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A Dose-Response Study of Arsenic Exposure and Markers of Oxidative Damage in Bangladesh

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Abstract

Objective—To evaluate the dose-response relationship between arsenic exposure and markers of oxidative damage in Bangladeshi adults.

Methods—We recruited 378 participants drinking from wells assigned to five water arsenic exposure categories; the distribution of subjects was as follows: 1) <10 µg/L (n=76); 2) 10–100 µg/L (n=104); 3) 101–200 µg/L (n=86); 4) 201–300 µg/L (n=67); and 5) > 300 µg/L (n=45). Arsenic concentrations were measured in well water, as well as in urine and blood. Urinary 8-oxo-2'-deoxyguanosine (8-oxo-dG) and plasma protein carbonyls were measured to assess oxidative damage.

Results—None of our measures of arsenic exposure were significantly associated with protein carbonyl or 8-oxo-dG levels.

Conclusions—We found no evidence to support a significant relationship between chronic exposure to arsenic-contaminated drinking water and biomarkers of oxidative damage among Bangladeshi adults.

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Keywords

arsenic; oxidative damage; 8-oxo-dG; protein carbonyls; Bangladesh

INTRODUCTION

Chronic exposure to arsenic (As)-contaminated drinking water is associated with increased risk for cancers of the skin, lungs, kidney, bladder, and liver (1). In addition, evidence suggests As exposure increases the risk of developing cardiovascular disease (2, 3, but also see 4, 5). Some research also supports a link between As and type 2 diabetes (6-8), although this subject is controversial (9, 10). In Bangladesh alone, tens of millions of people are exposed to As-contaminated drinking water, putting them at increased risk for the attendant health problems (11).

While the modes of action by which As causes disease are not completely understood, an increased level of oxidative stress is widely believed to be one possible mechanism (12). Additional potential mechanisms, many of which are not mutually exclusive, include altered DNA repair capacity (13, 14), epigenetic dysregulation (15-17), enzyme inhibition (18), endocrine disruption (19, 20), and chromosomal instability (21-23). Reactive oxygen species (ROS) have the potential to damage critical molecules in the cell, including DNA and proteins. Increased levels of free radicals and oxidative damage have been observed in various types of cancers (24, 25), as well as chronic conditions such as cardiovascular disease (26, 27) and diabetes (28). Thus, oxidative stress has been implicated in many of the conditions linked to As exposure, though the causal relationship between ROS and these health outcomes remains unclear.

In vitro and *in vivo* studies indicate that As induces oxidative damage. For example, treatment of human-hamster hybrid fibroblast cells, rat lung epithelial cells, and breast-cancer cells with sodium arsenite at doses ranging from 2–15 μ M has been shown to result in higher levels of ROS and 8-oxo-2'-deoxyguanosine (8-oxo-dG), a biomarker of DNA damage (29-31). Similarly, rats exposed to either 100 mg/L sodium arsenite or 200–400 mg/L dimethylarsenic acid (DMA) in drinking water display increased levels of various markers of oxidative stress, including glutathione disulfide (GSSG), malondialdehyde (MDA), a marker of lipid oxidation (32), and 8-oxo-dG (33, 34). It should be noted that the levels of As exposure used in these studies are orders of magnitude higher than those humans are exposed to via naturally contaminated drinking water.

Some, but not all, studies in humans have yielded evidence indicating that As exposure is associated with oxidative damage. Three population-based studies with sample sizes greater than 100 and detailed information about potential confounders have been performed to assess the relationship between As and DNA damage, as measured by urinary 8-oxo-dG. One reported a significant positive association between urinary As and 8-oxo-dG in 212 pregnant women in Matlab, Bangladesh (35). The other two studies found no such association. One of these null studies was performed in Arizona and Sonora, where levels of drinking-water As were relatively low (< 40 μ g/L, $n=124$) (36), and the other took place in the Andes of northern Argentina, where exposure was higher (median water As ~ 200 μ g/L,

n=108) (37). Finally, a recent study on the same Bangladeshi sample investigated in the present article reported that water, urinary, and blood As levels were all inversely associated with levels of blood glutathione (GSH), an important endogenous antioxidant (38). However, As exposure was not linked to levels of blood glutathione disulfide (GSSG), the oxidized form of GSH that increases under conditions of oxidative stress (38). In summary, evidence supporting a causal relationship between chronic As exposure and measures of oxidative damage in humans has not yet been firmly established.

