METAL MIXTURES IN URBAN AND RURAL POPULATION IN THE UNITED STATES: EVIDENCE FROM THE MULTI-ETHNIC STUDY OF ATHEROSCLEROSIS AND THE STRONG HEART STUDY

by

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ABSTRACT

Introduction: Natural and anthropogenic sources of metal exposure are different for residents of urban and rural areas. The objectives of this study were to evaluate metal-mixtures and allocate potential environmental sources of different urinary metals, and to compare metal-mixtures in two population-based studies in the United States: the Multi-Ethnic Study of Atherosclerosis (MESA) conducted in 6 urban areas, and the Strong Heart Study (SHS) conducted in 3 rural areas.

Methods: We studied 308 White, Chinese, Black and Hispanic adult participants in MESA (2000-2002 examination), and 105 American Indian adult participants in SHS (1998-2003 examination). Participants were selected at random and stratified by site for metal measurements. Nine metals (As, Cd, Mo, Pb, Sb, Se, U, W and Zn) were measured in spot urine specimens by inductively coupled plasma-mass spectrometry. For arsenic, we used the sum of inorganic and methylated species (∑As). We used principal component analysis (PCA), cluster analysis (CA), and linear discriminant analysis (LDA) to evaluate metal-mixtures. We accounted for urine dilution by standardizing metal concentrations by specific gravity.

Results: Levels of ∑As, Cd, U, W and Zn were higher in SHS participants as compared with MESA participants. PCA and CA revealed consistent patterns in SHS, suggesting 4 distinct principal components (PC) or clusters (∑As-U-W, Mo-Se, Pb-Sb, Cd-Zn). In MESA, CA showed 2 large clusters (∑As-Mo-Sb-U-W, Cd-Pb-Se-Zn), while PCA showed 4 components (Sb-Se-Zn, Pb-U-W, Cd-Mo, ∑As-Pb). After adjusting for rice intake in MESA, PCA and CA showed more similar findings. LDA indicated that ∑As was the most discriminant variable distinguishing MESA and SHS participants.

Conclusions: The ∑As-U-W urinary cluster and PC in SHS might reflect groundwater contamination in rural areas. The Cd-Zn cluster and PC in SHS could reflect common sources from processed meat and interactions in metabolic pathways for those metals. Among our 9 metals, ∑As had the highest discriminant ability to distinguish participants from MESA and SHS, reflecting disproportionate inorganic arsenic exposure in rural tribal communities compared to urban communities around the US.

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BACKGROUND

Exposure to metals is widespread in the environment. Experimental and epidemiologic evidence support that low-to-moderate chronic exposure to certain toxic metals play a role in the development of cardiovascular disease, kidney disease, neurocognitive outcomes and some cancers.[1-14] Biomarkers, including urine, are commonly used to assess metal exposure and internal dose as they integrate multiple exposure sources including air, water and food. [15,16] Metals in urine might be related with each other due to common environmental sources, similarities in metabolism, and excretion through urine. Multivariate analysis, including principal component analysis (PCA) and cluster analysis (CA), is a useful technique for identifying common patterns in data distribution. These methods can reduce the initial dimension of the variables, facilitate their interpretation, and thus can be used to identify common sources and/or metabolic pathways for urinary metals.[17-19]

Few studies have evaluated common sources of metal exposures in general populations, as most studies on metal mixtures have focused on determining common sources in either occupational populations or populations living in contaminated areas, and evaluated metals in plasma or scalp hair.[20-24] Urban or rural residency might be an important source of variation for metal exposures as natural and anthropogenic sources could be different. It is often assumed that urban areas are more contaminated than rural areas due to the high number of potential sources.[25-29] Groundwater sources contaminated with naturally occurring metals, on the other hand, are more commonly used for drinking water in rural areas.[30,31] Identifying common patterns of urinary metals in general

populations might help to evaluate metal exposure levels and could be easily applied to biomonitoring studies in general populations.

Our study population for this research was drawn from two separate populations, American Indian participants in the Strong Heart Study (SHS) in rural areas of Arizona, Oklahoma, and North/South Dakota, and White, Black, Hispanic and Chinese-American participants in the Multi-Ethnic Study of Atherosclerosis (MESA) in the urban setting of Winston-Salem, NC; New York, NY; Baltimore, MD; St. Paul, MN; Chicago, IL and Los Angeles, CA. Both studies are funded by the National Health Lung and Blood Institute and their main goals are to evaluate cardiovascular disease and its risk factors in populations from the United States.

The objective of this study was to characterize metal mixtures in urine and allocate potential environmental sources and/or metabolic pathways of different urinary metals in MESA and SHS. In addition to PCA and CA, we used linear discriminant analysis (LDA) to differentiate participants from both studies based on urinary metals, as well by other grouping characteristics (US regions, race/ethnicity and smoking status). To evaluate the consistency of the metal patterns across different communities, we compared the principal component (PC) score levels in each study area. We specifically hypothesized that arsenic, uranium and tungsten would cluster together due to common exposure from contaminated groundwater in the Southwestern and Midwest States. Understanding patterns of metal-mixtures in US communities can contribute to identify sources of metal exposures and to guide future assessment of the health implications of metal-mixtures.

METHODS

Study Population

The Multi-Ethnic Study of Atherosclerosis (MESA) is a population-based cohort study evaluating cardiovascular disease and its risk factors in participants aged 45 to 84 years who were free of cardiovascular disease at baseline (2000-2002) in 6 urban communities in the United States.[32] A pilot study recently measured baseline urinary metal concentrations in 310 participants randomly selected within each of the 6 study sites (90 White, 75 Black, 75 Hispanic and 70 Chinese participants), providing the opportunity to investigate geographic variation in metal exposure, as measured in urine. For this study, we excluded 2 participants with abnormal levels of tungsten in urine (37.5 and 230.0 times higher than the 90th percentile), leaving a total of 308 participants for this analysis.

The Strong Heart Study (SHS) is a longitudinal study of cardiovascular disease and its risk factors in 13 American-Indian communities from Arizona, Oklahoma, and North Dakota and South Dakota that started in 1989-1991.[33] In 1998-2003, family members from the original SHS participants were recruited and included a total of 110 extended families (Arizona, 35; Oklahoma, 36; and North Dakota and South Dakota, 39) totaling 3,665 participants from all three centers ranging in age from 14 to 93 years. Urinary metals were measured in 2,456 participants as part of an ancillary study to evaluate gene-environment interactions for diabetes and the metabolic syndrome. Among them, we randomly sampled one individual from each family within the same age range as MESA participants, resulting in a total of 105 participants for this analysis.

Urinary Metals

Urinary antimony (Sb), arsenic (As), cadmium (Cd), lead (Pb), molybdenum (Mo), selenium (Se), tungsten (W), uranium (U) and zinc (Zn) were measured in urine of MESA and SHS participants using inductively coupled plasma mass spectrometry (ICPMS) at the Trace Element Laboratory of the Karl-Franzens University, Graz, Austria following the same protocol.[34] The limits of detection (LOD) were 0.1 μg/L for As, 0.015 μg/L for Cd, 0.10 μg/L for Mo, 0.08 μg/L for Pb, 0.006 μg/L for Sb, 2 μg/L for Se, 0.008 μg/L for U, 0.005 μg/L for W and 10 μg/L for Zn. The percentages of participants with concentrations below the LOD are summarized in Table S1 and Table S2. For those samples below the LOD, we replaced their values by the LOD divided by the square root of two. For arsenic, we used the sum of inorganic and methylated species (∑As). In MESA, we accounted for arsenobetaine to remove the impact of relatively high levels of organic arsenic species from seafood.[35,36] In SHS, seafood intake is rare and there was no need to account for organic arsenic species in seafood.[37] For all metals, we accounted for urine dilution by standardizing their concentrations by specific gravity.

Other Variables

The interviews, physical examinations and collection of biospecimens were conducted in MESA and SHS by trained and certified staff using similar procedures. Sociodemographic (age, sex, race/ethnicity) and lifestyle (smoking status) information was collected using standardized questionnaires. Body mass index was calculated from measured weight (kg) divided by measured height (m^2).[32,33] Estimated glomerular filtration rate (eGFR) was calculated from recalibrated creatinine, age and sex using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.[38,39]

Statistical Analysis

Descriptive analyses were conducted for each study separately (MESA and SHS). Essential metals (Mo, Se, and Zn) were analyzed untransformed. Toxic metals (∑As, Cd, Pb, Sb, U, and W) were right skewed and log-transformed for all analyses. Pearson's correlation coefficient was calculated to examine the bivariate correlations between each pair of metals.

PCA is widely used to reduce data dimension and to extract a small number of latent factors (principal components, PCs) for analyzing relationships among the observed variables.[40] By describing uncorrelated linear combinations of the variables that account for most of the variance, PCA can reduce data complexity with minimum loss of original information.[40,41] Hierarchical cluster analysis, used in this study, can identify relatively homogeneous groups of variables using an algorithm that starts with each variable in a separate cluster and combines variables into agglomerative clusters until only one cluster is left.[42,43]

To assess commonalities of metal exposure in MESA and SHS, we used PCA and CA in each study. Within study, we compared the findings of PCA and CA to assess common patterns between the 2 methods. The concentrations of the 9 metals evaluated varied by different orders of magnitude. We therefore normalized each variable to unit variance and zero mean before conducting PCA and CA. To make the results more easily interpretable, the PCA with varimax normalized rotation was applied, which can maximize the variances of the factor loadings across variables for each factor.[44] We retained all principal factors with eigenvalues ≥1.0, as suggested by the Kaiser criterion.[45] For CA, dendrograms were constructed to assess the cohesiveness of the clusters formed,

in which correlations among elements can readily be seen. We then compared differences in metal-mixtures across study communities in MESA and SHS. For each community, PC scores were generated for each of the retained components in MESA and SHS, respectively.

