Excretion of Arsenic in Urine as a Function of Exposure to Arsenic in Drinking Water

Rebecca L. Calderon,¹ Edward Hudgens,¹ X. Chris Le,² Dina Schreinemachers,¹ and David J. Thomas³

¹Human Studies Division, National Health and Environmental Effects Research Laboratory, Research Triangle Park, North Carolina, USA; ²Department of Public Health Sciences, University of Alberta, Edmonton, Alberta, Canada; ³Experimental Toxicology Division, National Health and Environmental Effects Research Laboratory, Research Triangle Park, North Carolina, USA

Urinary arsenic (As) concentrations were evaluated as a biomarker of exposure in a U.S. population chronically exposed to inorganic As (InAs) in their drinking water. Ninety-six individuals who consumed drinking water with As concentrations of 8–620 μg/L provided first morning urine voids for up to 5 consecutive days. The study population was 56% male, and 44% was younger than 18 years of age. On one day of the study period, all voided urines were collected over a 24-hr period. Arsenic intake from drinking water was estimated from daily food diaries.

Comparison between the concentration of As in individual urine voids with that in the 24-hr urine collection indicated that the concentration of As in urine was stable throughout the day. Comparison of the concentration of As in first morning urine voids over the 5-day study period indicated that there was little day-to-day variation in the concentration of As in urine. The concentration of As in drinking water was a better predictor of the concentration of As in urine than was the estimated intake of As from drinking water. The concentration of As in urine did not vary by gender. An age-dependent difference in the concentration of As in urine may be attributed to the higher As dosage rate per unit body weight in children than in adults. These findings suggest that the analysis of a small number of urine samples may be adequate to estimate an individual’s exposure to InAs from drinking water and that the determination of the concentration of InAs in a drinking water supply may be a useful surrogate for estimating exposure to this metalloid.

Key words: arsenic, biomarker, drinking water, exposure, interindividual variability, intraindividual variability, United States. Environ Health Perspect 107:663–667 (1999).

http://ehpnet1.niehs.nih.gov/docs/1999/107p663-667/calderon.abstract.html

Chronic exposure of humans to inorganic arsenic (InAs) has been associated with increased risk of cancer (1–3), disorders of the peripheral vasculature (4), diseases of the cardiovascular (5) and cerebrovascular (6) systems, hypertension (7), diabetes (8,9), and reproductive failure (10). Accurate estimation of magnitude, pattern, and duration of exposure is critical for a better understanding of the dose–response relationships that underlie these adverse effects of chronic exposure to InAs. Typically, epidemiologic studies have relied on a few measures of arsenic (As) in drinking water to estimate exposure. The use of such studies to assess dose–response relationships has been questioned based on uncertainties about the accuracy of quantitation of As in the water supplies and in the reconstruction of exposure (11,12). Improved estimates of exposure to As may be obtained by quantitation of the amount of As excreted in the urine. However, there are uncertainties about the relationship between exposure to As and its clearance in urine as a marker of exposure to As in drinking water. Previous studies have been inconsistent in methodological uses for the collection of urine samples (e.g., spot urines vs. first morning urines, single collections vs. repeated samples) (13–15). Because these data have generally been analyzed and presented on a group basis, there is little information on intra- or interindividual variation in the output of As in urine independent of dose. In particular, there is little information on the extent of As exposure in U.S. populations that consume drinking water that contains As. The research described in this paper provides novel information on the pattern and extent of urinary As excretion in a U.S. population exposed to As in drinking water.

In the present study, we evaluated the excretion of As in urine in a Milliard County, Utah, population that is chronically exposed to InAs from drinking water. We found that the concentration of As excreted in urine was highly correlated with the concentration of InAs in the drinking water supply and the concentration of As in urine was remarkably stable throughout a single day and over a 5-day study period. These findings suggest that the analysis of a small number of urine samples may be adequate to estimate an individual’s exposure to InAs from drinking water and that the determination of the concentration of InAs in a drinking water supply may be a useful surrogate for estimating exposure to this metalloid.