In this study, we set out to evaluate the dose-response relationship between As exposure and oxidative damage. We conducted a cross-sectional study of 378 individuals chronically exposed to drinking water As in Araihaazar, Bangladesh. A wide range of water As concentrations is present in this area, and detailed information about exposure and potential confounders is available. We collected As measurements for drinking water as well as for blood and urine from each individual in the study. Finally, we measured two biomarkers of oxidative damage: (1) plasma levels of protein carbonyls, a measure of protein damage, and (2) urinary 8-oxo-dG, to investigate damage to DNA.

METHODS

Study Overview

The study site, Araihaazar, is located roughly 30 km east of Dhaka, Bangladesh. Araihaazar is one of 509 thanas, or administrative units, in Bangladesh. In the year 2000, the Health Effects of Arsenic Longitudinal Study (HEALS) cohort began, eventually recruiting roughly 30,000 adults (39). Every two years, both As exposure and the development of various health outcomes are assessed in study participants. A subset of the HEALS cohort study was recruited into the Folate and Oxidative Stress (FOX) study between February and August of 2008, forming the basis of this investigation.

Study Sample

Study participants between the ages of thirty and sixty-five years were selected based on their well water As concentrations, so that the final study sample represented the full range of water As values in the region. We set out to recruit seventy-five individuals in each of five water As categories: 1) $< 10 \mu\text{g/L}$ (corresponding to the World Health Organization's drinking water standard); 2) $10\text{--}100 \mu\text{g/L}$; 3) $101\text{--}200 \mu\text{g/L}$; 4) $201\text{--}300 \mu\text{g/L}$; and 5) $> 300 \mu\text{g/L}$. This approach was chosen to maximize the ability to study dose-response relationships. Exclusion criteria included 1) women currently pregnant; 2) individuals taking nutritional supplements; 3) individuals who had not been drinking water from their current well for at least 3 months; and 4) individuals with diseases known to be associated with oxidative stress, such as diabetes, cardiovascular, or renal disease. As a result of water remediation and well-switching interventions between the year 2000 and the time of recruitment for the FOX study, some water As categories included more or less than the seventy-five participants originally planned. The final study sample totaled three hundred and seventy-eight participants. Well water As concentrations were re-measured at the time of recruitment into the FOX study. The distribution of subjects within water As categories

was as follows: 1) $< 10 \mu\text{g/L}$ ($n=76$); 2) $10\text{--}100 \mu\text{g/L}$ ($n=104$); 3) $101\text{--}200 \mu\text{g/L}$ ($n=86$); 4) $201\text{--}300 \mu\text{g/L}$ ($n=67$); and 5) $> 300 \mu\text{g/L}$ ($n=45$).

At each recruitment visit, a trained staff member described the purpose of the study to the participant. A Bangladeshi field staff physician read an approved assent form to each participant, after which oral informed consent was obtained and a subsequent visit to the field clinic was scheduled. At the field clinic, a trained interviewer administered a detailed questionnaire to each participant and a physician collected a venous blood sample.

This study was approved by both the Bangladesh Medical Research Council (BMRC) and the Institutional Review Board at Columbia University Medical Center.

Collection of Biological Samples

Each participant came to our field clinic in Araihaazar where blood and urine samples were collected and processed. Spot-collected urine samples were stored in 50-mL acid-washed tubes and frozen at -20°C . Blood was collected into EDTA-containing tubes; not all participants were fasting at the time of collection. Plasma samples were immediately separated at our field clinic, and blood and plasma samples were frozen in -80°C freezers in Araihaazar. Samples were later transported in batches to Dhaka on dry ice, and stored in our Dhaka lab at -80°C . All samples were transported in coolers with dry ice to Columbia University, where they were analyzed.

Laboratory Assays

Water As—Our field sample collection and laboratory analysis procedures have been described elsewhere in detail (40, 41). Briefly, water samples were collected in 20-mL polyethylene scintillation vials. At least 48 hours prior to analysis, high-purity Optima HCl (Fisher Scientific, Pittsburg, PA, USA) was used to acidify the samples to 1% (42). After being diluted 1:10, water samples were analyzed using high-resolution inductively coupled plasma mass spectrometry (ICP-MS). A germanium (Ge) spike was added to correct fluctuations in instrument sensitivity. The detection limit of the method is typically $< 0.2 \mu\text{g/L}$. The intra- and inter-assay coefficients of variation (CVs) for the assay were 6.0% and 3.8%, respectively.