LDA identifies the variables that better discriminate two groups by seeking the linear combination of the discriminating variables that provides maximal separation between the groups compared. Maximal separation of groups is determined from an eigen analysis of $W^{-1}B$, where B is the between-group sum-of-squares and cross-products matrix, and W is the within-group cross-products matrix.[46] The method extracts N-1 discriminant functions, N being the number of groups to differentiate. In a two-group situation, the discriminant function has the following mathematical form:

$$D = d_1Z_1 + d_2Z_2 + \cdots + d_nZ_n$$

where D is the score on discriminant function, d is the weighting coefficient of a discriminating variable, Z is the standardized value of a discriminating variable used in the analysis, and underscore n is the number of variables, in our case the 9 metals. The magnitude of the weighting coefficients indicate how strongly the discriminating variables contribute to group separation.

All nine metals were used in discriminant function to assess which of them could better differentiate distinct groups. We conducted four discriminant analyses with the following grouping variables: study cohort (MESA and SHS), US regions (East (Baltimore, New York City and Winston Salem), Middle (Chicago, North and South Dakota, Oklahoma and St Paul), and West (Arizona and Los Angeles)), race/ethnicity (White, Black, Hispanic, Chinese American and American Indian) and smoking status

(current smokers and non-current smokers). When regions and race/ethnicity were used as grouping variables, we considered differences between two groups at a time for generating discriminant functions, i.e., between East and Middle US regions (group 1), between East and West US regions (group 2), and between Middle and West US regions (group 3); between American Indians and Whites (group 1), between Blacks and Whites (group 2), between Hispanics and Whites (group 3), and between Chinese Americans and Whites (group 4).

We ran several sensitivity analyses. First, we conducted PCA and CA in MESA adjusting for rice intake (Table S3, Figure S1). The aim was to control for the influence of rice intake on urinary ∑As and potentially better discriminate other sources of arsenic such as drinking water. This is because rice is a major source of ∑As from food and Chinese Americans and Hispanics have relatively high levels of rice intake compared with other race/enthinicity groups. Second, we repeated PCA and CA restricting to individuals with eGFR ≥60 ml/min/1.73m^2 and to non-current smokers, in separate analyses, with similar results (Table S4, Table S5, Figure S2). The aim was to reduce the possibility that declined kidney function might affect urinary metal concentrations, and to control for the impact of active smoking on urinary metals as cigarette smoking is a major source of various toxic metals.[47-49] Third, we repeated PCA and CA in SHS with a random sample size of 2 individuals in each family instead of 1, also with similar findings (Table S6, Figure S3). All statistical analyses and graphical displays were performed using R software (version 2.14.2).

RESULTS

Metal Levels in Urine

MESA participants were older, more likely to be men, never smokers, current alcohol drinkers, and had lower body mass index than SHS participants (Table 1). SHS participants had higher urinary concentrations of ∑As, Cd, U, W and Zn than MESA participants (Table 2, Figure 1). In unadjusted analyses, participants in the Middle and West regions had higher urinary ∑As, U, W and Zn than those in the East regions. Compared with non-current smokers, current smokers had higher levels of ∑As, Cd, Pb, U, W and Zn. Compared with Whites, American Indians, Blacks and Chinese Americans had higher levels of Cd and Zn. Mo was higher in Chinese Americans compared to other race/ethnic groups. Pb levels were higher in Whites and American Indians and ∑As, U and W were higher in American Indians compared to other race/ethnic groups.

We observed moderate positive correlations between Zn-Sb, Sb-W, Mo-W, U-W in MESA and between Mo-Se, Pb-Sb, and ∑As-U in SHS (Table S7).

Principal Component and Cluster Analyses

Four PCs explained 65.7% and 66.3% of the total variance in MESA and SHS (Table 3), respectively. In MESA, the 4 PC (% variance explained) were characterized by Sb-Se-Zn (20.9%), Pb-U-W (17.9%) with an inverse correlation of Pb with U and W, Cd-Mo (14.0%) with an inverse correlation of Cd and Mo, and ∑As-Pb (12.9%). In SHS, the 4 PC (% variance explained) were characterized by ∑As -U-W (18.6%), Cd-Zn (16.5%), Mo-Se (16.5%), and Pb-Sb (14.6%). PC score levels by communities in MESA and SHS were summarized in box plots (Figure-S4, S5). The distributions of the PC levels were

similar across communities both in MESA and SHS, except for higher levels of PC-1 (∑As -U-W) in SHS for Arizona, and higher levels of PC-2 (Pb-U-W) in MESA for LA.

In MESA we found two large clusters: ∑As-Mo-Sb-U-W and Cd-Pb-Se-Zn (Figure 2). In SHS, we found four clusters: Mo-Se, ∑As-U-W, Cd-Zn, and Pb-Sb (Figure 2).

Linear Discriminant Analyses

The weighting coefficients showed that the discriminant function contrasting MESA and SHS was positively weighted most by ∑As and Zn (Table 4). This indicated that ∑As and Zn, and especially ∑As, were the most discriminant variables. Figure 3 displays good group separation based on discriminant scores.

Comparing Middle and East US regions, ∑As was the most discriminant variable. Comparing East and West regions, the discriminant function was positively weighted by Sb and negatively weighted by ∑As and W, with W being the most discriminant variable. Comparing Middle and West regions, the discriminant function was positively weighted by ∑As and Sb, and negatively weighted by U and W, with W and U being the most discriminant variables. In a plot of discriminant functions 1 (East vs. West plus Middle) and 2 (West vs. Middle) (Figure S6), participants in the East, Middle and West regions overlapped, although participants in the West appeared to be differentiated better from participants in the other two regions.

Comparing American Indian and White participants, ∑As was the most discriminant variable. Comparing Black and White, the discriminant function was positively weighted most by Zn, and negatively weighted by Pb and Sb, with Zn being the most discriminant variable. Comparing Hispanic and White participants, the

discriminant function was positively weighted by Pb and W, and negatively weighted by ∑As and Zn, with Pb being the most discriminant variable. Comparing Chinese American and White participants, the discriminant function was positively weighted by Pb, and negatively weighted by ∑As and Cd, with ∑As being the most discriminant variable.

Comparing current smokers and non-smokers, the discriminant function was positively weighted by Cd and As, and negatively weighted by U and Sb, with U and Cd being the most discriminant variables.

DISCUSSION

We used PCA, CA and LDA to identify potential common environmental sources and/or metabolic pathways from metal-mixtures in urine in participants from two well-established cohort studies that cover 6 urban and 3 rural areas across the US, MESA and SHS. In SHS, PCA and CA provided consistent results. The ∑As-U-W cluster and PC in SHS might reflect groundwater contamination in rural areas, and the score level for this PC was higher for Arizona. The Cd-Zn cluster and PC in SHS could reflect common sources, for instance from organ or processed meat consumption,[50-52] or from interactions in metabolic pathways for those metals.[53-60] Chinese Americans and Black participants also had higher Zn levels, maybe also because of higher intake of organ meats, shellfish and processed meats. SHS participants had a higher burden of ∑As, Cd, U, W and Zn, as measured in urine, compared to MESA. Moreover, urinary ∑As concentrations completely distinguished participants from MESA and SHS in LDA, reflecting disproportionate inorganic arsenic exposure in rural tribal communities compared to urban communities around the US.

Arsenic, Uranium and Tungsten

Elevated levels of ∑As-U-W in groundwater are found in areas with high naturally occurring levels of these metals in rocks and soil, especially in certain areas, such as the Western US. [61-64] Drinking water from groundwater sources might be a common route of exposure for these metals in some communities. In SHS, there was a clear cluster and PC for these 3 metals. The higher PC scores in Arizona are consistent with high levels of ∑As, U, and W in groundwater in Arizona.[61,63, 64] In MESA, a cluster of ∑As, U and W could also be related to groundwater as evidence by the first cluster in CA, which included those 3 metals. In PCA, there was also a PC for tungsten and uranium, with a higher score for LA. The average uranium concentrations in drinking water are reported to reach 2.5 pCi/L in Arizona and 2.7 pCi/L in California.[65] Tungsten is not a substance that is typically measured in drinking water by the US Environmental Protection Agency, and tungsten levels in drinking water are generally unknown. Releases to groundwater typically occur in regions where natural formations of tungsten minerals are prevalent, including California, Arizona, North and South Dakota. The 2010 US Geological Survey (USGS) report shows that tungsten in soil collected from a depth of 0 to 5 centimeters ranges from 0.9 to 24.1 mg/kg in California and Arizona, and from 0.8 to 1.5 mg/kg in North and South Dakota, levels that are markedly higher compared to the overall median level (0.8 mg/kg).[66]

Among our 9 metals, ∑As had the highest discriminant ability to distinguish participants from MESA and SHS in LDA. This finding reflects disproportionate inorganic arsenic exposure through drinking water in rural tribal communities compared to urban communities around the US. Evidence from SHS

has shown that arsenic exposure is a risk factor for the development of cardiovascular disease and cancer, and possibly for the development of diabetes, chronic kidney disease and respiratory disease.[67-71] The health risks associated with arsenic at low-moderate levels in SHS are consistent with findings from other communities in the US,[72-76] and with findings at higher arsenic levels in Bangladesh, Chile, Mexico and Taiwan.[77-91] The markedly higher urinary ∑As levels in SHS than MESA participants represent an additional call for action to prevent inorganic arsenic exposure in drinking water in rural communities in the US, in particular tribal communities. In recent years, efforts have been made to warrant that all community water systems are compliant with the maximum contaminant level of 10 μg/L. Levels between 5 and 10 μg/L and even between 1 and 5 μg/L, are likely to disproportionately occur in many small community water systems compared to urban areas.[92,93] Moreover, private wells, which are more common in rural areas, are not required to comply with the legislation of the US Environmental Protection Agency. Additional efforts are urgently needed to minimize exposure to inorganic arsenic in small rural communities around the US.

