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## Combined exposure to lead, cadmium, mercury, and arsenic and kidney health in adolescents age 12-19 in NHANES 2009-2014

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### Abstract

**Background**—Occupational and environmental exposures to toxic metals are established risk factors for the development of hypertension and kidney disease in adults. There is some evidence of developmental metal nephrotoxicity in children and from animal studies; however, to our knowledge no previous studies have examined associations between co-exposure to nephrotoxic environmental metals and children’s kidney health.

**Objective**—The objective of this study was to assess the association between co-exposure to lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As), measured in urine and blood, and kidney parameters in US adolescents.

**Methods**—We performed a cross-sectional analysis of a subsample of 2,709 children aged 12–19 participating in the National Health and Nutrition Examination Survey (NHANES) between 2009 and 2014. We analyzed urine levels of 4 nephrotoxic metals selected a priori (As, Cd, Pb and Hg),  $U_{mix}$ , and 3 nephrotoxic metals in blood (Cd, Pb, and Hg),  $B_{mix}$ , using a weighted quantile sum (WQS) approach. We applied WQS regression to analyze the association of  $B_{mix}$  and  $U_{mix}$  with estimated glomerular filtration rate (eGFR), serum uric acid (SUA), urine albumin, blood urea nitrogen (BUN), and systolic blood pressure (SBP), adjusting for sex, race/ethnicity, age, head of household’s education level, height, BMI, serum cotinine, and NHANES cohort year.  $U_{mix}$  and urine albumin models were also adjusted for urine creatinine, and  $B_{mix}$  models were also adjusted for fish consumption. Subanalyses included stratification by sex and an arsenic-only model including six speciated forms of As measured in urine.

**Results**—In WQS regression models, each decile increase of  $U_{mix}$  was associated with 1.6% (95% CI: 0.5, 2.8) higher BUN, 1.4% (95% CI: 0.7, 2.0) higher eGFR, and 7.6% (95% CI: 2.4, 13.1)

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higher urine albumin. The association between  $U_{\text{mix}}$  and BUN was primarily driven by As (72%), while the association with eGFR was driven by Hg (61%), and Cd (17%), and the association with urine albumin was driven by Cd (37%), Hg (33%), and Pb (25%). There was no significant relationship between  $U_{\text{mix}}$  and SUA or SBP. In WQS models using the combined blood metals,  $B_{\text{mix}}$ , each decile increase of  $B_{\text{mix}}$  was associated with 0.6% (95%CI: 0.0, 1.3) higher SUA; this association was driven by Pb (43%), Hg (33%), and Cd (24%) and was marginally significant ( $p=0.05$ ). No associations were observed between  $B_{\text{mix}}$  and urine albumin, eGFR, BUN, or SBP.

**Conclusions**—The findings suggest metals including As, Pb, Hg, Cd and their combinations may affect renal parameters, although potential reverse causation cannot be ruled out due to the cross-sectional study design. Implications of early life low-level exposure to multiple metals on kidney function may have far-reaching consequences later in life in the development of hypertension, kidney disease, and renal dysfunction. Longitudinal studies should further evaluate these relationships.

### Keywords

arsenic; lead; cadmium; mercury; mixture; childhood; kidney; systolic blood pressure; blood urea nitrogen; glomerular filtration

## 1. INTRODUCTION

Hypertension and renal diseases are prevalent in the US population and worldwide, and have generally increased in children and adolescents since the 1980s [1]. While there is some evidence of overall stabilization in recent years, there remains a high prevalence among minority children [2]. Moreover, poorer kidney function is a major risk factor for cardiovascular disease, a leading cause of mortality in the US [3]. A growing body of evidence supports that adult cardiorenal health and disease status may be predicted by childhood and adolescence, including hypertensive blood pressure trajectories that emerge as early as 7 years of age and persist into poorer cardiometabolic risk indicators in adulthood [4, 5]. A recent study of 2,658,238 adolescents in Israel showed that well-established hypertension during adolescence was associated with a doubling of the risk for future end-stage renal disease in an otherwise healthy population [6]. Additionally, the American Heart Association (AHA) estimates that in 2015, 85.7 million American adults ages 20 and older had high blood pressure with associated direct and indirect costs (annual average) estimated at \$53.2 billion [7]. Thus, the potential burden of adolescent kidney dysfunction presents both immediate and later-life health concerns, and prevention of early life environmentally-associated kidney disease and dysfunction yields immense public health and economic advantages. Yet, the contributions of low-level environmental toxicant exposures on adolescent cardiorenal health and function are relatively understudied [8–10].

Toxic metals are paradigmatic nephrotoxicants that are understudied in human populations, and no prior studies have examined the mixed or additive effects of combined exposures with early life kidney parameters. It is estimated that in adult populations arsenic (As) and lead (Pb) exposures account for 7% and 5% of the population attributable risk for high blood pressure [11]. This is important since essential hypertension among children (in the absence of renal abnormality) is multi-factorial in origin attributable to complex cardiovascular and

renal factors [12]. Further, single toxicant exposures (i.e. Pb and As) provide some evidence for developmental nephrotoxicity demonstrated by altered estimated glomerular filtration rate (eGFR) and other kidney biomarkers such as serum creatinine and urea in children [13, 14]. The absence of studies considering metal co-exposure in the literature is important because the effects of putative toxicants may be additive in combination, or otherwise interdependent on co-exposures.

In this cross-sectional study we examined urine and blood metal levels in US adolescents age 12–19 participating in the National Health and Nutrition Examination Survey (NHANES). We selected 4 primary toxic metals (As, Cd, Pb, and Hg) based on 1) demonstrated developmental nephrotoxicity in animal models [15–17], and 2) evidence from existing literature that identified associations with kidney parameters in children [9, 10]. We applied a two-tiered approach in our modeling strategy, first developing a mixtures strategy to consider the combined effects of multiple exposures as measured in blood and urine in this sample, followed by a survey-weighted single chemical analysis. The former approach enables evaluation of exposure-related effects in the mixed context in which they occur, while the survey-weighted latter approach may yield effect estimates more generalizable to a larger population. We applied weighted quantile sum (WQS) regression in our mixtures modeling approach, which accounts for correlation among the components of a mixture via supervised dimensionality-reduction [18, 19]. This ensemble approach assesses the combined effects of multiple exposures via an empirically-weighted index which both standardizes exposure concentrations and weights the contribution of individual mixture components according to their relation to the kidney parameters of interest, which included eGFR, systolic blood pressure (SBP), blood urea nitrogen (BUN), serum uric acid (SUA), and urine albumin.