Blood As—Whole blood As was measured using ICP-MS with a dynamic reaction cell, or ICP-MS-DRC (43, 44), with the modifications to the blood sample preparation process suggested by the Laboratory for ICP-MS Comparison Program, Institut National de Sante Publique du Quebec. Briefly, whole blood samples were thawed, mixed, then diluted 50X with a solution containing 1% HNO_3 , 1% Methanol, 0.2% Triton-X-100, and 0.5% NH_4OH . Diluted samples were centrifuged for 10 minutes at 3,500 rpm, and the analysis was performed upon the supernatant. Matrix-induced interference was corrected using an iridium (Ir) internal standard and the instrument's DRC technology feature. Quality control samples with known As concentration were obtained from the Quebec Institute and run each time the instrument was calibrated, as well as after every 10 samples. For study samples, the intraprecision and interprecision coefficients were 2.1% and 4.9%, respectively. Total blood As was obtained by adding inorganic As, MMA, and DMA concentrations.

Urinary As—Urinary As was measured using the Aanalyst 600 graphite furnace system (PerkinElmer, Shelton, CT, USA), as described previously (45). The intraclass correlation coefficient between the samples calibrated by the Quebec laboratory and our laboratory's values was 0.99. Specific gravity was measured using a handheld refractometer (TS 400, Reichert, Depew, NY). In addition, urinary As metabolites were measured as previously described (46). The method involves the separation of arsenobetaine (AsB), arsenocholine (AsC), arsenate, arsenite, monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) using high-performance liquid chromatography, followed by detection using ICP-MS. The intra- and inter-assay coefficients of variation (CVs) for urinary As were 3.9% and 5.6%, respectively, for quality-control samples. For study samples, the intra- and inter-assay CVs were 3.8% and 5.1%, respectively. Total urinary As was obtained by adding inorganic As, MMA, and DMA concentrations.

8-oxo-dG—8-oxo-dG was measured in urine using the 'New 8-OHdG Check' ELISA kit at the Genox Corporation's laboratory (Baltimore, MD). Prior to analysis, samples were centrifuged at 6,000 g for 5 minutes in order to remove particulate matter. Samples were measured in triplicate. The detection level for this assay is 0.64 ng/mL urine. The average intra-assay coefficient of variation and inter-assay coefficient of variation were calculated using control samples run in triplicate on 21 plates; they were 7.5% and 7.7%, respectively.

Protein carbonyls—Plasma protein carbonyls were measured using non-competitive ELISA based on the method described by Winterbourn and Buss (47), as explained in detail in Zipprich et al (48). Briefly, the protein concentration of each sample was measured using a Bicinchoninic Acid Kit (Sigma-Aldrich, St Louis, MO). Proteins were derivatized using 2,4-dinitrophenylhydrazine. This was followed by the addition of a primary antibody (anti-dinitrophenyl-KLH Rabbit + IgG 1:1500) and then a secondary antibody (streptavidine-biotinylated horseradish peroxidase complex). 3,3', 5,5'-tetramethylbenzidine, or TMB, was used as a visualizing reagent, after which the reaction was stopped using sulfuric acid and plates were read at 450 nm. Samples were measured in duplicate. The average intra-assay and inter-assay coefficients of variation were calculated using controls run in duplicate on 12 plates; they were 2.0% and 13.8%, respectively.

Statistical Analysis

Descriptive statistics were calculated to describe the sample characteristics, overall and by sex. To examine bivariate associations between variables, Spearman correlations were used for two continuous variables, and t-tests were used to detect mean differences in continuous variables between two levels of binary variables, including sex. Prior to performing t-tests, the appropriate transformations were applied to variables with skewed distributions.