Cadmium and Zinc

Smoking is the major source of Cd in most populations.[94] Among non-smokers, leaf and root vegetables, organ meats and shellfish are major sources of Cd exposure.[94] In SHS, urinary Cd levels were relatively high and not too different by smoking status, suggesting that there is another major source of cadmium in the population.[95] We found a cluster and PC formed by Cd and Zn in SHS. This cluster could be due to environmental sources or to interactions in metabolic pathways. In food, Cd and Zn levels are higher in whole grains, seafood and meat products, including organ meats.[94,96,97] High intake of

meat products in SHS could represent a source of Cd and Zn and explain relatively high urinary Cd levels in SHS participants.[50] Another common source of Cd and Zn is related to mining activity. Cadmium mainly occurs in association with the sulfide ores of zinc, and thus cadmium becomes a by-product when zinc is mined.[98]

In addition, Cd and Zn are interrelated due to replacement and interactions of Cd to Zn in metabolism. Cd and Zn display similar chemical properties and are invariably related in the geosphere and biosphere.[99-101] Cd is able to replace or mimic Zn in the first or early step of transport and metabolism, but then it is incapable of mediating subsequent vital functions.[53,54] In metabolism, Cd and Zn are interrelated due to competition for transposter and induction of metallothionein (MT).[55] Cd uptake occurs predominantly through Zn-associated co-transport, and both ions compete for common binding sites and for membrane carriers such as divalent metal transporter-1 and luminal Zn transporter-1.[56] After its absorption, Cd is taken up by the hepatocytes, and then from the liver it circulates in blood bound to MT.[57] The Cd-MT complex is filtered in the renal glomerulus, reabsorbed in the tubular cells, and accumulated in the kidney with a biological half-time ranging from 10 to 30 years.[58] Zn is able to induce synthesis of MT in the liver and kidney.[55] Ample dietary intakes of Zn increase the induction of MT, and thus can increase the accumulation of Cd in the kidney.[59,60] In human tissues, Cd and Zn are correlated, with the highest correlation coefficients between Cd and Zn in human tissues being found in the kidney (rank correlation coefficient of 0.70).[97]

Urinary Zn levels also had the ability to distinguish participants from MESA and SHS in LDA. For American Indians living in rural areas or reservations, dietary choices are influenced by foods available at local

convenience stores or through the US Department of Agriculture commodity foods assistance program, such as processed meats.[50] It has been reported that SHS participants have a high intake of processed meats and other meat products.[51,52] Because meat products are generally high in Zn, it is possible that the markedly higher meat product consumption in SHS versus MESA could result in high levels of urinary Zn in this population. This explanation is only tentative and further studies are needed to explain the high body burden of Zn and the clustering of Cd and Zn in SHS participants.

Consistency between PCA and CA

Although results of PCA and CA showed some agreement with each other in MESA participants, the patterns of urinary metals were not as clear and consistent as in SHS participants. This phenomenon is possible for several reasons. First, PCA and CA depend on the direction and magnitude of correlations among urinary metals, but display them differently. In PCA, loadings for each metal within the same components can be either positive or negative, and negative loadings might indicate a different source than metals with positive loadings. For instance, Cd was positively correlated with PC-3, while Mo was negatively correlated with PC-3 in MESA. This can explain why we observed Cd and Mo in two separate clusters in CA. This was also the case for Pb-U-W. CA, on the other hand, combines the most similar urinary metals at a time, and thus metals within the same cluster tend to be positively correlated. Furthermore, the dendrogram distance represents the degree of correlations among metals. The lower the value on the distance, the stronger was the correlation. Clusters of metals were formed at a lower distance in SHS than MESA, implying stronger correlations among urinary metals in SHS.

Second, results from PCA and CA might be confounded by relatively higher rice intake in Chinese Americans and maybe Hispanics compared to other race/ethnic groups in MESA. Rice is a major contributor to inorganic arsenic, and rice consumption is imbalanced across race/ethnic groups, with Chinese Americans having the highest levels of consumption. In sensitivity analysis, we conducted PCA and CA in MESA with adjustment for rice intake. Results yielded more consistent patterns for PCA and CA, suggesting 4 PCs and clusters in MESA: Sb-U-W, Se-Pb-Zn, Cd-Mo, and ∑As (Table S3, Figure S1).

Limitations and Strengths

Our study has several limitations. First, urinary Pb and Se are usually considered less reliable biomarkers of exposure and internal dose of Pb and Se, respectively.[102-104] Second, as in most epidemiologic studies of urinary metals we used spot urine samples, which requires adjustment for urine dilution. There is scientific debate about whether it is better to adjust for urine dilution using urine specific gravity or urine creatinine.[105-107] In our study, we adjusted for urine specific gravity because urine creatinine is also a marker of creatinine production, and thus it is associated with age, gender, race/ethnicity, and muscle mass.[107] Specific gravity adjustment might be a more appropriate approach to control for urine dilution, especially when we consider the distinct participant characteristics in MESA and SHS.

Despite these limitations, strengths of this study include the inclusion of urban and rural populations in the U.S., the wide geographical and race/ethnic coverage, the rigorous laboratory methods with extensive quality control, the reliability of urine as biomarkers of exposure for most of the metals studied, and the simultaneous use of three

multivariable statistical approaches to disentangle differences in metal exposures across two studies conducted in urban and rural communities. This study also benefited from the similar time periods of collection of urine samples for metal analysis in both MESA and SHS, and the use of the same laboratory and analytical procedures. Additional research is needed in metal mixtures to confirm the potential sources and metabolic pathways as well as to evaluate the potential health impacts of metal co-exposures. For instance, while arsenic, tungsten and uranium are highly toxic metals, most studies in the US have evaluated health effects of each metal individually instead of in combination.

CONCLUSIONS

In conclusion, the present study showed marked differences in the distribution and correlations of selected urinary metals in urban and rural populations, as represented by participants of MESA and SHS. On average, ∑As, Cd, U, W and Zn were significantly higher in urine in SHS participants than in MESA participants. Groundwater contaminated with ∑As-U-W or with U-W could explain the PCA and CA findings in MESA and SHS. Diets rich in meat products, including organ meats, could explain the Cd-Zn cluster and PC in SHS as well as higher Zn levels in some race/ethnic groups compared to Whites. The separation of the two studies in LDA with highest weights of As and Zn suggests the impact of ground water contamination and dietary differences across these populations. In particular, the marked difference in arsenic exposure between MESA and SHS highlights the importance of preventing arsenic exposure in small rural communities affected by arsenic in drinking water.

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TABLES

Table 1. Characteristics of the study population by study cohort[a]

	MESA (n=308)	SHS (n=105)
Age, years	61.4 ± 9.6	55.1 ± 9.2
Sex: men	57.1	42.9
Race-ethnicity		
White	28.9	--
Black	22.7	--
Hispanic	24.4	--
Chinese American	24.0	--
American Indian	--	100
Cigarettte smoking		
Never	44.8	33.3
Former	41.6	27.6
Current	13.6	39.1
Current alcohol drinking	97.4	42.9
Body mass index, kg/m^2	27.5 ± 5.4	31.7 ± 7.7
BMI categories		
Normal	38.3	17.1
Overweight	31.8	24.8
Obese	29.9	58.1
Estimated GFR< 60[b]	7.5	10.5

[a] Values are expressed as percentage for categorical variables or means ± standard errors for continuous variables.
[b] Glomerular filtration rate < 60 mL/minute per 1.73 m^2.

