Biomarkers of Exposure: Arsenic Concentrations in Keratin in Populations Exposed to Arsenic in Drinking Water

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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Earth and Ocean Sciences in the Graduate School of Duke University

2014
ABSTRACT

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Abstract

Arsenic (As) exposure via groundwater consumption is a global health problem affecting millions. Monitoring exposure is a key step in understanding and predicating future health outcomes. This thesis explores the relationships between arsenic concentrations in toenails and arsenic in water. Three case studies were investigated, with residents from: North Carolina, USA (n=103); the Rift Valley, Ethiopia (n=60); and the Mekong Delta, Vietnam (n=65). Arsenic concentrations above the WHO’s recommended 10ppb limit were found in groundwater from the three research sites. Arsenic in toenails was analyzed by inductively coupled plasma mass spectrometry (ICP-MS).

In the Rift Valley of Ethiopia, 53% of the tested drinking wells (n=34) had As above the WHO’s limit. Arsenic concentrations in toenails (n=60) were significantly correlated to As concentrations in groundwater (r=0.72; p<0.001), reflecting the direct exposure of rural communities to As in well water, which is their principle water source. Male minors (<18 years old) were found to have greater nail–As concentrations compared with adults consuming equal amounts of As (p<0.05). Estimated As dose specifically from drinking water sources was also associated with nail concentrations (p<0.01).
In the Mekong Delta of Vietnam (Dong Thap Province), 36 out of the 68 tested wells had As content above the WHO’s recommended limit of 10ppb, with levels as high as 981 ppb. Arsenic contents in nails collected from local residents (n=62) were significantly correlated to As in drinking water (r=0.49, p<0.001). Demographic and survey data show that the ratio of As in nail to As in water varies among residents that reflects differential As accumulation in the exposed population. The data show that water filtration and diet, particularly increased consumption of animal protein and dairy and reduced consumption of seafood, were associated with lower ratios of As in nail to As in water and thus could play important roles in mitigating As exposure.

Sixty-one wells were tested from Union County, North Carolina, with 15 out of 61 wells exceeded the WHO’s 10 ppb limit. Arsenic values ranged from below the limit of detection (0.07) to 130ppb, with a mean of 11ppb (median=1.5ppb). Nails were collected from county residents (n=103) and were statistically correlated with As-water concentrations (r=0.48, p<0.001).

Integration of the data from the three cases studies across different populations and ethnicities show high correlation between As concentrations in groundwater and As in nails in all the three locations (r[Union County]= 0.48, p<0.001; r[Ethiopia]=0.72 p<0.001; r[Vietnam]=0.49, p<0.001). For As-nail to As-water pairs in which As in water was above 1ppb, these three locations are statistically indistinguishable from one another (r=0.62, p<0.001, n=176). These results support the hypothesis that nails can be used as a
biomarker of exposure regardless of geographic or ethnic differences in populations considered. Nutrition (meat, seafood, and milk consumption) rather than gender, ethnicity, or dose is suggested to be the major confounding issue affecting the magnitude of As exposure in the human body.
Dedication

To my family and friends who have given me constant love and support. I could not have done this without you. To Mimi and Michael Merola for making my education a priority and allowing me freedom to form my own opinions. To Tore for making me laugh. To Jimmy Munoz for teaching me that it was okay to follow any path I chose. And to Jamie Giannone, Todd Dowling, Jennifer Padgett, Elda Varela, and Mary Greene for sticking with me even when I disappeared for months at a time. I love you all.
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1. Introduction

Arsenic (As) contamination of drinking water currently affects millions of people globally, particularly as developing nations have moved away from bacteriologically-contaminated surface water sources and increased utilization of groundwater resources for drinking water. The health effects of As-rich drinking water are well known; however the literature on the direct exposure and the bioaccumulation of As in the human body is lacking. The objective of this dissertation is to fill that gap by investigating and quantifying As exposure. In particular, the study aims to evaluate 1) the degree to which populations consuming As in their drinking are exposed; and 2) what are the confounding factors that amplify or mitigate this As exposure. Given the potential for long latency periods between consumption of contaminated water and health effects, linking disease to water quality requires a sufficient understanding of exposure. This problem underscores the need to study exposure and its variations throughout populations.

Previous research has shown nail-As concentrations to be higher in populations consuming water with more than the WHO limit of 10ppb As than in populations consuming water with As lower than the limit (Hinwood et al., 2003; Karagas et al., 1996, 2000; Mandal et al., 2003; Samanta et al., 2004). Both Karagas et al. (1996, 2000) and Mandal et al. (2003) have demonstrated a potential linear relationship between exposure through drinking water and concentration in nail keratin.
In addition to basic exposure relationships, research has investigated the effect of confounding factors such as age, gender, and race on As-nail concentrations. Hinwood et al. (2003) saw a non-statistically significant trend of minors having greater As levels in nails than their adult counterparts. Current literature shows disagreement whether males or females exhibit greater As concentrations when controlling for As concentrations in water (Hinwood, 2003; Loffredo et al., 2003). Ethnicity has been shown to affect As-nail concentrations in unexposed (consuming water with As concentrations below 1ppb) populations living in the same location (Brima et al., 2006), and in low exposure (consuming water with <10ppb As) populations in different countries (Loffredo et al., 2003). Diet, water intake, and age were not accounted for in these studies.

1.1 Methodology overview

For each case study employed in this dissertation a common methodology was used and is described in detail in each chapter. IRB approval was obtained both from Duke University and in country when applicable. Groundwater samples and field parameters (pH, conductivity, ORP (oxidation-reduction potential), and DO (dissolved oxygen)) were collected on site. Water samples were collected and analyzed according to USGS protocols (USGS 2011). Arsenic speciation was performed according to methods in Bednar et al. (2002). Nails were cleaned using water, acetone, and a 1% solution.
Digestion was accomplished using $\text{H}_2\text{O}_2$ and $\text{HNO}_3$, after which As values were obtained using ICP-MS.

A survey was used in each case study to collect demographic, water consumption, and nutrition information. In the Mekong Delta case study, health information was also collected. Each study was pretested and adapted to reflect cultural differences in each location, then translated.

**1.2 Dissertation research and objectives**

This dissertation was designed to test the following hypotheses:

1) Toenails can be used in different populations to measure the magnitude of As exposure from drinking water.

2) Age, gender, ethnicity, water consumption, nutrition, and chemical speciation have previously been considered to affect the rate at which As can be eliminated from the body. As in nails can detect differences in the magnitude of exposure due to these factors and regardless of As concentration in drinking water.

3) Toenails can be used universally, across different populations to measure the magnitude of As exposure from drinking water.

To test these hypotheses three geographically different locations were selected to measure As in nails: Union County, NC, Rift Valley, Ethiopia, and Mekong Delta, Vietnam. In depth As exposure analysis was completed in the Rift Valley and the
Mekong Delta. Union County, whose As concentrations in water were comparatively lower than the other two case studies was used as a no- to low-level exposure comparison.

**1.3 Chapter 2 Synopsis: Arsenic exposure of rural populations from the Rift Valley of Ethiopia as monitored by toenails**

Chapter 2 investigates As exposure in the Rift Valley of Ethiopia, where its effect among vulnerable and rural populations is not well studied. This study examines As exposure and bioaccumulation from drinking water by monitoring human keratin in the form of toenails from exposed populations. Groundwater samples from drinking water wells (n=34) were collected along with toenail samples (n=58) from local communities and were analyzed for trace metals including As by inductively coupled plasma mass spectrometry (ICP-MS). Of the total number of wells tested, 53% had As level above the WHO maximum contamination level of 10 ppb. After linear regression analysis was preformed arsenic in toenails was significantly correlated to drinking water (r=0.72; p<0.001). This correlation improves for drinking water with As concentrations above 2 ppb (r=0.74; p<0.001). Male minors (<18 years old) were found to have greater nail–As concentrations compared with adults consuming equal amounts of As (p<0.05). Estimated As dose (self reported volume consumed * As concentration in drinking water) specifically from drinking water sources, was also associated with nail concentrations (p<0.01). Results demonstrate that As measurement in nails could be a
reliable method for estimating the magnitude of As exposure in residents living in rural areas of the Ethiopian Rift Valley.

1.4 Chapter 3 Synopsis: Arsenic exposure in the Mekong Delta

Chapter 3 gives an in depth look at As contamination of groundwater drinking resources in the Mekong Delta, Vietnam, in order to assess the occurrence of As in the groundwater and the magnitude of As exposure of local residents through drinking water. Groundwater (n=68) and toenail (n=62) samples were collected in Dong Thap Province adjacent to the Mekong River in southern Vietnam. Fifty-three percent (n=36) of the wells tested had As content above the WHO’s recommended limit of 10ppb. Samples were divided into a Northern (mean As = 4.0ppb) and a Southern (mean As = 329.0ppb) group; the Southern group was located closer to the Mekong River. Elevated As contents were associated with depth (<200m), groundwater salinity (low salinity), and redox state (reducing conditions). In 79% of the wells As was primarily composed of the reduced As(III) species. Arsenic contents in nails collected from local residents were significantly correlated to As in drinking water (r=0.49, p<0.001), and the relationship improved for pairs in which As in drinking water was higher than 1ppb (r=0.56, p<0.001). Demographic and survey data show that the ratio of As in nail to As in water varies among residents, which reflects differential As exposure in the population. The data show that water filtration and diet, particularly increased consumption of animal protein and dairy and reduced consumption of seafood, were associated with lower
ratios of As in nail to As in water and thus could play important roles in mitigating As exposure.

1.5 Chapter 4 Synopsis: Arsenic concentrations in toenails across different populations exposed to arsenic in drinking water

The final chapter of this dissertation compares the relationships between arsenic concentrations in keratin in the form of toenails and arsenic in water across several locations. Three diverse populations were analyzed: residents from North Carolina, USA (n=103); the Rift Valley, Ethiopia (n=60); and the Mekong Delta, Vietnam (n=65). Arsenic concentrations in groundwater above the WHO’s recommended 10ppb limit were found in all of the three locations albeit each had different mean As values and frequency of samples above 10ppb (Union County, NC mean=11ppb; Rift Valley mean=18.6ppb; Mekong Delta mean=211.2ppb). Arsenic-nail concentrations were correlated to As concentrations in groundwater in all three locations (r(Union County)= 0.48, p<0.001; r(Ethiopia)=0.72 p<0.001; r(Vietnam)=0.49, p<0.001). When only pairs in which As in water was above 1ppb are considered, these three locations are statistically indistinguishable from one another (r=0.62, p<0.001, n=176). These results support the hypothesis that nails can be used as a biomarker of exposure regardless of geographic or ethnic differences in populations considered. Nutrition (meat, seafood, and milk consumption) rather than gender or ethnicity is suggested to be the major confounding issue affecting As bioaccumulation in the human body.
2. Arsenic exposure of rural populations from the Rift Valley of Ethiopia as monitored by toenails

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2.1 Introduction

Arsenic (As) exposure is a global phenomenon; millions consume drinking water with As concentration exceeding the WHO recommended guideline of 10 ppb (WHO 2011). High levels of As exposure have been shown to cause acute health effects such as nausea, vomiting, abdominal pain, profuse diarrhea, renal failure, and shock (Huang et al. 1985), whereas chronic exposure is known to increase the risk of diseases such as lung, skin, kidney, urinary, bladder cancers, cardiovascular diseases, peripheral neuropathies, and diabetes, as well as many others (Abernathy et al. 1999; Chen CJ et al. 1992, 2005; Chouhan and Flora 2010; Kapaj et al. 2006; Kitchin 2001; Meliker et al. 2007; Morales et al. 2000; NRC 2001; Yoshida et al. 2004). Evaluating the symptoms and diseases directly associated with low-to moderate As levels, yet still exceeding the maximum contaminant level (MCL) threshold (10 ppb), is problematic because of the long latency period between chronic exposure and disease occurrence, many confounding factors, and multiple competitive risks (e.g., it has been shown that As can act as a synergistic carcinogen) (Bates et al. 1995, 2004; Chen CL et al. 2004; Chen Y et al. 2006; Ferreccio et al. 2000; Lindberg et al. 2010; Ratnaike 2003). Understanding the correlation between moderate As exposure from the ingestion of drinking water and
bioaccumulation in humans is challenging yet essential for evaluating health implications of long-term consumption of As-contaminated water.