## 2. METHODS

### 2.1 Study population

We analyzed NHANES data from 2009–2014, which were the study years with available blood and urine metals exposure and renal outcome data. Our analyses included eGFR, BUN, SUA, urine albumin and SBP measures. Participant selection is shown in Figure S1. Only subjects with complete data for all covariates (head of household education, race/ethnicity, BMI, height, sex, age, fish consumption, and serum cotinine), either complete data for the 4 metals in urine (As, Cd, Pb and Hg) or 3 metals in blood (Cd, Pb, and Hg), and at least one of the kidney outcomes were included in our analyses. We analyzed renal outcomes as continuous variables rather than categorically by clinical cutoffs due to the low prevalence of clinical kidney dysfunction in this cohort, and because our primary interest is in subclinical population-level shifts in adolescents. For example, we did not exclude individuals based on low eGFR since a single eGFR measurement is not sufficient to diagnose kidney disease (only 5 participants had eGFR levels  $< 75$  mL/min/1.73m<sup>2</sup>). Our study included 2,709 adolescents aged 12–19 years who participated during the 2009–2010, 2011–2012, and 2013–2014 cycles. Our study used publicly available data and was exempted from review by the Icahn School of Medicine at Mount Sinai's Institutional Review Board (#1702145). Statistical analyses were conducted in SAS 9.4.

## 2.2 Laboratory analyses

Our analysis included metal exposures and renal parameters measured over three NHANES cycle years: 2009–2010, 2011–2012, and 2013–2014. Briefly, serum creatinine, BUN and SUA were analyzed on a Beckman UniCel DxC800 Synchron by Collaborative Laboratory Services, LLC. Serum creatinine was analyzed using the Jaffe rate method. SUA was assessed using a timed endpoint method. Urea nitrogen was assessed using an enzymatic conductivity rate method. Urine albumin was analyzed using a fluorescent immunoassay method on a Sequoia-Turner digital fluorometer at the University of Minnesota. Urine creatinine was analyzed via an enzymatic method on a Roche Cobas 600 Analyzer at the University of Minnesota. Clinical reference ranges for all kidney parameters, as well as the percentage of the overall cohort above or below these ranges are presented in Table A3.

Whole blood Pb, Cd, and Hg as well as spot urine As, Pb, Cd, and Hg were analyzed via inductively coupled plasma-dynamic reaction mass spectrometry (ICP-DRC-MS) at the CDC's National Center for Environmental Health. In addition to total urinary As, speciated As including arsenous acid (As(III)), arsenic acid (As(V)), arsenobetaine (AsB), arsenocholine (AsC), dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA) were analyzed in urine using high performance liquid chromatography coupled with ICP-DRC-MS. Limits of detection (LOD) for blood and urinary metals, as well as the number and percent of samples below the LOD are presented in Table A4. Values below the LOD were imputed as the lowest LOD value for each metal.

## 2.3 Model selection and rationale

Our primary analysis included two WQS indices, stratified by tissue matrices: 1)  $U_{\text{mix}}$ , comprised of As, Cd, Pb and Hg levels measured in urine, and 2)  $B_{\text{mix}}$ , comprised of Cd, Pb, and Hg levels measured in blood. We prioritized these metals of interest based on developmental nephrotoxicity in animal models [15–17], as well as some epidemiological evidence in children [9, 10]. We additionally included As (measured in urine only) because of mounting recent evidence that As is nephrotoxic, supported by epidemiological studies [20]. Arsenic is rapidly cleared from blood, with approximately 70% of As excreted in urine, and therefore urine is the preferred matrix for capturing relevant As exposure [21]. Ultimately, our selection of total As in the main urine index ( $U_{\text{mix}}$ ) rather than one or more As species in our multi-metal index was based on several factors. The data represent a generally healthy population and inclusion of select or a single species of As may underestimate a chronic exposure. By selecting total As (comprised of all species) in human urine we consider this proxy measure assessment of combined exposure and metabolic processes [22]. We also pursued a subanalysis that examined an arsenic-only index comprised of a mixture of 6 arsenical species measured in urine (see Methods 2.6).

## 2.4 Single chemical analyses

We applied survey-weighted linear regression analyses for each of 4 metals in urine (As, Cd, Pb and Hg) and 3 in blood (Cd, Pb, and Hg), with separate models constructed for each metal. We examined associations between individual metals and eGFR, SUA, BUN, SBP, and urinary albumin. All models were adjusted for sex, race/ethnicity, age, serum cotinine (as a biomarker of nicotine exposure), head of household's education level, height, BMI, and

NHANES cohort year. Additionally, models with urinary metals were adjusted for urinary creatinine concentration (included as a covariate), and the model with Hg in blood was adjusted for fish consumption within the last 30 days, and blood metals models with urine albumin as an outcome were adjusted for urinary creatinine. Metal concentrations were deciled for these analyses. For all outcomes, natural log-transformed kidney parameters were normally distributed. Since height was used in the calculation of eGFR, it was not included as a covariate in these models. We calculated eGFR with serum creatinine and height measures using the Schwartz formula  $eGFR = [k \times \text{height in cm} / \text{serum creatinine in mg/dL}]$ , where  $k$  is equal to 0.7 for adolescent boys age 13 years and older, and 0.55 for boys under 13 years old and adolescent girls [23]. For SBP we used the average of the first three measures (out of four available, since the 4th measure was inconsistently obtained); subjects were excluded from SBP analyses if any of the first three measures were missing. Only subjects with complete data were included in a given model. Models were implemented in SAS 9.4 (Cary, NC) via the SURVEYREG procedure. The estimation of survey-weighted means describing metal levels and kidney parameters was similarly implemented in SAS 9.4 via the SURVEYMEANS procedure.

## 2.5 WQS statistical analyses

To estimate the combined effects of co-occurring metals exposure, while also assessing the contributing effects of individual metals, we employed a “mixtures” approach based on WQS regression analysis, which was developed to assess combined and discrete effects of multiple predictors in the context of correlated high-dimensional mixtures. WQS regression empirically estimates an index that identifies the influential exposure variables with non-negligible weights, and tests for associations between the exposure index and an outcome in a traditional linear framework, as:  $g(\mu) = \beta_0 + \beta_1 WQS + z' \phi$ . To assess potentially nonlinear trends, this model was extended to include an additional quadratic term for the WQS index.

