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Human nails as a biomarker of arsenic exposure from well water in Inner Mongolia: comparing atomic fluorescence spectrometry and neutron activation analysis

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Abstract

Arsenic (As) is found naturally in the geological strata within the Ba Men Region of Inner Mongolia, China. A study was conducted to compare the total As measurements from two analytical techniques: instrumental neutron activation analysis (INAA) and atomic fluorescence spectrometry (AFS), and to verify nails as an exposure biomarker in this population. In 1999, nail and water samples were collected in a pilot study. Fingernails and toenails were pooled from 32 participants and analysed for total As by both INAA and AFS. Mean nail As values were 14.8 ± 2.4 and 19.4 ± 2.8 $\mu g\, g^{-1}$ ($\pm$SEM) for INAA and AFS, respectively. Results from these two methods were significantly correlated ($r = 0.93$, $p < 0.0001$). In 2000, a second study was conducted and INAA was used to measure total As in toenails from 314 Ba Men residents. Well water samples were collected from 121 households and analysed by AFS. A significant correlation was observed between toenail and well water As ($r = 0.84$, $p < 0.0001$). Based on the results, INAA was significantly correlated with AFS and proved to be a reliable measure of nail As levels. In this population, toenail samples are a useful internal As exposure biomarker from drinking water sources.

Keywords: *Arsenic, nails, drinking water, neutron activation, analysis, atomic fluorescence spectrometry*

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Introduction

Arsenic (As) accumulates in geological strata because of the natural weathering of rocks and minerals. Many parts of the world use drinking water sources contaminated with As levels up to 3.2 $mg\, l^{-1}$, including India, Chile, Argentina and Mexico (Hopenhayn-Rich et al. 1996, Armienta et al. 1997, Thornton and Farago 1997,

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Guha Mazumder et al. 1998, Smith et al. 1998). In the USA, As has been found in several areas including the Southwest, with concentrations in Utah ranging from 8 to 620 $\mu g\,l^{-1}$ (Calderon et al. 1999). Chronic exposures to drinking water As (DWA) have been linked to a variety of adverse health effects including cardiovascular and pulmonary disease, neurological and developmental effects, and cancers of the skin, bladder, kidney, lung and liver (Ma et al. 1995, Smith et al. 1998, Guha Mazumder et al. 1998, NRC 1999, Morales et al. 2000, Rahman et al. 2001).

A useful biomarker for the assessment of the internal exposure to As in humans is needed for risk assessment. Nails may potentially be widely used as an internal biomarker of As exposure from drinking water. The advantages of using nails are slow growth rates, the affinity of As to accumulate, and the relative ease of collection and storage during field studies. A few studies have examined the use of nails as a biological marker of As exposure (Olguin et al. 1983, Agahian et al. 1990, Karagas et al. 1996, Karagas et al. 2000). In the current literature, As concentration in nails is measured using two different analytical detection methods. No study to date has compared these two commonly used analytical techniques.

In Ba Men, the presence of As occurs naturally in the ground water and DWA has been reported to range from non-detectable levels to 1.8 $mg\,l^{-1}$ in well water (Ma et al. 1999).

Ba Men is a good population for evaluating As biomarkers, such as nails, for the following reasons: (1) the timeframe of DWA exposure is well characterized, beginning with the use of deeper wells in the area after 1979–80; (2) the levels of DWA vary from low to high; and (3) most residents (80%) have their own wells, and drinking water is generally thought to be the only source of As in this population. The objectives of this As exposure study were (1) to compare the measurement of nail As by AFS and INAA, and (2) to investigate if nails could provide a useful internal biomarker of As exposure in this population.

Materials and methods

Initially, a 1999 pilot nail study ($n=32$) was conducted to measure nail As concentrations using two analytical methods, hydride generation atomic fluorescence spectrometry (HGAFS) and instrumental neutron activation analysis (INAA) and comparing these levels to the As levels in the well water. A follow-up study ($n=314$) was conducted in 2000 comparing well water values again with the nail As levels measured specifically by INAA.

