Arsenic Exposure from groundwater in Union County, North Carolina

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Abstract

Arsenic contamination of groundwater is a global problem affecting human health. The highest concentrations occur overseas in areas such as Bangladesh, India, Vietnam, and Thailand. The United States is not immune and also has hotspots of arsenic in dangerously high concentrations. The focus of this project was to evaluate the extent and concentration of arsenic in one such area, Union County, North Carolina, and to attempt to use arsenic in toenails as a biomarker of exposure. Arsenic concentration above the EPA's maximum contaminant level (MCL) of 10ppb was found in 22 out of 64 households tested (34%). The measurement of arsenic in toenails was successfully used as a biomarker of exposure. Data showed that men had a greater sensitivity to arsenic and their nail data had better correlation. Children also showed a greater sensitivity. Out of 69 participants in the toenail-biomarker study, 4 had arsenic concentrations in their toenails above a level deemed safe. Based on these results it is evident that arsenic contamination of drinking water in Union County is an issue of concern.

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Introduction

Arsenic has had a long association with human culture. It is one of the oldest poisons known to mankind. In Nriagu's *Arsenic Poisoning through the Ages*, he writes, "Arsenic, the king of poisons, has probably influenced human history more than any other element of compound" (2002). This is a result of its long history of use (Nriagu 2002; Mandel, 2002). Arsenic can be naturally associated with copper which early blacksmiths commonly worked with. In turn they had extremely high exposure levels, and some poisoned themselves. Nriagu, goes on to write that during the middle ages the words arsenic and poison were, for all intensive purposes, synonymous with one another. Today occupational exposure still occurs, however the extent is significantly less (Nriagu, 2002).

Figure 1. Global distributions of arsenic contamination of groundwater (Nordstrom 2002

GLOBAL ARSENIC CONTAMINATION IN GROUND WATER

Country/ region	Potential exposed population	Concentration (μg/liter)	Environmental conditions
Bangladesh	30,000,000	<1 to 2,500	Natural; alluvial/deltaic sediments with high phosphate,* organics
West Bengal, India	6,000,000	<10 to 3,200	Similar to Bangladesh
Vietnam	>1,000,000	1 to 3,050	Natural; alluvial sediments
Thailand	15,000	1 to >5,000	Anthropogenic; mining and dredged alluvium
Taiwan†	100,000 to 200,000	10 to 1,820	Natural; coastal zones, black shales
Inner Mongolia	100,000 to 600,000	<1 to 2,400	Natural; alluvial and lake sediments; high alkalinity
Xinjiang, Shanxi	>500	40 to 750	Natural; alluvial sediments
Argentina	2,000,000	<1 to 9,900	Natural; loess and volcanic rocks, thermal springs; high alkalinity
Chile‡	400,000	100 to 1,000	Natural and anthropogenic; volcanogenic sediments; closed basin lakes, thermal springs, mining
Bolivia§	50,000	–	Natural; similar to Chile and parts of Argentina
Brazil‖	–	0.4 to 350	Gold mining
Mexico	400,000	8 to 620	Natural and anthropogenic; volcanic sediments, mining
Germany	–	<10 to 150	Natural: mineralized sandstone
Hungary, Romania	400,000	<2 to 176	Natural; alluvial sediments; organics
Spain¶	>50,000	<1 to 100	Natural; alluvial sediments
Greece#	150,000	–	Natural and anthropogenic; thermal springs and mining
United Kingdom**	–	<1 to 80	Mining; southwest England
Ghana	<100,000	<1 to 175	Anthropogenic and natural; gold mining
USA and Canada	–	<1 to >100,000	Natural and anthropogenic; mining, pesticides, As_2O_3 stockpiles, thermal springs, alluvial, closed basin lakes, various rocks

*These estimates [from (*6*) except where noted] are highly uncertain, difficult to obtain, and changing as new water sources or treatment are established. †Additional estimate from (*15, 16*). ‡Installation of a new treatment plant has greatly decreased the exposed population. §Estimate from (*16*). ‖Source (*17*), no ground waters analyzed. ¶Source (*18*). #Source (*6*) and (*16*). **Source (*19*)

<u>Exposure</u>

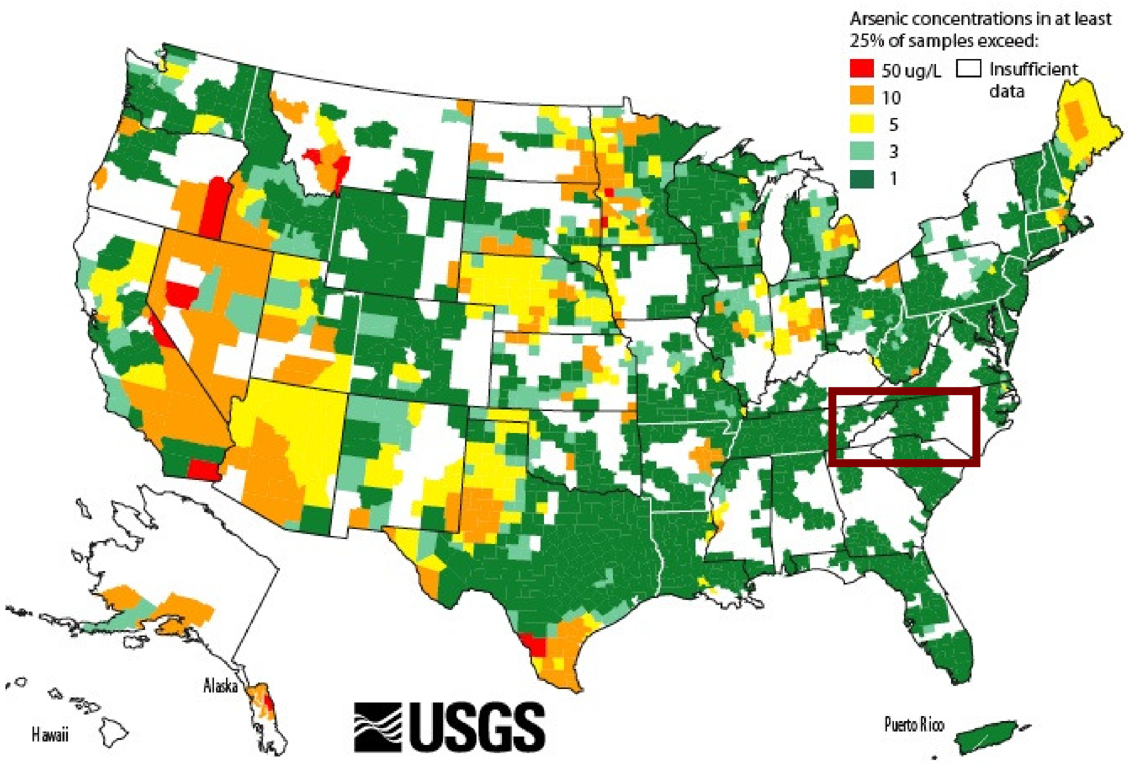
In modern times, the non-occupational exposure of primary concern is through the ingestion of contaminated drinking water (Hughes, 2002; Mandal, 2002; Nickson, 2000;

Saha, 1999). The highest levels in drinking water found globally occur in Bangladesh, and West Bengal, India (See Figure 1) (Nordstrom, 2002). In the United States, the EPA lowered the maximum contaminant level (MCL) for arsenic in drinking water from 50ppb from 10 ppb effective as of 2006 (EPA, Arsenic in Drinking Water). In theory this standard applies to all sources of drinking water in the United States. Public water sources are required to test not only for arsenic but for a host of other potential contaminants on a regular basis. Private drinking wells are also technically required to comply with these standards. However because these wells are on private property it is difficult, without regulation, to enforce these testing requirements and almost impossible, with or without regulation, to enforce compliance if tested wells are found to be contaminated.

In 2008, North Carolina passed the North Carolina Safe Drinking Water from Wells Act (Session Law 2006-202). In this bill, North Carolina recognized the important role that private ground water plays in supplying drinking water and the need for this water to meet the same water quality standards public drinking water must meet. As a consequence of this bill, new wells constructed must be tested for a host of potential contaminants, arsenic included, with oversight falling to the North Carolina Department of Environment and Natural Resources. This law has enormous potential to increase awareness of well contamination issues across the state; however, once a well is tested there are still no mechanisms in place to force individual homeowners to comply with standards.

No quantity of arsenic is good for human health, and North Carolina's health recommendation for arsenic in drinking water is 0.02ppb. The presence of arsenic in water and soil is directly related to the geology of the area. Figure 2 shows arsenic concentrations, by county, across the United States (Ryker, 2001). The red box highlights the piedmont area of North Carolina where there is a region of arsenic contamination. The study area is here, in Union County, North Carolina, which falls within the high arsenic risk area.

Figure 2. Arsenic concentration in groundwater across the United States by county (Ryker, 2001)



Arsenic is released to local aquifers from arsenic containing minerals in the underlying strata (Harvey, 2002; Mandel, 2002). The areas within the Piedmont region of North Carolina possess these minerals and there is a risk of human exposure from the consumption of contaminated well water (Daniel 2002; Ryker, 2001).

Arsenic naturally occurs in two forms, Arsenite (H_3AsO_3), and Arsenate ($H_2AsO_4^-$). For the purposes of this paper, Arsenite will be referred to as As^{III} and Arsenate will be referred to as As^{V}. There are important consequences of the chemical differences of these two species. As^{III} is uncharged and therefore more difficult to remove from drinking water. This difference also allows for the two types of arsenic to be analytically speciated, in order to get the relative amounts of each present in the water supply (Bednar, 2002).