Here,  $g(\mu)$  reflects a nonlinear link function allowing generalization to continuous, binary, and other distributions, though in this study only continuous outcomes were considered. As in typical regression approaches,  $\beta_0$  reflects the model intercept, while  $\beta_1$  reflects the parameter estimate for the co-exposure index, represented here as  $WQS$ ; the significance of this parameter thus reflects a straightforward test of associations between the co-exposure index and the outcome. The WQS index is constructed such that  $WQS = w_i q_{ij}$  where  $w_i$  indicates a vector of empirically-estimated weights for each mixture component, and  $q_{ij}$  indicates the quantiled rank assigned to each subject per variable. In these analyses, as with the single chemical analyses, concentrations were deciled, such that  $q_j = 0 - 9$ . Deciles were selected based on examination of the distribution of residuals for normality in models using varying quantile ranks of the exposures. Weight estimations were calculated across 100 bootstrapped samples, with weights constrained to sum to 1 and to vary between 0 and 1 to facilitate comparison.

We created two WQS indices stratified by tissue matrices:  $U_{\text{mix}}$ , comprised of As, Cd, Pb and Hg levels measured in urine, and  $B_{\text{mix}}$ , comprised of Cd, Pb, and Hg levels measured in blood (As was not measured in blood). WQS analyses were split into training and validation

sets (40:60), where the training dataset was used to estimate variable weights, and the validation dataset was used to test mixture significance. As with single chemical analyses, all outcome measures (eGFR, SUA, BUN, SBP, and urinary albumin) were natural log transformed. Models were adjusted for sex, race/ethnicity, age, head of household's education level, height, BMI, NHANES cohort year, and serum cotinine levels (to account for tobacco smoke exposure [24]) except in cases where outcomes were calculated with covariate terms. Additionally,  $U_{\text{mix}}$  models were adjusted for urinary creatinine concentration included as a covariate, and  $B_{\text{mix}}$  models adjusted for fish consumption within the last 30 days. As with the single chemical analyses, height was not used as a covariate for eGFR since this variable was a component of the outcome calculation. The percent change in a given outcome per unit change in the WQS index was calculated as  $(e^{\text{BetaWQS}} - 1) \times 100$ . Supplemental analyses examined the relationships stratified by sex.

## 2.6 Urinary As speciated mixture and BUN

To further examine the association of BUN and  $U_{\text{mix}}$ , we examined speciated As in urine, including arsenous acid (As(III)), arsenic acid (As(V)), arsenobetaine (AsB), arsenocholine (AsC), dimethylarsinic acid (DMA), and monomethylarsonic acid (MMA). Trimethylarsine oxide was excluded from analysis since this measure was not available for subjects in the NHANES 2013–14 cohort. The six speciated As measures were deciled and analyzed as a mixture for associations with BUN using WQS regression, as previously described. As in prior analyses, the model was adjusted for urinary creatinine, sex, race, age, head of household's education level, height, BMI, NHANES cohort year, and cotinine in serum.

## 3 RESULTS

Overall demographics of the 2,709 NHANES participants included in this study are shown in Table 1. The average age was 15.4 years and 48% were female. Approximately 50% of participants reported education level for head of household as at least some college education. Overall, 45% of participants indicated that they consumed fish within the last 30 days. Survey-weighted descriptive statistics for renal outcomes and metal levels by NHANES cohort year are shown in Table 2. Based on the clinical cutoffs and the single timepoint renal measurements for eGFR, SUA, BUN, and ACR, <0.5% of the cohort were outside of normal range for eGFR, 8% were outside of normal range for SUA, 8% were outside of normal range for BUN, and 18% are in the range of mild to moderately increased ACR with only 1% severely increased ACR (Table A3).

### 3.1 Association of urine metals co-exposure with renal parameters

WQS regression analyses of the index  $U_{\text{mix}}$ , the mixture of Hg, Pb, As, and Cd levels from urine, with each of the 5 renal parameters, showed evidence of association with BUN, eGFR, and urinary albumin, but not with SUA or SBP (Table 3). Specifically, per each decile increase in  $U_{\text{mix}}$ , BUN was 1.6% higher (95% CI: 0.5, 2.8), primarily due to the contribution of As (72%) and Pb (14%) (Figure 1a). Per each decile increase in  $U_{\text{mix}}$ , eGFR was 1.4% (95% CI: 0.7, 2.0) higher, attributable to the contribution of Hg (61%), Cd (17%), and As (13%) (Figure 1b). Per each decile increase of  $U_{\text{mix}}$ , we found urine albumin was 7.6%

(95%CI: 2.4, 13.1) higher. This relationship was driven by Cd (37%), Hg (33%), and Pb (25%) (Figure 1c).

In comparison, survey-weighted single chemical analyses suggested that BUN was increased by 1.2% (95%CI: 0.0, 2.5) with each decile increase of urinary As, but not Pb. Survey weighted single chemical analyses showed eGFR was 0.6% (95%CI: 0.1, 1.0) and 1.0% (95%CI: 0.4, 1.5) higher with each decile increase in Hg and Pb, respectively, with no significant associations observed with As and Cd. Similarly, urine albumin showed a 6.9% (95%CI: 2.7, 11.3) increase per decile increase Cd and a 3.1% (95%CI: 0.3, 6.0) increase per decile increase of Pb. We found no significant association between urine Hg with albumin levels.

### 3.2 Association of blood metals co-exposure with renal parameters

Next, we examined associations of  $B_{mix}$ , comprised of Hg, Pb, and Cd measured in blood, with each of the 5 renal parameters. The index  $B_{mix}$  was associated with SUA, but not urine albumin, BUN, eGFR, or SBP (Table 3). Specifically, per each decile increase in  $B_{mix}$ , SUA was 0.6% (95%CI: 0.0, 1.3) higher, with weights indicating Pb (43%) Hg (33%) and Cd (24%) all contributed to this association.

Survey-weighted single chemical analyses indicated that SUA was 0.8% (95%CI: 0.5, 1.2) higher per decile increase in blood Pb, the highest contributing metal to the  $B_{mix}$  mixture, but we found no significant association with blood Hg or Cd (Table A1). Additionally, a decile increase in blood Pb was associated with 0.5% (95%CI: -0.9, 0.0) lower eGFR.

### 3.3 Stratification by sex

Examination of potential sex-specific associations with  $U_{mix}$  or  $B_{mix}$  indicated that co-exposure effects differed between males and females (Table 4). A significant association between BUN and  $U_{mix}$  was observed among males only, where each decile increase of  $U_{mix}$  was associated with 2.6% (95%CI: 1.2, 4.0) higher BUN. Conversely, a significant association between eGFR and  $U_{mix}$ , was observed only in females, where eGFR was 2.0% (95%CI: 0.8, 3.2) higher for each decile increase of  $U_{mix}$ . Similarly, the association of SUA and  $B_{mix}$  was present in females, with 1.7% (95%CI: 0.9, 2.5) higher SUA levels for each decile increase in  $B_{mix}$ , but this association was not significant among males. A positive association of urine albumin and  $U_{mix}$  was detected in both males and females, with a respectively higher urine albumin of 12.5% (95%CI: 4.9, 20.7) and 11.8% (95%CI: 4.0, 20.2) for each decile increase of  $U_{mix}$ .