Methods

Site selection. Study subjects were recruited from long-term residents of Millard County, Utah. The primary industry in this region is agricultural, and surface water is only used for crop irrigation. Drinking water supplies are obtained from public and private wells. The source of the InAs in drinking water is As that occurs naturally in the soil and rock strata. The likely sources of soil As are several extinct volcanos within the study area. The state of Utah has measured InAs concentrations in the wells used for drinking water and livestock in this area for more than 30 years. The InAs concentrations of wells in this area have been stable for many years and range from 2 to 260 μg/L (16).

Subject selection. The study population included mostly members of the Church of Jesus Christ of Latter-Day Saints. Possible confounding factors such as smoking, alcohol use, and caffeine consumption were minimal in this population. Subjects had not changed their place of residence in the previous 5 years. Approximately half of the subjects were recruited within the town of Delta, Utah, which obtains its drinking water from two wells with InAs concentrations below 20 μg/L. The remaining subjects were recruited from the area surrounding Delta, where the concentration of InAs in well water was > 20 μg/L. Multiple subjects were recruited from families. When possible, two adults and up to four children from 8 to 18 years of age were selected from a family. A family was excluded if one of its members was employed in the mining or smelting industries. Because all family members were sampled concurrently, subjects had to be available for an entire week of sampling. Each family’s drinking water was analyzed for InAs concentration. The final decision on study eligibility was based on the measurement of InAs concentration.

Address correspondence to R.L. Calderon, Human Studies Division, NHEERL, U.S. EPA, Research Triangle Park, NC 27711 USA. Telephone: (919) 966-7358. Fax: (919) 966-0655. E-mail: Calderon.Rebecca@epamail.epa.gov

We thank M. Schmitt (U.S. EPA), J.W. Southwick and J. Cowan (SRA Technologies), and L. Scanlon (Utah State Department of Health and Laboratory Science).

The research described in this article has been supported by U.S. EPA contract 68D20187. It has been subjected to agency review and does not necessarily reflect the views of the agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Received 5 January 1999; accepted 22 April 1999.
in the household water source, as performed by the state of Utah. The study group included families using the Delta municipal water system. The protocol for this study was approved by an institutional review board and all adult subjects signed an informed consent form. Parents signed assent forms for pediatric subjects.

**Exposure assessment.** After obtaining consent, each subject completed an exposure assessment questionnaire. This questionnaire requested demographic information, medical history, and length of residence in their present home. All subjects estimated their drug, alcohol, or tobacco usage and daily water consumption. Data on other environmental or occupational exposures and other exposures to As compounds (such as veterinary medicines or pesticides) were obtained with this questionnaire.

**Dietary intake and As intake estimation.** Study subjects kept a diet diary for 6 consecutive days starting on Monday. Each family was given measurement aids to estimate the amounts of different foods and beverages consumed. The food diaries used a mixture of different units such as cups, fluid ounces, cans, and gallons to collect consumption information. Total liters of drinking water consumed per day by each subject were estimated from water drunk during the day and the estimated amount of water contained in some foods prepared at home (e.g., beverages prepared from concentrates, soups, pasta, rice, and cooked oatmeal). Daily estimates of As consumption were calculated as the product of the number of liters consumed per day and the As concentration in the subject's drinking water source. Daily estimates were summed to calculate As intake over the 5-day study period. No As estimates were calculated for commercially precooked and prepared foods, locally produced meats and dairy products, and bottled or canned drinks prepared outside the study area. The contribution of canned, fresh, or home-grown vegetables washed or cooked in drinking water were ignored in this study because of the uncertainty about the amount of water absorbed during preparation.