Numerous studies have used keratin biomarkers in human subjects (e.g., hair and nails) for delineating the accumulation of As in the human body resulting from As exposure (Garland et al. 1993; Hinwood et al. 2003; Karagas et al. 1996, 2000; Pearce et al. 2010; Samanta et al. 2004; Slotnick and Nriagu 2006; Yoshida et al. 2004). Here we present a unique case study in which the majority of participants living in the Rift Valley of Ethiopia only consume water from a single water source—a community well located in their village, which allows us to minimize error due to multiple drinking water sources. This study presents data of the relationships between As in drinking water and nails from rural communities in the Rift Valley of Ethiopia, where well water is the only available drinking water source and the participants report long residency times. We hypothesize that the magnitude of As exposure from drinking water for these populations can be reliably determined by nail–As analysis. In addition, basic demographic information, residency time, water consumption habits, and meat consumption patterns were collected and analyzed in order to understand the role they might have on As bioaccumulation in the residents (Survey attached in Appendix A).

Groundwater from the Ethiopian Rift Valley is known to contain high levels of fluoride, which is a serious health concern (Gizaw 1996; Rango et al. 2010, 2012; Reimann et al. 2003; Tekle-Haimanot et al. 2006). Yet, in addition to fluoride, recent
studies have also documented As concentrations in drinking water above the WHO limit of 10 ppb (Godebo et al. 2013; Rango et al. 2010). Although fluoride may garner more attention because of its early and visible health effects, the potential effects of moderately elevated As ingestion are more insidious, causing long-term health effects that are difficult to correlate directly to water exposure. Evaluation of As exposure can be affected by multiple confounding factors that are not always visible during brief exams in the fields. In addition, other As exposure and health outcome monitoring factors such as smoking, sun exposure, nutrition, water consumption rates, age, gender, genetic factors, and water chemistry could affect the exposure evaluation (Brima et al. 2006; Gebel 2000; Hossain et al. 2012; Karagas et al. 2000; Pierce et al. 2011; Vahter et al. 2002; Yoshida et al. 2004; Zablotska et al. 2008). Although there are some disagreements in the literature (Smith et al. 2000), evidence points toward malnutrition, particularly diets lacking animal protein, folic acid, calcium, vitamin A, and fiber, as being associated with increased incidences of As-induced skin lesion (Anetor et al. 2007; Zablotska et al. 2008). Nutrients such as animal proteins, folic acid, vitamin A, and fiber may therefore play a protective role in preventing negative health outcomes associated with As exposure by increasing the rate at which the methylation process, and therefore the elimination of As from the body, can occur (Brima et al. 2006; Mitra et al. 2004; Pierce et al. 2011). Arsenic is primarily excreted from the body through urine (Ratnaike 2003). In order for this to occur, As is methylated through a series of oxidative methylation and
reduction reactions (Figure 1).

Figure 1: Arsenic reduction and oxidative methylation steps in the body. Inorganic As(V) (IAs^5) is reduced to inorganic As(III) (IAs^3) by glutathione (GSH)
which is then oxidized to glutathione disulfide (GSSG). Inorganic As(III) then undergoes oxidative methylation to monomethyl-As(V) (MMA\textsuperscript{5}) by S-adenosyl methionine (SAM) which in the process is then transformed to S-adenosyl-homocysteine (SAH). This process repeats to form monomethyl-As(III) (MMA\textsuperscript{3}), dimethyl-As(V) (DMA\textsuperscript{5}), and dimethyl-As(III) (DMA\textsuperscript{3}).

This study is focused on measuring As in nail samples of rural populations in the Ethiopian Rift Valley. Keratin, with its large quantity of the sulfhydryl-rich amino acid cysteine group, binds As and subsequently removes it from the metabolic process, preserving it in the nail material (Slotnick and Nriagu 2006; Yoshida et al. 2004). Nails in particular are thought to be a reliable biomarker because of their ease in collection and storage, and the lack of external contamination compared to other keratin mediums (Garland et al. 1993; Hinwood et al. 2003; Karagas et al. 2000; Slotnick and Nriagu 2006). The slow growth of toenails represents an exposure window ranging from several months to a year and provides the base for understanding chronic accumulated exposures (Schroeder and Balassa et al. 1966; Slotnick and Nriagu 2006; Yoshida et al. 2004). In addition, the slower growth of toenails compared with fingernails corresponds with a greater As content per equal mass of nail (Brima et al. 2006; Karagas et al. 1996; Slotnick and Nriagu 2006), which makes its analysis more sensitive to changes in As in drinking water.

This study provides systematic measurements of As and its major inorganic species in drinking water wells combined with As measurements in toenails of residents who consume well water from selected villages in the Ethiopian Rift. The study aims to
establish the relationship between As in water and nails for quantifying the magnitude of As exposure, understanding the factors that may modify this exposure to As, and the role that As may be playing in the health of people in the region.

2.2 Methods

2.2.1 Site Selection

The study area with a length of ~210 km is located in the main Ethiopian Rift Valley. In each town, local water officials were contacted and acted as guides to locate the drinking water wells. Wells were selected based on accessibility and whether or not they were functioning. As part of this study, 34 wells were tested. Figure 2 shows the location of these wells and their As concentrations.

At each location, researchers selected participants by intercepting local residents who were present at the well at the time of sampling. The survey questionnaire and study were conducted after ethical approval from the Duke University Institutional Review Board (IRB). An additional permission to carry out the project was also obtained from the Addis Ababa University and local institutes in the studied region (schools, water bureaus, and hospitals) after an explanation of the objectives and the method of study. Anonymity was also ensured to the investigated subjects.

Participants completed a questionnaire (Appendix A) to document not only their general demographics such as age, gender, and residence time at the location, but also their water consumption volumes and locations, tobacco usage, general health status,
and a basic nutrition assessment. In terms of nutritional assessment three major foods, animal protein, seafood consumption, and milk consumption, were focused on for two reasons a) their documented relationship with As excretion rates and b) they are foods consumed in many populations exposed to As making the instigated affects potentially generalizable to other populations. Participants were asked explicitly how often they consumed these foods. Results generated provide frequency of food consumption however not quantity. Because all nail samples were collected within a 4-week period, seasonal variations in concentrations due to nail growth rate variations were not considered in our analysis.

2.2.2 Drinking water collection and analysis

Well water samples were collected at the well site following US Geological Survey protocols along with pH, electrical conductivity, oxidation–reduction potential, dissolved oxygen, and temperature (USGS 2011). Samples were filtered at the well sites using 0.45 mm syringe filters and preserved using nitric acid. Samples were then shipped back to Duke University for analysis; more details of the analytical methodologies can be found in Ruhl et al. (2010). Major elements were determined by direct current plasma optical emission spectrometry (DCP-OES) and anions by ion chromatography (IC). Trace elements were analyzed by inductively coupled plasma mass spectrometry (ICP-MS). Speciation of As was performed in the field using methods
described in Bednar et al. (2002) applying EDTA and anion exchange to separate the uncharged As(III) species and preserved separately in the field.

2.2.3 Nail collection and analysis

Toenails were collected from all 10 toes from participants using clean new clippers and stored in a Ziploc bag until the samples could be cleaned and analyzed. Visible dirt was removed by hand. Samples were cleaned with successive rinses of acetone, a 1% Triton X solution, and an additional acetone rinse. Each rinse was sonicated for 30 min and followed with a sonicated water rinse for 30 min. After cleaning, the samples were dried overnight at 60 °C. Samples were digested using ultra pure concentrated HNO₃ and 30% H₂O₂ in a ratio of 1ml HNO₃: 100 μl H₂O₂: 10mg of nail using methods modified from Chen KLB et al. (1999), Karagas et al. (2000), and Samanta et al (2004). After digestion, samples were sealed in Teflon containers and heated at a low temperature to ensure each sample’s uniformity. An aliquot was then diluted with ultrapure water and run on the ICP-MS. Linear regression analysis was completed to analyze the relationships between nails and water, while t-tests were employed to analyze nutrition.
2.3 Results

Arsenic was detected in all 34 selected wells, ranging from 0.6 to 73.4 ppb with a mean of 18.6 ppb; 19 out of 34 (53%) of the wells tested had As concentrations above the WHO limit of 10 ppb. The spatial distribution of As concentrations in well waters as well as the concentration frequencies are shown in Figures 2 and 3, respectively.

Figure 2: Location of drinking water wells in the Ethiopian Rift Valley and the spatial distribution of their As concentrations
Figure 3: Histogram of As in drinking water wells tested. Out of 34 wells, 19 were above the WHO’s threshold drinking limit of 10ppb.

A total of 60 participants (43 males and 17 females) completed the study questionnaire, and 58 donated nail samples. The mean age of participants was 21 years, ranging from 8 to 58 years of age (male average age: 22; female average age: 21). The data show that As concentrations in nails were positively correlated with As concentrations in water ($r=0.72$; $R^2=0.52$; $p<0.001$). All participants who donated nails had lived in the area for at least 1 year (mean=20 years, ranging from 4 to 57 years) and
had been consuming water from the specific community well during that time period. The water and nail data were not normally distributed, and required log-transformation before statistical analysis.

The correlation between As–nail concentration and water concentration improved for water exposures above 2 ppb (Figure 4a). The correlation obtained for

![Figure 4: (a) Log-As concentration in nail versus log-As concentration in drinking water. For groundwater above 2ppb (red circles), higher correlation was obtained between As in nails and As in water (r=0.74; R²=0.55; p<0.001) relative to groundwater below 2ppb (blue diamonds). (b) Log-As concentration in nail versus log-As concentration in drinking water for adults (>18 years old; red circles; r=0.72; R²=0.52; p<0.001) and minor males (blue squares; r=0.92; R²=0.85; p<0.001). The two populations are significantly different from each other (p<0.05).](image)

nail–water pairs for population consuming drinking water with As >2 ppb was higher than the bulk data with an r value of 0.74 (R²=0.55; p<0.001).

The data show no statistically significant difference in nail concentrations between males and females, which may be a result of the limited number of females in
the study. Upon arrival at each well location, researchers approached those present, explained the study, and asked for volunteers. Although males and females were both present at the wells, males were more likely to volunteer to participate, creating an inevitable male bias in the samples collected. There was a small but significant difference between adult males and minor males (defined as <18 years of age; p<0.05; Figure 4b). Minors were found to have greater As accumulation in their nails as compared with their adult counterparts, despite equal amounts of As in their drinking water, which has been reported in another study investigating the correlation between As exposure and nail concentrations (Hinwood et al. 2003).

In the oxidizing groundwater of the Ethiopian Rift valley (Rango et al. 2010), the predominant As species is arsenate (H₂AsO₄⁻, As(V)) relative to the reduced form arsenite (H₃AsO₃, As(III)). Arsenite is known to be more toxic to human health (Bednar et al. 2002; Schroeder and Balassa 1966; Smith et al. 1992). Our water quality data are consistent with Rango et al. (2010, 2012) and Godebo et al. (2013) and show that the majority of the study wells tested predominately comprised As(V); yet, wells with higher concentrations of As contained relatively more As(III) than those with lower As concentrations. Figure 5 shows the As speciation data from groundwater samples investigated in this study. Groundwater with As(V) comprising >90% of the total As is
Figure 5: Ratios of As(V)/As(III) versus concentration of As in well water. Wells with predominance of As(V) that comprised at least 90% of the total dissolved As are marked by red circles relative to wells where As(V) was <90% (i.e., predominance of As(III)) marked as blue squares.