Linear regression models were used to examine the relationship between each As exposure variable and protein carbonyls, with and without adjustment for potential confounding factors. Because 8-oxo-dG had a skewed distribution, with 18% (n=68) of values below the detection limit of 0.64 ng/mL urine, we set below-the-detection-limit values to half the detection limit, or 0.32 ng/mL urine; dichotomized the variable at its median, adjusted for urine concentration (10.9 ng/mL urine); and used logistic models to assess the association

between 8-oxo-dG levels and each of the As variables, adjusting for covariates. We derived odds ratios with 95% confidence intervals from the model parameters. Candidate control variables included those suggested in the literature or our previous studies or those that showed associations with both As exposure and an outcome variable at a significance level of 0.10 in the bivariate analysis. Control variables were included in the final models if adding them resulted in a change to B of at least 10%. Urinary 8-oxo-dG and urinary As levels were adjusted for urine concentration by applying the following equation: [(sample mean for specific gravity – 1)/(individual specific gravity – 1)]×(individual urinary analyte value) (49). Models with and without adjustment for urine concentration were run. As the main predictors in our models, markers of As exposure were investigated both as continuous and categorical variables, divided into water groups or quintiles. Variables with skewed distributions were transformed to reduce the impact of extreme values or improve the fit of models. Blood and urinary As were transformed using the natural logarithm, and water As was square root transformed.

RESULTS

Subject Demographics

Demographic information on the subjects is presented in Table 1. The sample included a similar number of males ($n=184$) and females ($n=194$). The median age of our participants was 42 years and most had only a few years of education (median duration: 4 years for males, 1 year for females). Median water As levels were 114.0 $\mu\text{g/L}$, but ranged from 0.4 – 700 $\mu\text{g/L}$. Smoking was common among males (55.6%) but not among females (2.6%), and betel nut was used equally by both sexes (~35%). Fifty-eight percent of individuals in our study owned a television.

No potential confounders were associated with markers of oxidative stress at the $p<0.05$ level. However, we observed a trend toward an inverse correlation between years of education and plasma protein carbonyl levels (Spearman's $r=-0.09$, $p=0.07$). There was also a trend toward television owners being more likely to have urinary 8-oxo-dG values above the median (53.6% of owners vs 44.9% of non-owners, $\chi^2=2.78$, $p=0.10$).

Plasma Protein Carbonyls

No significant associations between water, blood, or urinary As and plasma protein carbonyl levels were detected, either in bivariate analysis or in linear regression analysis, with As variables included as continuous and categorical variables. The results of linear regression analyses with As exposure variables included as continuous variables are presented in Table 2. No potential confounders were associated with both protein carbonyl and arsenic levels. We fit the models adjusted for current smoking status and found no appreciable changes in the results. Mean protein carbonyl values for each water As exposure group are shown in Table 3.

Urinary 8-oxo-dG

No significant positive associations between As exposure and urinary 8-oxo-dG levels above the median were detected, whether As variables were included in the models as continuous

or categorical variables. The results of logistic regression analyses with As exposure variables included as continuous variables are presented in Table 4. Among the potential confounders, only television ownership was associated with both 8-oxo-dG and arsenic levels and thus included in the models. When current smoking status was included in the models, no appreciable differences in the results were found. Models with and without adjustment for urine concentration gave similar results, except for the unadjusted model that included urinary As as a predictor of urinary 8-oxo-dG (Table 4). A strong association was found between these two variables ($p < 0.0001$), as would be expected since they are both strongly influenced by urine concentration. Mean 8-oxo-dG levels values for each water As exposure group are shown in Table 3.

DISCUSSION

Given the premise that oxidative damage to the cell's structural molecules lies on a causal pathway leading from As exposure to associated diseases, one would expect to observe elevated levels of oxidative damage in individuals who have been chronically exposed to As but have not yet become ill. In this cross-sectional, dose-response study of generally healthy individuals, we found no evidence of an association between water, blood, and urinary As levels and plasma protein carbonyl or 8-oxo-dG levels.

This study has a number of strengths. First, a wide range of water As levels, from 0.4–700 $\mu\text{g/L}$, was examined. Second, subjects were selected by exposure level to yield a relatively even distribution across a wide range of water As concentrations, an asset when studying potential dose-response relationships between exposure and markers of oxidative damage. Third, three measures of As exposure were examined (well water, blood, and urinary As levels), in order to optimize our assessment of exposure status. Fourth, extensive information on possible confounding variables including age sex, BMI, education level, cigarette and betel nut use, and television ownership (an indicator of socioeconomic status), was collected. Fifth, two different measures of oxidative damage were examined, one targeting damage to proteins (plasma protein carbonyls) and the other to DNA (urinary 8-oxo-dG). These biomarkers measure oxidative damage over different time periods: 8-oxo-dG has a circulating half-life of hours (50) and protein carbonyls reflect exposure over a period of weeks (51). Interestingly, we found no significant association between the two biomarkers themselves (Spearman's $r = -0.006$, $P = 0.91$), adding support to previous reports of a lack of association between levels of oxidative damage to different types of biomolecules in humans (52, 53). Our data suggest that a single oxidative damage biomarker may be insufficient for drawing conclusions about oxidative damage in general. Finally, with 378 participants, our study was considerably larger than previous population-based studies of As exposure and oxidative damage.