Table 2. Geometric means of metal levels in urine (μg/L) by participant characteristics[a]

	Cohort			Region				Smoking			Race/ethnicity					
	MESA	SHS	p value	East	Middle	West	p value	Non-smoker	Smoker	p value	White	American Indian	Black	Hispanic	Chinese American	p value
∑As	0.22	6.75	<0.001	0.19	0.78	0.80	<0.001	0.43	1.22	<0.001	0.19	6.77	0.18	0.234	0.34	<0.001
Cd	0.61	0.79	0.017	0.60	0.72	0.61	0.18	0.58	1.02	<0.001	0.54	0.79	0.72	0.49	0.77	0.002
Mo	48.77	42.34	0.13	46.06	46.21	49.49	0.71	47.32	46.39	0.84	44.32	42.34	43.02	48.56	60.81	0.012
Pb	1.62	2.22	0.052	1.65	1.96	1.58	0.38	1.61	2.51	0.01	2.60	2.22	1.71	1.12	1.23	<0.001
Sb	0.099	0.13	0.065	0.099	0.11	0.10	0.78	0.10	0.12	0.5	0.11	0.13	0.082	0.097	0.11	0.19
Se	53.41	51.76	0.48	51.73	53.77	53.04	0.72	53.60	50.52	0.23	55.46	51.76	49.91	52.15	55.89	0.31
U	0.013	0.041	<0.001	0.011	0.017	0.027	<0.001	0.016	0.023	0.01	0.011	0.041	0.011	0.012	0.018	<0.001
W	0.042	0.15	<0.001	0.028	0.056	0.12	<0.001	0.054	0.080	0.038	0.043	0.15	0.032	0.031	0.074	<0.001
Zn	421.01	970.04	<0.001	432.23	640.49	564.63	0.004	534.93	664.19	0.046	386.76	970.04	457.15	396.55	451.71	<0.001

[a] Metal levels are standardized by specific gravity to account for urine dilution. P-values are estimated based on ANOVA.
∑As refers to the sum of inorganic and methylated arsenic species. In MESA we accounted for organic arsenic exposure from seafood based on arsenobetaine, a specific arsenic biomarker of seafood intake.

Table 3. Standardized rotated factor loadings from PCA in MESA and SHS (loadings are bolded if > 0.40)

	MESA				SHS			
Factor loadings	Component	Component	Component	Component	Component	Component	Component	Component
Varimax raw	1	2	3	4	1	2	3	4
∑As	-0.064	0.045	-0.023	**0.87**	**0.62**	0.12	-0.12	0.02
Cd	0.10	0.099	**0.76**	0.019	0.06	0.05	0.06	**0.66**
Mo	0.16	0.13	**-0.61**	0.075	0.10	**0.72**	0.18	-0.21
Pb	0.34	**-0.50**	0.090	**0.42**	0.13	-0.25	**0.53**	-0.01
Sb	**0.43**	0.28	0.011	0.028	-0.12	0.16	**0.73**	0.03
Se	**0.51**	-0.096	-0.011	-0.15	-0.10	**0.59**	-0.17	0.36
U	-0.035	**0.62**	0.17	0.15	**0.50**	-0.01	0.27	0.12
W	0.23	**0.49**	-0.11	0.085	**0.55**	-0.07	-0.15	-0.07
Zn	**0.59**	-0.048	0.039	-0.092	-0.02	-0.18	0.014	**0.61**
Eigenvalue	1.88	1.61	1.26	1.16	1.68	1.49	1.49	1.31
Total variance (%)	20.86	17.92	14.01	12.87	18.64	16.52	16.51	14.59
Cumul. (%)	20.86	38.78	52.79	65.66	18.64	35.16	51.68	66.26

For adjustment of urine dilution and interpretation of ∑As, see Table 2

Table 4. Results of linear discriminant analysis

Parameters	Cohort	Region			Smoking status	Race			
	SHS: MESA	Middle:East	East:West	Middle:West	Current: Non-smokers	American Indian:White	Black:White	Hispanic:White	Chinese American:White
∑As	0.97	0.87	-0.49	0.47	0.64	1.04	-0.05	-0.64	-0.53
Cd	-0.08	-0.01	-0.0004	0.34	0.69	-0.02	0.33	0.15	-0.43
Mo	-0.28	-0.10	0.06	0.06	0.17	-0.25	-0.005	0.09	-0.28
Pb	-0.15	-0.004	0.03	0.01	0.30	-0.19	-0.64	1.03	0.55
Sb	-0.13	-0.21	0.42	0.42	-0.45	-0.11	-0.42	-0.10	0.34
Se	-0.08	0.08	-0.09	-0.03	-0.31	-0.08	-0.35	0.10	0.16
U	0.04	-0.003	-0.30	-0.67	-0.79	-0.14	0.16	0.11	-0.38
W	0.06	0.24	-0.72	-0.85	0.10	0.11	-0.26	0.45	-0.25
Zn	0.34	0.11	0.10	0.26	-0.11	0.25	0.69	-0.43	-0.22
Wilks' lambda	0.23	0.82	0.64	0.85	0.88	0.14	0.89	0.83	0.72
P value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.003	<0.001	<0.001

For adjustment of urine dilution and interpretation of ∑As, see Table 2.

FIGURES

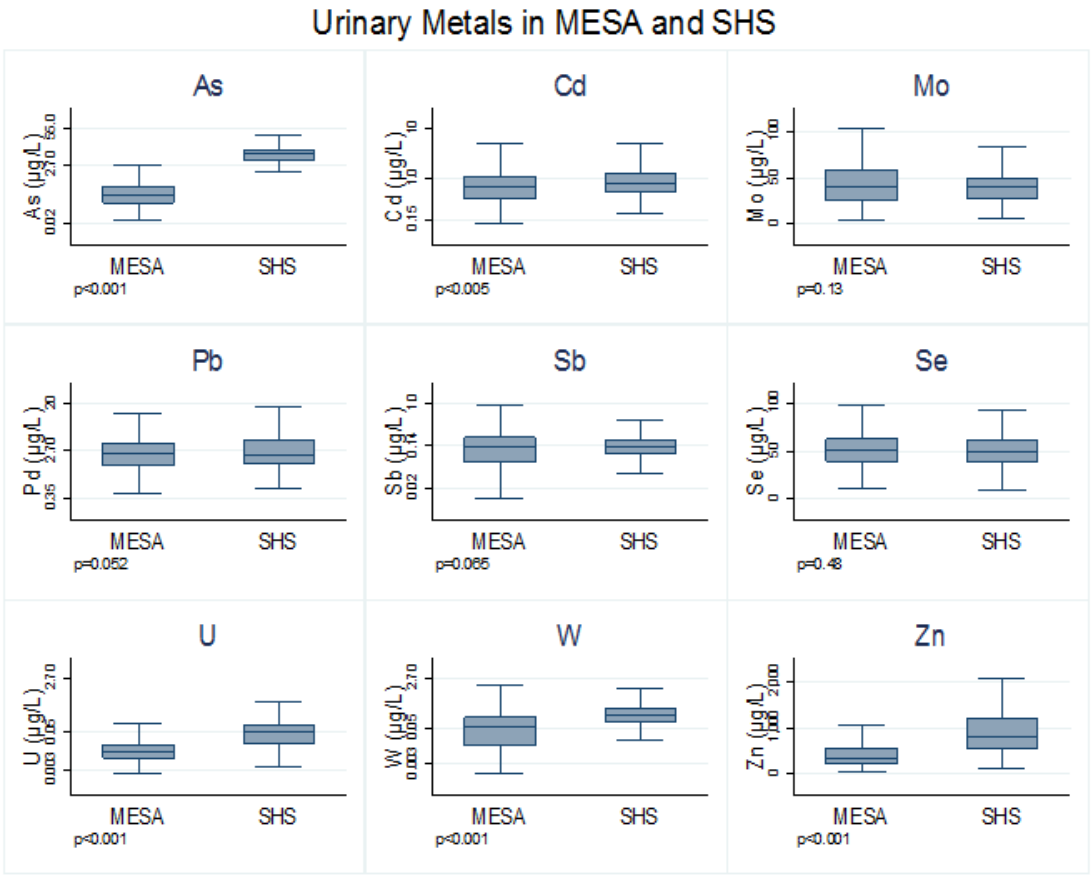

Figure 1. Box plots of urinary metals (µg/L) standardized by specific gravity in MESA and SHS. As refers to the sum of inorganic and methylated arsenic species (after correction for arsenobetaine levels in MESA because of frequent seafood intake).