### 3.4 Association of speciated urinary As with BUN

To further examine the association of BUN with  $U_{mix}$ , we examined an As-speciated WQS mixture that included speciated levels of As(III), As(V), AsB, AsC, DMA, and MMA in urine (Table A2). Each decile increase of the speciated urinary As index was associated with 2.2% (95%CI: 0.7, 3.8) higher BUN, driven by DMA (41%), AsC (33%), and AsB (24%).

## 4 DISCUSSION

Several studies have examined whether individual metal exposures are associated with adverse effects on kidney function, biomarkers of kidney damage, or blood pressure changes in children or adolescents (reviewed in [9, 10]); however, to our knowledge, this is the first study to examine the association between combined metals exposures (measured in blood or urine) and renal parameters in adolescents. Our modeling strategy was inherently two-tiered and stratified, in that we pursued both mixtures and single-chemical analyses, and discrete analyses of urine and blood exposure biomarkers. In these contexts, we identified relationships between adolescents' co-exposures to toxic metals and renal parameters and found that: i) metals assessed in urine (e.g. As, Pb, Hg, and Cd) were associated with increased eGFR, BUN, and urine albumin; ii) blood levels of Pb, Hg, and Cd were associated with increased SUA; iii) there were sex-specific associations of urine metal levels (e.g. Cd and Pb) with increased eGFR, BUN, as well as with blood metals and increased SUA.

Our findings suggest an association between combined exposures to Hg, As, Cd, and Pb, as measured in urine, and higher eGFR. We observed positive associations with eGFR when urine metals were combined as an index of co-exposure, and when assessed separately. One explanation of the findings is that joint metals exposure may lead to glomerular hyperfiltration. Although prior studies of metals and glomerular filtration report mixed findings [25–29], there is some evidence of associations between trace metal exposure (to As, Cd, and Pb for example) and hyperfiltration [30–33]. Glomerular hyperfiltration is an absolute increase in GFR well described for diabetes, obesity, and other conditions associated with damage to the glomerular filtration barrier, as well as physiological processes such as high protein loading. Hyperfiltration may precede albuminuria clinically, and is often a precursor of chronic kidney disease (CKD) [34]. As evidenced in studies of adults [35], hyperfiltration may precede later life reduction in GFR and renal decline by several years. Thus, early life hyperfiltration may be an important indicator of lifelong renal health. Another possible interpretation of our findings is that the observed positive associations are indicative of reverse causality, previously observed for single urinary metal analyses in NHANES [33, 36]. In this case, reduced renal function may affect metal excretion. Our findings are similar to those reported by Buser *et al.* (2016) who showed that increased urinary Cd and Pb levels were associated with increased eGFR, whereas an inverse relationship was observed with Cd levels measured in blood [36]. Similarly, these authors concluded that this relationship may either reflect hyperfiltration due to renal function decline or an adverse effect of low-level Cd exposure on kidney function. Still, there is a need for future studies to examine the contributions of combined metals exposure to eGFR in prospective cohorts and populations, and to examine the longitudinal impact of early life hyperfiltration on adult renal function.

We also observed an association between co-exposure to As and Pb measured in urine with higher BUN. To our knowledge, no prior studies have analyzed the combined effect of these metals on serum urea in children or adolescents. In single-metal analyses, we observed a marginally significant association with urine As and BUN, but no association with Pb. These findings underscore the potential for unique health effects resulting from exposures to



combinations of metals (i.e. metal co-exposures) that would otherwise be missed in single-metal analyses, and highlight the utility of WQS regression to detect complex associations. Additionally, our findings align with a study of rats treated with As via drinking water which showed increased BUN and serum creatinine, as well as elevated markers of liver toxicity [37]. In our analysis of the urinary “arsenic index”, urinary DMA, AsC, and AsB contributed the highest weights to the arsenic index associated with BUN. Interestingly, among these top-weighted components DMA and AsB were also described in a previous study of the NHANES population [22]. This supports the hypothesis that both organic and inorganic species may play a role in kidney dysfunction although the mechanisms remain unclear. Our findings further highlight the utility of the WQS co-exposure approach to detect complex associations.

In single-metal analyses, urine Pb and Cd concentrations were also associated with increased urine albumin. Two previous studies including adults (> 20 years) participating in NHANES 1999–2006 and 2007–2012 showed that chronic exposure to low levels of Cd was associated with changes in albumin and eGFR, and with coexposure to Pb, an increased risk for albuminuria and CKD [38, 39]. It is well-established that one of the earliest signs of Cd-induced renal damage is characterized by the presence of tubular proteinuria such as  $\beta$ 2-microglobulin as well as glomerular proteinuria such as albuminuria [40–42]. Intriguingly, we observed a significant association between the urinary metals mixture and urine albumin in males mainly driven by Cd concentrations. However, prospective longitudinal studies are needed to clarify the link between Cd exposure and renal biomarkers in adolescents, including sex-specific associations.

We observed no association between metals exposure and SBP. This is consistent with a number of studies on childhood environmental exposure and BP reviewed elsewhere [9]. However, we and others have previously demonstrated associations between in utero Pb exposure and subsequent childhood BP [43, 44]. Thus, it may be that prenatal and early childhood metal exposures contribute to changes in SBP that are no longer influenced by exposures in adolescence; however, prospective longitudinal studies are needed to explore this possibility.