**Sample collection and processing.** All study subjects, regardless of age, followed the same procedure to obtain a urine specimen. Subjects were asked to provide the first urine voided upon waking [first morning void (FMV)] for 5 consecutive days starting on Tuesday. On one of the 5 sampling days, all voids were requested for a 24-hr period; each void was collected in a separate container. Each subject was given a commodity specimen collection system (Sage Products, Inc., Crystal Lake, IL), a water-resistant laboratory marker, and a resealable plastic bag to contain the sample during transport. The subjects placed the bowl-shaped device into the commode and collected the void (up to a maximum of 700 mL of urine). After the lid was snapped tightly over the collection vessel, the plastic support webbing was removed from the vessel. On the day when more than one sample was collected, the subject recorded the approximate time of collection on the lid of the collection vessel. Each collection vessel was placed in the heavy duty plastic bag and the seal was closed. Urine samples were stored in a cold chest with frozen refrigerant packs provided for each family. Normally, the FMV was collected by the researchers each morning. On days when multiple voids were collected, the second and subsequent specimens were delivered to the field laboratory in the afternoon and on the following morning.

In the laboratory, urine samples were transferred to labeled 500-mL graduated polypropylene conical centrifuge tubes. The volume was determined and recorded in the sample log. Bladder cells were separated from urine by means of centrifugation at 500 × g for 10 min. After several minutes of centrifugation, urine aliquots were removed from the top of the sample with a disposable serological pipette and transferred to 15-mL polypropylene plug seal centrifuge tubes. To avoid oxidation of As in urine by residual acid, centrifuge tubes used in this study were not acid washed. These samples for As analysis were quick-freeze on dry ice and shipped on dry ice.

**Arsenic in water.** The analysis of InAs in the drinking water source was performed by the Utah State Department of Health, Division of Epidemiology and Laboratory Services (Salt Lake City, UT). Tap water samples were collected in the homes of potential subject families using acid-washed containers provided by the state laboratory and transported to Salt Lake City for analysis. Total As was determined using an ELAN 6000 ICP-mass spectrometer system (Perkin-Elmer, Norwalk, CT) using U.S. Environmental Protection Agency method 200.8 (17).

**Arsenic in urine.** Arsenite, arsenate, monomethylarsonic acid (MMA), and dimethylarsonic acid (DMA) were measured by high performance liquid chromatography (HPLC)/hydride generation/atomic fluorescence detection (HG/AFD) according to the procedure of Le and Ma (18). Briefly, the HPLC consisted of a Gilson model 307 HPLC pump (Gilson, Middleton, WI), a Rheodyne 6-port sampling valve (Rheodyne, Cotati, CA) with a 20-µL sample loop, and a 150 mm × 4.6 mm, 3-µm particle size C18 column (Phenomenex, Torrance, CA). Arsenicals were eluted with a mobile phase of 5 mM tetrabutylammonium hydroxide, 4 mM malonic acid, and 5% methanol (pH 5.8). The flow rate was 1.5 mL/min. The column was maintained at 70°C inside a model CH-30 column heater controlled by a model TC-50 temperature controller (Eppendorf, Westbury, NY). The mobile phase was preheated to the temperature of the column by using a 50-cm coil of stainless steel capillary tubing placed inside the column heater.

An HG atomic fluorescence detector (Excalibur 10.003; P.S. Analytical, Kent, UK) was used to detect As. The combination of HPLC and HG/AFD has been described previously (19). Effluent from the HPLC column meets at two T-joints with continuous flows of 3 M hydrochloric acid, and a solution of 1.3% sodium borohydride in 0.1 M NaOH is introduced with a peristaltic pump (Gilson). The resulting arsines are separated from liquid waste in a gas/liquid separator and carried by a continuous flow of argon to the atomic fluorescence detector. A Pentium computer (Digital, Maynard, MA) with Varian (Victoria, Australia) Star Workstation software and an analog-digital converter board was used to acquire and process signals from the detector.