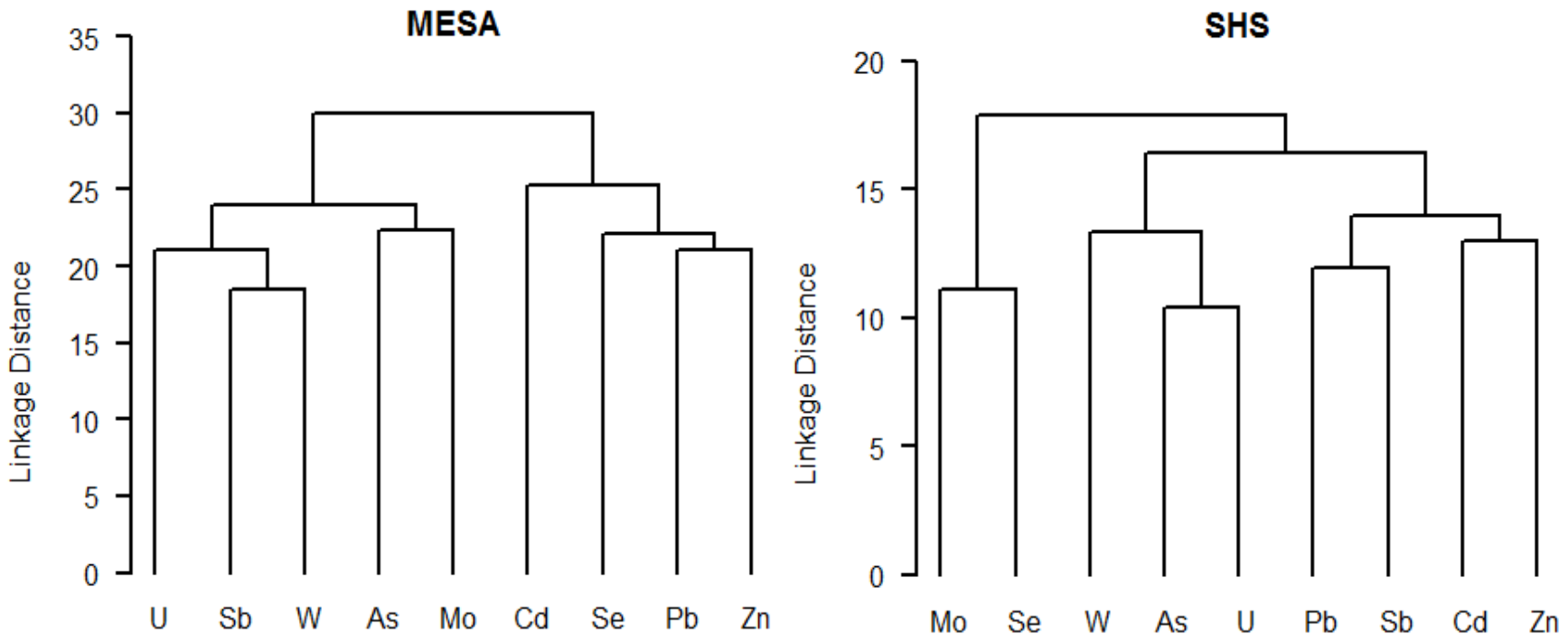

Figure 2. Dendrogram of metals in urine in MESA and SHS. All metals were standardized by specific gravity. As refers to the sum of inorganic and methylated arsenic species (after correction for arsenobetaine levels in MESA because of frequent seafood intake).

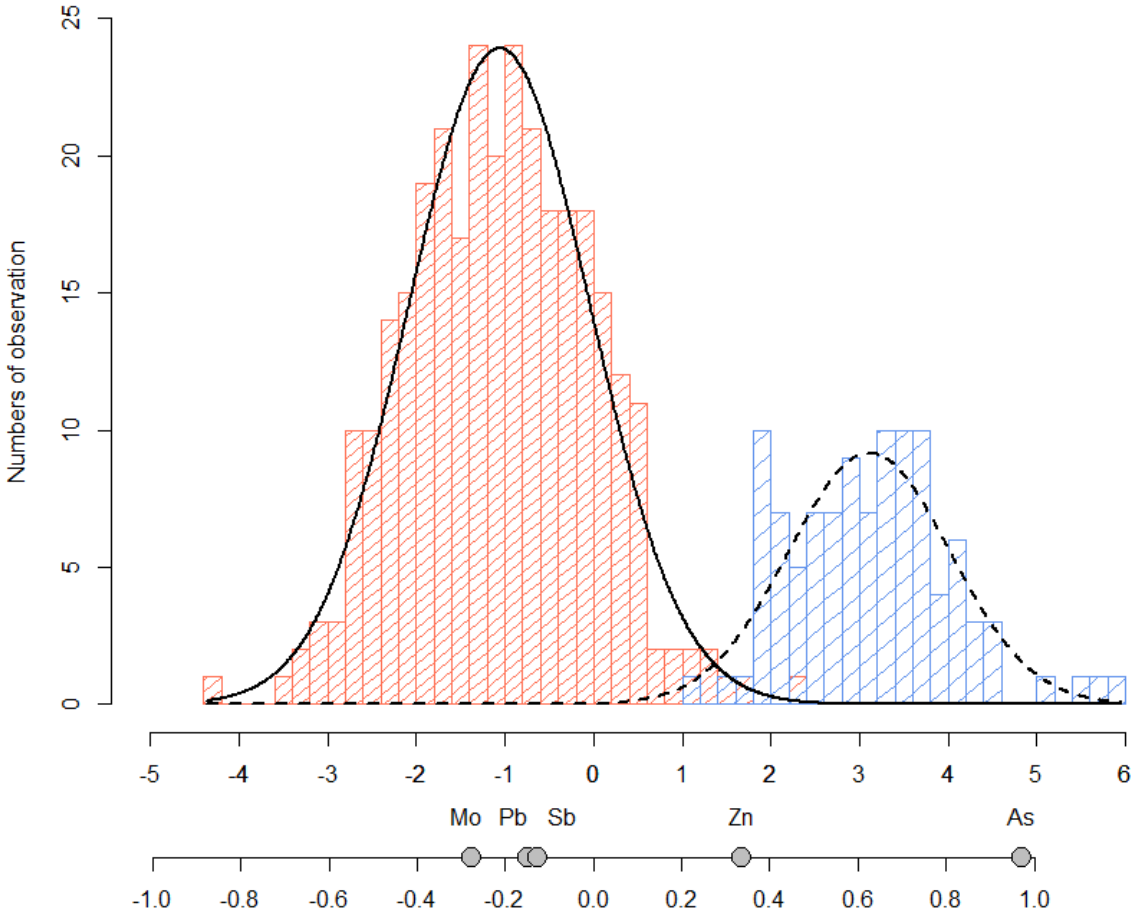

Figure 3. Linear discriminant analysis. Group seperation, efficiency of seperation, and independent variables which contributed most to the seperation, according to loadings in discriminant functions (Cd, W, and U are not included as their loadings are very close to 0).

APPENDIX

Table S1. Metal levels in urine (μg/L) in MESA[a]

	∑As	Cd	Mo	Pb	Sb	Se	U	W	Zn
Sample size	308	308	308	308	308	308	308	308	308
Geometric mean	0.22	0.61	48.8	1.6	0.10	53.4	0.01	0.04	421.0
GM SD	2.6	2.6	41.2	4.5	4.1	21.1	2.3	4.9	358.8
Percentile									
10th	0.06	0.23	17.7	0.08	0.01	28.6	0.008	0.004	116.0
25th	0.11	0.38	25.5	1.4	0.06	38.5	0.008	0.01	192.5
50th	0.23	0.67	40.8	2.3	0.14	51.1	0.01	0.06	331.8
75th	0.45	1.1	58.4	3.6	0.21	62.8	0.02	0.13	557.3
90th	0.72	1.7	87.4	6.4	0.34	84.9	0.04	0.26	773.0
Maximum	4.5	9.6	408.0	49.4	4.80	125.3	0.33	4.80	3069.1
LOD	0.1	0.015	0.1	0.08	0.006	2.0	0.008	0.005	10.0
Percent < LOD	0	2.3	0	15.3	12.0	0	37.0	25.3	0

Abbreviations: LOD, limit of detection. For adjustment of urine dilution and interpretation of ∑As, see Table 2.

[a] Values are expressed as means for essential metals and geometric means for toxic metals.

Table S2. Metal levels in urine (µg/L) in SHS

	∑As	Cd	Mo	Pb	Sb	Se	U	W	Zn
Sample size	105	105	105	105	105	105	105	105	105
Geometric mean	6.8	0.79	42.3	2.2	0.13	51.8	0.041	0.15	970.0
GM SD	2.0	2.6	21.4	3.4	1.8	19.6	3.3	2.6	695.3
Percentile									
10th	3.1	0.33	19.9	0.82	0.06	28.5	0.01	0.06	373.9
25th	3.9	0.53	27.6	1.5	0.10	38.7	0.02	0.08	532.6
50th	6.5	0.76	38.9	2.2	0.13	49.6	0.05	0.14	795.8
75th	9.6	1.3	50.2	4.2	0.18	61.8	0.08	0.26	1187.9
90th	16.9	2.3	70.0	7.7	0.26	79.3	0.19	0.59	1939.7
Maximum	63.6	7.4	128.2	292.1	0.71	104.1	0.48	1.3	5297.0
LOD	0.1	0.015	0.1	0.08	0.006	2	0.008	0.005	10
Percent < LOD	0	1.9	0	4.8	0	0	15.2	0.95	0

Abbreviations: LOD, limit of detection. For adjustment of urine dilution and interpretation of ∑As, see Table 2.
[a] Values are expressed as means for essential metals and geometric means for toxic metals

Table S3. Standardized rotated factor loadings from PCA in MESA (adjusting for rice intake) (loadings are bolded if > **0.40**)

Factor loadings	Component	Component	Component	Component
Varimax raw	1	2	3	4
∑As	0.01	0.02	-0.01	**0.97**
Cd	0.12	0.07	**0.76**	-0.01
Mo	0.20	0.08	**-0.61**	0.05
Pb	-0.25	**0.62**	0.07	0.16
Sb	**0.44**	0.27	0.01	-0.01
Se	0.10	**0.44**	-0.04	-0.13
U	**0.56**	-0.25	0.19	0.12
W	**0.57**	0.01	-0.10	-0.04
Zn	0.18	**0.53**	0.02	-0.05
Eigenvalue	1.77	1.75	1.21	1.20
Total variance (%)	21.27	18.18	13.97	11.28
Cumul. (%)	21.27	39.46	53.42	64.71

For adjustment of urine dilution and interpretation of ∑As, see Table 2.