Study subjects

All study subjects were farmers from Ba Men, Inner Mongolia, China. Questionnaires were administered to the subjects for both pilot and follow-up studies to obtain demographic information, As exposure, water consumption, diet, smoking, occupation, pesticide use and relevant medical information. The study was designed to recruit an equal distribution of males and females with similar age distributions and exposure histories. We also enrolled an equal distribution of smokers even though this population tends to have a larger proportion of males than females that smoke. Thirty-two subjects participated in the pilot study in 1999. Well water and nail samples were collected from each participant. In the follow-up study conducted in 2000, 314 subjects contributed nail samples, and well water was collected from their households

($n = 121$). Based on *a priori* selected well water concentrations, the study subjects were divided into low (below detection limit–21 $\mu g\,l^{-1}$), medium (100–300 $\mu g\,l^{-1}$), and high (434–690 $\mu g\,l^{-1}$) As exposure groups. This study was conducted according to the recommendations of the World Medical Association Declaration of Helsinki (1989) for the protection of human subjects. All study subjects gave informed consent to participate in this study. The research protocol for this study was approved by the US Environmental Protection Agency.

Water sample collection and analysis

All drinking water samples were collected in acid-washed containers from the water tanks of the study subject's homes. Water tanks are used in homes to store well water until the time of consumption. Water samples were collected, stored at $-20^{\circ}C$ and transported on ice via air to the University of Alberta in Edmonton, Canada, for analysis. Total As was blindly determined using hydride generation atomic fluorescence spectrometry (HGAFS) (Le and Ma 1998). The detection limit (LOD) for HGAFS is 0.2 $\mu g\,l^{-1}$.

Nail sample collection and analysis

In the pilot study, toenail and fingernail samples from each subject were mixed to provide a large enough sample size for comparison of two analytical methods, AFS and INAA. However, in the follow-up study, only toenail samples were collected from each of the 314 individuals. All nail samples were stored in ziplock plastic bags, shipped back to the USA, and cleaned by sonication in HPLC-grade water, followed by an acetone wash to remove water and organic contamination from the nail surface (Das et al. 1995). In the pilot study, nails were analysed blindly for total As by HGAFS at the University of Alberta and by INAA at North Carolina State University's Nuclear Services Department (Raleigh, NC, USA) (Heydorn 1984, Le and Ma, 1998, Le et al. 2000). In the follow-up study, toenails were blindly analysed for total As using instrumental neutron activation analysis (INAA) by North Carolina State University's Nuclear Services Department (Heydorn 1984). The LOD for INAA is 0.001 $\mu g\,g^{-1}$. The accuracies for both the HGAFS and INAA methods were confirmed using standard reference material obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) and both are within 10% of the certified values.

Statistical analysis

The mean, standard error of the mean and Pearson correlation coefficients were determined using the Prism statistical software for the pilot nail study (GraphPad Software, Inc., San Diego, CA, USA). For the follow-up study, associations among As in nails ($\mu g\,g^{-1}$), As in drinking water ($\mu g\,l^{-1}$), and well depth (m) were evaluated by Pearson's correlation coefficient. Differences in age, sex and smoking distributions within the three As exposure groups were determined with the Kruskal–Wallis test (age) and the Chi-square test (sex and smoking). Linear regression was used to assess the effects of drinking water As on nail As, controlling for age, sex, smoking and water intake. Statistical analyses were performed using SAS statistical software (SAS Institute 2001, Cary, NC, USA).

Results

Thirty-two study subjects living in Ba Men participated in the 1999 pilot study. The study included nail and water samples from 19 study subjects exposed to high concentrations of DWA (506.5 ± 23.6 $\mu g\,l^{-1}$, mean $\pm$ SEM) and from 13 study subjects exposed to low concentrations of DWA (4.3 ± 1.0 $\mu g\,l^{-1}$), representing the referent group (Table I). All study participants had been consuming well water in this area for an average of 17 years. The mean value for total As for all water samples analysed by AFS was 302.5 ± 45.9 $\mu g\,l^{-1}$. The mean values for total As in the nail samples were 19.4 ± 2.8 and 14.8 ± 2.4 $\mu g\,g^{-1}$ for analysis by AFS and INAA, respectively. Levels of DWA measured by AFS and nail As by AFS and INAA, respectively, are shown in Figure 1a, b. Significant correlations were observed between DWA and nail samples analysed by both methods, $r = 0.88$ ($p < 0.0001$) for AFS, and $r = 0.84$ ($p < 0.0001$) for INAA. Figure 1c shows the correlation between the two analytical methods used for detecting As in nails ($r = 0.93$, $p < 0.0001$).