In addition to determining the relationship between As in water and nails, we evaluated the As dose for the participants by using the amount of water consumed by each individual daily and the As concentrations in the drinking water (Figure 6). Individuals were asked to self-report the amount of water they consumed. Dose was
defined as \( d = c \times v \), where \( d \) = dose, \( c \) = As concentration in water in parts per billion, and \( v \) = self-reported volume of water consumed per day in liters. Water consumption ranged from 0.5 l/day to 3 l/day, with a mean of 1.54 l/day, which is slightly below the WHO worldwide average of 2 l/day (WHO 2011). None of the participants in the study

![Figure 6: As-water dose (defined as As concentration in drinking water * self-reported volume of water consumed) versus As concentration in participants’ nails. The data show a clear relationship between the amount of As consumed and As bioaccumulation \((r=0.67; \ p<0.01)\).](image)
reported using bottled water, and 11 of the 59 participants reported rare usage of additional water sources (usually surface water sources) beside their well water source.

Basic nutrition information such as frequency of meat and milk consumed was collected as part of this study. In addition, participants were asked to report what foods they consumed most often. Participants’ diets consisted primarily of maize, injera (made of teff grain), and occasionally wheat. Because regular meat and dairy consumption was less common, a 24-h food recall survey would not have accurately reflected consumption of these foods, and therefore individual participants were asked their consumption patterns for these items explicitly. Mean meat intake for participants was 14.6 times/year (median 4 times/year). Dairy consumption was more common than meat consumption; participants reported consuming dairy ~3 times/week on average (median 1.5 times/week). None of the participants reported any seafood consumption, which could have potentially increased exposure to organic As present in seafood (Gebel 2000; Liao et al. 2008).

2.4 Discussion

The As concentration in nail samples collected was significantly correlated with the As in participants’ drinking water, showing that As is in fact accumulating in people in this region consuming well water. It is important to note that bioaccumulation does not directly equate to negative health outcomes; however, it is a vital first step in assessing which populations should be targeted when evaluating health outcomes from
As exposure.

Karagas et al. (2000) found that there is a significantly improved correlation between nails and water when individuals from New Hampshire, USA, were exposed to As in water concentrations above 1 ppb. This may be evidence for a drinking water exposure threshold at which the body begins to accumulate As that is reflected in the As level in the nails. Our data validate this trend, and the nail–water correlation improves for As water concentrations above 2 ppb (Figure 4a). These results suggest that below 1 ppb the primary source of As exposure is not water, but some other source such as diet. The threshold our data show is slightly greater than that of Karagas et al. (2000) (2 versus 1 ppb); however, that is likely because of the low number of wells we measured with As near or below 1 ppb. It is important to note that As is already accumulating in the body below the water concentration that WHO considers safe (10 ppb), and negative health effects caused by exposures in this range need to be investigated in the future.

One major limitation of this study was a lack of female participants. When researchers arrived at each well site, the study was explained to individuals present and the first few individuals to volunteer were recruited into the study. Although unintentional, males were more likely to volunteer. Males <18 years old were found to have greater As accumulation in their nails compared with their adult counterparts, a relationship that has been noted in previous studies (Garland et al. 1993). Nail growth rate is maximized around ages 10–14 years (Dawber et al. 2001). Faster nail growth
would result in lower As concentrations per equal mass of nail. In comparing As concentrations between adult males and minor males, we assume that nail growth is equal and therefore the rate of As deposited is the same in both subgroups. However, if children’s nails grow faster than their adult counterparts, nails may actually be underestimating the As bioaccumulation in minors compared with adults. Minors (of both genders) in our study had higher concentrations in general than adults (of both genders); however, for females this difference was not statistically significant.

The As in the samples of groundwater was overwhelmingly As(V) (as compared with As(III)). Although As(V) is thought to be slightly less toxic to humans than As(III), consumption never the less is harmful (Bednar et al. 2002; Schroeder and Balassa et al. 1996; Smith et al. 1992). In addition, wells with the highest levels of As had a relatively higher As(III) fraction (Figure 5). This means that individuals from the Rift Valley who are at a greater risk due to consuming larger concentrations of As are also at an increased health risk from consuming relatively greater amounts of As(III). Our data did not show any evidence for higher As in nails for individual consuming either As(III) or As (V); however, the small number of As–nail to predominantly As(III) pairs in our sampling pool makes this distinction difficult. Because of the lack of heterogeneity in As speciation in the water samples tested, the population in this study is not a good candidate for investigating the role that As speciation may play in As bioaccumulation in the nail.
Individuals were asked to self-report the amount of water they consumed in order to calculate a dose. Although recall error in the estimation of personal consumption is often a major source of bias, individuals in this region commonly used jerry cans and other water containers to transport water from the well to their home each day, and hence participants had a good understanding of how much water was brought into their home each day, and reported using only one water source. Our data suggest a clear relationship between dose of As from direct water intake and exposure. Although the average daily water intake for the study population was below the level at which the WHO bases their As limits in water, the data show that individuals consuming this amount are still being exposed at a non-negligible level, and that this exposure is reflected by As increasing in the nail even at concentrations of <10 ppb in drinking water. Although not all exposure would occur from direct intake of As in water (Hossain et al. 2012), our data demonstrate that for the studied population, exposure from water alone correlates with As bioaccumulation. Consequently, this study shows that As intake from contaminated drinking water is playing a major role in As exposure.

The role of nutrition in health outcomes related to As exposure is an important research question that is still debated; although some studies question the protective role that nutrition may play in preventing negative health outcomes (Smith et al. 2000), others have shown a decrease of skin lesions associated with better nutritional status, particularly with regard to calcium, animal protein, folate, and fiber intakes (Mitra et al.)
Animal protein is a source of methyl donors that play a role in the excretion of As from the body (Anetor et al. 2007; Brima et al. 2006; Gebel 2000; Styblo et al. 1997; Vahter 2002). Although many studies have measured the concentrations of As compared with the incidence of health outcomes associated with As exposure, and even in some cases the concentrations of As in the food, no other studies to our knowledge have yet examined the relationships between nail biomarker As concentrations and nutrition. This association is an important step in understanding the relationship between bioaccumulation and nutrition.
Of the 55 participants, 36 (65%) consumed meat less than 5 times per year. Our data show that As–nail concentrations of individuals who consume meat once per month or less is higher compared with those who consume once per week or more for the same As concentrations in drinking water (p<0.2; Figure 7). It should be noted that this difference was not significantly different at the p<0.05 level, but it is significant with an 80% confidence interval. Given the variability in individual metabolism, this

Figure 7: Boxplot showing that the ratios of As in nails to As in water differ in participants by their meat consumption patterns: meat consumption once per month or less (n=32) versus meat consumption more than once per month (n=14). This difference is statistically significant with an 80% CI (p=0.02).
difference is reasonable. These data variations are consistent with the observations that meat consumption may lower incidences of As-induced skin lesions (Brima et al. 2006; Gebel 2000; Smith et al. 2000; Styblo et al. 1997; Vahter 2002).

In conclusion, this study investigated the bioaccumulation of As in residents of rural communities of the Ethiopian Rift valley of Eastern Africa who were exposed to different levels of As in drinking water. Our study monitored large variations in As concentrations in well water (<1 to 70 ppb). However, As bioaccumulation in the body, as revealed by As concentrations in nails of residents who consume well water, was also found in individuals who consume drinking water below the WHO guideline value of 10 ppb. A systematic correlation was found between As in drinking water and As in nails. We identified an empirical threshold of 2 ppb from which As bioaccumulation strongly corresponded to As levels in drinking water. In addition, our results show that poor nutrition and drinking water consumption habits also affect As bioaccumulation. This study found that As–nail analysis is an important and useful tool in assessing the body burden of As, particularly in those rural communities where health information is not often available. Future work should assess the types of the specific health outcomes in populations exposed to such intermediate As levels in drinking water and show measureable As bioaccumulation.
3. Arsenic exposure in the Mekong Delta

3.1 Introduction

The Mekong Delta is a biologically diverse and water-rich area, and together with the Red River in northern Vietnam comprises one of the most productive agricultural regions in Southeast Asia (Berg et al. 2001, 2007). Projected hydropower generation and dam construction on the upstream Mekong River pose risks for water availability in the downstream Mekong Delta. Consequently, groundwater is expected to become a pivotal irrigation and drinking water resource in this region. It is estimated that the demand for groundwater will increase by up to 6.5 times by 2020 (510-520x10⁹ m³/year) in comparison to the 1990-2000 period (Van et al., 2004). In addition to water availability, quality of groundwater in the Mekong Delta may limit its potential to substitute for declining surface water resources. In particular, previous studies have highlighted the occurrence of high salinity and arsenic in groundwater that could limit agricultural production and pose human health risks (Buschmann et al., 2009).

Understanding of the relevant geochemical conditions in which contaminants are mobilized to groundwater and the magnitude of exposure of the local residents to these contaminants are important for evaluating the risks associated with anticipated growth in populations transitioning from surface water to groundwater use in the Mekong Delta.

Arsenic in Vietnam has been measured in groundwater in both the Red River
and the Mekong River Deltas (Berg et al., 2001, 2007, 2008; Buschmann et al., 2009; Nguyen and Itoi, 2009; Winkel et al., 2010). Elevated As levels typically occur in shallow groundwater from both the Holocene and Pleistocene aquifers. In the Red River Delta, Winkel et al. (2010) demonstrated over-pumping of the As-free deep groundwater as the cause of drawdown of shallow As-rich, groundwater to deep previously uncontaminated aquifers resulting in contamination of deep aquifers and degraded drinking water resources.

Arsenic contamination in groundwater from the Mekong Delta is naturally occurring and caused by chemical and microbial induced reductive dissolution of iron-oxides from the alluvial sediments in the delta (Rowland et al., 2008; Quicksall et al., 2008; Fendorf et al., 2010; Winkel et al., 2010). The World Health Organization (WHO’s) recommends As concentrations be below 10 ppb in drinking water (WHO, 2011), but As concentrations in groundwater over 800 ppb have been reported in the region (Winkel et al., 2011; Stollenwerk et al., 2007). In addition, other inorganic contaminants with potential health effects including Mn and Ba have been found in the region (Buschmann et al., 2007, 2008). Bushchmann et al. (2008) noted that high As levels occur selectively in low-saline drinking water wells close to the Mekong River, while groundwater located greater distances from the Mekong River is characterized by higher salinity and lower As content.

In spite of the extensive literature on the toxic effects of As, it can be difficult to
establish a direct link between health effects such as cancer and As exposure from drinking water in a given population due to the long latency period between the window of exposure and the development of health outcomes. Keratin-rich tissues, such as hair and nails, have been shown to be the preferred material to monitor long-term exposure to As in drinking water. While blood and urine are useful biomarkers for smaller exposure windows, nails reflect an integrated exposure time ranging from 3 months to a year (Schroeder and Balassa, 1966; Slotnick and Nriagu, 2006; Yoshida et al., 2004). Toenails are thought to be better than fingernails at capturing As exposure due to the fact that their slower growth rate provides greater As levels per mass compared to fingernails. Both are thought to be better than hair because variations in individual hair growth rates vary more across populations compared to individual nail growth rates (Karagas et al., 1996, 2000; Slotnick and Nriagu, 2006).

Although elevated As in drinking water sources of the Mekong and Red River Deltas in Vietnam have been identified as a major health concern, no exposure study through the monitoring of As in nails has been conducted in the Mekong Delta Region. Nguyen et al. (2009) found a correlation between As concentrations in potable groundwater from the Red River Delta, and As concentrations in women’s hair, while Berg et al. (2007) found correlation between As concentrations in drinking water and As concentrations in hair in the Mekong Delta (in both Vietnam and Cambodia), and also in the Red River Delta. The current investigation aims to fill the literature gap by focusing
on As occurrence and human exposure in the Mekong Delta by measuring As concentrations in drinking water and in nails of local residents consuming groundwater as their major drinking water source.

The objectives of this study are to: (1) evaluate the As occurrence in the Mekong Delta groundwater; and (2) assess the magnitude of exposure in populations consuming As in their drinking water. Groundwater and nail data from Dong Thap Province in southern Vietnam were compared to As data of previous studies. By understanding the extent of As distribution in groundwater and its accumulation in the local populations in the Mekong Delta this paper provides the foundation for evaluating the health risks associated with the increased utilization of groundwater, which will likely result from the projected reduction of future Mekong River flows.

3.2 Methods

IRB approval was obtained from Duke University, Ho Chi Minh Science University, and the Department of Natural Resource and Environment of Dong Thap Province. The study site spans approximately 70km north to south in the Dong Thap province of Vietnam. Wells were selected based on government approval, as well as consent from individual well owners. In total 68 groundwater wells were tested as well as 5 surface water samples from the Mekong River.