Table S4. Standardized rotated factor loadings from PCA in MESA (estimated glomerular filtration rate < 60 mL/minute per 1.73 m^2) (loadings are bolded if > **0.40**)

Factor loadings	Component	Component	Component	Component
Varimax raw	1	2	3	4
∑As	-0.07	0.04	-0.04	**0.84**
Cd	0.10	0.10	**0.75**	-0.01
Mo	0.17	0.14	**-0.61**	0.05
Pb	0.31	**-0.54**	0.10	**0.44**
Sb	**0.43**	0.23	0.02	0.10
Se	**0.52**	-0.08	-0.02	-0.21
U	-0.01	**0.61**	0.18	0.18
W	0.20	**0.48**	-0.10	0.08
Zn	**0.57**	-0.09	0.04	-0.08
Eigenvalue	1.91	1.54	1.26	1.18
Total variance (%)	21.24	17.13	14.05	13.16
Cumul. (%)	21.24	38.37	52.41	65.58

For adjustment of urine dilution and interpretation of ∑As, see Table 2.

Table S5. Standardized rotated factor loadings from PCA in SHS (estimated glomerular filtration rate < 60 mL/minute per 1.73 m^2) (loadings are bolded if > **0.40**)

Factor loadings	Component	Component	Component	Component
Varimax raw	1	2	3	4
∑As	-0.08	**0.63**	0.10	0.05
Cd	0.16	0.05	**0.58**	-0.25
Mo	0.11	0.11	0.18	**0.72**
Pb	**0.57**	0.07	-0.19	-0.10
Sb	**0.69**	-0.15	0.04	0.15
Se	-0.16	-0.07	**0.67**	0.24
U	0.34	**0.44**	0.12	-0.03
W	-0.12	**0.60**	-0.22	0.04
Zn	0.05	0.12	0.28	**-0.57**
Eigenvalue	1.67	1.66	1.48	1.24
Total variance (%)	18.58	18.41	16.42	13.80
Cumul. (%)	18.58	36.99	53.41	67.21

For adjustment of urine dilution and interpretation of ∑As, see Table 2.

Table S6. Standardized rotated factor loadings from PCA in SHS (N=2 for each family) (loadings are bolded if > **0.40**)

Factor loadings	Component	Component	Component	Component
Varimax raw	1	2	3	4
∑As	**0.63**	-0.08	0.11	-0.05
Cd	0.12	0.0008	0.02	**0.61**
Mo	0.09	0.12	**0.69**	-0.16
Pb	-0.04	**0.72**	0.05	-0.06
Sb	0.03	**0.67**	-0.05	0.10
Se	-0.08	-0.11	**0.64**	0.25
U	**0.56**	0.04	-0.28	0.13
W	**0.51**	0.04	0.16	-0.07
Zn	-0.07	0.02	0.0009	**0.71**
Eigenvalue	1.80	1.51	1.47	1.33
Total variance (%)	19.95	16.77	16.22	14.73
Cumul. (%)	19.95	36.72	53.05	67.78

For adjustment of urine dilution and interpretation of ∑As, see Table 2.

Table S7. Pearson's correlation matrix for the metal concentrations[a]

	∑As	Cd	Mo	Pb	Sb	Se	U	W	Zn
∑As		0.17	0.21	0.02	-0.07	0.10	0.48*	0.26	0.04
Cd	0.04		-0.007	0.12	0.20	0.07	0.29	0.06	0.19
Mo	0.19*	-0.24*		-0.08	0.11	0.41*	0.03	0.14	-0.03
Pb	0.17	0.04	-0.06		0.32*	-0.27	0.28	0.08	0.18
Sb	0.18	0.004	0.19*	0.14		-0.08	0.28	-0.07	0.15
Se	0.07	0.06	0.15	0.18	0.19*		0.02	-0.04	0.07
U	0.21*	0.06	0.10	-0.17	0.26*	0.002		0.21	0.10
W	0.22*	-0.03	0.31*	-0.04	0.45*	0.16	0.39*		0.09
Zn	0.07	0.08	0.20*	0.28*	0.36*	0.27*	0.07	0.21*	

For adjustment of urine dilution and interpretation of ∑As, see Table 2.

[a] The left lower part is correlation coefficient for MESA; the right upper part is for SHS

*P<0.05 (2-tailed)

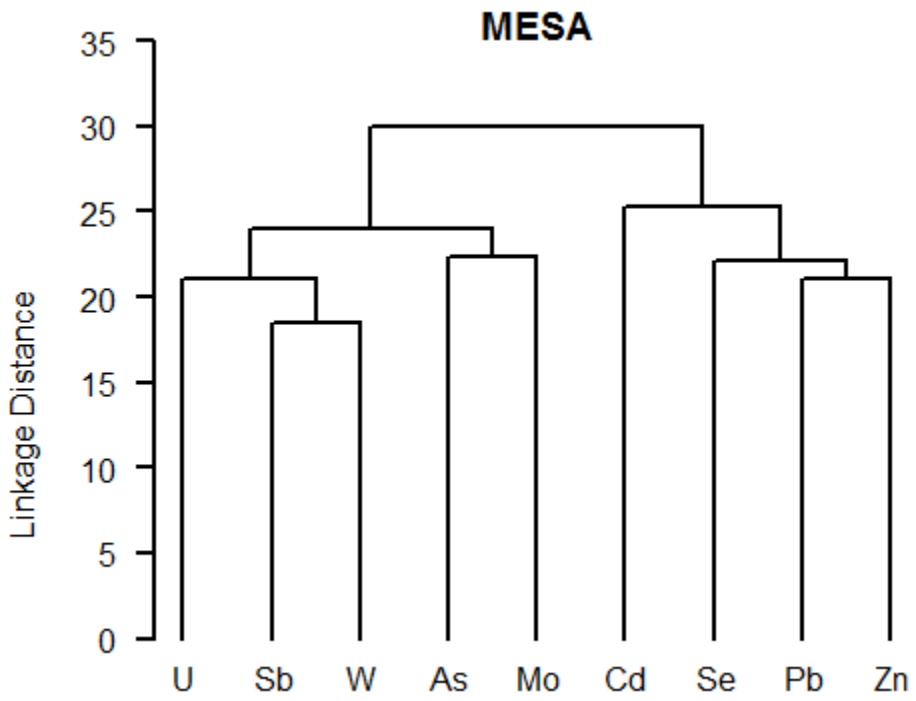

Figure S1. Dendrogram of metals in urine in MESA (adjusting for rice intake) As refers to the sum of inorganic and methylated arsenic species (after correction for arsenobetaine levels in MESA because of frequent seafood intake).

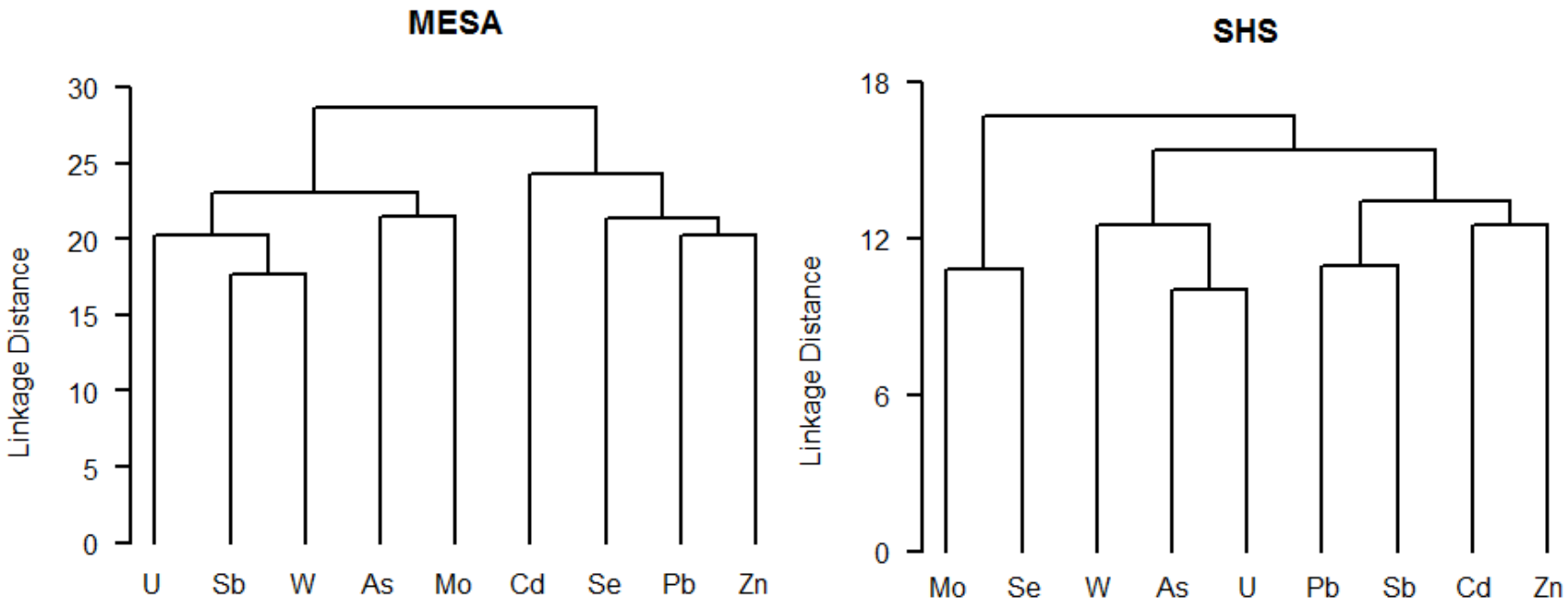

Figure S2. Dendrogram of metals in urine in MESA and SHS (estimated glomerular filtration rate < 60 mL/minute per 1.73 m^2). As refers to the sum of inorganic and methylated arsenic species (after correction for arsenobetaine levels in MESA because of frequent seafood intake).