The follow-up study conducted in 2000 included 314 study subjects, 167 males and 147 females, with a mean age of 35 years (Table II). Their daily water consumption was approximately 1.8 litres and the average length of As exposure was approximately 15 years. Drinking water As was divided into categories based on As concentrations *a priori* with an overall mean of 245.8 ± 21.0 $\mu g\,l^{-1}$. Age distribution was similar within the three As exposure groups ($p = 0.92$), as were the sex and smoking distributions ($p = 0.18$ and 0.72, respectively). Toenail As concentrations ranged from 0.17 to 65.9 $\mu g\,g^{-1}$ and As concentrations increased in a dose–response manner with the three DWA categories (Table II). Figure 2a presents the correlation between As concentrations in water and nail samples ($r = 0.84$, $p < 0.0001$). Well depth was also significantly correlated with As concentrations in both water ($r = 0.87$, $p < 0.0001$, Figure 2b) and nails ($r = 0.75$, $p < 0.0001$, Figure 2c).

The dependent variable in the linear regression models (Table III), As concentration in nails, was log transformed to normalize residuals. Because water samples were taken from three groups of wells with low, medium and high As concentrations, the exposure in model 1 was represented by two indicator variables, representing medium and high As wells, using samples from the low As wells as a referent. Model 1 shows the significant effects from both medium and high As levels in drinking water on As concentration in nails. Males had significantly higher As toenail levels than females.

Table I. Characteristics of the study population, Ba Men, Inner Mongolia, 1999 ($n = 32$ individuals).

	Low (0.3–9.8 $\mu g\,l^{-1}$)	High (264–648 $\mu g\,l^{-1}$)	Total (0.3–648 $\mu g\,l^{-1}$)
n (subjects)	13	19	32
Sex (M/F)	8/5	14/5	22/10
Age[b]	38.5 ± 3.7	38.0 ± 3.6	38.2 ± 2.6
Smokers (%)	30.8	47.4	40.6
DWA[b] ($\mu g\,l^{-1}$)	4.3 ± 1.0	506.5 ± 20.7	302.5 ± 45.9
Nail As AFS[b] ($\mu g\,g^{-1}$)	3.4 ± 0.7	32.0 ± 1.8	19.4 ± 2.8
Nail As INAA[b] ($\mu g\,g^{-1}$)	0.7 ± 0.07	23.8 ± 2.1	14.8 ± 2.4
As exposure years[a,b]	17.5 ± 0.9	16.8 ± 0.9	17.1 ± 0.7
	17.0 (8–21)[c]	18.0 (10–23)[c]	17.5 (8–23)[c]

[a]As exposure years or years using current well water supplies.
[b]Mean $\pm$ SEM.
[c]Median (range).