Our study had many strengths. We assessed the relationship between multiple metal co-exposures and adolescent kidney function in a large, nationally representative population with carefully collected exposure, covariate and kidney parameter data. We included a breadth of kidney parameters and adjusted for factors that can influence metals exposure or kidney parameters among adolescents. For example, we adjusted for cotinine, a biomarker of nicotine exposure [24] which can co-occur with exposures to Pb, Cd, and As and other nephrotoxic contaminants found in tobacco products. Further we stratified by sex to examine sex-specific effects of metals with kidney parameters. Our statistical approach included both blood and urine metal biomarkers, separated into indices based on the respective matrix, as well as a separate arsenic-only index of subspecies measured in urine. We acknowledge that these distinct exposure biomarker matrices (blood and urine) and the specific mixture subcomponents (metals or speciated arsenic compounds) represent varying toxicological profiles for metabolism and excretion. For example, the biological half-lives of Hg, Cd, and Pb are respectively longer (days to years [45, 46] [47] [48]) than that of As (hours) which is

rapidly excreted in urine [21]. We note that given these considerations, a direct comparison of the two matrices is inappropriate. A strength of WQS regression is that the weights of individual metals shift with each model, allowing for different resultant indices reflecting effect sizes or potential underlying mechanism(s). Our findings support prior observations that associations of single metal exposures with renal outcomes is matrix-dependent [33]; we also observed this for mixed metal exposures. There are many ways we could have performed this analysis and we present the  $U_{\text{mix}}$  and  $B_{\text{mix}}$  approach as one. We are further encouraged by the consistency of results in the individual metal analyses and mixtures based approaches. Our selection of a mixtures-based strategy allowed us to simultaneously assess the combined effects of multiple co-exposures in this sample, which we complement with survey-weighted single-chemical analyses which emphasize mostly consistent effects that may be expected to generalize to a larger population. The WQS statistical approach ensures that our results are not confounded by issues of exposure collinearity and enables identification of major contributors (i.e. higher weighted metals) to the association with kidney parameters.

Our study has several limitations. Due to the cross-sectional study design, we cannot draw conclusions regarding temporality of the relationship between metals exposure and renal outcomes. Reverse causality remains a concern given that as renal function declines, changes in the excretion rate of metals in urine could contribute to elevated urinary metal levels [49]. This is a particular concern for Cd and As since these metals are eliminated from the body primarily in urine [31, 36]. Thus, as discussed above, observed associations between higher levels of urinary metals and renal parameters could be interpreted in two ways: 1) exposure to these metals resulted in altered renal function, or 2) urine metal levels are elevated due to increased filtration among individuals who already have poorer renal function. Another limitation is that NHANES collected a single spot urine sample for metals and urine albumin analysis. We acknowledge that blood and urine sample collection times were not standardized in NHANES and that metal levels as well as renal biomarkers can fluctuate over time [50]. Similarly, due to the cross-sectional study design, measures were collected at only a single time point, and longitudinal assessment of eGFR or blood pressure are required to define clinical kidney disease or pediatric hypertension, respectively. Challenges remain in defining the best approaches in studies of metals and kidney function [51], and interpretation of findings is especially in limited cross-sectional studies where directionality cannot be determined.

## 5 CONCLUSION

To our knowledge, this is the first study to examine combined metal co-exposures measured in blood or urine with renal parameters in adolescents. The findings suggest metals including As, Pb, Hg, Cd and their combinations may affect renal parameters, although potential reverse causation cannot be ruled out due to the cross-sectional study design. Our results support previous observations that urinary metal assessment presents challenging interpretations in studies of kidney function. Implications of early life low-level exposure to multiple metals on kidney function may have far-reaching consequences later in life in the development of hypertension, kidney disease, and renal dysfunction. Longitudinal studies

should further evaluate whether the combined effects of metals and other nephrotoxics may be risk factors of early life subclinical dysfunction.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## 9.: APPENDIX A

**Table A1.**  
**Comparison of results from the survey-weighted single chemical analyses and WQS regression of the matrix specific metal mixtures per renal outcome for the urine and blood mixtures ( $U_{mix}$  and  $B_{mix}$ ).**

The parameter estimate ( $\beta$ ) is reported in bold for significant single chemical or WQS mixture effects. The components with the highest weights are reported for mixtures with significant effects (ns: not significant).

Outcome	Matrix	Single chemical regression survey-weighted			WQS regression		Components with highest weight
		Metal	$\beta_{metal}$ (95%CI)	P-value	$\beta_{WQS}$ (95%CI)	P-value	
SUA	Urine	Cd	-0.0006 (-0.007, 0.006)	0.844	-0.0073 (-0.017, 0.002)	0.133	n/a
		Pb	-0.0054 (-0.012, 0.001)	0.115			
		Hg	-0.0051 (-0.011, 0.001)	0.103			
	As	0.0058 (-0.001, 0.012)	0.085				
	Blood	Cd	-0.0019 (-0.006, 0.003)	0.409	<b>0.0063 (0.000, 0.013)</b>	<b>0.051</b>	<b>Pb, Hg, Cd</b>
	Hg	0.0031 (-0.001, 0.007)	0.112				
BUN	Urine	Cd	-0.0073 (-0.018, 0.003)	0.171	<b>0.0159 (0.005, 0.027)</b>	<b>0.006</b>	<b>As, Pb</b>
		Pb	0.0040 (-0.005, 0.013)	0.389			
		Hg	0.0068 (-0.001, 0.015)	0.095			
		As	0.0120 (0.000, 0.024)	0.059			

Outcome	Matrix	Single chemical regression survey-weighted			WQS regression		Components with highest weight
		Metal	$\beta_{\text{metal}}$ (95%CI)	P-value	$\beta_{\text{WQS}}$ (95%CI)	P-value	
	Blood	Cd	0.0021 (-0.004, 0.008)	0.491	0.004 (-0.003, 0.011)	0.265	n/a
		Pb	0.0014 (-0.005, 0.008)	0.657			
		Hg	0.0059 (0.000, 0.012)	0.069			
eGFR	Urine	Cd	0.0057 (0.000, 0.012)	0.068	<b>0.0137 (0.007, 0.020)</b>	<b>&lt; 0001</b>	<b>Hg Cd As</b>
		<b>Pb</b>	<b>0.0098 (0.004, 0.015)</b>	<b>0.001</b>			
		<b>Hg</b>	<b>0.0056 (0.001, 0.010)</b>	<b>0.015</b>			
		As	0.0017 (-0.005, 0.008)	0.595			
	Blood	Cd	-0.0025 (-0.007, 0.002)	0.248	-0.0032 (-0.009, 0.002)	0.241	n/a
		<b>Pb</b>	<b>-0.0046 (-0.009, 0.000)</b>	<b>0.049</b>			
		Hg	-0.0008 (-0.005, 0.004)	0.725			
SBP	Urine	Cd	0.0001 (-0.002, 0.003)	0.914	-0.0007 (-0.005, 0.003)	0.713	n/a
		Pb	-0.0009 (-0.004, 0.002)	0.507			
		Hg	0.0001 (-0.002, 0.002)	0.925			
		As	0.0010 (-0.002, 0.004)	0.495			
	Blood	Cd	0.0010 (-0.001, 0.003)	0.216	0.001(-0.001, 0.003)	0.338	n/a
		Pb	-0.0003 (-0.002, 0.001)	0.712			
		Hg	0.0002 (-0.002, 0.002)	0.883			
Urine Albumin	Urine	<b>Cd</b>	<b>0.0668 (0.026, 0.107)</b>	<b>0.002</b>	<b>0.0731 (0.024, 0.123)</b>	<b>0.004</b>	<b>Cd Ha Pb</b>
		<b>Pb</b>	<b>0.0305 (0.003, 0.058)</b>	<b>0.030</b>			
		Hg	0.0195 (-0.007, 0.053)	0.219			
		As	0.0149 (-0.022, 0.052)	0.427			
	Blood	Cd	-0.0004 (-0.026, 0.025)	0.974	0.0035 (-0.025, 0.031)	0.807	n/a
Pb		-0.0072 (-0.028, 0.014)	0.502				