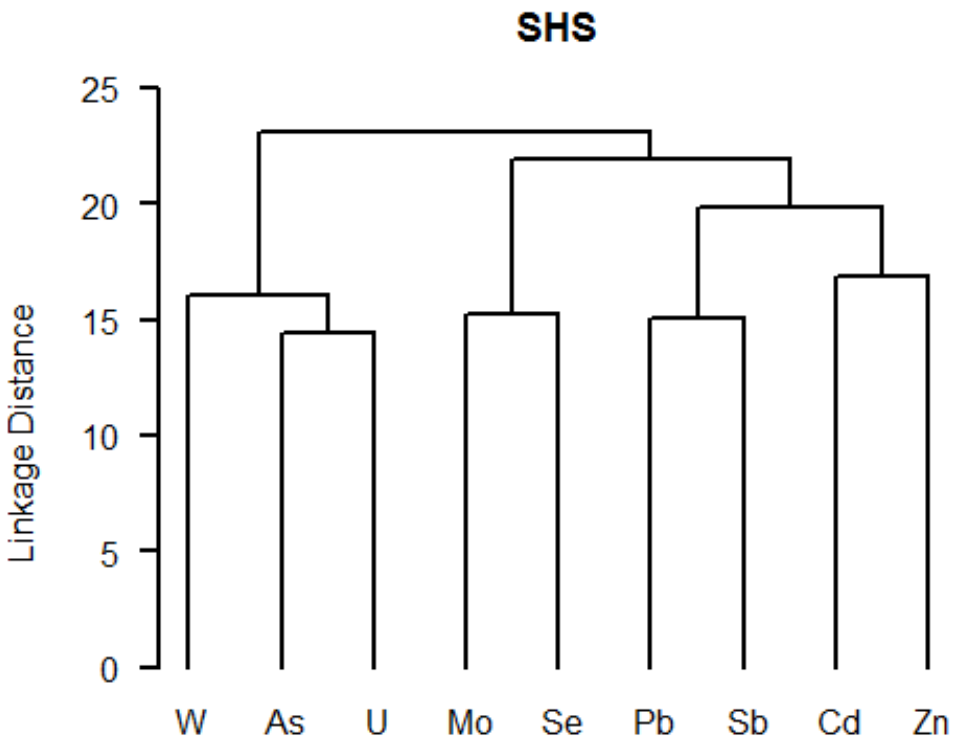

Figure S3. Dendrogram of metals in urine in SHS (N=2 for each family)

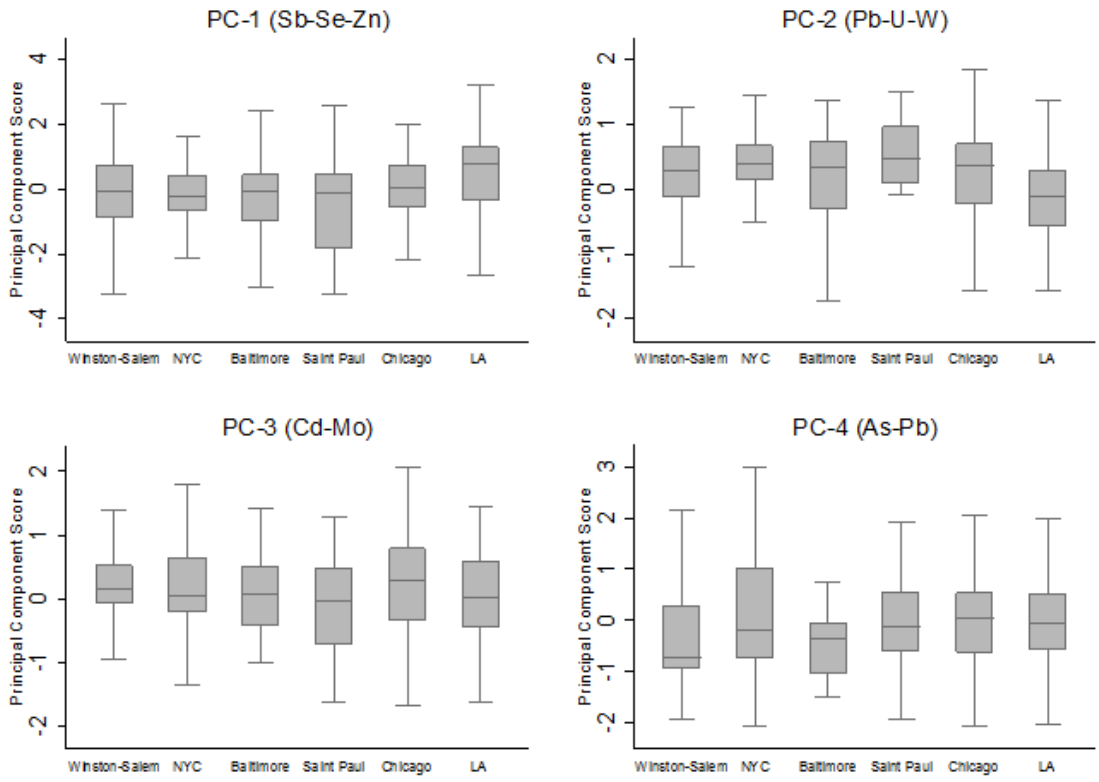

Figure S4. Box plots of principal component (PC) scores for 6 communities in MESA. As refers to the sum of inorganic and methylated arsenic species (after correction for arsenobetaine levels in MESA because of frequent seafood intake).

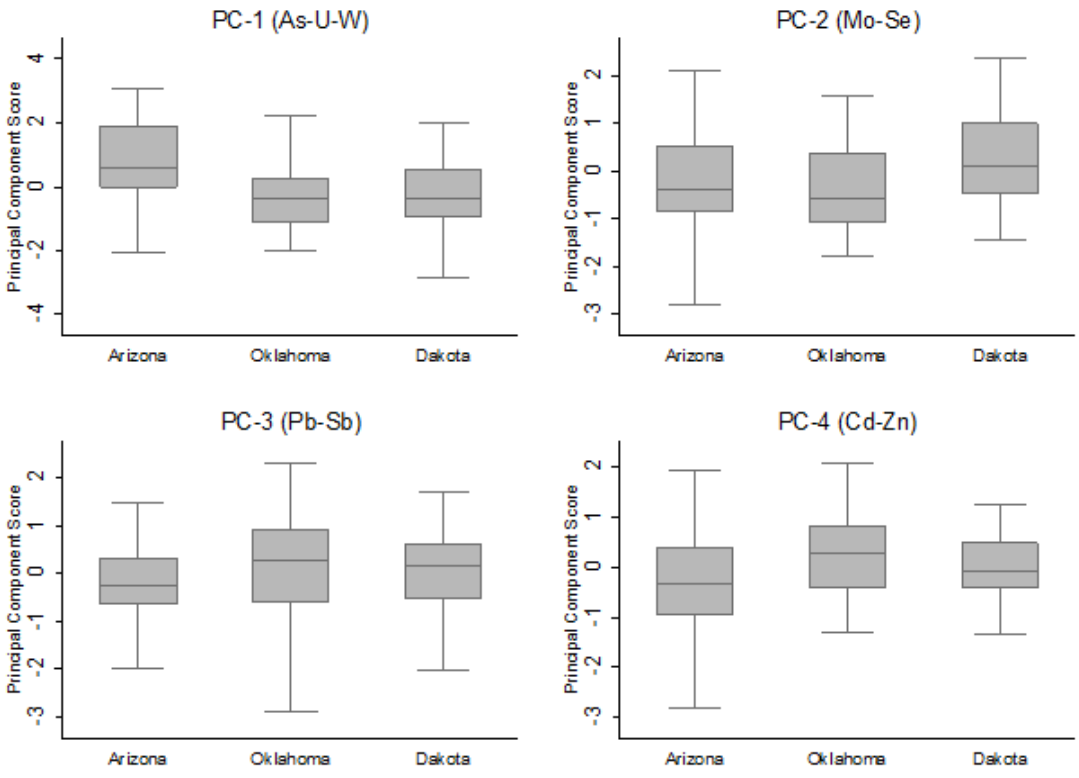

Figure S5. Box plots of principal component (PC) scores for 3 communities in SHS. As refers to the sum of inorganic and methylated arsenic species (after correction for arsenobetaine levels in MESA because of frequent seafood intake).