There are also several potential limitations of this study. First, we measured oxidative damage in plasma and urine samples but damage leading to As-related skin lesions and cancers may be tissue specific. It is possible that oxidative damage is occurring in tissues not sampled in this study. However, previous positive reports of As-associated oxidative damage have also examined biomarkers in plasma (54, 55) and urine (35, 56), as was done here. In addition we note that peripheral blood mononuclear cells are a target for As toxicity

(57), so if chronic As toxicity to these cells occurs via oxidative damage one might expect to observe evidence of it in plasma. Moreover, urinary 8-oxo-dG is viewed as a biomarker of “whole-body” damage (58), so if oxidative damage to DNA is significantly increased in other target tissues one would expect this to be reflected in levels of this marker in urine.

The second limitation is that our study focused on whether drinking water As affected biomarkers of oxidative damage but it has been shown that exposure to As can also occur through food items; this contribution is relatively speaking greater when water As levels are low (59). In this study data on dietary As exposure were not available. However, we did measure As levels in both blood and urine which reflect exposure from all sources including the diet.

A third potential limitation is that the two markers of oxidative damage examined plasma protein carbonyls and urinary 8-oxo-dG, were not associated with variables that might be expected to affect oxidative stress levels such as current smoking status (60-62). An association between smoking and these biomarkers has not been detected in many other studies however (63-68). In a low-income setting such as Araihaazar it is likely that many sources of oxidative damage such as air pollution (69, 70), are present. Under these conditions the level of damage associated with smoking may be relatively insignificant. Like us other researchers have also failed to find an association between age and plasma protein carbonyls (71, 72) as well as urinary 8-oxo-dG (73). Similarly, past studies have failed to find an association between sex and plasma protein carbonyls (74) and urinary 8-oxo-dG, after controlling for urine concentration (75).

The mixed data from human population studies reported here and elsewhere contrasts with the stronger and more consistent evidence that As exposure results in oxidative damage obtained from *in vitro* (29-31) and animal experiments (32-34). Though one important difference that may explain this discrepancy is the ready availability of target tissues in cell culture and animal experiments, it has also been shown that As exposure raises the levels of oxidative damage in blood (32) and urine (34) samples from rats. Thus, based on laboratory experiments, one might expect that oxidative damage markers in blood and urine in human studies would also rise with As exposure. The doses of drinking water As used in animal experiments are often orders of magnitude higher and of shorter duration than those encountered by humans in natural settings (e.g., 29, 32-34). It is possible that humans can better adapt to lower levels of As exposure over time and to some extent compensate for As-induced metabolic changes thereby limiting oxidative damage (76). In addition species-specific differences in As metabolism and related health-effects have been well documented (77-79), and it is possible that such differences also apply to As-induced oxidative damage.

In summary in this study designed to detect dose-response effects of As on two biomarkers of oxidative damage in humans we found no evidence that exposure to As increases levels of oxidative damage. No association was found between As and plasma protein carbonyl or 8-oxo-dG levels. A previous study on this sample has shown that As exposure is linked to a more oxidized redox state as measured by Eh values based on blood GSH and GSSG (38). It is possible that rather than manifesting in oxidative damage As-induced changes in the redox state could influence the activity of redox-sensitive enzymes and thereby have downstream

effects such as altered DNA methylation (80) or changes in gene expression (81). Future studies should clarify the downstream effects of the more oxidized cellular environment associated with As exposure.

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CLINICAL SIGNIFICANCE

Experimental evidence implicates oxidative damage in arsenic toxicity. In this study of 378 healthy Bangladeshi adults chronically exposed to a wide range of water arsenic levels no link between arsenic exposure and oxidative damage to DNA or proteins was observed.