The aquifer in this region is highly fractured (See Figure 3), which further

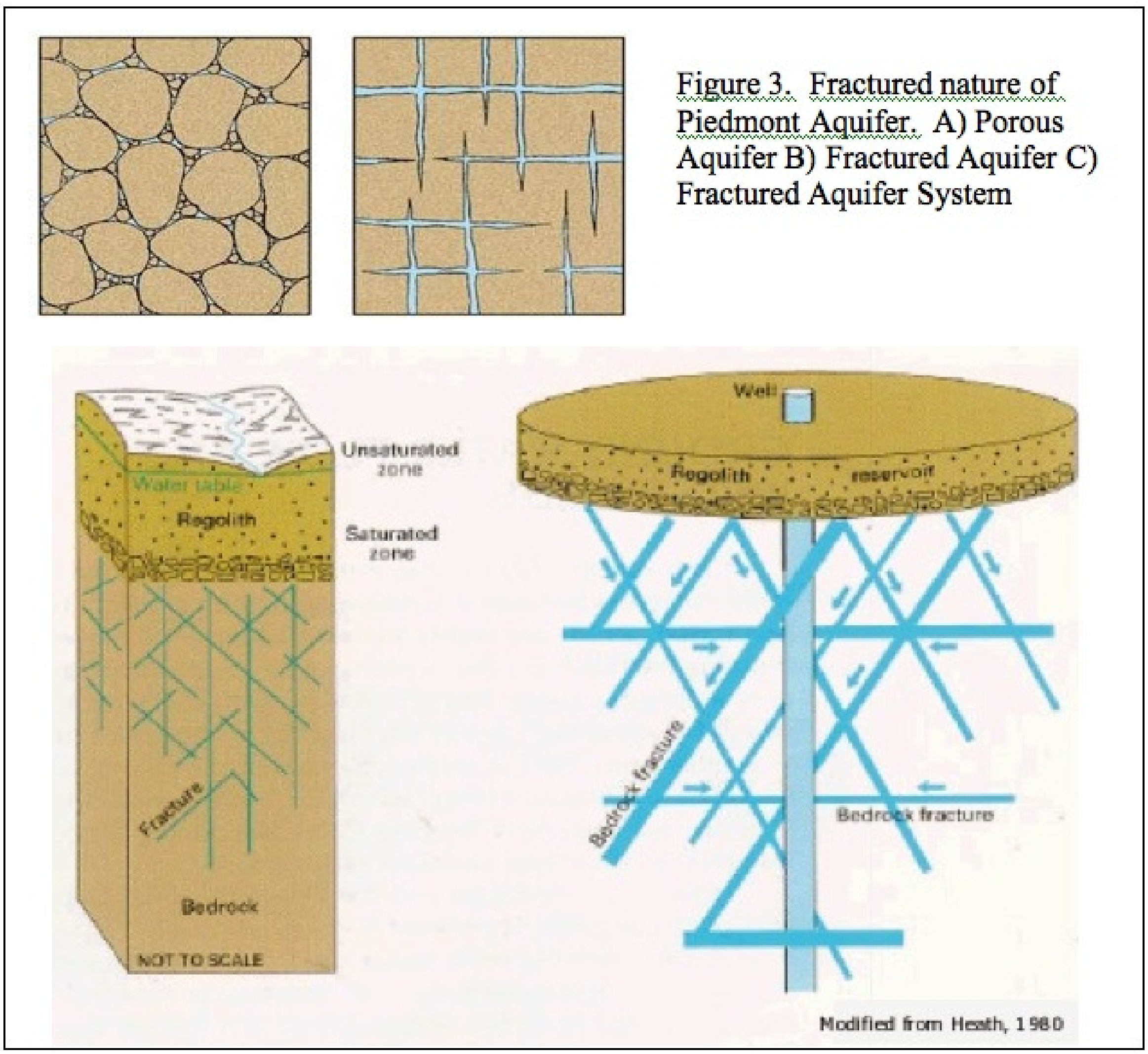


Figure 3. Fractured nature of Piedmont Aquifer. A) Porous Aquifer B) Fractured Aquifer C) Fractured Aquifer System

complicates the regulation and mitigation of the effects of arsenic. In general aquifer geology can run the spectrum from being highly porous to being highly fractured (Canace, 2000; Water: Department of Primary Industries and Water, 2009). As demonstrated in Figure 3a the pore space in porous aquifers is interconnected, which means water can flow freely. This signifies that if a contaminant is present in one area of the aquifer it can flow freely to all parts of the aquifer. In a fractured aquifer (Figure 3b) the fractures do not necessarily interconnect, which means that if a contaminant is present in one area of the aquifer, it can flow throughout its individual fracture system, but it can not contaminate fractures to which it is not connected. In practice this means that two neighboring households, both obtaining water from privately owned wells, could be receiving their water from separate factures systems with completely different water chemistries and subsequently different levels of contamination. Figure 3c demonstrates how a well might intersect a fracture system.

<u>Health Effects</u>

Exposure to arsenic has serious consequences for health. Symptoms of acute arsenic exposure include: nausea, vomiting, bloody urine, abdominal pain, severe diarrhea, anuria, shock, convulsions, coma, and death. High concentrations of arsenic need to be present for these symptoms to manifest themselves. The levels that would potentially be present in North Carolina would likely not be high enough to cause these acute symptoms, however chronic effects of arsenic would be of concern. Chronic exposure can lead to skin lesions, peripheral vascular disease, hypertension, Blackfoot disease, and various forms of cancer, commonly including but not limited to: bladder, liver, lung, kidney and skin cancer (Bernstam, 2002; Chen,1995; Garland, 1993; Rahman, 1999; Ratnaike, 2003; Sun, 2003). Bernstam, et al., 2002 showed that skin lesions can occur at levels as low as 100ppb.

Several potential mechanisms of toxicity have been suggested. Arsenic has been linked with the inactivation of 200 different enzymes (Garland, 1993; Saha, 1999; Ratnaike, 1999). Part of the difficulty involved with identifying a mechanism of action is the high interspecies variability, species-specific nature of arsenic with respect to health outcomes, and conflicting and unreliable animal models (Kwok, 2007; Mandal, 2002; Bernstam 2002).

Arsenic can directly bond and interfere with phosphorous linkages and thiol groups (Bernstam, 2002; Hughes, 2002; Ratnaike, 2003). It is also hypothesized that arsenic may be a carcinogenic amplifier, not directly causing cancer but increasing the damage that other carcinogens, such as smoking, may cause. Hypertension, a risk factor for cardiac disease which is the leading cause of death in the United States, has been linked with arsenic exposure (Chen, 1995; Kwok, 2007; Navas-Acien, 2005). The two species of arsenic are thought to act through different biological mechanisms. It is thought that As^{V} interferes with phosphate channels and replaces phosphorus in important biological processes (Bernstam 2002). As^{III} on the other hand is believed to, "diffuses passively into cells and tends to exert its toxic effects by reacting with molecules containing spatially proximate thiols" (Bernstam, 2002).

<u>Biomarkers for Arsenic Exposure</u>

The complex nature of arsenic makes problem identification complicated. Estimating exposure across the community based on the testing of one or two wells is not sufficient because of the fractured nature of the aquifer and the use of water filters. Even estimating exposure by measuring arsenic levels from the sink of individuals' households is not adequate because water-drinking habits will significantly affect the exposure estimate.

A biomarker can be used to better quantify individual exposure. Arsenic in the blood, urine and keratin, have all been tested as biomarkers of exposure (Chowdhury, 2000; Mandal, 2004). The body is able to rid itself of inorganic arsenic by the methylation of the arsenic in the kidneys, and elimination in the urine (Karim, 2000; Slotnick, 2006). However arsenic does not remain in the blood or urine for long, and has residence times of approximately 2-3 hours in blood and 3-4 days in urine (Adair, 2006; Karim, 2000).

Short residence times make these mediums useful for acute exposures but not practical for chronic, low-concentrations exposures. Human keratin, which includes, hair, fingernails, and toenails, are all proven markers of exposure for arsenic and other trace metal exposure (Chowdhury, 2000; Mandal, 2004). Toenails[1] are preferable to hair and fingernails because they have a more uniform growth rate across populations, have less external arsenic contamination, and have a slower growth rate (Batista, 2008; Slotnick 2006). The slow growth rate is especially important as it translates into a higher concentration of arsenic per unit mass of toenail and a more uniform assessment of the time of exposure; hair grows between 6 and 36 mm/month, while toenails only grow between 0.9 and 1.5 mm/month. (Adair, 2006; Aguiar, 2001; Batista, 2008; Chen, 1999; Hinwood, 2003; Slotnick, 2006**).** It is hypothesized based off literature values, the concentrations of arsenic in the toenails of an unexposed population will be between 0.34

[1] From this point on the use of term *nail* and *toenail* will be interchangeable.

and 1.08 μg-As/g-nail, and will represent an exposure of 2-12 months (Chen, 1999; Chowdhury, 2000; Hinwood, 2003).

Arsenic is deposited at the nail bed, likely in the form of As^{III}, upon the formation of the nail approximately two weeks after exposure (Adair, 2006; Ratnaike, 2003, Slotnick, 2006). Once deposited the arsenic is removed from the metabolic process and therefore the removal process (Adair, 2006). Arsenic (As^{III} especially, the more toxic of the As species) has an affinity for thiols (Bernstam, 2002; Hughes, 2002; Ratnaike, 2003). The hard keratin of nails has high levels of the amino acid cysteine (as much as 22%) that contains this sulfhydrl group (Bernstam, 2002; Hughes, 2002; Mandal, 2004; Ratnaike, 2003). The deposition of metals in hair and nails is a natural way for the body to rid itself of them. As such, nails have been used to measure many of these elements in exposed populations, including: Ni, Se, Pb, Fe, Ca, Cr, Mn, Zn, Hg, Cd, and many more (Batisita, 2008; Nowak, 2000; Sukumar, 2006).

Age, sex, gender, geographic location, season, quantity of water consumed, and medical conditions affecting nail growth rate are all important variables that affect metal uptake into the nail (Hopps, 1977; Slotnick 2006). Nail concentrations of these metals reflect all exposure sources, not just drinking water; therefore information from participants must be collected regarding these confounding factors. Arsenic has been shown to inactivate over 200 different enzymes (Ratnaike, 2003). While the relationship between arsenic exposure and nail concentrations is evident, biomarker-disease relationships have not been as clear. Previous studies have been conflicting and it is possible that variables such as good nutrition can act as effect modifiers for the disease outcome further emphasizing the need for participant information (Slotnick, 2006).