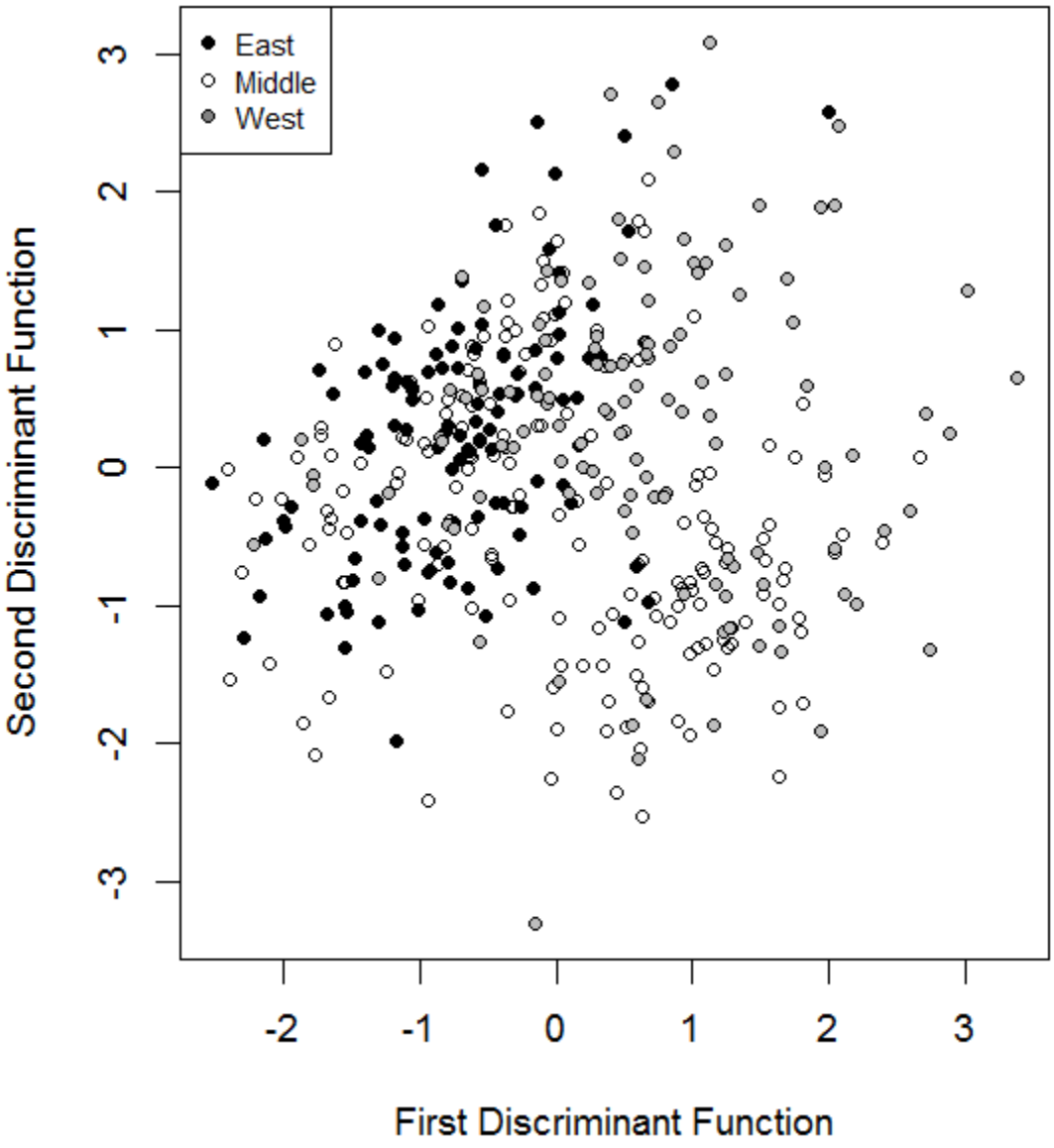

Figure S6. Linear discriminant analysis. Group seperation based on the first two discriminant functions.

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EDUCATION

Johns Hopkins Bloomberg School of Public Health Baltimore, MD 09/2013 – 05/2015
Master of Science (Sc.M.): Department of Epidemiology. Concentration: Cardiovascular Disease Epidemiology. *Selected coursework*: Epidemiologic Methods, Biostatistics, Cardiovascular Disease Epidemiology, Principles of Genetic Epidemiology, and Environmental and Occupational Epidemiology. (Academic Advisor: Yiyi Zhang, M.H.S., Ph.D.; Thesis Advisor: Ana Navas-Acien, M.D.,Ph.D.)

Peking University Health Science Center Beijing, China 09/2008 – 07/2013
Bachelor of Medicine (B.M.): School of Public Health. Concentration: Preventive Medicine.

RESEARCH EXPERIENCE

Johns Hopkins University, Department of Epidemiology, Baltimore, MD 09/2014 – 05/2015
Research Assistant, **Atherosclerosis Risk in Communities (ARIC)**
(PI: Kunihiro Matsushita, M.D.,Ph.D.)

- Perform cross-sectional, longitudinal and survival analysis in investigating the interaction of physical activity and obesity on myocardial injury
- Conduct cross-sectional analysis on the association of kidney function and albuminuria with arterial stiffness
- Conduct survival analysis on the association of subclinical atherosclerosis and incident end-stage renal disease
- Develop research proposals and prepare manuscripts

Johns Hopkins University, Department of Epidemiology, Baltimore, MD 03/2014 – 05/2015
Research Assistant, **Multi-Ethnic Study of Atherosclerosis (MESA) and the Strong Heart Study (SS)**
(PI: Ana Navas-Acien, M.D.,Ph.D.)

- Conduct principal component analysis, cluster analysis and linear discriminant analysis to explore co-exposures of urine metals (zinc, selenium, molybdenum, cadmium, lead, arsenic, antimony, tungsten and uranium) and urban versus rural differences in the Multi-Ethnic Study of Atherosclerosis (MESA) and the Strong Heart Study (SHS)
- Perform multivariable regression analysis to investigate the geographical differences in urine metals (cadmium, antimony, tungsten and uranium) and the associations between urine metals and ambient $PM_{2.5}$ and nitrogen oxides as part of the Multi-Ethnic Study of Atherosclerosis and Air Pollution (MESA Air)
- Develop research proposals and prepare manuscripts

Peking University, School of Stomatology, Beijing, China 10/2013 – 02/2014
Research Assistant, **Clinical Investigations on Oral and Maxillofacial Surgery**.
(PI: Zili Li, M.D.)

- Assisted in the design and registry of the clinical trial on computer planning and intraoperative navigation in orthognathic surgery

- Performed regression and correlation analysis to evaluate three dimensional-facial asymmetry of people with normal occlusion in North China
- Contributed to the design and data analysis of validity and reliability studies of three-dimensional craniofacial models

Peking University, Department of Environmental and Occupational Health Sciences, Beijing, China 02/2013 – 07/2013
Research Assistant, ***In vivo*** **experiment to study biomarkers of genotoxicity, oxidative stress and inflammation after tracheal instillation of PM2.5 black carbon.**
(Falcuty Advisor: Guang Jia, M.D, Ph.D.)
- Conducted tracheal instillation, organ harvest, plasma preparation, inflammation markers and micronucleus assays
- Synthesized and interpreted epidemiological studies, toxicological studies and governmental reports on black carbon

Peking University, Department of Epidemiology, Beijing, China 12/2011 – 06/2012
Research Assistant, **Systematic Review of multiple projects.** (PI: Siyan Zhan, M.D, Ph.D.)
- Systematic review on the economic burden of post stroke cognitive impairment
- Systematic review and meta-analysis on epidemiologic conditions and economic burden of hemophilia in Mainland China, Taiwan, Indian, Russia and Turkey
- Systematic review and meta-analysis on the relationship between polymorphism of HLA-DRB1, DQB1 and anti-tuberculosis drug induced liver injury

PROFESSIONAL EXPERIENCE

Johns Hopkins University, Department of Epidemiology, Baltimore,MD 03/2015 – 05/2015
Teaching Assistant, Environmental and Occupational Epidemiology.
(Course Instructors: Ana Navas-Acien, M.D.,Ph.D. and Eliseo Guallar, M.D.,Dr.Ph.)
- Gave lectures, developed lab sessions, and graded assignments

Johns Hopkins University, Department of Epidemiology, Baltimore,MD 07/2014 – 08/2014
Teaching Assistant, Principles of Epidemiology.
(Course Instructors: Rosa Crum, M.D.,Ph.D. and Gregory Kirk, M.D.,Ph.D.)
- Led group discussions during lab sessions, hosted office hours and graded assignments

Beijing Shijitan Hospital, Beijing, China 02/2011 – 05/2012
Intern Physician, Practicum for Undergraduate Student Majoring in Preventive Medicine
- Completed medical training: rotation among various divsions of the hosptial including internal medicine, surgery, pediatrics, obstetrics/gynecology, emergency medicine, family medicine and infectious disease

SKILLS

- Languages: Mandarin Chinese (native), English (full proficiency)
- Software: STATA 13, SAS, R, ArcGIS, Microsoft Office softwares

HONORS AND AWARDS

China Medical Board Next Generation Fellowship 2013-2015
Peking University Annual Merit Based Scholarships for Medical Students 2008-2012

THESIS AND DISSERTATIONS

2011 Bachelor's Thesis: Comparative study of inflammation, oxidative stress and genotoxicity in male ICR mice following tracheal instillation of PM2.5 black carbon and ozonized PM2.5 black carbon, collected in Peking University Bachelor Thesis Database

2014 Pang Y, Gao X, Jia G. Advance in genotoxicity of carbon black. Chin J Prev Med 2014, 48(7): 1-4.

VOLUNTEER SERVICES

2012 Volunteer, The 5th Beijing International Heart Forum, Beijing, China.Interpreter.

2011 Volunteer, Sunshine Doctors, Beijing, China. Providing health examinations to children of migrant works living in rural areas of Beijing.

2011 Volunteer Team Leader, Google Public Welfare in China, Beijing, China. In charge of publicity, volunteer training and offering health education classes for children of migrant workers.