Groundwater from private and monitoring wells, as well as surface waters from the Mekong River were collected following USGS protocols (USGS, 2011). Samples to be
analyzed for trace metals were filtered at the site location using 0.45 μm syringe filters and preserved using nitric acid and then shipped to Duke University for analysis. Samples were analyzed for major elements using direct current plasma optical emission spectrometry (DCP-OES), anions by ion chromatography (IC), and trace metals by inductively coupled plasma mass spectrometry (ICP-MS). More detailed methods can be found in Ruhl et al. (2010). Speciation of As was performed in the field and preserved according to methods in Bednar et al. (2002). Parameters collected in the field include pH, temperature, conductivity (EC), dissolved oxygen (DO), and oxidation-reduction potential (ORP).

Toenails clippings were collected from individuals whose water had been sampled. Researchers approached participants, explained the study and obtained consent. Individuals were then surveyed to collect basic demographic information as well as water consumption patterns and basic health issues. Toenails were then clipped using new clean stainless steal clippers and stored in Ziploc© bags and shipped to Duke University to be analyzed according to methods described in in Merola et al. (2013). In total 62 nail samples were collected.

Toenails were cleaned in the laboratory with successive sonicated rinses of acetone, a 1% titron-X solution, and another acetone rinse, with water rinses in between. Nails were then dried for at least 24 hours at 60°C and then digested with HNO₃ and 30% H₂O₂. An aliquot of digested solution was then diluted and run on the ICP-MS
(Chen et al., 1999; Karagas et al., 2000; Merola et al., 2013; Samanta et al., 2004). Linear regression analysis was completed to analyze the relationships between nails and water, while t-tests were employed to analyze factors potentially altering As magnitude such as nutrition and health outcomes.

### 3.3 Results and Discussion

#### 3.3.1 Arsenic occurrence in groundwater from Dong Thap Province, Vietnam

Groundwater samples from Dong Thap had elevated levels of arsenic. Fifty-three percent (36 out of 68 wells) of the study wells had As levels above the WHO’s recommended 10ppb limit. The spatial distribution of the sampling locations as well as the As concentrations are shown in Figure 8a. In general, two subgroups of groundwater with respect to As were identified (Figure 9): (1) groundwater from the northern part of the region near Tan Hong, further from the river with overall lower As concentrations (n=23; ranging from below limit of detection to 22.2ppb; median value 2.0ppb; mean value 4.0ppb); and (2) groundwater from the southern section of the region, located closer to the Mekong River and possessing higher As concentrations near Thanh Binh (n=45; ranging from 0.1 to 981.4ppb; median value 271.5ppb; mean value 329.0ppb). In contrast, the median As concentration of samples collected directly from the Mekong River was much lower (median=1.1ppb; mean=1.1ppb; n=5; ranging from 0.78-1.35ppb) but not negligible (detection limit=0.05 ppb).
Legend

- Study Area
- Major Rivers
- River Samples

Map A:
Arsenic Concentration (ppb)

- 0 - 10
- 10 - 50
- 50 - 100
- 100 - 250
- 250 - 1340

Map B:
Sample Density
Wells/Sqkm

- High : 2.5
- Low : 0

Map C:
Arsenic Concentration (ppb)

- <1
- 1-10
- 10-50
- 50-100
- 100-400
- 400-750
Figure 8: A) Arsenic variations in sampling sites of this study. Samples were divided into two subgroups: a Northern Subgroup located away from the Mekong River with lower As concentrations, and a Southern Subgroup located closer to the Mekong River with much higher As concentrations. B) Sample density of data points collected from multiple research studies (Buschmann et al., 2007, 2008; Nguyen and Itoi, 2009; NWD, 2014; Papacostas, et al., 2008; Sthiannopkao et al., 2008) in the Mekong Delta. C) Interpolated As concentrations across the Mekong Delta.

Figure 9: Histogram of As concentrations in the Northern and Southern subgroups. Fifty-three percent of all wells had As content above the WHO's 10ppb recommend drinking water limit, where most of the higher concentrations were found in the Southern group.

The Mekong Delta region is comprised of alluvial Holocene sediments (depth of 8 - 40m) overlying Pleistocene sediments (50 – 80m; Figure 10) (Nguyen and Itoi 2009).
Arsenic above 10 ppb was typically found in relatively shallow wells from both the Holocene and Pleistocene aquifers. Two deep wells (>200m) of the Lower Pliocene aquifer were exceptional and also showed elevated As. Both deep wells are located in the Northern subgroup.

Figure 10: Depth of the wells versus the arsenic concentration in groundwater, sorted by the groundwater location. The approximate depths of the different sub-aquifers in the Mekong Delta region (DWRPIS, 1992) are included.

Groundwater from both the Northern and Southern sampling subsets showed large chloride variations with values ranging from 2.7 to 1527 mg/L, which is consistent with salinity levels reported in Berg et al. (2007). With one exception elevated As concentrations were not found in wells with chloride concentrations above 200 mg/L.
While elevated chloride levels were also found in shallower wells (<100m depth), chloride and arsenic showed no relationship, indicating different modes of contamination.

Figure 11: Arsenic versus chloride concentrations in the study groundwater, sorted by the groundwater location. Groundwater from the southern area (red squares) is characterized by higher arsenic contents relative to the northern area (blue squares). No correlation between arsenic and salinity was observed.
Previous studies conducted in the region have suggested that As released from the delta sediments is due to the reductive dissolution of the iron bearing minerals (Berg et al., 2001; Winkel et al., 2011; Nguyen and Itoi, 2009). The As contents of groundwater in our study were consistently associated with low Eh values (approximately -100mv), which infer reducing conditions (Figure 12). During sediment transport under oxic

![Graph showing redox potential measured by Eh (mv) versus arsenic concentrations in groundwater samples collected in this study, sorted by the groundwater location.](image)

*Figure 12: Redox potential measured by Eh (mv) versus arsenic concentrations in groundwater samples collected in this study, sorted by the groundwater location.*
Arsenic concentrations were the highest in groundwater with negative Eh values that reflects anoxic conditions mostly in the southern area (red squares) relative to the northern area (blue squares).

Conditions oxyanion As species are bound to Fe-oxides, peat, clay, and other humic substances. Under the delta reducing conditions As is mobilized to the ambient groundwater (Berg et al., 2001, 2008; Bissen and Frimmel, 2003; Harvey et al., 2002; Nguyen and Itoi, 2009; Nickson et al., 1998; Polizzotto et al., 2005). Groundwater from the Northern subgroup tends to be less anoxic with a higher Eh values and lower pH levels relative to the As-rich Southern sub-group (Figure 13).
Figure 13: Redox potential measured by Eh (v) versus pH. Groundwater from the Northern subgroup (red squares) is less anoxic (higher Eh values) in contrast to samples from the southern subgroup (blue squares). The southern subgroup is more anoxic and has higher As concentration.

Arsenic in groundwater was composed of a mixture of As(III) and As(V) species. On average, As (III) constituted 79% of the total As, while As (V) consisted of only 21% of the total As; no differences in As species distribution were observed between the
Northern and Southern sub-areas. This trend is similar to previous reports conducted in the Red River Delta which found that As(III) constitutes 90% of the total As (Nguyen et al., 2009).

### 3.3.2 Arsenic occurrence in groundwater from other areas of the Mekong Delta

An extensive literature review was conducted to compile a large water quality database (n=7,346) and to integrate the As data distribution in groundwater across Mekong Delta region from Vietnam and Cambodia (Figures 8b and 8c) (Buschmann et al., 2007, 2008; Nguyen and Itoi, 2009; NWD, 2014; Papacostas, et al., 2008; Sthiannopkao et al., 2008). A density map of the distribution of wells shows that more wells have been investigated in Cambodia compared to Vietnam, particularly in the Phnom Phenh region (Figure 8b). Figure 8c illustrates the distribution of As contents in the groundwater and shows the proximity of the high-As groundwater to the Mekong River.

While the highest As values are found closest to the Mekong River, measurable As levels (>1ppb) were recorded in almost all groundwater samples in the database. GIS analysis of the region shows that the average As value decreases with distance from the Mekong River (Table 1).

<table>
<thead>
<tr>
<th>Average As Concentration (ppb)</th>
<th>Distance From Mekong River (km)</th>
</tr>
</thead>
<tbody>
<tr>
<td>132.9</td>
<td>0-1 km</td>
</tr>
<tr>
<td>Distance</td>
<td>As Concentration</td>
</tr>
<tr>
<td>----------</td>
<td>------------------</td>
</tr>
<tr>
<td>1-2 km</td>
<td>128.2</td>
</tr>
<tr>
<td>2-3 km</td>
<td>107.6</td>
</tr>
<tr>
<td>3-4 km</td>
<td>103.6</td>
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<td>17.3</td>
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<td>10-15 km</td>
<td>5.3</td>
</tr>
<tr>
<td>15-20 km</td>
<td>3.9</td>
</tr>
<tr>
<td>20+ km</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Within 1 km from the river the average As is ~133 ppb. In most cases groundwater within 10 km of the Mekong River had As contents above the WHO's 10 ppb limit, while groundwater located further way had lower concentrations.

Combining the interpolated As concentration map with population data (CIESIN, 2014) suggests that approximately 12.7 million people in the region are living in areas with average As-groundwater concentrations above the WHO's 10 ppb level, while 4.12 million people are consuming As concentrations above 1 ppb but below 10 ppb.

### 3.3.3 Arsenic in nails from exposed population

A total of 65 individuals donated nails (26 males; 39 females), while only 45 of those chose to complete the accompanying survey. Gender was recorded regardless of survey completion. The average age of all participants was 45 years old (n=43) (mean male age 51; mean female age 41). Only 4 minors (defined as participants under 18 years old), 2 males and 2 females, participated in the survey and nail donation. All participants reported living in their current home for at least one year, which negates
concerns about capturing exposures from other locations. On average participants lived in their current home for 20 years with residence times ranging from 1 to 74 years.

Arsenic concentrations in nails were significantly linearly correlated to As concentrations in drinking water \( (r=0.49, R^2=0.24, p<0.001; \text{Figure 14}) \). Studies have shown

![Figure 14: Nail-As concentrations (µg-As/g-nail, log scale) versus arsenic concentration in drinking water (µg/L; log scale). Nail-As values are significantly correlated with arsenic concentrations in drinking water \( (r=0.49, p<0.001) \). Dark squares are nail-water pairs measured in groundwater with As content above 1 µg/L.](image-url)
while blue circles are pairs in groundwater with As below 1 µg/L. The correlation between As-nail and As-water improves when only samples above 1 µg/L were considered ($r=0.56$, $p<0.001$).

there may be a threshold level at which As begins accumulating in the nail (Karagas et al., 2000; Merola et al., 2013). Successive linear regressions were performed to determine if this trend was present in this dataset. Changes in both the correlation coefficient and slope variable were considered when samples below certain thresholds were removed from the dataset. The threshold at which the correlation was maximized was 1ppb ($r=0.56$, $R^2=0.31$, $p<0.001$), which agrees with Karagas et al (2000). The data show no nail-As concentration differences based on age or gender, however as there were only 4 minors enrolled in the study, this dataset does not have the power to evaluate true age differences.