While toenails are agreed to be the preferred matrix to measure arsenic for a biomarker assessment to exposure, the high person to person variability makes correlation between nail concentrations and water concentrations difficult, as there are many potential confounders. Table 1 was taken from a review article written by

Table 1. Studies reporting individual level exposure-biomarker associations for arsenic and selenium (Slotnick, 2006)

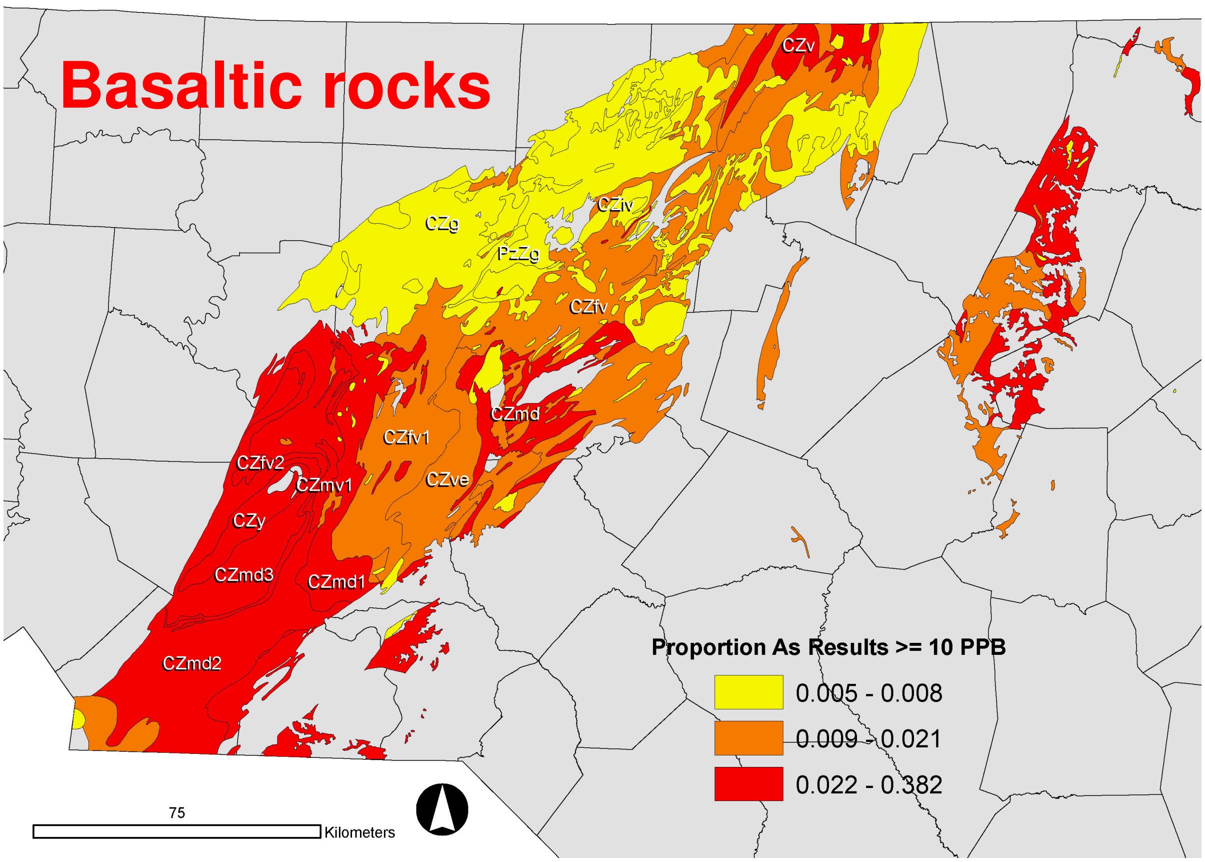
Element	Study size (N)	Exposure measure	Exposure concentration[a] (mean (SD) or range)	Association	Study
As	208	Drinking water	0.29 (0.04) µg/L	$r = 0.46$ ($P < 0.001$)	Karagas et al. (2000)
As	153	Drinking water	BDL–73 µg/L	$r = 0.55$ ($P < 0.05$)	Hinwood et al. (2003)
As	47	Drinking water	248 (59) µg/L	$R^2 = 0.68$ ($P < 0.03$)	Mandal et al. (2003)
As	153	Residential soil	1.7–9900 mg/kg	$r = 0.50$ ($P < 0.05$)	Hinwood et al. (2003)
As	153	Household dust	BDL–1300 mg/kg	$r = 0.46$ ($P < 0.05$)	Hinwood et al. (2003)
As	969	Diet	10.22 (6.26) µg/day	$r = 0.33$ ($P < 0.0001$)	MacIntosh et al. (1997)
As	524	Soil	26 µg/g	$r = 0.232$ ($P < 0.001$)	Wilhelm et al. (2005)
As	524	Household dust	11.6 µg/g	$r = 0.299$ ($P < 0.001$)	Wilhelm et al. (2005)
Se	96	Selenium supplementation	2–600 µg/day	$r = 0.32$ ($P = 0.05$)	Hunter et al. (1990)
Se	18	Selenium supplementation	113.0 (43.0) µg/day	$r = 0.50$ ($P = 0.03$)	Hunter et al. (1990)
Se	166	Diet: food records	47.9 (22.2) µg/day	$r = 0.18$ (not significant) overall; $r = 0.60$ ($P < 0.01$) for dietary supplement users	Ovaskainen et al. (1993)
Se	77	Diet: duplicate meals	2.99 µmol/day	$\beta = 1.55 \times 10^{-2}$, $R^2 = 0.45$	Longnecker et al. (1996)
Se	44	Diet: duplicate meals	1.74 (91) µg/day	$r = 0.59$ ($P < 0.01$)	Swanson et al. (1990)

[a]BDL, below detection limit.

Slotnick and highlights the low correlation coefficients (r values) associated with these studies (2006).

Spatial determinants are clearly the important factors concerning whether or not one is at risk for arsenic exposure. This study area was picked because analysis determined that, based on the geology of the region, there was a probability of 0.022 to 0.382 that an individual household was likely to be able the EPA's MCL (See Figure 4).

Figure 4. Proportion of households with arsenic in the drinking water and its relation to geology



Besides spatial determinants, there are other potential variables that can impact arsenic's effect on health, including: age, gender, smoking, amount of time drinking the contaminated water, and the quantity of water consumed (Slotnick, 2006). With regard to age, the young and the elderly will be at the greatest risk (Nickson, et al., 2000). In young populations the body burden is greater even if an equal amount of arsenic is consumed as in an adult. In elderly populations, impaired health from other competing issues can weaken defense and potentially limit the body's ability to methylate and remove arsenic from the system (Slotnick, 2006; Mandal, 2002). Besides age, smoking has also been shown to work synergistically with arsenic to increase cancer risks (Mostafa, et al., 2008).

Social behaviors seem may also increase risk of exposure. For example, people who are outside of the home for large portions of the day and obtain their drinking water from alternative sources are at less risk than those who are may be retired and spending large quantities of their time at home, or people who work exclusively from home (Slotnick, 2006). The quantity of water consumed is also important (Slotnick, 2006). Sodas, juices, and other store bought drinks are increasing more popular in American culture. Therefore, even if a person spends all of their time in the home, they might have a very low risk for arsenic exposure if they do not consume any tap water.

Study Design

Before any research was conducted an IRB was obtained from Duke University's Office of Research Support.

Subject Selection

Geographic information systems (GIS) software was used to identify specific communities for the study. This analysis was conducted by Joshua Tootoo, GIS Analyst and Associate in Research as part of the Children's Environmental Health Initiative. There were several components of the GIS analysis. The first component was to identify residential plots with private wells. Residential plots that are serviced by city and community water providers were identified and the remaining residential plots were flagged as potentially utilizing private well water.

Survey Design

Houses likely to use private well water and located in Union County in the region shown in Figure 4 were randomly selected and sent recruitment letters (See Appendix A). If a participant decided to be involved, they were asked to answer a short survey (See Appendix B) aimed at minimizing the confounding variables, such as age, gender, and drinking habits, that would lead to bias in the study design. Verbal consent was obtained from one adult head of household before the survey was administered, and written consent was also obtained from each individual who elected to participate in the nail biomarker element of the study (Adult and minor consent, parental consent and minor assent are included in Appendices C, D1, D2, and D3).

The survey collected demographic information such as age, gender and ethnicity. Each participant was also asked how much water they consumed both inside and outside the home, and how many meals were cooked in the home per week. Other questions included were, if they had ever had their water tested, and if so, if they knew of any problems with their water supply. This question was to help us see if there was a bias towards people who already knew they had a problem with their water. Another important question was regarding what variety of water treatment, if any, they employ in their home. While the aim of this survey was to minimize bias, it in itself creates the potential for additional sources of it, in the form of interviewer and recollection biases. To avoid this as much as possible questions were worded such that whenever possible answers were concrete and not open ended to minimize the chance for misclassification bias. The portion of the survey most at risk for recall bias was the quantity of water consumed estimation. Another survey issue is that only one member of each household was asked to complete the survey and asked to estimate water consumption rate for the other members of their household. For this reason the quantity of water consumed was not included in this initial analysis of exposure.

Samples of well water, sink water, and nails will be collected from households living the geologically defined region of potentially high arsenic levels. These results will then be compared with information about drinking water habits from a survey to assess whether the individuals living in this region have been exposed to arsenic.

<u>Sample Collection:</u>

Participants were mailed one Ziploc© bag per household member interested in participating with written consent forms and a letter asking each participant to clip his or her toenails and place these clippings into the provided bag.

Water samples were collected from each household's well and sink. Geochemical properties of the water was collected at the well and included: pH, electro-conductivity, oxidation reduction potential, and dissolved oxygen levels. The water to be tested for arsenic was collected in acid-washed bottles with (100µL) Nitric Acid for preservation. Anion and alkalinity samples were also collected. Arsenic was able to be prepared for speciation in the field following the methods set forth in (Bednar, 2002). At this time, participants were asked to have their nail samples ready and they were collected by the researchers, along with signed consent forms, and verbal assent for minors under the age of 10. Once analytical analysis of the water and nails had been completed participants were mailed a letter detailing the results of their well tests for arsenic, their nail concentration, the safety range for arsenic in human nails, and a pamphlet detailing the health risks associated with arsenic in drinking water. A follow up consultation was given to all participants approximately one to two weeks after the results were sent to make sure they had no further questions or concerns.

<u>Debriefing</u>

Once samples had been analyzed the results were returned to the individual household (See Appendix F). Included with their water and nail results was a pamphlet with information about the health risks associated with consuming arsenic contaminated drinking water. An unformatted copy of this pamphlet is included in Appendix G. Each household received a consultation to address any further questions or concerns they may have had.

Analytical Methods

Water analysis

A 2mL aliquot of each sample was diluted with 10mL ICP-MS solution internal standard consisting of Bi, Tm, In at 10 ppb in 2% HNO_3 was added using a repipet dispenser. Six external standards were created using SRM 1643e at various concentrations also spiked with 10mL of the internal standard. Samples were run on the ICP-MS and corrected for drift according to the procedure outlined in Data Analysis.

Biomarker Analysis

Procedures for the cleaning and digesting of toenails for use as a biomarker vary highly. For this experiment several procedures were combined according to methods primarily proscribed by: Chen 1998, Slotnick, 2006, Sekhar 2002, Samanta 1999, and Martínez 2005.

Toenail Cleaning

All samples were transferred into new 15mL polypropylene vials and 2mL acetone was added to each tube. Samples were then sonicated for 30 minutes, after which they were rinsed three times with ultrapure water. This process was repeated using an approximately 1% Neutrad® solution (2mL), a phosphorus free lab detergent and then repeated a third time again with acetone. If it seemed any residual cleaning solution was left in the vials, samples were re-rinsed with ultrapure water. Samples were dried in an oven until dry (approximately 24-36 hours) at 60°C.