Peak areas were used for quantitation. A minimum of three analyses was performed on each urine sample. The detection limits (3σ) for the four As species are 0.3 µg/L for arsenite, 0.5 µg/L for arsenate, 0.3 µg/L for MMA, and 0.5 µg/L for DMA. The limits of quantitation for these four compounds are 1.0, 1.6, 1.0 and 1.6 µg/L, respectively. Standard reference material urine (SRM 2670; National Institute of Standards and Technology (NIST), Gaithersburg, MD) was used to validate analysis. SRM 2670 consists of two bottles of urine—one containing an elevated concentration and one containing a normal concentration of As. The certified total As concentration in the elevated urine is 480 ± 100 µg/L. Arsenic in normal urine is not certified; however, the NIST provides a reference value of 60 µg/L. Replicate analyses of these standard reference materials using the method described above give concentrations of 517 ± 29 µg/L and 60 ± 7 µg/L, respectively, which are in good agreement with the certified and reference values. The total As concentration in urine samples presented in this paper is the sum of the concentrations of arsenite, arsenate, MMA, and DMA.

Creatinine in urine samples was determined by using an HPLC method with an ultraviolet/vis detection technique (20). Urine samples were diluted 50-fold with deionized water and a 10-µL aliquot was injected onto a 3.9 mm × 300 mm Bondelone C18 column (Phenomenex). The eluent was 50 mM sodium acetate (pH 6.5) in 98.2 (vol/vol) water/acetonitrile. The flow rate for the mobile phase was 1.0 mL/min. A
system consisting of a model DX300 pump (Dionex, Sunnyvale, CA), a 712 WISP autosampler (Waters, Milford, MA), and a model 484 tunable absorbance detector (Waters) was used. Detector wavelength was set at 254 nm. Peak area was measured for quantitation of creatinine.

Statistical methods. To determine if the FMV is representative of the combined 24-hr voids, the total As concentration [total micrograms arsenic per milligram creatinine (TAs/c)] in the FMV was compared with the mean TAs/c of all subsequent voids using graphical methods, Pearson correlation, and linear regression. To test for variation in the TAs/c among the FMV collected over the 5-day study period, the percent differences in TAs/c were calculated between each day’s FMV and the mean value for the FMV for the 5-day study period. The daily distributions of these percent differences were compared graphically with box plots. The associations between TAs/c in urine and the concentration of InAs in drinking water or the estimated intake of As were examined graphically and by statistical models. For these analyses, both the independent (drinking water As, estimated As intake) and dependent (urinary As) variables were log transformed. A mixed model (SAS Proc Mixed; SAS Institute, Cary, NC) was used to model the relation between the concentration of InAs in drinking water and the TAs/c. This procedure accommodates repeated sampling with missing observations. Variables for gender and age (< 18 years of age, ≥ 18 years of age) were included in this analysis. To determine whether reduction of the number of urine samples used for quantitation of total As affected the accuracy of the exposure assessment, we compared the between-subject variability and the standard error of the predictors by using separate models for 1, 2, 3, and 5 voids per subject. The effect from estimated As intakes from drinking water used was analyzed in a mixed model, including five voids per subject. These results were compared with those from the model that used the concentration of InAs in drinking water.

Table 1. Demographics of the study population.

| Characteristic | No. | %  |
|----------------|-----|----|
| Families       | 28  |    |
| Subjects       | 96  |    |
| Age            |     |    |
| < 18 years     | 42  | 43.8|
| ≥ 18 years     | 54  | 56.2|
| Gender         |     |    |
| Male           | 54  | 56.3|
| Female         | 42  | 43.8|
| Exposure level (µg/L) |    |    |
| 0–30           | 43  | 44.8|
| 31–50          | 14  | 14.6|
| 51–130         | 26  | 27.1|
| > 130          | 13  | 13.5|