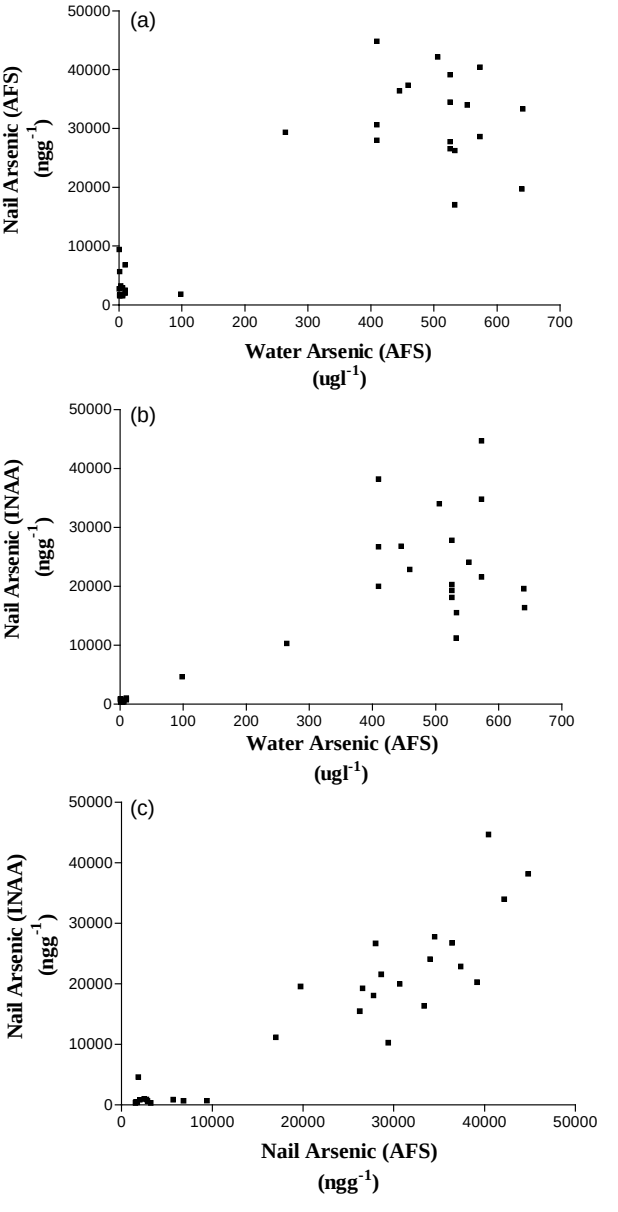

Figure 1. (a) Concentration of arsenic in well water analysed by AFS and concentration of arsenic in nails analysed by AFS, $r = 0.88$, $p < 0.0001$; (b) concentration of arsenic in well water analysed by AFS and concentration of arsenic in nails analysed by INAA, $r = 0.84$, $p < 0.0001$; (c) concentration of arsenic in nails analysed by AFS and INAA, $r = 0.93$, $p < 0.0001$.

Since well depth was highly correlated with As concentration in drinking water ($r = 0.87$, $p < 0.0001$), a model was included using well depth as a continuous, surrogate exposure variable (model 2). Model 2 shows the significant effect of well depth on nail As concentrations. Age and water intake had no effect in either model. Since most smokers were men (89%), a separate model was used including men only, to

Table II. Characteristics of the follow-up study population, Ba Men, Inner Mongolia, 2000 ($n=314$ individuals, $n=121$ households).

	Low ([a]BDL–21 $\mu g\,l^{-1}$)	Medium (100–300 $\mu g\,l^{-1}$)	High (434–690 $\mu g\,l^{-1}$)	Total ([a]BDL–690 $\mu g\,l^{-1}$)
n (subjects)	105	106	103	314
n (households)	41	44	36	121
Sex (M/F)	55/50	54/52	58/45	167/147
Age[c]	35.2±1.4	35.6±1.4	34.5±1.4	35.1±0.8
Smokers (%)	28.6	39.6	30.1	32.8
Water As levels[c] ($\mu g\,l^{-1}$)	11.5±1.0	200.7±8.8	567.9±12.2	245.8±21.0
Toenail As levels[c] ($\mu g\,g^{-1}$)	1.2±0.1	9.8±0.5	24.8±1.1	11.9±0.7
Well depth[c] (m)	12.6±0.2	22.3±1.4	28.9±0.8	21.2±2.7
Water consumption[c]	1.6±0.1	1.9±0.1	1.9±0.1	1.8±0.1
($l\,day^{-1}$)	1.5 (0.3–4.0)[d]	1.5 (0.5–5.0)[d]	1.5 (0.2–6.0)[d]	1.5 (0.2–6.0)[d]
As exposure years[b,c]	16.9±0.3	14.6±0.3	14.7±0.3	15.4±0.2
	18.0 (8–24)[d]	15.0 (3–21)[d]	15.0 (4–20)[d]	16.0 (3–24)[d]

[a]BDL, below detection limit.
[b]As exposure years or years using current well water supplies.
[c]Mean±SEM.
[d]Median (range).

determine the effect from smoking. Smoking was excluded from models 1 and 2 because it had no significant effect on the outcome variable in men ($p=0.17$).

Discussion

As in urine (metabolites), blood and hair, each has its advantage and disadvantage for measuring the biologically relevant dose. Urine is easy to collect and provides a profile of As metabolism. Because As is rapidly cleared from the body, urinary As measures only a relatively short-term exposure (Calderon et al. 1999, Karagas et al. 2000). Blood could be useful for studies attempting to make links with other endpoints such as oxidative stress markers, but As is also cleared relatively quickly from the blood (Karagas et al. 2000). In addition, collecting blood samples, an invasive procedure, may be difficult in some populations such as children, and storage of blood samples

Table III. Relationship of nail arsenic (log transformed, $\mu g\,g^{-1}$) to drinking water arsenic measures, controlling for smoking, age, and water intake in Ba Men, Inner Mongolia, 2000.