Toenail Digestion

Two digestion methods were utilized, the second of which being an improvement on the duration of time needed for digestion to occur. In the first method, dry samples were weighed into Teflon vials. Weights varied from 0.1080-2.1975g. Ultrapure concentrated nitric acid (HNO_3) (1mL) was added to each vial and the samples were allowed to digest for 24 hours. Next 30% hydrogen peroxide (H_2O_2) (1mL) was added and the samples were again allowed to digest for 24 hours. This was repeated making for a total of 2mL HNO_3 and 2mL of H_2O_2. Samples were then allowed to digest for one week. At this point samples were mostly digested; however some solid protein was sttill present.

Samples were dried down on low heat and redigested with 2mL of HNO_3. Samples were dried down once more, upon which time 1mL HNO_3 was added along with 4mL of quartz distilled ultrapure deionized water (DI-H_2O). To minimize error in ICP-MS analysis it was important to have approximately equal concentrations of the nail matrices. Therefore the samples were then grouped by original weight and aliquots of varying volumes of each were taken and diluted with varying volumes of a solution of 1:4 DI-H_2O: HNO_3, in a manner so that the overall matrix concentration was equal. ICP-MS solution (10mL) internal standard consisting of Bi, Tm, In at 10 ppb in 2% HNO_3 was added using a repipet dispenser. External standards (SRM 1643e) and blanks were also prepared and analyzed.

In the second method, dry nail samples were weighed into Teflon vials. Weights varied from 0.0085-0.3436g. Samples were grouped by weight and HNO_3 was added in varying volumes which were determined by the initial weight of the samples. The ratio of 20mg sample: 1mL HNO_3 : 100µL H_2O_2 was used for samples below 0.1328g. For samples above this weight, a ratio of 20mg sample: 0.75mL HNO_3: 75µL H_2O_2. HNO_3

was added first and the samples were allowed to digest for 24 hours followed by the addition of the half of the total H_2O_2 volume and another 24 hours of digestion.

At this point, samples were almost completely digested and resembled a thick yellow suspension like substance. Samples were heated lightly to “sweat” the Teflon containers and homogenize the samples. Then, once cool, the remainder of the H_2O_2 was added to each sample making the ratio 1mL HNO_3: 100uL H_2O_2. Samples were again heated overnight on low heat, without drying them down. External standards (SRM 1643e) and blanks were also prepared and analyzed.

An aliquot of 500uL of each sample was taken to be analyzed. For samples that did not have a full 500uL, the entire sample was analyzed, measured by weight. This aliquot was diluted with 1.5mL of DI-H_2O and 10 mL of the internal standard.

Data Analysis

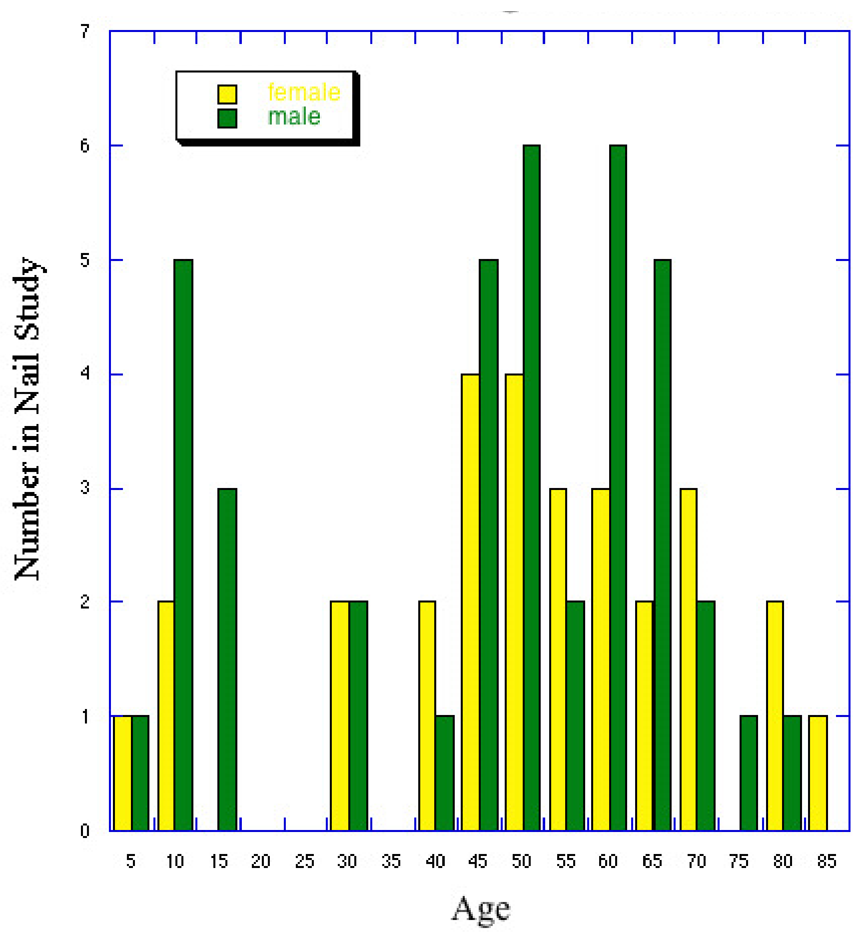
The data was collected and processed through a java-program that corrected for drift. The java-program automatically accounted for instrumental drift over time in two ways. Using the external standard, particularly the highest concentration run prior and post samples, it pro-rates the samples by 10% of the drift throughout the experiment. A second drift control that is utilizes is by accounting for drift by comparing values from the internal standard. In the second digestion method one blank found arsenic. This was subtracted from the data.

The presence of $AsCl^+$ was a confounder of concern. To alleviate this, equal amounts of sample are preferable to produce more consistency. Sekhar (2002) offers a method to correct for this. If interference from polyatomic $ArCl^+$ was an issue, there would not only be interference at mass 75 (where it would interfere with arsenic) but also at 77 ($^{40}Ar^{37}Cl$). Sekhar calculated the intensity of arsenic by comparing the relative amounts of ^{77}Se (relative abundance of 7.63%) with ^{78}Se (relative abundance of 23.78%) taking into account the ratio between their natural relative abundances. This method was utilized for this experiment however the relative abundance of ^{77}Se (relative abundance of 7.63%) was compared to ^{82}Se (relative abundance of 8.72%). If Cl^- was causing a major interference one would expect to see a significantly larger amount of ^{77}Se in the samples compared to ^{82}Se.

Results

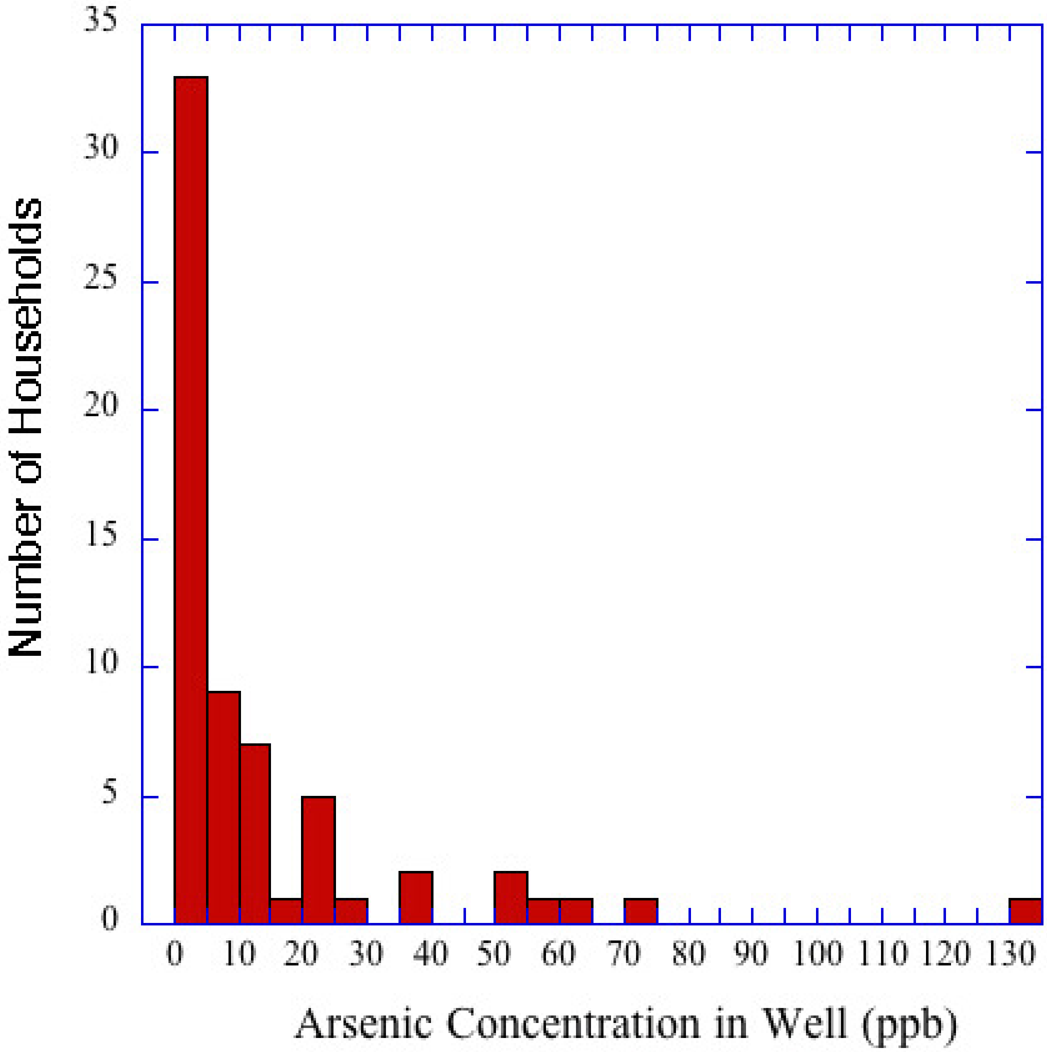
In total 64 households responded to the recruitment letter. Of those 40 households were asked to participate in nail analysis. The remaining 24 only had their water tested. The 40 nail-households translated into a total of 99 people. Figure 5 shows the age and gender demographics for those 99 people. People were asked to self-select out of the study if they suffered from certain medical conditions (See Appendix C), leaving only 76 nail samples. Of those, 69 were of a large enough sample mass to be used.