Outcome	Matrix	Single chemical regression survey-weighted			WQS regression		Components with highest weight
		Metal	$\beta_{\text{metal}}$ (95%CI)	p-value	$\beta_{\text{WQS}}$ (95%CI)	p-value	
		Hg	0.0018 (-0.022, 0.026)	0.881			

**Table A2**

Linear associations between the speciated As index measured in urine and BUN. Parameter estimates, 95% CIs, p-values, and component weights of the As index are reported as percentages.

Outcome	WQS Mixture result	Component Weights	
Blood Urea Nitrogen	$\beta_{\text{WQS}} = 0.0220$ , 95% CI (0.0068, 0.0372) $p = 0.005$	DMA	41%
		AsC	33%
		AsB	24%
		As(V)	1%
		As(III)	0%
		MMA	0%

**Table A3**

Number and percent of overall cohort outside of normal clinical range for eGFR, SUA, BUN, and ACR, along with the distribution of subjects within the overall cohort across clinical categories.

		<i>N</i> (% of overall cohort)
<i>eGFR clinical cutoffs</i>	<b>normal</b> 75 mL/min/1.73m <sup>2</sup>	2684 (100%)
	<b>abnormal</b> < 75 mL/min/1.73m <sup>2</sup>	1 (0%)
	<b>chronic kidney disease</b> 60 mL/min/1.73m <sup>2</sup>	4 (0%)
<i>SUA clinical cutoffs</i>	<b>normal</b> 12–18yo: 3.5–7.3 mg/dL 19yo: male: 3.6–8.4 mg/dL 19yo: female: 2.9–7.5 mg/dL	2481 (92%)
	<b>below normal range</b> 12–18yo: < 3.5 mg/dL 19yo: male: < 3.6 mg/dL 19yo: female: < 2.9 mg/dL	99 (4%)
	<b>above normal range</b> 12–18yo: > 7.3 mg/dL 19yo: male: > 8.4 mg/dL 19yo: female: > 7.5 mg/dL	109 (4%)
<i>BUN clinical cutoffs</i>	<b>normal</b> 12–15yo: 7–18 mg/dL 15–19yo: 6–23 mg/dL	2484 (92%)
	<b>below normal range</b> 12–15yo: < 7 mg/dL 15–19yo: < 6 mg/dL	190 (7%)
	<b>above normal range</b> 12–15yo: > 18 mg/dL	15 (1%)

	15–19yo: > 23 mg/dL	
<i>ACR clinical cutoffs</i>	<b>normal</b> < 20 mg/g	2175 (81%)
	<b>mild</b> 20–30 mg/g	165 (6%)
	<b>moderate</b> 30–300 mg/g	315 (12%)
	<b>severe</b> >300 mg/g	36 (1%)

**Table A4**

Range of the lower limits of detection (LOD) for each of the urine and blood metals that comprise the  $U_{mix}$  and  $B_{mix}$  mixtures and speciated urinary As, as reported by NHANES for cohorts between 2009 and 2014. Percent values below LOD are reported as a percentage of the combined cohort.

Mixture	Component (units)	LOD (range)	% below LOD
$B_{mix}$	Blood cadmium (ug/L)	0.10–0.16	46%
	Blood lead (ug/dL)	0.07–0.25	2%
	Blood mercury, total (ug/L)	0.16–0.28	23%
$U_{mix}$	Urinary cadmium (ug/L)	0.04–0.06	26%
	Urinary lead (ug/L)	0.03–0.10	4%
	Urinary mercury (ug/L)	0.05–0.13	19%
	Urinary arsenic, total (ug/L)	0.26–1.25	1%
Speciated urinary As mixture	Urinary arsenous acid (ug/L)	0.12–1.20	58%
	Urinary arsenic acid (ug/L)	0.79–1.00	98%
	Urinary arsenobetaine (ug/L)	0.40–1.19	63%
	Urinary arsenocholine (ug/L)	0.11–0.60	94%
	Urinary dimethylarsonic acid (ug/L)	1.70–1.91	52%
	Urinary monomethylarsonic acid (ug/L)	0.20–0.90	99%

## Abbreviations

<b>As(III)</b>	arsenous acid (arsenite)
<b>As(V)</b>	arsenic acid (arsenate)
<b>AsB</b>	arsenobetaine
<b>AsC</b>	arsenocholine
<b>As</b>	arsenic
<b>BUN</b>	blood urea nitrogen
<b>Cd</b>	cadmium

<b>DMA</b>	dimethylarsinic acid
<b>eGFR</b>	estimated glomerular filtration rate
<b>Hg</b>	mercury
<b>MMA</b>	monomethylarsonic acid
<b>NHANES</b>	National Health and Nutrition Examination survey
<b>Pb</b>	lead
<b>SBP</b>	systolic blood pressure
<b>SUA</b>	serum uric acid