Variable	Parameter estimates (p)	
	Model 1 ($n=313$)	Model 2 ($n=313$)
Medium As wells[a]	2.080 (<0.0001)	– (–)
High As wells[a]	3.060 (<0.0001)	– (–)
Well depth (m)	– (–)	0.163 (<0.0001)
Sex[b]	0.154 (0.02)	0.188 (0.03)
Age (years)	−0.0008 (0.72)	0.0003 (0.92)
Water intake ($l\,day^{-1}$)	0.034 (0.27)	0.004 (0.93)

Referent: [a]low As wells; [b]females.

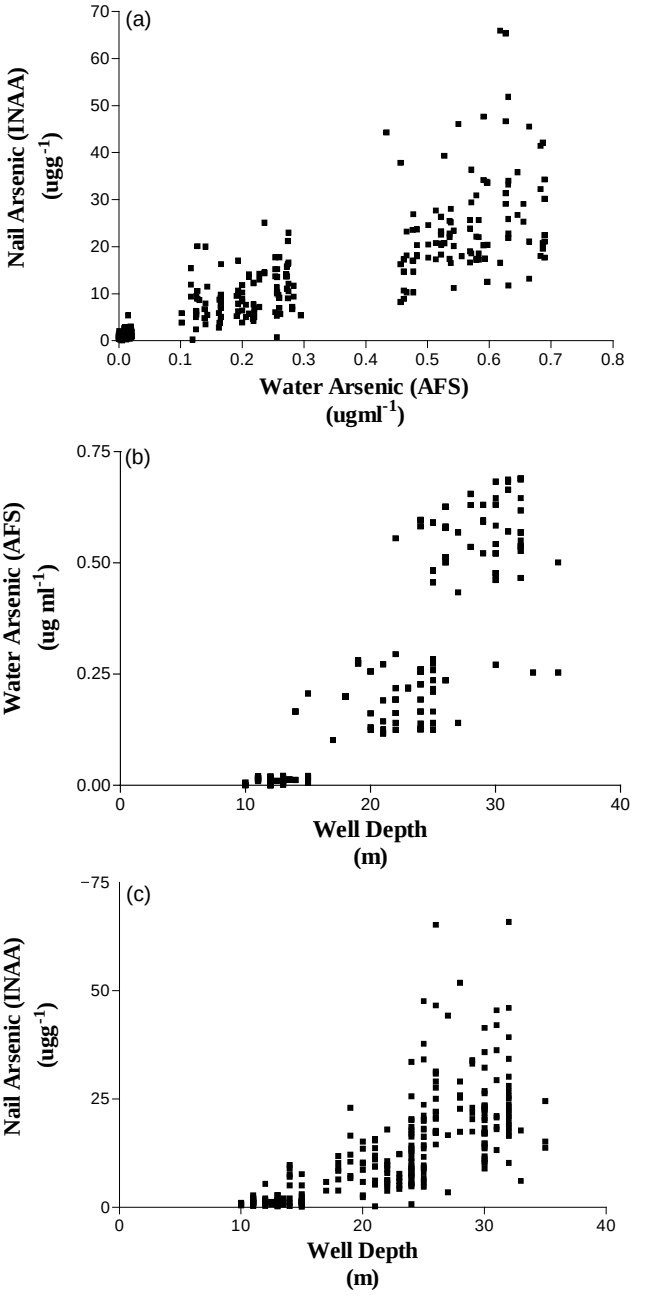

Figure 2. (a) Concentration of arsenic in well water analysed by AFS and concentration of arsenic in nails analysed by INAA, $r = 0.84$, $p < 0.0001$; (b) depth of well and concentration of arsenic in well water analysed by AFS, $r = 0.87$, $p < 0.0001$; (c) depth of well and concentration of arsenic in nails analysed by INAA, $r = 0.75$, $p < 0.0001$.

may present problems when conducting international studies. Hair is a less invasive biomarker for assessing chronic As exposures and has been used in forensic studies of As poisoning. However, hair is more likely to encounter external As contaminants

(airborne As particles) and requires a rigorous analytical procedure (Shapiro 1967, Henke et al. 1982, Karagas et al. 2001).