Diet, water consumption, filtration capacity, and occupational information were collected from participants to evaluate the effect they might have on As exposure (Table 2). To evaluate the possible differential As bioaccumulation, we normalized the As content in the nail to As level of co-existing groundwater. The differential As bioaccumulation factor ($\chi$) is defined as $\chi = \text{nail concentrations (µg-As/g-nail)} / \text{As in groundwater (ppb)}$. This variable allows analysis of factors affecting the magnitude of exposure however would be greatly improved by the ability to account for the amount of water consumed and the weight of participants, however this data was not available for all individuals. Thirty-one participants reported their occupation; these
were regrouped into two categories: (1) an outdoor exposure group (n=18); and (2) an indoor exposure group (n=10). Individuals working outdoors may consume more water and have a greater potential for As exposure. The outdoor exposure group included farmers, laborers and livestock tenders, whereas the indoor exposure group included homemakers, teachers and a student. Results indicated that the outdoor exposure group had a high bioaccumulation factor (mean $\chi=0.47$), while the indoor exposure group had a much lower value (mean $\chi=0.05$; $p = 0.067$).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Exposed population ($\chi$)</th>
<th>Low exposure population ($\chi$)</th>
<th>p Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Occupation</td>
<td>Outdoor exposure group</td>
<td>Limited exposure group</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.47</td>
<td>0.05</td>
<td>$p=0.067$</td>
</tr>
<tr>
<td></td>
<td>n=18</td>
<td>n=10</td>
<td></td>
</tr>
<tr>
<td>Household Treatment</td>
<td>No treatment</td>
<td>Some treatment</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.32</td>
<td>0.03</td>
<td>$p=0.057$</td>
</tr>
<tr>
<td></td>
<td>n=28</td>
<td>n=7</td>
<td></td>
</tr>
<tr>
<td>Personal water consumption habits</td>
<td>Exposed group</td>
<td>Low exposure group</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>0.02</td>
<td>$p=0.03$</td>
</tr>
<tr>
<td></td>
<td>n=31</td>
<td>n=9</td>
<td></td>
</tr>
<tr>
<td>Seafood consumption</td>
<td>Frequent consumption</td>
<td>Limited consumption</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.38</td>
<td>0.02</td>
<td>$p=0.02$</td>
</tr>
<tr>
<td></td>
<td>n=28</td>
<td>n=10</td>
<td></td>
</tr>
<tr>
<td>Meat consumption</td>
<td>Frequent consumption</td>
<td>Limited consumption</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>0.50</td>
<td>$p=0.02$</td>
</tr>
<tr>
<td></td>
<td>n=19</td>
<td>n=21</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Variations of As-nails to As-water ratios (bioaccumulation factor) in residents from the Mekong Delta.
<table>
<thead>
<tr>
<th>Milk consumption</th>
<th>Frequent consumption</th>
<th>Limited consumption</th>
<th>p=0.03</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.02</td>
<td>0.31</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=8</td>
<td>n=34</td>
<td></td>
</tr>
</tbody>
</table>

Most individuals were not using any treatment for the water they consumed (n=28). Testing sand filtration systems in other parts of Vietnam have found 90% removal of the total As in drinking water (Nguyen et al., 2009; Berg et al., 2006). In our study, 25% of households used some form of treatment (n=7). Treatment methods varied and included: carbon filters, settling and boiling combinations, and sand filters.

Considering the treatment availability for the investigated household, the average As bioaccumulation factor for individuals not using any treatment was much higher ($\chi^2=0.32$) relative to those with treatment ($\chi^2=0.03$, $p=0.057$). To further investigate this relationship, we evaluated the personal water consumption habits and compared the nail concentrations for individuals who reported only consuming unfiltered well water (exposed group) versus individuals who reported drinking filtered well water, occasional use of city water, or use of bottled water (low exposure group). The difference between these two groups was statistically significant ($p=0.03$); the average bioaccumulation factor for the exposed group was much higher ($\chi^2=0.33$) compared to the low exposure group (0.02). In sum, our data show that evaluation of As exposure requires a careful examination of the personal drinking habits that could mask the overall correlation between As in nail versus As in drinking water.
When conceptualizing As exposure it is important to consider confounding issues that might arbitrarily raise or lower As values in the nails. Important variables include age, gender, race, volume of water consumed, source of water, treated versus untreated water consumption, and dietary factors. Diet is of particular interest when understanding exposure because of the complexity it adds to understanding effects. Studies have shown that increased consumption of animal proteins, folic acid, calcium, and vitamin A is associated with a decrease in As induced skin lesions (Anetor et al., 2007; Mitra et al., 2004; Pierce et al., 2010; Zablotska et al., 2008). Merola et al., (2013) showed an inverse trend between As concentration in nails and greater rates of animal protein consumption. It has been suggested that increasing the consumption of these nutrients may increase the rate at which As can be metabolized in the body, eliminating it faster and therefore buffering against negative health effects (Brima et al., 2006; Pierce et al., 2011; Mitra et al., 2004).

Participants were asked to report the frequency of consumption of foods that may affect As metabolism and therefore concentration in nails. In particular, we focused on seafood, meat, and milk consumption. Increased seafood consumption was found to correspond with an increase in nail-As concentration, likely from the organic As in seafood (Gebel 2000; Liao et al. 2008). Twenty-eight participants reported consuming seafood daily, while 10 participants stated less frequent seafood consumption (between 1-3 times per week). The mean seafood consumption rate was 5 times per week. The
average As bioaccumulation factor for those consuming seafood daily was higher (\(\chi=0.38\)) than those with less frequent seafood consumption (\(\chi=0.01; p=0.02\)). This artificial increase in As may not have any effect on the health of the participants since the As consumed would predominately be organic As instead of the more toxic inorganic form, but it highlights the sensitivity nails possess for monitoring As exposure.

Increased meat consumption has been linked with a decrease in As related health problems, by increasing the rate at which As can be removed from the body (Mitra et al., 2004; Pierce, 2010; Scott et al., 1993). The frequency of meat consumption was measured however not the quantity. Participants were categorized into two exposure groups (1) a high meat consumption group that was defined as consuming meat at least 2-3 times per week or more (n=19); and (2) a low meat consumption group defined as consuming meat once per week or less (n=21). On average participants in our study consumed meat twice per week. Participants who consumed meat more frequently had statistically significant lower As-nail/As-water ratios compared to those who consumed meat less frequently (p=0.02). The average As bioaccumulation factor for those in the high meat consumption group was 0.01 relative to 0.50 in the low-meat consumption group.

Calcium like animal protein consumption has been shown to have an inverse correlation with negative health outcomes related to As consumption (Mitra et al., 2004). To evaluate calcium intake we surveyed the frequency of milk consumption among the
participants. Milk is not the only source of calcium but a source that is can be
generalizable to other populations. On average, the participants in our study reported
consuming milk approximately twice per month (median was no milk consumption).
Thirty-four participants reported no milk consumption, while 8 participants reported
milk consumption rates ranging from daily to less than once per month. Here again, we
show the effect on As bioaccumulation; the difference in the As bioaccumulation factor
was statistically significant (p=0.03) with higher levels for those not consuming milk
(mean $\chi=0.31$; $n=34$) relative to those who consumed milk with lower As
bioaccumulation (mean $\chi=0.02$; $n=8$). These results highlight the important role diet may
play on the exposure and regulation of As metabolism.

It is important to note that the bioaccumulation factor values for the specific yet
different exposure groups we identified were similar ($\chi=0.3$) and higher by a factor of 10
relative to the non-exposed groups. Since the bioaccumulation factor is the slope of the
relationship between As in nails to As in drinking water, we propose that this slope can
be used to delineate the selective bioaccumulation of exposed and/or higher risks groups
relative to the rest of populations. Thus As content in nails could represent not only the
overall exposure of populations to As in drinking water, but can also detect specific
populations with higher bioaccumulation factors and thus higher risks. While the
overall slope of As-nail to As-water in the entire population was 0.003, the higher
exposed and/or less preferred nutrition groups had a higher slope value of ~0.3.
As part of our study, participants were asked to report health issues including the occurrence of skin rashes, upper and lower abdominal pain, changes in hearing or vision, numbness or tingling in the extremities, breathing problems, joint pain, delays in wound healing, speed of hair growth, tiredness, and frequency of diarrhea. These health outcomes were designed as a basic general health assessment that could be conducted by non-medical professionals. In total, 13 health variables were collected; a positive response was defined when participants reported no problem with the health issue in question, while a negative response was defined when participants reported suffering from the particular health issues. On average, each participant (n=41) reported 2.5 negative health responses; participants’ responses ranged from no negative health issues to up to 6 negative health issues. While nail-As values are not able to signal disease occurrence, the data show a general trend of higher As-nail to As-water ratios in individuals reporting some negative health outcomes (Table 3). We show that As bioaccumulation factor in nails was higher (with varying degrees of statistical significance) among individuals reporting skin changes and rashes, lower abdominal pain, upper abdominal pain, vision changes, numbness, and joint pain. While these responses were not significant at the 95% confidence interval, most were significant at the 70-80% confidence interval or greater (Table 3). In contrast, no correlations were observed between As bioaccumulation factor and health issues of hearing loss, breathing difficulty, the rate of wounds healing, speed of hair growth, tiredness, and diarrhea. A
major hindrance was the small subgroups reporting these health issues, however we believe this potential relationship is incredibly important and further validates the strength of using nails as a biomarker of As exposure.

**Table 3: Relationship between health outcomes that were self-reported by participants and As-nail to As-water ratios.**

<table>
<thead>
<tr>
<th>Health outcome with relationship to $\chi$</th>
<th>Mean $\chi$</th>
<th>Mean $\chi$</th>
<th>p Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No effect reported</td>
<td>Effect Reported</td>
<td></td>
</tr>
<tr>
<td>Skin changes</td>
<td>No changes</td>
<td>Changes</td>
<td>0.196</td>
</tr>
<tr>
<td></td>
<td>n=33</td>
<td>n=5</td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>No pain</td>
<td>Pain</td>
<td>0.192</td>
</tr>
<tr>
<td></td>
<td>n=30</td>
<td>n=7</td>
<td></td>
</tr>
<tr>
<td>Upper abdominal pain</td>
<td>No pain</td>
<td>Pain</td>
<td>0.285</td>
</tr>
<tr>
<td></td>
<td>n=26</td>
<td>n=9</td>
<td></td>
</tr>
<tr>
<td>Vision</td>
<td>No vision changes</td>
<td>Vision loss</td>
<td>0.199</td>
</tr>
<tr>
<td></td>
<td>n=26</td>
<td>n=10</td>
<td></td>
</tr>
<tr>
<td>Numbness</td>
<td>No numbness</td>
<td>Numbness</td>
<td>0.206</td>
</tr>
<tr>
<td></td>
<td>n=24</td>
<td>n=11</td>
<td></td>
</tr>
<tr>
<td>Joint Pain</td>
<td>No pain</td>
<td>Pain</td>
<td>0.295</td>
</tr>
<tr>
<td></td>
<td>n=28</td>
<td>n=5</td>
<td></td>
</tr>
<tr>
<td>Health outcomes with inverse or no relationship to $\chi$</td>
<td>Heal normally</td>
<td>Delayed healing</td>
<td>0.416</td>
</tr>
<tr>
<td></td>
<td>n=23</td>
<td>n=17</td>
<td></td>
</tr>
<tr>
<td>Hair growth</td>
<td>Normal</td>
<td>Slow</td>
<td>0.308</td>
</tr>
<tr>
<td></td>
<td>n=31</td>
<td>n=9</td>
<td></td>
</tr>
<tr>
<td>Tiredness</td>
<td>Normal</td>
<td>Unusually tired</td>
<td>0.300</td>
</tr>
</tbody>
</table>

51
### 3.4 Conclusion

Our study shows that about 16 million people living in the Mekong Delta in Vietnam and Cambodia are at risk for elevated levels of As in their drinking water. Most of the As occurs in shallow and reduced groundwater, which is common in many deltaic aquifer environments of southeast Asia (Harvey et al., 2002; Nickson et al., 1998; Polizzotto et al., 2005). Populations living closer to the river have the greatest risk for exposure to elevated As, yet As levels in groundwater above 1 ppb were found also in areas over 20km away from the Mekong River. The positive correlation between As in nails and As in water ($r=0.49, p<0.001$; Figure 14) clearly shows bioaccumulation of As in residents in the area, including those who consume drinking water with As concentrations below the WHO’s limit of 10ppb. Consequently, our data show that bioaccumulation of As is occurring for all the populations who consume groundwater, including those who are consuming levels considered safe (the range of 1ppb to 10ppb). We use the ratios of As-nail to As-water to evaluate the differential As bioaccumulation.