Results

Thirty families were initially recruited to participate in the study. Two families were lost to follow-up and 28 families with a total of 96 participants completed the sampling protocol (Table 1). Because of the emphasis on families, approximately half of the subjects (44%) were younger than 18 years of age. Males comprised more than half of the study population (56%); there was a reluctance among teenage females to participate. None of the subjects reported smoking and none of the subjects had consumed alcohol within 48 hr of submitting a urine sample. Creatinine measurements ranged from 159 to 6,285 mg/L with adults averaging 1,601 (±931) and children averaging 1,539 (±740) mg/L. The concentration of InAs in home drinking water of study participants ranged from 8 to 620 µg/L. In addition, the drinking water supplies of work sites of several participants were sampled to compare exposure with As at home and at work. In all cases, the concentrations of InAs in home drinking water supplies exceeded those in the workplace. The As concentrations in home drinking water supplies were grouped into four categories: 8–30 µg/L (45%), 31–50 µg/L (15%), 51–130 µg/L (27%), and > 130 µg/L (13%). The pattern of the TAs/c in the FMV was compared with those for other urine voids taken over a 24-hr period to determine whether the TAs/c in urine was stable throughout the day. Figure 1 compares the TAs/c in the FMV with the mean TAs/c in subsequent voids on the same day. The Pearson correlation (0.95) and the linear regression with an intercept and slope of 0.001 and 0.96, respectively, indicate that for a given day, TAs/c in the FMV is representative of a subject’s TAs/c for all subsequent voids.

To test for between-day variation in the TAs/c, the TAs/c for each FMV for each subject was compared to that subject’s 5-day average TAs/c in the FMV. Figure 2 presents the distribution of the percent differences between the 5-day mean TAs/c with the TAs/c for each FMV. This plot includes 21 subjects with fewer than five FMVs. Restriction of the analysis to the 75 subjects with five FMV voids yields a similar distribution of percent differences between the mean value and each day’s value (data not shown). In most epidemiologic studies, the concentration of InAs in drinking water has been used as a measure of exposure. Here, we compared the use of the concentration of InAs in drinking water with estimated As intakes to determine the better metric for assessing exposure. Figures 3 and 4 present

Figure 1. Total urinary As, adjusted for creatinine (µg/mg), comparing first void to mean of subsequent voids on the day of 24-hr urine collection. Intercept = 0.001; slope = 0.959; correlation = 0.95.

Figure 2. Distribution of the percent difference from the 5-day mean for total urinary As, adjusted for creatinine (µg/mg). The line inside the box indicates the median. The bottom and top of the box indicate the 25th and 75th percentiles, respectively. The whiskers extend to a length of 1.5 times the interquartile length.

Figure 3. Total urinary As, adjusted for creatinine (µg/mg), mean of five daily first voids, plotted versus the As concentration in the drinking water (µg/L). The equation for the regression line is mean total As/creatinine = 10.257 × (As concentration)0.83, where -2.57 and 0.83 are the intercept and slope, respectively, for the regression of the log10-transformed data.

Figure 4. Total urinary As, adjusted for creatinine (µg/mg), mean of five daily first voids, plotted versus the estimated As intake (µg/day) from drinking water. The equation for the regression line is mean total As/creatinine = 10.1.99 × (As concentration)0.33, where -1.99 and 0.33 are the intercept and slope, respectively, for the regression of the log10-transformed data.
the relation between the log of mean TAs/c for five FMVs with the log of the concentration of InAs in drinking water and with the log of the estimated As intake. The plots show that the concentration of InAs in drinking water provides a better fit for the data than does the estimated As intake.