Nails have large amounts of scleroproteins, such as keratin, which contain sulfhydryl groups and As has a high affinity for these sulfhydryl groups (Karagas et al. 2000). Because the germinal nail matrix is fed by a rich blood supply, As deposition in the nails occurs shortly after ingestion. Nails have a slow growth rate (4–5 mm month^{-1} for fingernails and 1.1 mm month^{-1} for toenails) and are less likely to be contaminated by external As exposures (Henke et al. 1982). Collecting nails is a convenient, non-invasive measure of chronic As exposure from drinking water.

Some studies have found higher As levels in nails associated with DWA than in other tissues (Olguin et al. 1983). Karagas et al. (1996, 1998) reported a significant correlation ($r=0.67$, $p=0.009$) in As concentrations between nail and water samples in a small set ($n=21$) of individuals in New Hampshire where the water As concentration ranged from non-detectable to 0.137 mg l^{-1}. Karagas et al. (2000) also reported significant correlations between toenail and water As concentrations ($r=0.46$, $p<0.001$) in a US population-based case-control study of non-melanoma skin cancer conducted in New Hampshire. In a follow-up study of the same population to examine long-term reproducibility, the authors reported a significant correlation between As exposure and internal biomarkers of As exposure ($r=0.33$, $p=0.0016$) (Karagas et al. 2001). In both reports, the correlation between As concentrations in water and toenails were greatly increased for the individuals with water As >1 μg l^{-1} ($r=0.65$, $p<0.001$ and $r=0.64$, $p=0.006$) in comparison with the group with water As <1 μg l^{-1}. In a study conducted over 6 years, the authors found that INAA analysis of toenail clipping taken at two separate times from the same individuals for 16 trace elements, including As, was reproducible and reliable. The highest correlation among the 16 trace elements was observed for As ($r=0.54$) (Garland et al. 1993).

Our 1999 pilot study found that the concentration of nail As levels from AFS was consistently higher than the nail As levels from the INAA method. For our analysis, determining the absolute magnitude of As levels was less important than assessing the relationship between As in nails and As levels in drinking water. We used the INAA method to analyse the toenail samples in the follow-up study. INAA has certain advantages over the AFS method, including a limited non-destructive sample preparation, an instrumental method which reduces the sample preparation time and handling thus reducing potential errors, and high sensitivity for low level detection in individuals. In addition, INAA allows for multi-element analysis from the same sample, and is cost-effective.

The population in Ba Men provided a good opportunity to evaluate whether nail As could be a good biomarker of As exposure via drinking water. The Ba Men residents have been exposed to a wide range of As concentrations mainly via drinking water. In 1979–80, the residents of Ba Men switched from shallow (3–5 m) shared wells to deeper (>15 m), hand-pumped, private artesian wells, which are more likely to contain elevated levels of As (Ma et al. 1999). More than 80% of residents have their own well, so that it is possible to estimate the individual As exposure via drinking water. No known industrial sources have been reported in Ba Men and the farmers do not use pesticides containing As. Due to its inland location in Inner Mongolia, Ba Men residents very seldom consume seafood, which is known to contain organic

arsenicals. Thus, the data presented here support the hypothesis that As from drinking water is the main contributor to As exposure in this population.

In our study, As water concentrations were 50-fold greater in the high exposure group compared with the low exposure group, while the nail concentrations only showed a 20-fold increase. This may be due in part to individuals having a saturated body burden of As. Recall that this population has been chronically exposed (20+ years) to highly elevated levels of As in the well water. If this proves to be true, it will be important for future research to determine the saturation levels at which the nail samples can no longer absorb and store As in humans.

We found that age and water intake had no effect on the level of As observed in this population. Gender in both models is modestly significant. This trend may be explained by the greater amounts of water consumed by males in this agrarian population. Model 2 shows that well depth can be used as a surrogate instead of measured well water As levels in this population. The well depth in the Ba Men region of China changed over the years from shallow (5 m) wells to wells >15 m depth. This change corresponded with an increase in As levels in the drinking water (Ma et al. 1999). Both models show a highly significant effect on the As levels in toenails. Future studies will use a continuous variable for As concentrations in well water in this population.

In conclusion, quantification of As concentrations using INAA in nail samples provides a practical, non-invasive and reliable method for detection of As exposure on an individual level. The use of nails as a biomarker of exposure to As via drinking water can be used in studies that need to have an accurate, long-term chronic exposure measurement. Reliable, individual measures of As exposure should aid future investigations of As health effects from drinking contaminated water in the USA as well as in other countries. Nails may be a good internal biological marker of DWA exposure in a human population.

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