<table>
<thead>
<tr>
<th></th>
<th>None reported</th>
<th>Frequent</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>n=29</td>
<td>n=11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.332</td>
<td>0.066</td>
<td>p=0.065</td>
</tr>
<tr>
<td></td>
<td>n=31</td>
<td>n=5</td>
<td></td>
</tr>
<tr>
<td>Hearing</td>
<td>No hearing loss</td>
<td>Hearing loss</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.307</td>
<td>0.014</td>
<td>p=0.036</td>
</tr>
<tr>
<td></td>
<td>n=32</td>
<td>n=4</td>
<td></td>
</tr>
<tr>
<td>Breathing problems</td>
<td>No problems</td>
<td>Trouble breathing</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.380</td>
<td>0.040</td>
<td>p=0.039</td>
</tr>
<tr>
<td></td>
<td>n=27</td>
<td>n=9</td>
<td></td>
</tr>
</tbody>
</table>
The data show higher As-nail to As-water ratios (~0.3) in sub-groups with higher potential exposure (water use, occupation, diet). Thus we propose that the As in the nails methodology could be used for delineating specific and vulnerable exposed populations with higher risks for As bioaccumulation relative to the rest of the population. Our results show differential As bioaccumulation on the local population based on occupation, diet (more bioaccumulation for seafood, less accumulation for meat (protein) and milk (calcium), and water treatment. These observations indicate that the exposure of the local population to As in their drinking water could be reduced through treatment of the groundwater, diet, and occupation. A reduction in the As bioaccumulation could help to mitigate the long term negative health issues caused by long-term exposure to As in drinking water in the Mekong Delta.
4. Biomarkers of Exposure: Arsenic concentrations in toenails across different populations exposed to arsenic in drinking water

4.1 Introduction

Arsenic (As) contamination of groundwater sources is a well-known problem affecting millions of people who consume elevated As and are therefore at risk for As-related health problems. The World Health Organization’s (WHO) recommended drinking water limit is 10ppb in drinking water, however lower levels can also induce health issues and thus no As level of consumption is considered safe given that As is a known mutagen, carcinogen, and teratogen (Mandal and Suzuki, 2002; WHO, 2011).

Arsenic has been linked to a wide variety of health affects. Chronic exposure is associated with an increased risk of different types of cancer (bladder, kidney, urinary, lung, and skin), peripheral neuropathies, cardiovascular diseases, skin lesions, diabetes, and more (Abernathy et al., 1999; Chen et al., 1992; Chen et al., 2005; Chouhan and Flora, 2010; Kapaj et al., 2006; Kitchin, 2001; Mandal and Suzuki, 2002; Meliker et al., 2007; Morales et al., 2000; NRC, 2001; Yoshida et al., 2004.)

Arsenic in urine and keratin, in the form of hair or nails, are useful biomarkers of exposure to detect As bioaccumulation in humans (Hughes, 2006; Slotnick and Nriagu, 2006). Inorganic As is removed from blood within a few hours; so while blood can be useful for detecting acute poisonings, it is not useful for long term exposure monitoring (Hughes, 2006). There are many benefits to urinary analysis of As; urinary excretion is
the predominate method for eliminating As from the body and different metabolites can be detected and measured. Keratin in the form of finger nails or toenails does not provide the ability to distinguish As speciation in the body, but is stable over longer periods of time, non-invasive, and easily transportable, making it ideal for monitoring As bioaccumulation, particularly where field collection and analysis are not geographically close to one another (Slotnick and Nriagu, 2006; Garland et al., 1993; Hinwood et al., 2003; Karagas et al., 2000; Yoshida et al., 2004). While urinary analysis reflects exposure from several days, keratin values reflect a 6 to 12 months exposure window, making it ideal for chronic and long term As monitoring (Brima et al., 2006; Hughes, 2006; Karagas et al., 1996; Schroeder and Balassa, 1966; Slotnick and Nriagu, 2006; Yoshida et al., 2004). Consequently, these factors have led numerous studies to use keratin to delineate As bioaccumulation in exposed populations (Garland et al., 1993; Hinwood et al., 2003; Karagas et al., 1996, 2000; Merola et al., 2013; Samanta et al., 2004; Slotnick and Nriagu, 2006; Yoshida et al., 2004).

In addition to complications arising from synergistic health affects, confounding issues related to bioaccumulation of As obscure the assessment of As exposure. The literature highlights factors such as climate, genetics, age, gender, smoking, sun exposure, nutrition, water consumption rates, and water chemistry (Brima et al., 2006; Gebel, 2000; Hossain et al., 2012; Karagas et al., 2000; Pierce et al., 2010; Slotnick and Nriagu, 2006; Vahter, 2002; Yoshida et al., 2004; Zablotska et al., 2008). Nail growth can
be affected by seasonal and geographical climate variations as nails grow faster in warm climates (Fleckman, 2005; Slotnick and Nriagu, 2006). Faster nail growth would equate to less As deposition at the base of the nail, in essence lowering the overall As concentrations compared to the average. Nail-As concentrations have also been seen to vary according to ethnicity (Brima et al., 2006; Loffredo et al., 2003). Brima et al. (2006) investigated three unexposed populations of different ethnicities living in the United Kingdom and found that they had statistically different average nail-As concentrations. The hypothetical mechanism being either varying metabolic rates of As among various ethnicities, or potentially differing amounts of sulfhydryl concentrations in the keratin.

Gender has also been seen to affect As concentrations in biomarkers however, the current literature shows disagreement between whether males or females exhibit greater As concentrations when controlling for As concentrations in water (Hinwood, 2003; Loffredo et al., 2003). Loffredo et al. (2003) showed the differences in gender vary by ethnicity when measuring urinary As, although water intake, diet, and age were not accounted for in this study.

Age is an important variable since children may have greater As concentrations compared to their adult counterparts due to difference in body burdens, or, in young children, play behavior such as putting objects in their mouths (Hinwood et al., 2003; Slotnick and Nriagu, 2006). Studies have reported children with elevated nail-As levels compared to adults (Hinwood et al., 2003). Nail growth rates are maximized around
ages 10-14, which causes the assumption of an approximately average nail growth rate to break down (Dawber et al., 2001; Slotnick and Nriagu, 2006). Assuming an average nail growth for all ages would result in an underestimation of As exposure in children due to their faster nail growth.

The last confounding issues of particular importance when considering As in nails is nutrition. Increased consumption of animal protein, folic acid, calcium, vitamin A, and fiber have been linked with a decreased incidence of As related skin lesions (Anetor et al., 2007; Mitra et al., 2004; Pierce et al., 2010; Zablotska et al., 2008). The mechanism for this decrease is that these foods and nutrients increase the rate by which the As methylation process occurs; therefore removing it from the body faster (Brima et al., 2006; Pierce et al., 2010; Mitra et al., 2004; Gebel, 2000; Styblo et al., 1997).

The literature indicates that understanding the correlation between levels of As in water and the magnitude of exposure in humans is essential for evaluating health implications of long-term consumption of As contaminated water and is the focus of this study. The goal of this paper is to evaluate As exposure in various populations with different social (e.g., water source, nutrition, ethnicity) and physical (e.g., water chemistry, aquifers) conditions using toenails as a biomarker of exposure. The study provides systematic measurements of As in drinking water wells combined with co-As measurements in toenails of people that consume that water in three populations with different geological and social conditions: (1) private homeowners from Union County,
North Carolina, USA; (2) rural communities from the Rift Valley, Ethiopia; and (3) rural communities and farmers from the Mekong Delta region of Vietnam. The study aims to compare the relationships between As in water to As in nails in the three different field studies and to quantify the magnitude of As accumulation in the different populations. The study also evaluates the confounding factors that may effect and/or modify the exposure and accumulation As in the human body. We have explored the ethnicity, geographic location, age, gender, quantity of water consumed, nutrition, and As speciation variations and their relationships with the As-nail to As-water ratios.

4.2 Methods

Three case study locations were selected in Union County, North Carolina, USA, the Rift Valley, Ethiopia, and in the Mekong Delta, Vietnam. IRB approval was obtained from both Duke University local collaborators before research was begun.

4.2.1 Recruitment and Survey

4.2.1.1 Ethiopia

At each location, researchers selected participants by intercepting local residents who were nearby the well at the time of sampling. The project was explained, a water sample collected, and the survey was administered.

4.2.1.2 Union County

Several methods were used to recruit participants. The first 40 households participating were recruited by identifying residential plots that are serviced by city and
community water providers and excluding them, then flagging the remaining residential plots as potentially utilizing private well water and sending them recruitment material. Later, the study was advertised by local mayors in the county, and on local television and print-news outlets. In addition, postcards alerting county residence of the study were sent to registered voters. Participants then contacted us to participate, and the study was explained in further detail. One member of the household was surveyed in depth (with respect to water consumption habits, and nutrition), while age and gender data was collected for the entire household.

4.2.1.3 Mekong Delta, Vietnam

Households with potentially high As levels in their groundwater wells were identified and pre-approved for participation by local government officials. After a home was identified, a translator would explain the study to the homeowners and obtain consent followed by administration of the survey and groundwater testing.

4.2.2 Water Collection and Analysis

All water samples were collected according to USGS protocols (USGS, 2011). At each ground water well field parameters (pH, oxidation reduction potential (ORP), conductivity, and dissolved oxygen) were collected. Trace elements were analyzed using inductively coupled plasma mass-spectrometer (ICP-MS). Major cations were analyzed using direct coupled plasma optical emission spectrometry (DCP-OES). Arsenic speciation was performed in the field according to methods in Bednar et al. (2002). Major
anions were analyzed using ion chromatography (IC). Water analysis methods are described in Ruhl et al. (2010).

4.2.3 Nail Collection and Analysis

Nail clippings from all 10 toes were collected in the field and brought back to Duke University where they were cleaned, digested, and analyzed. Detailed methods can be found in Merola et al. (2013). Visible dirt was removed by hand. Samples were cleaned with successive rinses of acetone, a 1% Titron X solution, and a second acetone rinse. Each rinse was sonicated for 30 minutes, followed with a sonicated water rinse for 30 minutes, and dried overnight. Samples were digested using ultra pure concentrated HNO₃ and 30% H₂O₂. Cleaning and digestion procedures were developed using methods modified from Chen et al. (1999), Karagas et al. (2000), and Samanta et al. (2003).

4.3 Results and Discussion

Three geographically diverse case studies were selected for this study. Figure 15 shows the locations of each case study and the As distribution in the three sites. The first
Figure 15: Map showing all three study populations in relation to one another. Arsenic concentrations in groundwater for each sample location is shown.

case study is located in Union County, North Carolina, USA. This region is located in the Carolina Slate Belt, where previous studies have identified elevated As levels in groundwater associated with specific rock types (Kim et al., 2011; Sanders et al., 2012).

Sixty-one wells were tested from this area. Arsenic values ranged from below the limit of detection (0.07) to 130ppb, with a mean of 11ppb (median=1.5ppb). Fifteen out of the 61 wells (24.6%) were above the WHO’s drinking water limit. One hundred three
participants donated nail samples and were surveyed to some degree. Table 4 shows the breakdown of gender and age for the different and combined populations.

Table 4: Population breakdown for each case study and compiled populations. Number of participants, age, and gender are shown. In addition nutrition data is shown. Meat, seafood, and milk consumption averages are given for each case study and compiled populations.