The results of the mixed model regression analysis using log TAs/c are presented in Table 2. The dependent variable is the log mean TAs/c for five FMVs. Models I–IV include the log of the concentration of InAs in drinking water; model V uses the log-estimated As intake. Gender and age are included in all models. These analyses found that either the concentration of InAs in drinking water or the estimated As intake is a good predictor of TAs/c excretion. Age had a small effect on the relation but gender had no effect. The values for the intercept of these models are -2.6 (models I–IV) and -2.1 (model V). A comparison of models I–IV shows that the standard errors of the predictors decrease slightly and the 95% confidence intervals narrow with an increasing number of voids. Because there are only small gains in the statistical power of the analyses with an increasing number of urine voids, it is unlikely that much is gained in practical terms when the number of FMVs for an individual is increased beyond two or three.

The estimates of between-subject variation of models IV (0.033) and V (0.078) can be compared to determine whether the concentration of As in drinking water or the estimated As intake from drinking water is a better predictor for TAs/c. The lower coefficient for model IV suggests that concentration of As in drinking water is the better predictor. Akaike’s information criterion is another measure indicating that model IV better fits the data. This criterion has the value 177 for model IV and 140 for model V. An increase in the value is taken to be an improvement of fit for the model.

Discussion
In a U.S. population that has continuously consumed As-containing drinking water and for which there is excellent demographic data, the concentration of InAs in drinking water is a good predictor of the TAs/c in urine. The estimated intake of As calculated from the amount of water consumed directly or used in the preparation of food proved a less satisfactory predictor of the TAs/c. The failure of the estimated intake of As to improve the prediction of TAs/c likely reflects limitations in the methods used to estimate water consumption or the amount of water used in food preparation. Notably, the present study did not attempt to estimate exposure to As from the consumption of other foods or from inhalation. Because this population is relatively stable and the same wells have been used for many years by the families who participated in this study, it is likely that individuals are in a steady state condition relative to As ingestion and excretion.

Within-day variation in TAs/c was relatively small for members of this study population and appeared to be independent of exposure as measured by As concentration in drinking water. Similarly, analysis of the between-day variation in TAs/c for this population indicated that the concentration of InAs in urine remained relatively stable over the 5-day study period. Hence, it may be possible to reduce the number of urine samples required to give a reasonably good estimate of the extent of exposure to As to one or two FMVs. Gender did not affect the relation between the concentration of As in drinking water and the TAs/c. This finding is in contrast to some earlier studies in which distinct gender-related differences have been reported in the magnitude and pattern of As excretion in urine (13,21). Age was a significant covariable in the relation between the concentration of As in drinking water and the TAs/c. The effect of age may be related to a difference in dosage level in adults and children (22). In contrast, a recent study in a population in northern Argentina chronically exposed to InAs in drinking water did not find a consistent age-dependent difference in the TAs/c (23).

This study provides novel data on the pattern of As clearance in urine in a U.S. population exposed to InAs in drinking water. It demonstrates that the concentration of As in urine is relatively stable throughout the day and over a period of 5 days. The stability of the output of As into urine suggests that members of this population were likely to be at steady state in terms of exposure to InAs.

The high correlation between the concentration of InAs in drinking water supplies and the concentration of As in urine suggests that over the range of exposures examined in this study, drinking water was the predominant source of exposure to As. The absence of a discernable contribution of food As to the urinary output of As in individuals who consume drinking water with low (<10 μg As/L) As contents suggests that food As contributes little to the intake of inorganic As in this population. Average daily intake of As from a North American diet is estimated to be approximately 38 μg (24). However, if 10% of this As is present as inorganic, methyl, or dimethyl arsenic, then approximately 4 μg As would be contributed to the As in urine that would be detected by the analytical methods used in the present study. Hence, the contribution of food As might easily be obscured by the contribution of As obtained from the daily ingestion of 2 L of drinking water containing 10 μg of InAs/L. Analyses of the relation between the intensity of exposure to InAs in drinking water and the pattern of methylated arsenicals in urine in this population are currently under way.