Figure 5. Age and gender distribution of all household members participating in water testing



Of the 69 participants in the biomarker study, there were 40 males and 29 females. Eight males were minors, while only 2 females were minor. Most participants were Caucasian, with the exception of one African America, one Hispanic, and one participant of mixed Hispanic-Caucasian descent. Three participants were current smokers.   Although quantity of water consumed was not included in any analysis, the

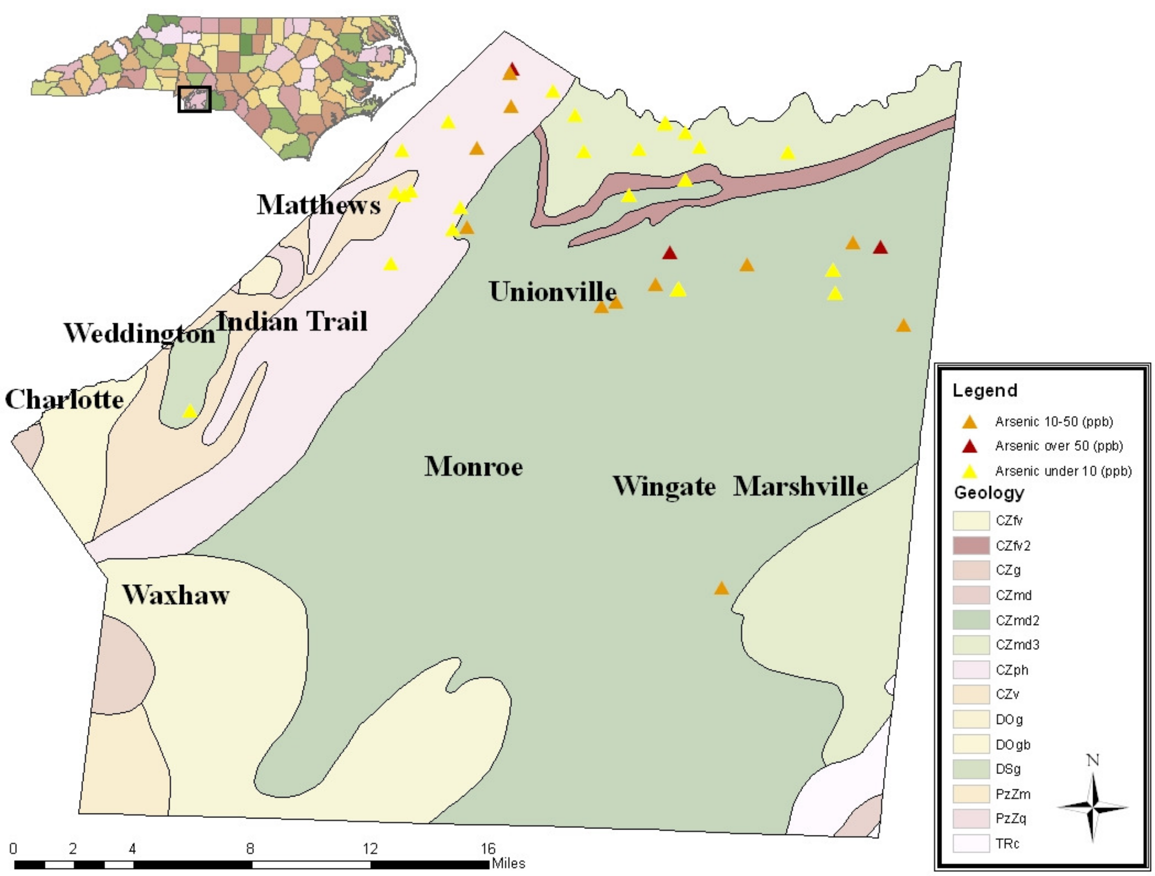
Figure 6. Distribution of arsenic concentrations found in individual wells tested



average total volume of water consumed was approximately 8 cups per day with an average of approximately 5 of those cups being consumed in the home. The average household cooked approximately 10 meals in the home per week.

Of the 64 households tested 34% (22 out of 64) were above the EPA's 10ppb MCL (See Figure 6). Figure 7 shows a map of the household locations in relation to one

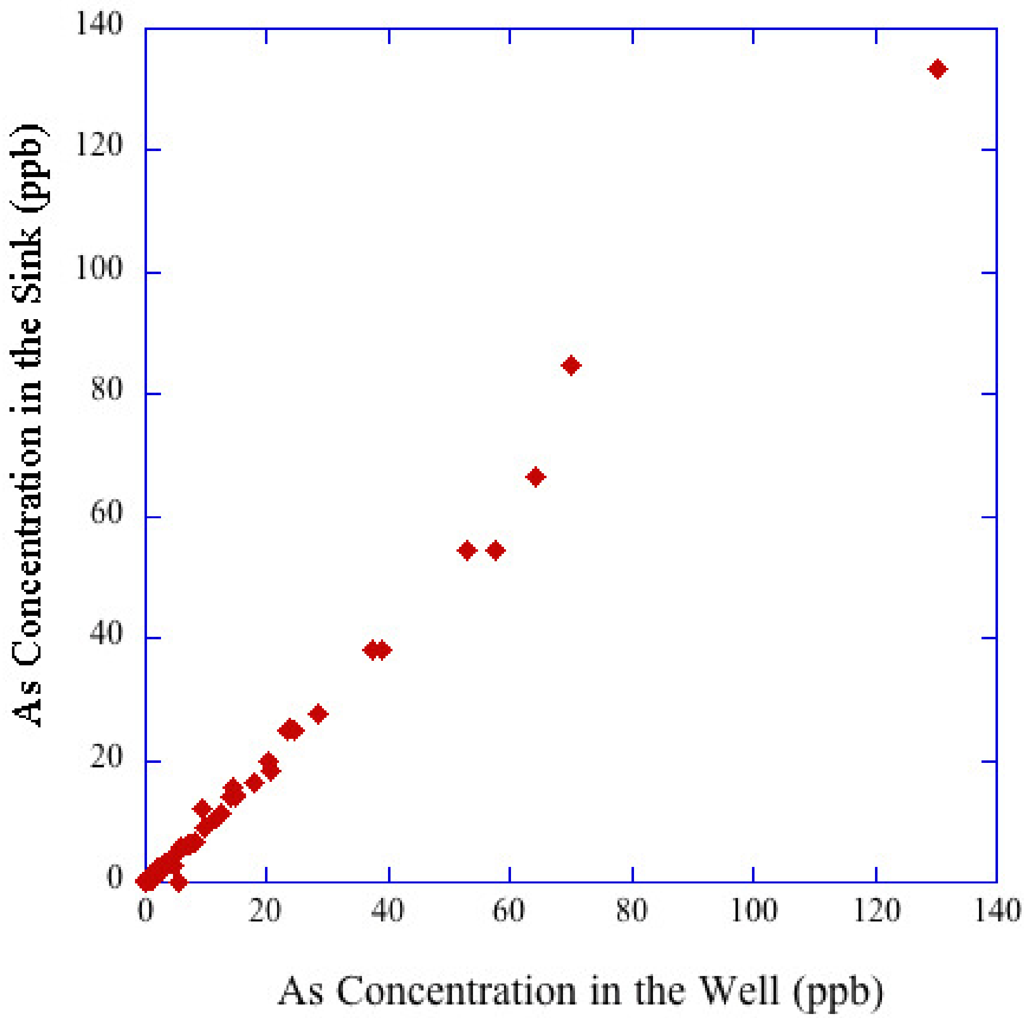
Figure 7. Spatial distribution of arsenic concentrations found in individual wells tested



another and whether they were above the current EPA limit of 10ppb, or the old EPA limit of 50ppb. As is evident from this map, there is no spatial trend in arsenic concentration.

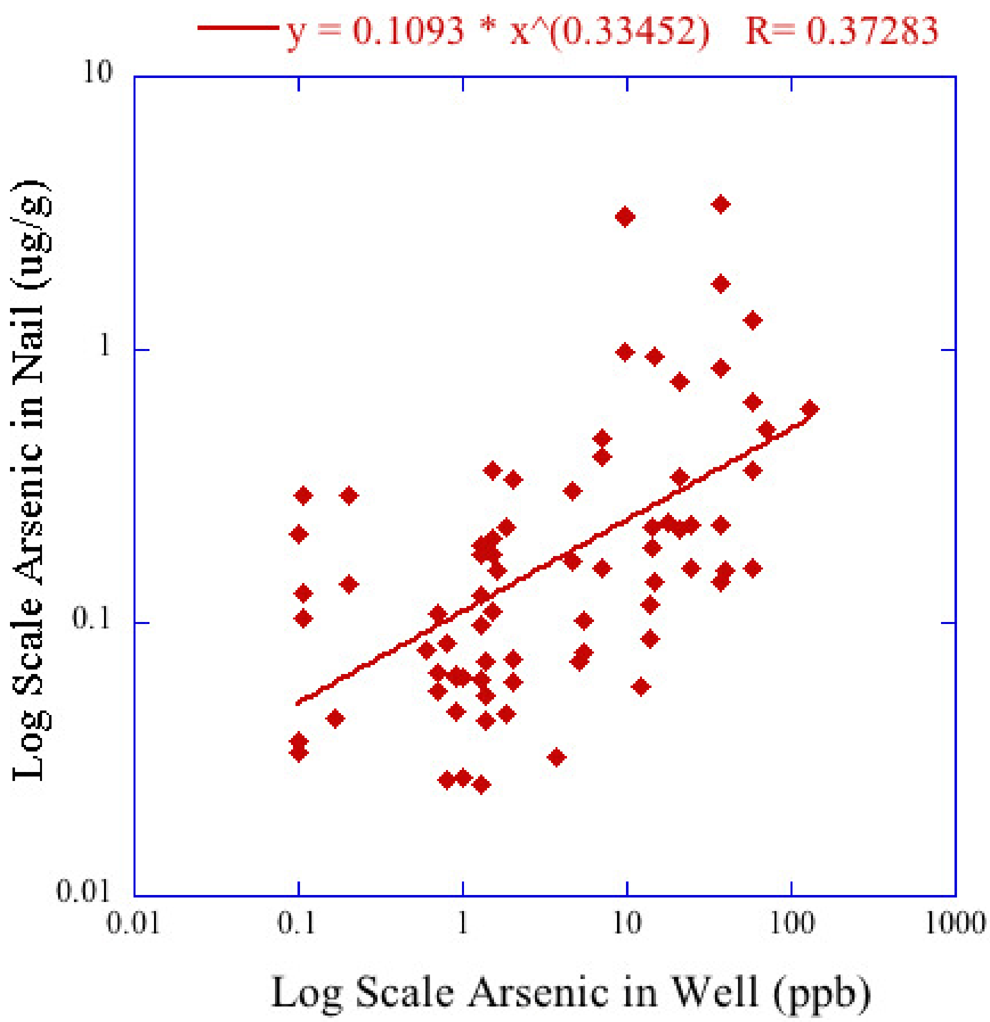
Comparison of the arsenic concentrations in the well versus concentrations in the sink show that the two were highly correlated (Figure 8). Therefore households were consuming the level of arsenic present in their well when they drank their tap water. Any treatment systems in place were not removing this arsenic from their water source.