<table>
<thead>
<tr>
<th></th>
<th>All case studies</th>
<th>North Carolina</th>
<th>Ethiopia</th>
<th>Vietnam</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>228</td>
<td>103</td>
<td>60</td>
<td>65</td>
</tr>
<tr>
<td>Male</td>
<td>126 (55)</td>
<td>57 (55)</td>
<td>43 (72)</td>
<td>26 (40)</td>
</tr>
<tr>
<td>Female</td>
<td>102 (45)</td>
<td>46 (45)</td>
<td>17 (28)</td>
<td>39 (60)</td>
</tr>
<tr>
<td>Mean age, years (median, range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>40 (42, 4-89)</td>
<td>49 (52, 4-89)</td>
<td>21 (20, 8-58)</td>
<td>45 (44, 13-78)</td>
</tr>
<tr>
<td>Male</td>
<td>38 (34, 4-89)</td>
<td>47 (51, 4-89)</td>
<td>22 (21, 8-58)</td>
<td>50 (48, 25-78)</td>
</tr>
<tr>
<td>Female</td>
<td>43 (44, 5-86)</td>
<td>52 (54, 5-86)</td>
<td>21 (17, 8-45)</td>
<td>42 (40, 13-74)</td>
</tr>
</tbody>
</table>

Nutrition

Meat Consumption, mean per week

<table>
<thead>
<tr>
<th></th>
<th>All participants</th>
<th>North Carolina</th>
<th>Ethiopia</th>
<th>Vietnam</th>
</tr>
</thead>
<tbody>
<tr>
<td>All participants</td>
<td>2.3</td>
<td>6.2</td>
<td>0.3</td>
<td>1.7</td>
</tr>
<tr>
<td>Participants above 1ppb As in water</td>
<td>1.7</td>
<td>6.2</td>
<td>0.3</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Seafood Consumption, mean per week

<table>
<thead>
<tr>
<th></th>
<th>All participants</th>
<th>North Carolina</th>
<th>Ethiopia</th>
<th>Vietnam</th>
</tr>
</thead>
<tbody>
<tr>
<td>All participants</td>
<td>3.5</td>
<td>1.0</td>
<td>0</td>
<td>5.7</td>
</tr>
<tr>
<td>Participants above 1ppb As in water</td>
<td>4.0</td>
<td>1.2</td>
<td>0</td>
<td>5.4</td>
</tr>
</tbody>
</table>

Milk Consumption, mean per week

<table>
<thead>
<tr>
<th></th>
<th>All participants</th>
<th>North Carolina</th>
<th>Ethiopia</th>
<th>Vietnam</th>
</tr>
</thead>
<tbody>
<tr>
<td>All participants</td>
<td>2.3</td>
<td>5.5</td>
<td>2.7</td>
<td>0.4</td>
</tr>
<tr>
<td>Participants above 1ppb As in water</td>
<td>2.1</td>
<td>4.7</td>
<td>2.8</td>
<td>0.4</td>
</tr>
</tbody>
</table>

The second case study is in the Rift Valley region of Ethiopia (Merola et al. 2013).

Here 34 drinking water wells were evaluated and As in wells was from 0.6 to 73.4ppb (mean=18.6ppb; median=10.2ppb). Nineteen out of the 34 wells (53%) had As levels
above the WHO’s 10ppb limit. Sixty participants completed the study questionnaire however only 58 donated nail samples.

The third case study was conducted in the Mekong Delta region of Vietnam (Merola et al., in review). Sixty-eight groundwater samples were collected and As concentrations above 10ppb was found in 53% (36 out of 68) of samples. Values ranged from below the limit of detection to 981.4ppb (mean 211.2ppb; median=17.5ppb). Sixty-five individuals donated nail samples but only 45 participated in the accompanying survey (gender was recorded regardless of survey participation). Figure 16 illustrate the As concentration distribution in the three locations.

Figure 16: Histogram of As concentrations for each case study.
Overall, data from 228 participants was analyzed for this study. Figure 17 shows As concentration in nails (µg-As/g-nail) versus As concentration in groundwater (ppb) in the three case studies. Each of the nail-water relationship is statistically significant ($r$ (Union County) = 0.48, $p<0.001$; $r$ (Ethiopia) = 0.72, $p<0.001$; $r$ (Vietnam) = 0.49, $p<0.001$). However the three groups are statistically different from one another ($p<0.05$). Given this distinction,
distinct from one another ($r_{\text{Union County}} = 0.48, p<0.001$; $r_{\text{Ethiopia}} = 0.72, p<0.001$; $r_{\text{Vietnam}} = 0.49, p<0.001$).

one would assume that either geography (leading to different rates of nail growth due to climate) or ethnicity are variables that affect bioaccumulation of As in nails. Yet, we show below that this observation may not be correct.

Karagas et al. (2000) suggested that there may be some limit of detection or background level of As in nails from which As can either not be accurately measured or is simply not associated with As levels in drinking water. In that particular study, this limit was suggested to be 1ppb. We tested the possible minimum threshold value by evaluating the linear regressions of the nail-water pairs in the three case studies. We found that the correlations between As-nail to As-water statistically increase upon using such minimum thresholds values; 2ppb in the Ethiopian population and 1ppb in both the North Carolina and Mekong Delta populations. When we combined the pairs from the three case studies, the limit was also found to be 1ppb. Applying the As-nails to As water relationships for cases above 1ppb, the three case studies are no longer statistically different from one another and exhibit a common correlation (Figure 18). These results
suggest that previous studies may be biased since they included “unexposed” or “low exposure” populations consuming As in water below 1ppb that could mask the true relationship between As-nail to As-water. Our data also demonstrate that geography does not play a role in As bioaccumulation. To our best knowledge, this is the first
study in which nail values from different populations are compared and are not statistically different from one another.

The combined data from the three sites show statistically significant correlation between As-nail to As-water, including samples below 1ppb. This correlation is significant regardless of any confounding factors including: water chemistry, age, gender, ethnicity, geographic location, diet, or water consumption habits. Figure 19 shows the overall correlation of As-nail to As-water concentrations ($r_{\text{all values}} = 0.56$, $p<0.001$, $n=228$). Above the 1 ppb threshold the correlation is strengthen ($r_{\text{above 1ppb}} = 0.62$, $p<0.001$, $n=176$).
Figure 19: Arsenic concentrations in nails (µg-As/g-nail) versus As concentration in groundwater (ppb) for all populations. The nail-water relationship is statistically significant when all data points are considered however correlation increases above 1ppb ($r_{(all\ values)}=0.56, p<0.001$; $r_{(above\ 1ppb)}=0.62, p<0.001$).

In order to evaluate confounding variables effect on the concentration of As in the nail independent of As exposure from drinking water, we normalized the As content in the nail to the As level in the water consumed, creating a differential bioaccumulation factor $\chi$; where $\chi$= nail concentrations (µg-As/g-nail) divided by As in groundwater (ppb). Differences in $\chi$ were considered in relationship to age, gender, As speciation, and
dose (self reported quantity of water consumed * As concentration in drinking water). This variable allows us to analyze factors regardless of water As concentration however to be more accurate individual quantity of water consumed for each and body weight would also be included. There were no statistical differences found for any of these variables (Appendix B: Figures 20-23). While previous studies have reported differences in gender and ethnicity (Loffredo et al., 2003; Brima et al., 2006) the combined data presented in this study revealed no relationships between age, gender, and ethnicity. While this does not mean that those relationships don’t potentially exist, we believe our data points to previous relationships reported being due to a lack of sample size.

In contrast, nutrition seems to be a measureable confounding variable that can explain differences in As-nail concentrations. Table 4 shows average meat, seafood, and milk consumption for each case study as well as for the combined database. Meat consumption was investigated because animal protein donates methionine, which will increase the rate of As metabolism. Studies have shown that increased meat consumption resulted in lowered incidences of As-induced skin lesions (Anetor et al., 2007; Brima et al., 2006; Gebel, 2000; Styblo et al., 1997; Vahter et al., 2002). When meat consumption frequency for all three case studies were analyzed, individuals consumed meat on average approximately twice per week. Individuals who consumed meat everyday had lower χ levels than individuals consuming meat less often (χ=0.095 versus 0.206, p=0.07). Table 5 shows differences in χ due to nutrition in all participants as well
as $\chi$ values in participants consuming water above 1ppb. When only participants consuming water above 1ppb were considered, the statistical difference was lower, although still different at the 90% confidence interval ($\chi=0.025$ versus 0.013, $p=0.095$). Furthermore, the difference in $\chi$ values with respect to meat consumption continues to hold when

**Table 5: Nutritional affect on $\chi$ bioaccumulation values for all participants. Increases in meat and milk consumption lower $\chi$ values while increases in seafood consumption raise $\chi$ values.**

<table>
<thead>
<tr>
<th>All Data</th>
<th>Variable</th>
<th>Exposed</th>
<th>Low exposure</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Meat Consumption</td>
<td>Less than everyday</td>
<td>Everyday</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.206</td>
<td>0.095</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>n=97</td>
<td>n=29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Everyday</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Seafood Consumption</td>
<td>Everyday</td>
<td>Less than everyday</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.391</td>
<td>0.245</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>n=27</td>
<td>n=45</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-3 times per week or more</td>
<td>Less than 2-3 times per week</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.364</td>
<td>0.210</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>n=42</td>
<td>n=30</td>
</tr>
<tr>
<td></td>
<td>Milk Consumption</td>
<td>Never consume milk</td>
<td>Consume milk</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.242</td>
<td>0.136</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>n=44</td>
<td>n=83</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Above 1ppb As in water</th>
<th>Variable</th>
<th>Exposed</th>
<th>Low exposure</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Meat Consumption</td>
<td>Less than everyday</td>
<td>Everyday</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.025</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>n=84</td>
<td>n=14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Less than once per week</td>
<td>Once per week</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.029</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n=52</td>
<td>n=46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>------</td>
<td>------</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Seafood Consumption</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Everyday</td>
<td>0.046</td>
<td>0.015</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than Everyday</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-3 times per week or more</td>
<td>0.036</td>
<td>0.013</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 2-3 times per week</td>
<td>0.013</td>
<td>0.015</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk Consumption</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never consume milk</td>
<td>0.031</td>
<td>0.018</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consume Milk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

including individuals who consume meat once per week versus those with less than once per week for participants that consume drinking water with As above 1ppb ($\chi=0.029$ versus 0.017, $p=0.097$).

Individuals were asked to report seafood consumption because it is a potential source of organic As (Gebel, 2000; Liao et al., 2008). The data on seafood consumption excludes the Ethiopia population (given the lack of seafood eating in the population) and considers seafood consumption approximately 3-4 times per week. Seafood consumption seems to affect the $\chi$ values in individuals consuming seafood everyday relative to less than everyday, and in individuals consuming seafood 2-3 times per week, and less than 2-3 times per week. Those consuming seafood everyday compared to less than everyday had $\chi$ values of 0.046 and 0.015 respectively; while those consuming seafood 2-3 times per week or more compared to less than 2-3 times per week had lower
χ values of 0.036 and 0.013. These differences were only statistically significant (p=0.05) in individuals consuming water above 1ppb.

Finally, milk consumption, used as an index for calcium consumption, also had an affect on the bioaccumulation factor. Participants who consumed milk had lower χ values than participants who did not. Individuals who never consume milk had a mean χ value of 0.242 versus 0.136 for participants who consume milk at some frequency (p=0.021). While only statistically significant at the 90% confidence interval, this relationship is also true for participants consuming water above 1ppb As; those consuming no milk had a mean χ value of 0.031 while those consuming milk had a χ of 0.018 (p=0.094).

In conclusion, our results show that nails can be used as a reliable biomarker for exposure to As regardless of geography, age, gender, or ethnicity of populations in question. The reliability of nails as a tracer for As bioaccumulation seems to diminish below the 1 ppb threshold minimum As content in groundwater. The only meaningful variable that has any affect on As concentrations in nails is nutrition, in that increased meat and milk consumption lowers the nail-As concentrations while increased seafood consumption increases nail-As concentrations. These results have important implications for As monitoring as more detailed dietary intake monitoring may be necessary for correctly evaluate the exposure of populations to As in drinking water.
Appendix A

A survey was used to collect demographic, water consumption, diet, and health information. Appendix A shows a generic survey adapted for residents living in Union County, NC (although health questions were not used in this case study). Questions were tailored to each location based on focus group and pretesting results. For example sand filters were a treatment option in Vietnam however this particular technique is not used in North Carolina and therefore not included as an option.
ID creation

What type of residence is this?
- House
- Townhouse/Duplex
- Apartment
- Mobile home
- Other ________________

In what year was your home built?
- Participant Knows ________________
- I don't know

Can you approximate the age of the home? For example, do you think the home is more than 10 years old, more than 20 years old, more than 30 years old, etc.? Give the largest number of years for which you feel confident. For example, if you know the house is at least 10 years old, but you are not sure if it is 20 years old, please say 10 years.
- Participant knows ________________
- I don't know

How long have you lived at this residence?
- Participant knows ________________
- I don't know

Can you approximate the number of years you have lived at this residence? For example, have you lived here for more than 10 years, more than 20 years, etc.? Give the largest number of years for which you feel confident.
- Participant knows ________________
- I don't know

Including you, how many people live at this residence?