References and Notes
1. Bates MN, Smith AH, Hopenhayn-Rich C. Arsenic ingestion and internal cancers: a review. Am J Epidemiol 134:402–478 (1991).
2. Chen C-J, Chen CW, Wu MM, Kuo T-L. Cancer potential in liver, lung, bladder and kidney due to ingested inorganic arsenic in drinking water. Br J Cancer 66:888–892 (1992).
3. Chiu H-Y, Hsuw Y-M, Liaw K-F, Horng S-F, Chang M-H, Pu Y-S, Lin J-S-N, Hung C-H, Chen C-J. Incidence of internal cancers and ingested inorganic arsenic: a seven-year follow-up study. Cancer Res 59:1296–1300 (1999).
4. Tsen C-H, Chong C-K, Chen C-J-L, Lin B-J, Tai T-Y. Abnormal peripheral microcirculation in seemingly normal subjects living in blackout-disease-hyperendemic villages in Taiwan. Int J Microcirc 15:21–27 (1995).

Table 2. Mixed model regression results.

| Predictor variables | Coefficient (SE) | p-Value | CI     |
|---------------------|------------------|---------|--------|
| Model I, one void   |                   |         |        |
| Log of As concentration in drinking water (μg/L) | 0.659 (0.049) | 0.0001  | 0.563–0.756 |
| Gender (female vs. male) | 0.032 (0.047) | 0.50    | -0.060–0.124 |
| Age (< 18 years vs. ≥ 18 years) | 0.118 (0.048) | 0.01    | 0.025–0.211 |
| Model II, two voids |                   |         |        |
| Log of As concentration in drinking water (μg/L) | 0.639 (0.043) | 0.0001  | 0.554–0.723 |
| Gender (female vs. male) | 0.029 (0.042) | 0.51    | -0.054–0.110 |
| Age (< 18 years vs. ≥ 18 years) | 0.113 (0.043) | 0.01    | 0.029–0.197 |
| Model III, three voids |             |         |        |
| Log of As concentration in drinking water (μg/L) | 0.626 (0.043) | 0.0001  | 0.543–0.710 |
| Gender (female vs. male) | 0.034 (0.043) | 0.44    | -0.051–0.118 |
| Age (< 18 years vs. ≥ 18 years) | 0.092 (0.044) | 0.04    | 0.006–0.178 |
| Model IV, five voids |                   |         |        |
| Log of As concentration in drinking water (μg/L) | 0.655 (0.040) | 0.0001  | 0.576–0.733 |
| Gender (female vs. male) | 0.027 (0.041) | 0.50    | -0.062–0.107 |
| Age (< 18 years vs. ≥ 18 years) | 0.090 (0.041) | 0.03    | 0.009–0.170 |
| Model V, five voids |                   |         |        |
| Log of estimated As intake from drinking water (μg/day) | 0.358 (0.043) | 0.0001  | 0.274–0.441 |
| Gender (female vs. male) | -0.019 (0.060) | 0.76    | -0.136–0.099 |
| Age (< 18 years vs. ≥ 18 years) | 0.127 (0.064) | 0.048   | 0.001–0.252 |

Abbreviations: CI, 95% confidence interval; SE, standard error.
5. Chen CJ, Chiu H-Y, Chiang M-H, Lin L-J, Tai T-Y. Dose-response relationship between ischemic heart disease mortality and long-term arsenic exposure. Arterioscler Thromb Vasc Biol 16:504-510 (1996).

6. Chiu H-Y, Huang W-I, Su C-I, Chang S-F, Hsu Y-H, Chen C-J. Dose-response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. Stroke 28:1717-1723 (1997).

7. Chen CJ, Hsuheh YM, Lai M, Shyu MP, Chen SY, Wu MM, Kuo TL, Tai TY. Increased prevalence of hypertension and long-term arsenic exposure. Hypertension 25:53-60 (1995).

8. Lai M, Hsuheh YM, Chen C-J, Shyu MP, Chen SY, Kuo TL, Wu MM, Tai TY. Ingested inorganic arsenic and prevalence of diabetes mellitus. Am J Epidemiol 139:484-492 (1994).