Figure 8. Correlation between arsenic concentrations found in the well and in the sink



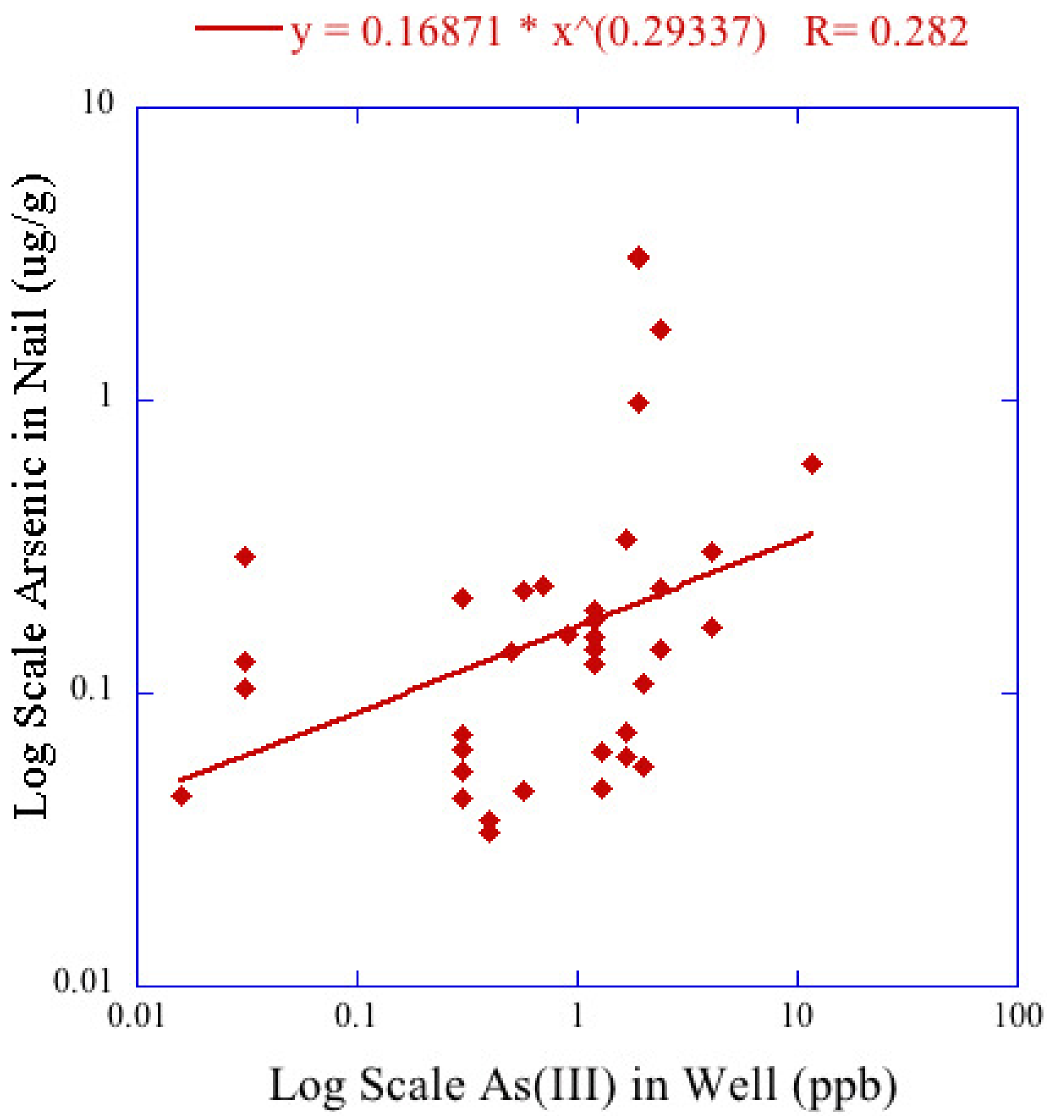
This information would be alarming without any information about individual exposure. Figure 9 shows the concentration of arsenic in each household's well versus the arsenic concentration found in the nail. There is a linear correlation between the two, and there is agreement with the r values found in previous biomarker studies of arsenic and toenails (See Table 1). In total, 4 out of the 69 participants in the study were over the safe-level of 1.08µg-As/g-nail-As in the nail, three of these participants being children, a vulnerable subpopulation.

Figure 9. Correlation between arsenic found in the well and concentration of arsenic found in the nail



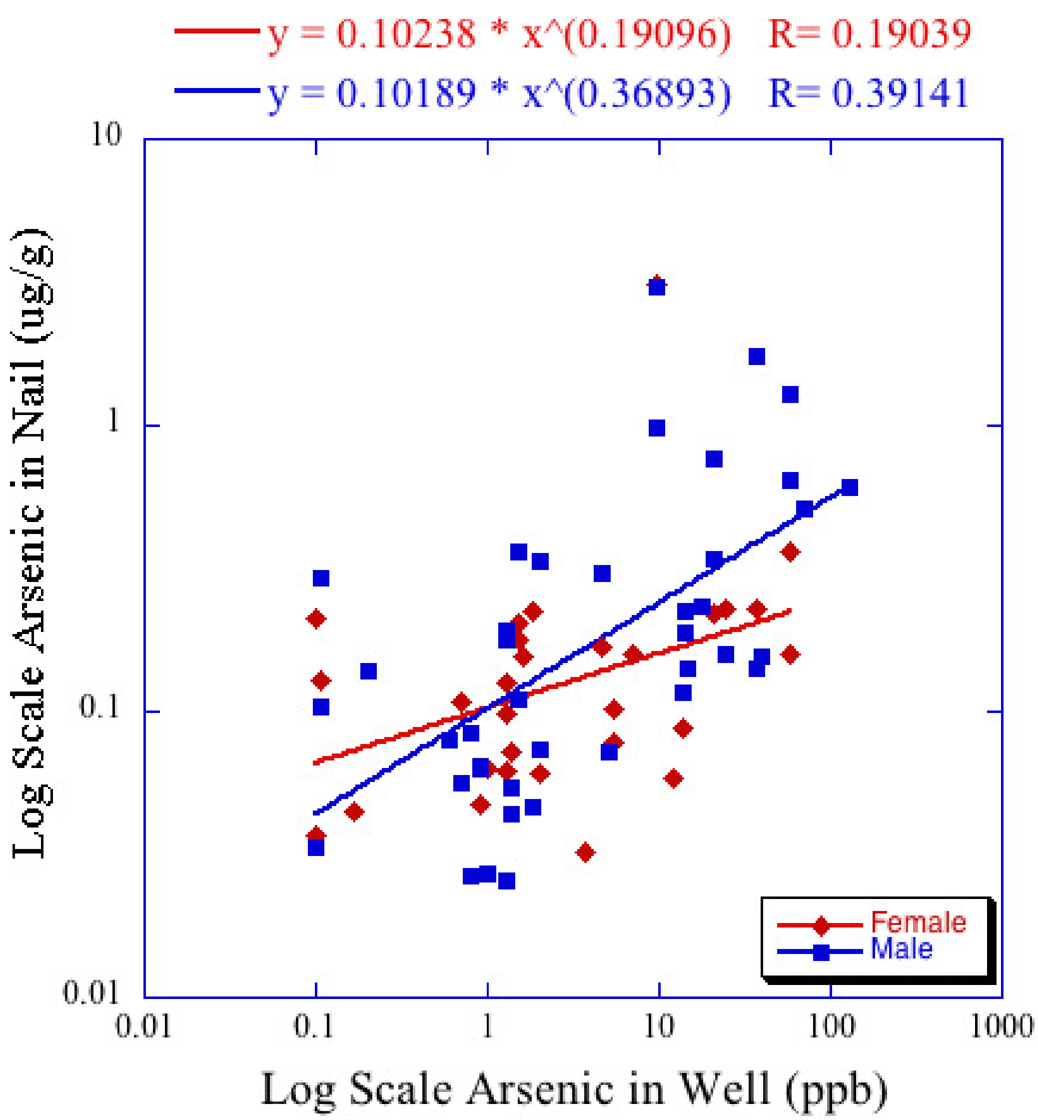
The relationship between nail-arsenic concentration and As^{III}, the more toxic arsenic species was also examined (Figure 10). A positive correlation was found between these two variables, which reinforces the proposed biological mechanism of As^{III} being preferentially deposited at the nail bed. Unfortunately this analysis suffers from a lack of data. Due to technical difficulties, the first batches of households' well samples were not able to be speciated.

Figure 10. Correlation between As^{III} concentrations found in the well and concentrations of arsenic found in the nail



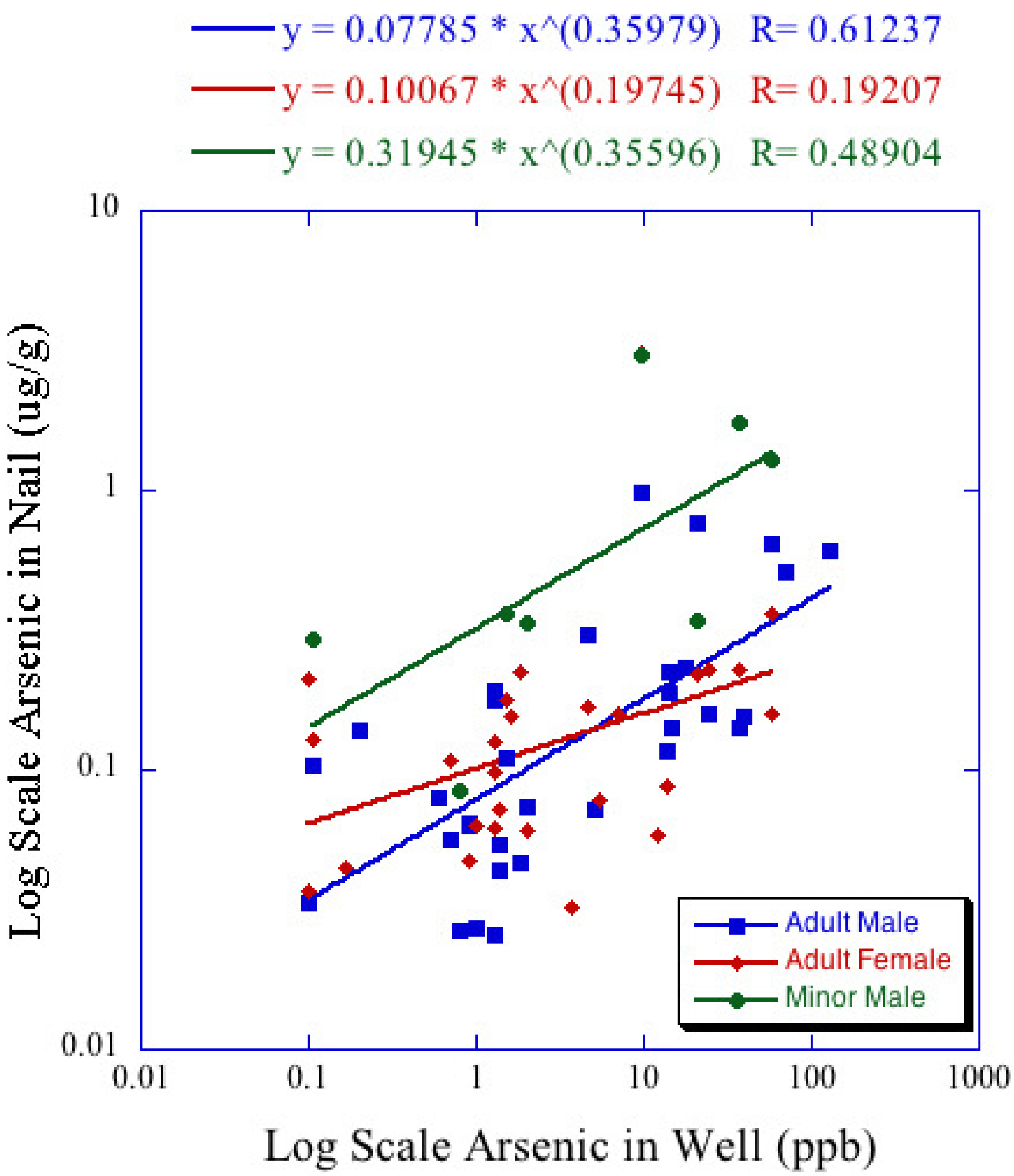
The effect of gender was another confounder that was important to observe. Males seem to have a greater sensitivity to arsenic exposure, and this exposure is also better correlated to well concentrations with an r value of 0.39141 versus the female r value of 0.19039 (Figure 11).