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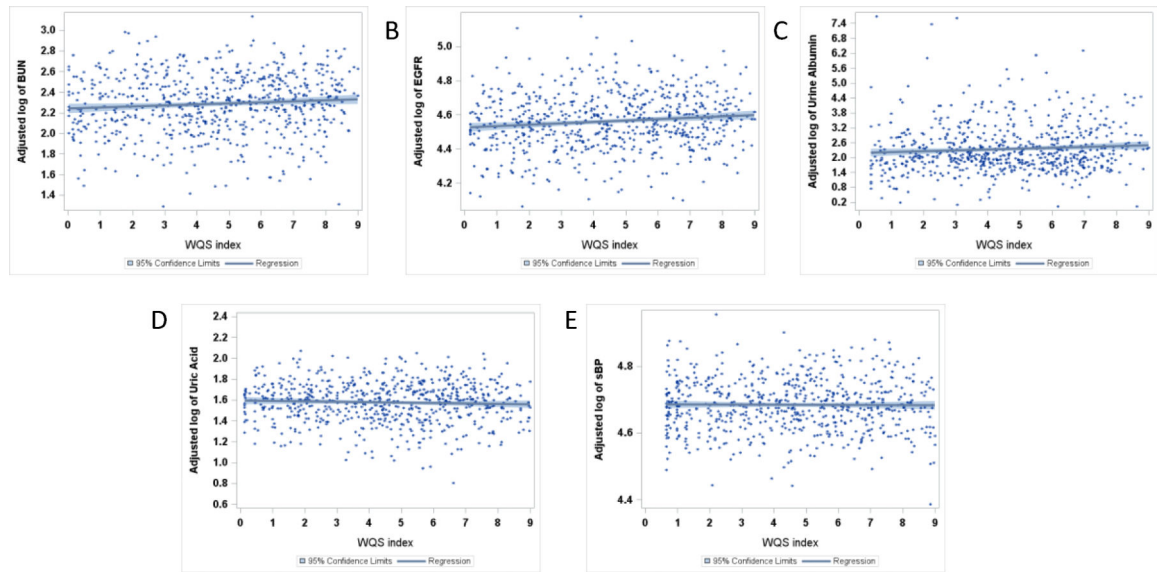
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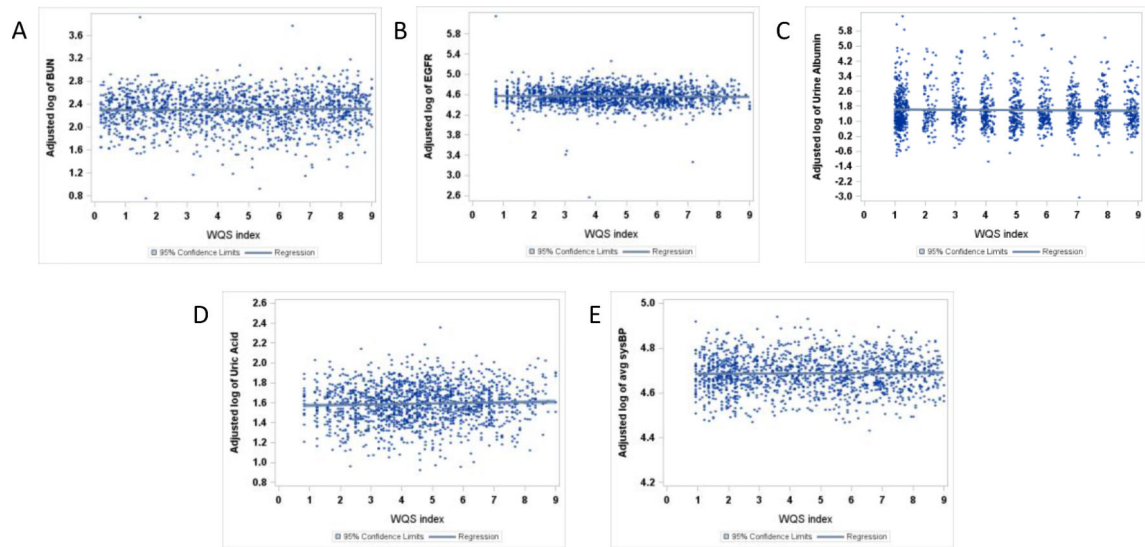
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### Highlights

- Co-exposure to metals is associated with altered renal parameters in adolescents.
- Urine As, Pb, Hg, and Cd were associated with increased eGFR, BUN, and albumin.
- Combined blood levels of Pb, Hg, and Cd were associated with increased SUA.
- Sex-specific effects were observed with increased eGFR, BUN, and SUA.



**Figure 1.** Covariate adjusted plots with confidence limits for the mean predicted value of the metals index in urine,  $U_{mix}$ , and the ln-transformed measures for BUN (A), eGFR (B), urine albumin (C), SUA (D), and SBP (E).



**Figure 2.** Covariate adjusted plots with confidence limits for the mean predicted value of the metals index in blood,  $B_{\text{mix}}$ , and the ln-transformed measures for BUN (A), eGFR (B), urine albumin (C), SUA (D), and SBP (E).

**Table 1.**  
**Demographics for 2,709 adolescents aged 12–19 years participating in NHANES 2009–2014.**

The table below details the mean (SD) or percentage of cohort for each variable are shown by NHANES cycle year and overall.

	NHANES cycle year			
	2009–10 <i>n</i> =1,102	2011–12 <i>n</i> =1,015	2013–14 <i>n</i> =592	Overall <i>n</i> =2,709
Age (yrs)	15.5 (2.2)	15.4 (2.3)	15.4 (2.3)	15.4 (2.3)
Height (cm)	165.8 (9.8)	165.4 (9.7)	164.6 (9.7)	165.4 (9.8)
BMI (kg/m <sup>2</sup> )	24.2 (5.8)	24 (6.1)	24.6 (6.8)	24.2 (6.2)
Cotinine, serum (ng/mL)	14.5 (51.4)	13.4 (58.8)	12.4 (52.4)	13.6 (54.5)
<i>Sex</i>				
Male	54%	52%	48%	52%
Female	46%	48%	52%	48%
<i>Race</i>				
Hispanic	39%	29%	37%	35%
Black	22%	30%	21%	25%
White	32%	22%	27%	27%
Other	7%	19%	16%	13%
<i>Parental Education</i>				
HS Grad or less	53%	51%	51%	52%
Some College	29%	28%	30%	29%
College Grad	18%	21%	19%	19%
Fish consumption in last 30 days	42%	49%	44%	45%

**Table 2.**  
**Survey-weighted renal outcomes and metal levels for 2,709 adolescents aged 12–19 years participating in NHANES 2009–2014.**

The mean (SD) of cohort for each outcome and exposure variable are shown by NHANES cycle year and overall.

