubon Ratchathani (northeastern)	General adult (non-exposure to groundwater drinking)	42	AM	19.87±50	μg/L	Wongsasuluk, P., Chotpantarat, S., Siriwong, W., & Robson, M. (2018). Using hair and fingernails in binary logistic regression for bio-monitoring of heavy metals/metalloid in groundwater in intensively agricultural areas, Thailand. Environmental Research, 162, 106-118. doi:https://doi.org/10.1016/j.envres.2017.11.024
	General adult (exposure to groundwater drinking)	58	AM	21.14±50	μg/L	

GM: geometric mean AM: Arithmetic mean

Appendices

Flow of specimen collection



*P1 : Urine sample n =1-50, P2 : n= 51-100, n3 :n= 101-150, P4 : P4 :n= 151-200, P5 : n= 201-250

- Record gestational age of pregnancy, maternal age and specimen collecting date in specimen collection form

Specimen collection form

Hospital name ……..… Province ……
Trimester ……. (1) GA < 14 weeks …….. (2) GA 14-28 weeks
…….... (3) GA > 28 weeks …….. (0) Cannot identify
Total number of specimen ……
Specimen collection date …../……./……. - ……/……/……

No. of sample	**Pool**	**Specimen collection date**	**GA (week)**	**Maternal age (years)**
1	1			
2	1			
3	1			
4	1			
5	1			
6	1			
7	1			
8	1			
9	1			
10	1			
11	1			
12	1			
13	1			
14	1			
15	1			
16	1			
17	1			
18	1			
19	1			
20	1			
21	1			
22	1			
23	1			

No. of sample	Pool	Specimen collection date	GA (week)	Maternal age (years)
24	1			
25	1			
26	1			
27	1			
28	1			
29	1			
30	1			
31	1			
32	1			
33	1			
34	1			
35	1			
36	1			
37	1			
38	1			
39	1			
40	1			
41	1			
42	1			
43	1			
44	1			
45	1			
46	1			
47	1			
48	1			
49	1			
50	1			

Summary of specimen collection form

Hospital name …… Province……

Specimen collection date …../……./……. - ……/……/……

Trimester	Pool	total n /pool	Code
1	1		
1	2		
1	3		
1	4		
1	5		
2	1		
2	2		
2	3		
2	4		
2	5		
3	1		
3	2		
3	3		
3	4		
3	5		

Lab coordinator
name……Tel……

EMORY UNIVERSITY

Institutional Review Board

May 26, 2017

Thanawadee Chantian, MD
Rollins School of Public Health

RE: Determination: No IRB Review Required
Title: ***Exposures to Emerging Environmental Toxicants among Pregnant Women of Thailand; Evidence from Analysis of Pooled Urine Samples***
PI:

Dear Dr. Chantian:

Thank you for requesting a determination from our office about the above-referenced project. Based on our review of the materials you provided, we have determined that it does not require IRB review because it does not meet the definition of "research" with human subjects or "clinical investigation" as set forth in Emory policies and procedures and federal rules, if applicable. Specifically, in this public health practice project, you will collect and analyze pooled urine samples from pregnant Thai women that are left over from clinical care to determine environmental toxicant exposure. You will report results to the Department of Disease Control, Thailand.

Please note that this determination does not mean that you cannot publish the results. This determination could be affected by substantive changes in the study design. If the project changes in any substantive way, please contact our office for clarification.

Thank you for consulting the IRB.

Sincerely,

Jessica Baker, CIP
Education and QA Research Protocol Analyst