Table 1

Descriptive statistics for FOX participants

	Total Sample N=378	Males n=184	Females n=194	<i>p</i>^a
	Median (Range)			
Age (years)	42 (30–63)	44 (31–63)	40 (30–62)	0.006
Education (years)	3 (0–16)	4 (0–14)	1 (0–16)	0.11
BMI (kg/m ²)	19.7 (13.8–35.3)	19.0 (14.2–32.3)	20.7 (13.8–35.3)	0.003
Water As (µg/L)	114.0 (0.4–700.0)	114.1 (0.4–700.0)	113.1 (0.4–492.8)	0.67
Blood As (µg/L)	12.5 (2.5–53.1)	14.1 (3.7–53.1)	11.5 (2.5–37.9)	<0.001
Urinary As (µg/L) ^b	170.4 (12.9–1088.3)	171.2 (15.2–1015.0)	170.1 (12.9–1088.3)	<0.001
8-oxo-dG (ng/mL urine) ^b	10.9 (0.5–301.3)	10.9 (0.6–71.9)	11.0 (0.5–301.3)	<0.001
Protein carbonyls (ng/g proteins)	243.0 (75.1–383.0)	249.2 (75.1–362.9)	235.1 (165.5–383.0)	0.39
	%			
Current smoker	28.2	55.6	2.6	<0.0001
Current betel nut user	35.8	35.0	36.5	0.77
Television Owner	58.2	59.2	57.2	0.69

^a *P*-values obtained from testing for differences between the sexes using *t*-tests for continuous variables and χ^2 tests for binary variables.

^b Adjusted for specific gravity

Table 2Associations between arsenic exposure and plasma protein carbonyls using multiple linear regression models^a

	Full Sample N=378	Males n=184	Females n=194
	B (95% CI)		
Water As (µg/L)	0.17 (-0.56, 0.90)	0.41 (-0.59, 1.41)	-0.12 (-1.16, 0.92)
Blood As (µg/L)	3.44 (-5.85, 12.7)	0.86 (-12.3, 14.0)	5.22 (-8.25, 18.7)
Urinary As (µg/L): not adjusted for specific gravity	-0.94 (-4.65, 2.77)	-2.57 (-8.37, 3.22)	0.14 (-4.69, 4.96)
Urinary As (µg/L): adjusted for specific gravity	-0.37 (-4.94, 4.20)	-0.40 (-7.44, 6.64)	-0.45 (-6.47, 5.57)

^aBlood As and urinary As were log transformed prior to inclusion in models. Water As was square-root transformed.

Table 3

Mean levels of plasma protein carbonyls and urinary 8-oxo-dG for each of five water As exposure groups^a

Water As Exposure Group	Plasma Protein Carbonyls	Urinary 8-oxo-dG
Group 1: < 10 µg/L As n=76	253.14 ± 40.43	13.09 ± 34.16
Group 2: 10–100 µg/L As n=104	233.96 ± 42.24	10.47 ± 7.30
Group 3: 101–200 µg/L As n=86	238.10 ± 42.66	12.80 ± 8.37
Group 4: 201–300 µg/L As n=67	245.48 ± 43.62	11.96 ± 10.35
Group 5: > 300 µg/L As n=45	253.99 ± 44.41	10.10 ± 5.52

^a8-oxo-dG levels were adjusted for urine concentration using specific gravity. The standard deviation of each mean value is shown.

Table 4

Odds ratios for the association between each arsenic exposure measure and 8-oxo-dG levels above the median derived from logistic regression models^a

	Full Sample N=377	Males n=184	Females n=193
OR (95% CI)			
Urinary Analytes Unadjusted for Specific Gravity			
Water As (µg/L)	1.02 (0.99, 1.06)	1.02 (0.97, 1.06)	1.03 (0.98, 1.09)
Blood As (µg/L)	0.79 (0.51, 1.21)	0.94 (0.52, 1.71)	0.65 (0.34, 1.23)
Urinary As (µg/L)	3.58 (2.70, 4.76)*	4.04 (2.62, 6.23)*	3.26 (2.25, 4.75)*
OR (95% CI)			
Urinary Analytes Adjusted for Specific Gravity			
Water As (µg/L)	1.03 (1.00, 1.07)	1.02 (0.97, 1.07)	1.05 (1.00, 1.10)
Blood As (µg/L)	0.89 (0.58, 1.36)	0.96 (0.53, 1.74)	0.87 (0.46, 1.63)
Urinary As (µg/L)	1.18 (0.95, 1.47)	1.06 (0.77, 1.47)	1.29 (0.97, 1.73)

*
p<0.0001

^aWater As was transformed using the square root. Blood As and urinary As were transformed using the natural logarithm. All models were adjusted for television ownership.