Figure 11. Correlation between arsenic concentrations found in the well and concentrations of arsenic found in the nail by gender



This correlation gets stronger when you separate the subpopulations by age (Figure 12). Males are still the most sensitive and have the strongest correlation to arsenic in the well but it is interesting to note that male children are more sensitive than adult males to arsenic exposure. A lack of minor females (only 3 participants) made it difficult to look at them as their own subgroup with regard to exposure.

Figure 12. Correlation between arsenic concentrations found in the well and concentrations of arsenic found in the nail by gender and age

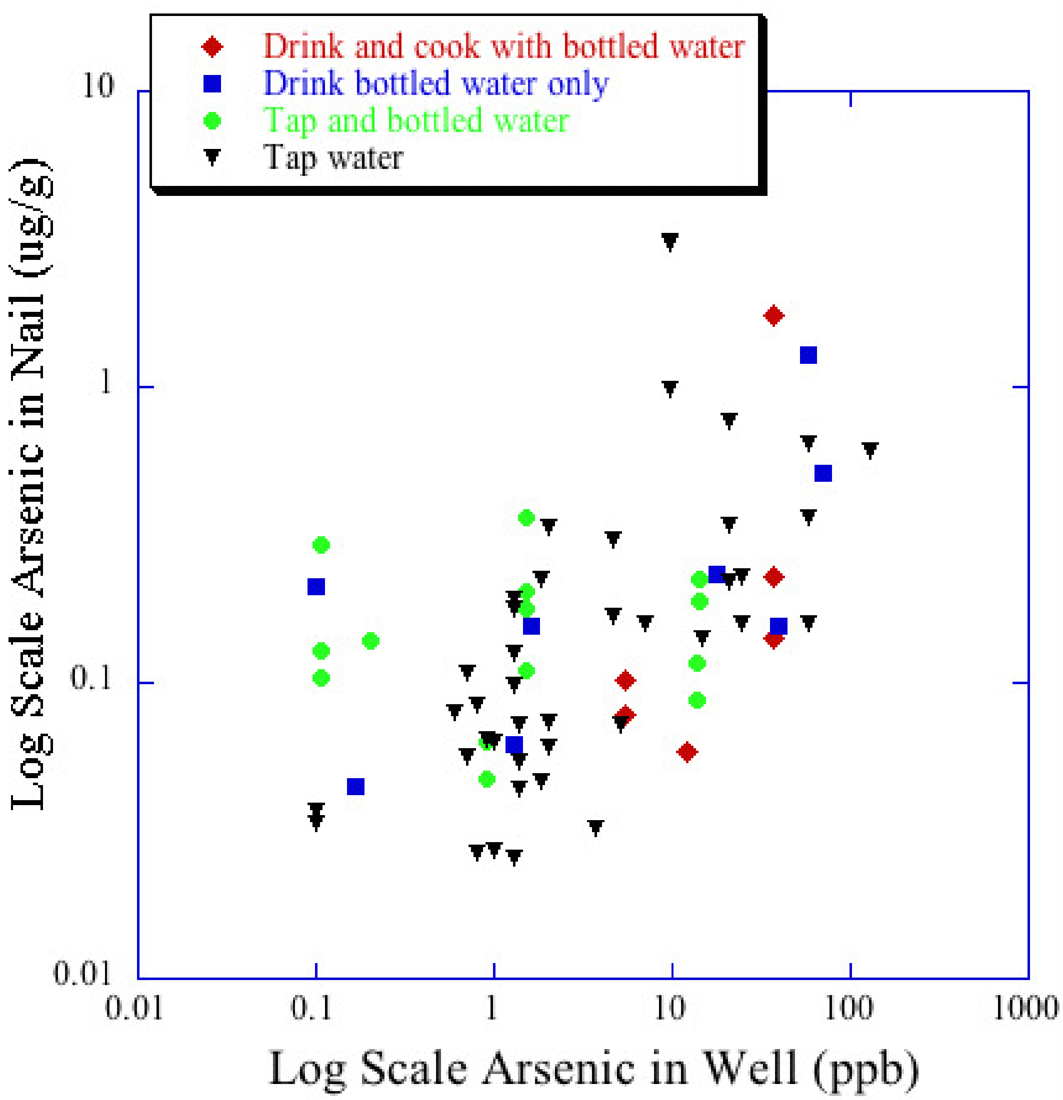


The quantity and type of water consumed can also confound exposure. If a person lived in a home with high arsenic in their water, but drank and cooked exclusively with bottled water, it is possible for that person to have had a lower exposure than someone with less arsenic in their well. Exposure was broken up into four categories:

- Individuals who drank and cooked with tap water;
- Individuals who drank bottled and tap water in the home, but cooked with tap water;
- Individuals who only drank bottled water, but cooked with tap water;
- And individuals who drank and cooked with bottled water.

Using this classification scheme, there was no evident general trend (Figure 13).

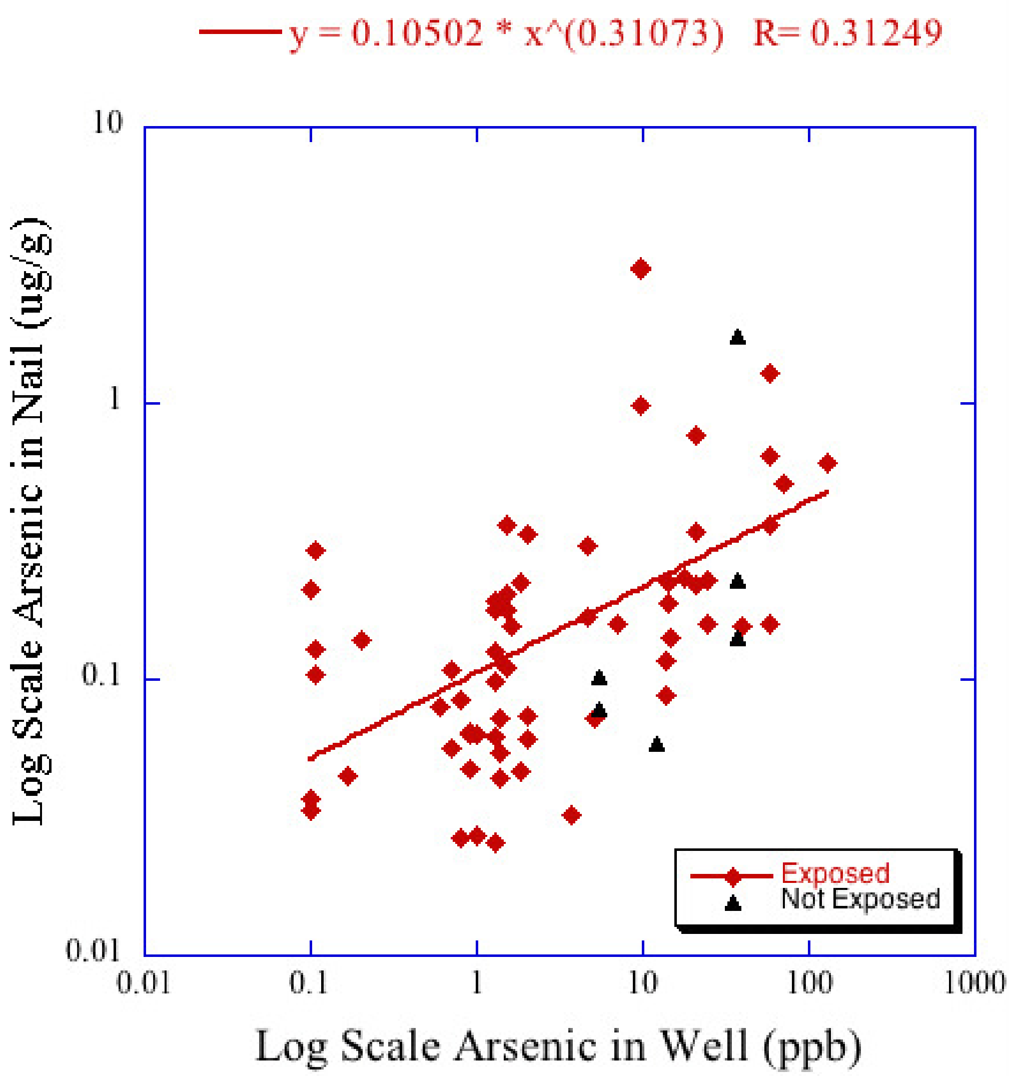
Figure 13. Correlation between arsenic concentrations found in the well and concentrations of arsenic found in the nail by exposure classification



However this exposure breakdown is subject to very wide variations in exposure and possible misclassification. For example, it is possible for a person who drank one bottle of water per week and the remaining water from the tap to be classified the same as some one who drank one glass of tap water per week and the remaining water from bottled water.