Please answer the following question:

<table>
<thead>
<tr>
<th>Gender</th>
<th>Age</th>
<th>Individual ID</th>
<th>Race</th>
<th>Race do you identify with?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Are you male or female?</td>
<td>How old are you?</td>
<td>A1, B1, C1, D1</td>
<td>M, F, MM, MF</td>
<td></td>
</tr>
</tbody>
</table>

Participant ID:
- ☐ Male
- ☐ Female

<table>
<thead>
<tr>
<th>Pregnant?</th>
<th>Pregnant in the last year</th>
<th>Occupation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Are you currently pregnant?</td>
<td>Have you been pregnant in the last year?</td>
<td>What is your occupation?</td>
</tr>
</tbody>
</table>

Participant ID:
- ☐ Yes
- ☐ No

What is the age of your well?
- ☐ Participant knows age ________________
- ☐ I don’t know

Can you approximate the age of the well? For example, do you think the well is more than 10 years old, more than 20 years old, more than 30 years old, etc.? Give the largest number of years for which you feel confident. For example if you know the well is at least 10 years old, but you are not sure if it is 20 years old, please say 10 years.
- ☐ Participant can estimate ________________
- ☐ I don’t know

Have you ever had your well tested for any contamination?
- ☐ Yes
- ☐ No
- ☐ I don’t know

When was the test done? Please enter the year. If the participant is not sure write not sure. If it is an approximation say approx.

What contaminants were tested?
What were the results of the test?

- I don't remember
- Everything was fine
- One or more of the contaminants concentration was too high. (Which one?)
- Other

Do you presently use any of the following treatment technologies for any water consumed in your home? (Check all that may apply)

- Carbon filter (Includes Brita filters and similar devices, filters on refrigerator water dispensers, etc.)
- Water softener
- Reverse osmosis
- Other
- I don't use any treatments on my water.

Where is this device installed?

- Manual filtering system such as a Brita filter
- Refrigerator water supply
- Kitchen tap
- Other taps within home
- Whole house treatment
- Other

Approximately how many glasses of water the size of a soda can do you drink on an average day? Do not include water used in cooking, but do include water used in making other beverages such as coffee, iced tea, lemonade, etc.
What is the primary source of the water you drink?
- Tap water from your home that is unfiltered
- Tap water from your home that is filtered
- Bottled water
- Water from another source (workplace tap, restaurant, etc.? please explain) ________________
- Other ____________________

How many meals do you or your family members cook in your home during the week?

What is the primary source of water you cook with?
- Tap water from your home that is unfiltered
- Tap water from your home that is filtered
- Bottled water
- Other ____________________

I’m now going to ask you about what you ate or drank yesterday. This question is to get a snapshot of the water you might consume on any given day. I am going to talk you through your day and ask you to remember what you ate or drank.

What does your diet usually consist of? What foods do you eat most often?
How often do you eat seafood?
- Never
- Less than Once a Month
- Once a Month
- 2-3 Times a Month
- Once a Week
- 2-3 Times a Week
- Daily

How often do you eat meat not including seafood?
- Never
- Less than Once a Month
- Once a Month
- 2-3 Times a Month
- Once a Week
- 2-3 Times a Week
- Daily

How often do you drink milk?
- Never
- Less than Once a Month
- Once a Month
- 2-3 Times a Month
- Once a Week
- 2-3 Times a Week
- Daily

Do you consume other dairy products?
- Yes __________________
- No
Health Information

Do you have skin changes (thick, dry skin with plaques, changes in pigmentation) on your feet, hands, trunk, or groin?

- Yes; on feet ________________
- Yes; on hands ________________
- Yes; on trunk ________________
- Yes; on groin ________________
- No

How long have you had these changes?

- I don’t have any
- 1-3 days
- About a week
- About a month
- 2-3 months
- A year
- 2-3 years
- As long as I can remember
- Other ____________________
Abdominal pain

Do you ever have abdominal pain?
- Yes
- No

How often do you get this pain?
- Never
- Less than Once a Month
- Once a Month
- 2-3 Times a Month
- Once a Week
- 2-3 Times a Week
- Daily
- It never goes away

On a scale of 1 to 10, how bad is this pain, 1 being no pain and 10 being excruciating pain.

Do you have a good appetite? One a scale of 1 to 5, one being no appetite at all and 5 being a good appetite.
- 1
- 2
- 3
- 4
- 5

Has the appetite level you picked above changed at all? If yes do you remember when this change occurred?
- Yes — see next question
- No
If yes do you remember when this change occurred?

- I don’t have any changes
- 1-3 days
- About a week
- 2-3 weeks
- About a month
- 2-3 months
- About a year
- 2-3 years
- Other ___________

Do you ever feel pain in your right or left upper abdominal region?

- Yes; right upper abdomen
- Yes; left upper abdomen
- No

What does it feel like?

- Pressure
- Enlargement
- Heaviness
- Pain
- Other

How often do you feel this pain?

- Never
- Less than Once a Month
- Once a Month
- 2-3 Times a Month
- Once a Week
- 2-3 Times a Week
- Daily
- It never goes away
Upper abdomen

On a scale from 1 to 10, 1 being no pain and 10 being excruciating pain, how bad is the pain in your upper abdomen?

<table>
<thead>
<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>No</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

How long does it usually last?
1 being it passes quickly 5 being it never goes away.

Nausea questions

Do you ever feel nauseas?

- Yes
- No

How often do you feel nausea?

- Never
- Less than Once a Month
- Once a Month
- 2-3 Times a Month
- Once a Week
- 2-3 Times a Week
- Daily
- It never goes away
**On a scale from 0 to 10, 0 being no nausea and 10 being constant nausea, how bad is your nausea?**

![Nausea Scale]

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>How long does it usually last?</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 being it passes quickly 5 being it never goes away.</td>
<td>☑</td>
<td>☑</td>
<td>☑</td>
<td>☑</td>
<td>☑</td>
</tr>
</tbody>
</table>

**Sensation loss**

**Do you have a problem with any of the following?**

<table>
<thead>
<tr>
<th>Problem</th>
<th>How long have you had this problem?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
</tr>
<tr>
<td>Hearing</td>
<td></td>
</tr>
<tr>
<td>Loss of taste</td>
<td></td>
</tr>
<tr>
<td>Blurred vision</td>
<td></td>
</tr>
<tr>
<td>Tingling and numbness of your limbs</td>
<td></td>
</tr>
</tbody>
</table>
### How bad are each of these on a scale of 0-10;
0 Being it doesn’t affect me, and 10 being total loss of sensation

<table>
<thead>
<tr>
<th></th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hearing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of taste</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blurred vision</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tingling and numbness of your limbs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Do you ever get shortness of breath or a cough?
- Yes; shortness of breath
- Yes; a cough
- No

### How bad is it usually? 1 being not very bad, 5 being extremely bad
- 1
- 2
- 3
- 4
- 5

### When does it usually occur? (In the morning, at work, etc.)

84
How often does it happen?

- Never
- Less than Once a Month
- Once a Month
- 2-3 Times a Month
- Once a Week
- 2-3 Times a Week
- Daily
- It never goes away

When did this start happening?

- I don’t get shortness of breath or coughs
- 1-3 days ago
- About a week ago
- 2-3 weeks ago
- About a month ago
- 2-3 months ago
- About a year ago
- 2-3 years ago
- Other

When you were pregnant did you ever have a seizure?

- Yes
- No

How often did you have seizures?

- Never
- Less than Once a Month
- Once a Month
- 2-3 Times a Month
- Once a Week
- 2-3 Times a Week
- Daily
Have you ever had a miscarriage?
- Yes
- No

Do you have joint stiffness or chronic joint pain?
- Yes; joint stiffness
- Yes; joint pain
- No

How long have you had this stiffness?
- I don’t have stiffness
- 1-3 days
- About a week
- 2-3 weeks
- About a month
- 2-3 months
- About a year
- 2-3 years
- Other ____________

Please answer the following questions:

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Do your wounds take a long time to heal?</td>
<td>☑️</td>
<td>☐️</td>
</tr>
<tr>
<td>Do you lose your hair easily?</td>
<td>☑️</td>
<td>☐️</td>
</tr>
<tr>
<td>Do your nails chip easily?</td>
<td>☑️</td>
<td>☐️</td>
</tr>
<tr>
<td>Do you become tired quickly?</td>
<td>☑️</td>
<td>☐️</td>
</tr>
</tbody>
</table>
How often do you have diarrhea?
- Never
- Less than Once a Month
- Once a Month
- 2-3 Times a Month
- Once a Week
- 2-3 Times a Week
- Daily
- It never goes away

Do you currently use tobacco products?
- Yes; smoking products
- Yes; chewing products
- Yes; sniffing products
- No

How many times per day/week do you use these products?

<table>
<thead>
<tr>
<th></th>
<th>Include units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking products</td>
<td></td>
</tr>
<tr>
<td>Chewing products</td>
<td></td>
</tr>
<tr>
<td>Sniffing products</td>
<td></td>
</tr>
</tbody>
</table>

In the past did you ever consume tobacco products?
- Yes; smoking products
- Yes; chewing products
- Yes; sniffing products
- No
In the past how many times per day/per week do you use these products?

<table>
<thead>
<tr>
<th></th>
<th>Include units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking products</td>
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<tr>
<td>Chewing products</td>
<td></td>
</tr>
<tr>
<td>Sniffing products</td>
<td></td>
</tr>
</tbody>
</table>
Appendix B

Figures demonstrating age, gender, dose, and arsenic (As) chemistry have no
effect on the relationship between As concentration in nail-keratin and As concentration
in water.

Figure 20: No relationship is exists between As in nail versus As in groundwater
differentiated by age. Data points represent adults (defined as >18 years old) (red), and
minors (blue). There was no statistically significant difference (p>0.05) comparing all adults
(red line) to all minors (blue line).
Figure 21: No relationship is exists between As in nail versus As in groundwater differentiated by gender. Data points represent males (red), and females (blue). There was no statistically significant difference (p>0.05) based on the comparison of all males (red line) to all females (blue line).
Figure 22: As concentration in nails versus dose. Dose was defined as self reported quantity of water consumed per day in liters * concentration of As in groundwater in ppb. No relationship was observed.
Figure 23: As concentration in nails versus the percent of total groundwater As present as As(III). No relationship was observed.
References


Hughes, M. Biomarkers of exposure: A case study with inorganic arsenic. Environ Health Perspect. 114, 1790-1796.


**Biography**

Rose Brittany Merola was born in Hunterdon, NJ, on May 3, 1985, and grew up in Hope, NJ. She attended Bucknell University, in Lewisburg, PA where she majored in Chemistry (BA) and Spanish (BA) and graduated in May 2007. Upon completion of her undergraduate degree, Brittany entered the Master of Environmental Management Program at Duke University. She graduated in May 2009 with a focus in Ecotoxicology and Environmental Health. In January 2010 she began her doctorate at Duke in the Earth and Ocean Science Department. Brittany founded the US Chapter of Medical Geology. She received 1st place in the WRRI Annual Conference and NCWRA Symposium Poster Competition (2011), the International Medical Geology Association’s Outstanding Presentation Award (2011), and 2nd prize in the Student Oral Presentation Competition at the 5th International Conference on Medical Geology (2013). Brittany has authored or coauthored the following publications: The effect of non-fluoride factors on risk of dental fluorosis: Evidence from rural populations of the Main Ethiopian Rift (2014); Arsenic exposure of rural populations from the Rift Valley of Ethiopia as monitored by keratin in toenails (2013); The isotopic imprints of mountaintop mining contaminants (2013); Groundwater quality and its health impact: An assessment of dental fluorosis in rural inhabitants of the Main Ethiopian Rift (2012); Cumulative impacts of mountaintop mining on an Appalachian watershed (2011); Comparison of the electronic spectra and
reduction processes in the Uley Nontronites, NAu-1 and NAu-2 (2009); and Spectroscopic investigations of Fe$^{2+}$ complexation on nontronite clay (2007).