9. Rahman M, Tondel M, Ahmad SA, Axelson O. Diabetes mellitus associated with arsenic exposure in Bangladesh. Am J Epidemiol 148:198-203 (1998).

10. Ihrig MM, Shailat SI, Baynes C. A hospital-based case-control study of stillbirths and environmental exposure to arsenic using an atmospheric dispersion model linked to a geographical information system. Epidemiology 9:250-254 (1998).

11. Geesler W, Kuehnelt D, Igolic KJ. The mercuric-bromide-stain method and the Natelson method for the determination of arsenic: implications for assessment of risks from exposure to arsenic in Taiwan. In: Arsenic, Exposure and Health Effects (Abemathy C, Calderon RL, Chappell W, eds). London:Chapman and Hall, 1997:17-32.

12. Brown KG, Chen C-J. Significance of exposure assessment to analysis of cancer risk from inorganic arsenic in drinking water in Taiwan. Risk Anal 15: 475-484 (1995).

13. Del Razo LM, Garcia-Vargas GG, Vargas H, Albores A, Gonesbatt ME, Montero R, Ostrowsky-Wegman P, Kelsh M, Cebrian ME. Altered profile of urinary arsenic metabolites in adults with chronic arsenism. A pilot study. Arch Toxicol 71:211-217 (1997).

14. Hopenhayn-Rich C, Smith AH, Goeden HM. Human studies do not support the methylation threshold hypothesis for the toxicity of inorganic arsenic. Environ Res 60:161-177 (1993).

15. Hsu K-H, Fronies JR, Chen C-J. Studies of arsenic ingestion from drinking water in northeastern Taiwan: Chemical speciation and urinary metabolites. Arsenic Exposure and Health Effects (Abemathy C, Calderon RL and Chappell W, eds). London:Chapman and Hall, 1997:309-339.

16. Lewis DR, Southwick JW, Scianan LR, Ranch J, Calderon RL. Feasibility of conducting epidemiologic studies of arsenic in the U.S.: Millard County, Utah. J Environ Health 60:14-19 (1998).

17. Creed JT, Brockhoff CA, Martin TD. Methods for the Determination of Metals in Environmental Samples, Supplement I. EPA/600/R-94/111, 200.8. Washington, DC.U.S. Environmental Protection Agency, 1994:1-58.

18. Le XC, Ma M. Short Column Liquid Chromatography with Hydride Generation Atomic Fluorescence Detection for the Speciation of Arsenic. Anal Chem 70:1926-1933 (1998).

19. Ma M, Le XC. Effect of arsenosugars ingestion on urinary arsenic speciation. Clin Chem 44:539-550 (1998).

20. Archari R, Meyersohn M, Conrad KA. HPLC analysis of creatinine in human plasma and urine. J Chrom Sci 21:278-281 (1983).

21. Del Razo LM, Hernandez JL, Garcia-Vargas GG, Ostrosky-Wegman P, deNava CC, Cebrian ME. Urinary excretion of arsenic species in a human population chronically exposed to arsenic via drinking water: a pilot study. In: Arsenic Exposure and Health (Chappell WR, Abemathy CD, Cothran CR, eds). Northwood, UK:Science and Technology Letters, 1994:51-59.

22. Roseberry AM, Burmaster DE. Log-normal distribution for water intake by children and adults. Risk Anal 12:99-104 (1992).

23. Concha G, Nermell B, Vahter M. Metabolism of inorganic arsenic in children with chronic high arsenic exposure in Northern Argentina. Environ Health Perspect 106:355-359 (1998).

24. Debeke RW, McKenzie AD, Lacroix DMA, Cleroux C, Bowe S, Graham RA, Conacher HBS, Verdier P. Survey of arsenic in total diet food composites and estimation of the dietary intake of arsenic by Canadian adults and children. JAGAC Int 76:14-25 (1995).