	NHANES cycle years			Overall <i>n</i> =2,709
	2009–10 <i>n</i> =1,102	2011–12 <i>n</i> =1,015	2013–14 <i>n</i> =592	
<i>Outcomes</i>				
Avg Sys Blood Pressure (mmHg)	108.51 (21.79)	109.49 (19.73)	108.19 (13.54)	108.84 (19.43)
eGFR (mL/min/1.73 m <sup>2</sup> )	145.41 (70.72)	144.17 (49.16)	142.55 (35.32)	144.32 (56.67)
Blood urea nitrogen (mg/dL)	10.48 (5.15)	10.63 (4.43)	10.78 (7.47)	10.6 (5.49)
Uric acid (mg/dL)	5.08 (1.69)	5.05 (1.71)	5.12 (1.67)	5.07 (1.71)
Albumin creatinine ratio (mg/g)	30.8 (255.55)	27.13 (109.93)	31.55 (159.35)	29.47 (186.00)
Urine albumin (ug/mL)	37.78 (293.69)	33.77 (147.14)	34.3 (122.43)	35.44 (211.44)
<i>Urinary metals</i>				
Cadmium (ug/L)	0.10 (0.08)	0.10 (0.15)	0.08 (0.14)	0.09 (0.13)
Lead (ug/L)	0.41 (0.45)	0.36 (0.54)	0.29 (0.48)	0.36 (0.52)
Mercury (ug/L)	0.53 (0.97)	0.42 (0.93)	0.27 (0.8)	0.41 (0.94)
Arsenic, total (ug/L)	10.66 (19.14)	18.28 (93.28)	12.16 (36.71)	13.62 (61.96)
<i>Blood metals</i>				
Cadmium (ug/L)	0.20 (0.56)	0.20 (0.35)	0.19 (0.45)	0.20 (0.46)
Lead (ug/dL)	0.77 (0.86)	0.65 (1.11)	0.64 (1.08)	0.70 (1.01)
Mercury, total (ug/L)	0.80 (1.88)	0.62 (1.42)	0.55 (1.12)	0.68 (1.56)

**Table 3.**  
**Association of  $U_{\text{mix}}$  or  $B_{\text{mix}}$  metals indices with renal parameters.**

Results of WQS regression analysis per renal outcome for the urine and blood mixtures ( $U_{\text{mix}}$  and  $B_{\text{mix}}$ ). Parameter estimates ( $\beta_{\text{WQS}}$ ), 95% confidence intervals (CI), and p-values of the WQS mixture reported for each model. Component weights reported as percentages for mixtures with significant effects (n/a: not applicable).

Mixture	Outcome	WQS Mixture result	Component Weights	
$U_{\text{mix}}$	BUN	0.0159 (0.005, 0.027) $p = 0.006$	As Pb Hg Cd	72% 14% 11% 3%
	eGFR	0.0137 (0.007, 0.020) $p < 0.0001$	Hg Cd As Pb	61% 17% 13% 9%
	SUA	-0.0073 (-0.017, 0.002) $p = 0.130$	n/a	
	SBP	-0.0007 (-0.005, 0.003) $p = 0.710$	n/a	
	Urine albumin	0.0731 (0.024, 0.123) $p = 0.004$	Cd Hg Pb As	37% 33% 25% 6%
$B_{\text{mix}}$	BUN	0.0040 (-0.003, 0.011) $p = 0.265$	n/a	
	eGFR	-0.0032 (-0.009, 0.002) $p = 0.241$	n/a	
	SUA	0.0063 (0.000, 0.013) $p = 0.052$	Pb Hg Cd	43% 33% 24%
	SBP	0.0010 (-0.001, 0.003) $p = 0.338$	n/a	
	Urine albumin	0.0035 (-0.025, 0.031) $p = 0.807$	n/a	

**Table 4.**  
**Association of urine or blood metals indices with renal parameters – stratified by sex.**

Results of WQS regression analyses stratified by sex per renal outcome. The parameter estimates for the WQS mixture ( $\beta_{WQS}$ ) is reported in bold for significant WQS effects. The components with the highest weights are reported for mixtures with significant effects (n/a: not applicable).

Mixture	Outcome	Sex	N	$\beta_{WQS}$ (95%CI)	p-value	Components with highest weight	
U <sub>mix</sub>	SUA	Full Cohort	1,121	-0.0073 (-0.017, 0.002)	0.133	n/a	
		Female	555	-0.0025 (-0.016, 0.011)	0.729	n/a	
		Male	566	-0.0013 (-0.018, 0.016)	0.878	n/a	
	Urine albumin	Full Cohort	1,130	<b>0.0731 (0.024, 0.123)</b>	<b>0.004</b>	Cd, Hg, Pb	
		Female	559	<b>0.1116 (0.042, 0.185)</b>	<b>0.002</b>	Cd, As, Pb	
		Male	571	<b>0.1181 (0.048, 0.188)</b>	<b>0.001</b>	Cd, Pb, As	
	BUN	Full Cohort	1,121	<b>0.0159 (0.005, 0.027)</b>	<b>0.006</b>	As, Pb	
		Female	555	0.0092 (-0.011, 0.029)	0.372	n/a	
		Male	566	<b>0.0257 (0.012, 0.039)</b>	<b>&lt;.001</b>	As	
	eGFR	Full Cohort	1,121	<b>0.0137 (0.007, 0.020)</b>	<b>&lt;.0001</b>	Hg, Cd, As	
		Female	555	<b>0.0196 (0.008, 0.031)</b>	<b>0.001</b>	As, Hg, Pb, Cd	
		Male	566	0.0129 (-0.001, 0.027)	0.072	n/a	
	SBP	Full Cohort	1,001	-0.0007(-0.005, 0.003)	0.713	n/a	
		Female	488	-0.0016 (-0.007, 0.003)	0.520	n/a	
		Male	513	0.0001 (-0.005, 0.005)	0.962	n/a	
	B <sub>mix</sub>	SUA	Full Cohort	2,634	<b>0.0063 (0.000, 0.013)</b>	<b>0.051</b>	Pb, Hg, Cd
			Female	1,266	<b>0.0168 (0.009, 0.025)</b>	<b>&lt;.0001</b>	Pb, Hg
			Male	1,368	0.0076 (-0.001, 0.016)	0.073	n/a
Urine albumin		Full Cohort	2,636	0.0035 (-0.025, 0.031)	0.807	n/a	
		Female	1,264	-0.0101 (-0.056, 0.036)	0.667	n/a	
		Male	1,372	-0.015 (-0.044, 0.014)	0.313	n/a	
BUN		Full Cohort	2,634	0.004 (-0.003, 0.011)	0.265	n/a	
		Female	1,266	0.0035 (-0.008, 0.015)	0.540	n/a	
		Male	1,368	0.0051 (-0.007, 0.017)	0.409	n/a	
eGFR		Full Cohort	2,634	-0.0032 (-0.009, 0.002)	0.241	n/a	
		Female	1,266	<b>-0.0111 (-0.018, -0.004)</b>	<b>0.002</b>	Pb, Cd	
		Male	1,368	-0.0008 (-0.007, 0.005)	0.795	n/a	
SBP		Full Cohort	2,414	0.0010 (-0.001, 0.003)	0.338	n/a	
		Female	1,158	-0.0013 (-0.004, 0.002)	0.403	n/a	
		Male	1,256	-0.0011 (-0.005, 0.003)	0.565	n/a	