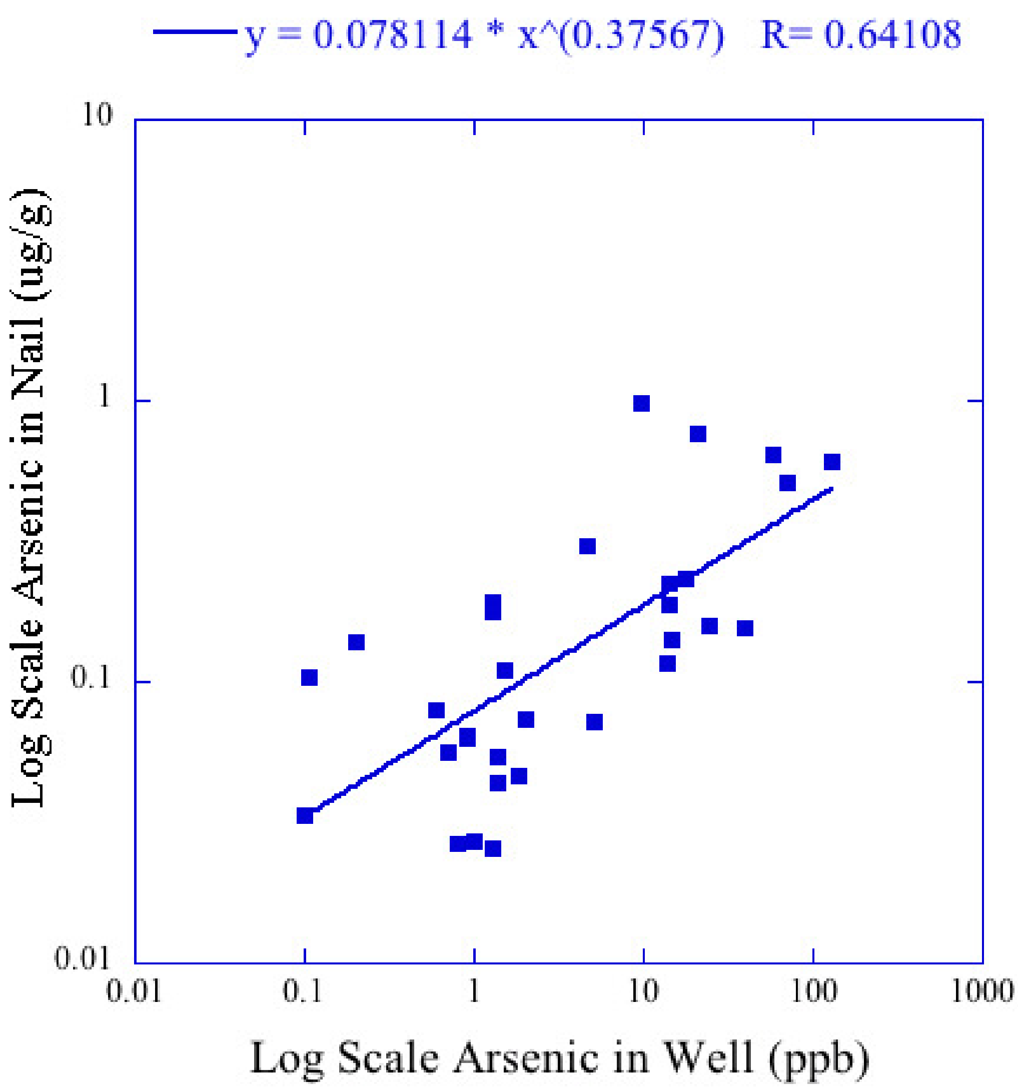
For this reason, Figure 14 combines these exposure classifications. Anyone who

Figure 14. Correlation between arsenic concentrations found in the well and concentrations of arsenic found in the nail by combined exposure classification



drank any amount of tap water was classified as exposed, while those who drank and cooked with only bottled water were classified as unexposed (Figure 14). While a positive linear correlation is present, this solution does not solve the misclassification bias, and it suffers from a lack of unexposed participants. Only two households (6 participants) used bottled water exclusively.

Figure 15. Correlation between arsenic concentrations found in the well and concentrations of arsenic found in the nails of exposed adult males



This classification scheme was not useless. When you use the exposed category classification and select only adult males, a strong linear correlation is present. This is demonstrated in Figure 15. Using this categorization to look at male exposure data provides the highest correlation coefficient, r = 0.64108.

Conclusion

This study has demonstrated that there are elevated levels of arsenic in the drinking water of privately owned wells in Union County, North Carolina. Thirty-four percent of households randomly tested are above the EPA's MCL. The spatial distribution of arsenic found in these households shows no trends. This is to be expected with the fractured nature of the region, and further supports the evidence that the arsenic present is directly related to the geology of the region and not from anthropogenic sources. The lack of a spatial gradient or trend can make regulating arsenic in the region difficult as policy makers can not focus on a particular localized hotspot. The fact that concentrations between well and sink are highly correlated, in conjunction with the low number of people who consume bottled water exclusively, means that if arsenic is present in the well, it is highly likely that the household members drinking that water will be exposed to these elevated levels.

While direct linkages between nail concentrations of arsenic and negative health effects are still lacking, the fact that 4 participants are over the safety level for nail concentrations is significant. More alarming is the fact that 3 out of 4 of those people were children, a subpopulation known to be more sensitive to negative health effects from arsenic (Sukumar, 2006). One of those children was part of a household that had high levels of arsenic in their water, but that was classified as having no exposure. The other children in that household also had high levels of arsenic. This emphasizes the fact that the measurement of arsenic in nails catches all exposure routes, not just the arsenic consumed through drinking water. Interestingly, all were young children, and all bathed in the contaminated water. It is possible that exposure may have come from the consumption of bath water and in the future survey questions should address bathing habits for all household members.

While the relationship between As^{III} and nail concentrations of arsenic showed a general positive trend, this analysis suffered greatly from a lack of data. Before any in depth investigation can be made on this subject, more data needs to be collected to increase the power of this analysis.

A gender effect was observed. As nail measurements are a biomarker of exposure and not of effect, the greater male sensitivity to this biomarker does not in itself mean that men will necessarily have greater negative health effects. However as there is no safe level of arsenic consumption, it stands to reason that if males have a greater arsenic body burden they will also see more negative health effects. Epidemiological studies collecting more health data should be conducted to explore this area further.

A solution to many of these issues is a classification scheme with more precise reporting so to know exactly how many bottles of water are consumed versus glasses of tap water. Total water consumed is very important, and although that information was collected, it is subject to a high level of recall bias and thus was not included in this preliminary analysis. Lastly, small children bathing in water with high arsenic concentrations may be exposed by drinking bathwater and the survey questions should address this potential confounder.

In conclusion, this study was successful in establish a framework for future arsenic groundwater testing and illuminating what future steps can be taken to further explore the health effects of this contaminant in Union County.

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Appendix A: Recruitment Letters
(all letters will be on Duke Letterhead)

Household Letter

<<current date>>, 2008

«First_Name» «Last_Name»
«ADDR1»
«ADDR2»
«ADDR3»

Dear «First_Name» «Last_Name»,

We are currently looking for residents in your area who own homes with private water wells to take part in this research study.

Researchers from Duke University are conducting a study that examines levels of arsenic in well water in North Carolina and to see if you may have been exposed to arsenic through drinking this water. Research has shown that exposure to arsenic can be measured by examining a person's toenail clippings

If you take part in the study we will ask you to clip your toenails into a plastic bag for collection and allow researchers to analyze the clippings. Also members of our research team will take samples of water from your well. The researchers will need approximately 45 minutes to take the well water samples. The appointment will be scheduled at a time that is convenient for you. We will send you a letter that will explain the results of the test for arsenic. You will also receive information on how to interpret these results and on methods you could use to improve your water quality, if applicable. This testing and information will be provided free of charge to everyone who participates in the study.

In addition, you will be asked to complete one survey. It will ask questions about the people in your home and about the house and the well, for example: How many people live in your house? How old is your well? The survey can be administered over the phone or online. The survey will take approximately 10 minutes. You may stop being in the study at any time.

To participate or for further information, please contact us toll free at 1-866-788-2558.

Sincerely,

Dr. Avner Vengosh
Associate Professor
Nicholas School of the
Enivironment and Earth Sciences
Duke University

R. Brittany Merola
Master of Environmental Management Candidate
Nicholas School of the Environment and Earth Sciences
Duke University

Appendix B: Survey

Initial Survey Protocol
Conducted via phone
Phone interviews are directly inputted into online survey by interviewer

This is the survey you will be required to complete as part of your participation in the study. The survey contains 20-32 questions, depending on how many people live in your home, and should take approximately 12 minutes.

Answers to this survey will help us better understand private well water quality in North Carolina. In order for your participation to have the greatest impact on our study, we ask that you answer all of the questions.

If you have any questions about the survey, please call the toll-free information line at 1-866-788-2558, or me directly at 919-681-8228. You may also send me an email at rose.merola@duke.edu. Thank you again for your participation.

May I have your name please?____

In case we get disconnected, may I have a phone number where I could call you back?

____

County: Do you live in Union County?

Yes (1) **No (0)**

Source: Households may get their drinking water from a variety of sources. The most common sources are:

(1) Public water (often called city water or municipal water),
(2) community water provided by a private company (examples in North Carolina include Heater Utilities or Aqua North Carolina), and
(3) private wells.

What is the source of your water?_____________________________________

Property: Do you **rent (0)** **or own (1)** your home?

Letter: Did you receive a letter from our team**? No (0)** **Yes (1)**

Verify **address:**

User ID:________________________________

Thank you. You are eligible for participation in the study. The next step is for me to explain the study, in particular the benefits and risk associated with participation. Feel free to ask questions as we go along. At the end of this description, you will be asked whether you agree to participate. Participation is entirely voluntary and you may withdraw from the study at any time.

The study is run by Brittany Merola and Dr. Avner Vengosh from Duke. This research will help us better understand the risks of contaminants that could be present in private well water in North Carolina. This research examines levels of several inorganic elements in well water and toenails. We are particularly interested in arsenic. You will be mailed a Ziploc bag for each member of your family who would like to participate in this study. Each person participating will receive a bag for her/his nail clippings. Next the researchers will take samples of water from your well and from your sink. They will need approximately 45 minutes to take the samples. We will mail you a report of the findings for arsenic, along with information on how to interpret the results. You will also receive a report of the findings for all inorganic elements detected in your well and nails.

In addition, one adult in each household will be asked to complete a survey. It will ask questions about the people in the household and about the house and the well, for example: How many people live in your house? Or How old is your well?

You do not have to be in the study if you don't want to and you may stop being in the study at any time. You do not have to be in the study in order to have your well tested. The State Department of Public Health can test your well for arsenic. In addition, many laboratories are certified by the State to test your well water for a variety of inorganic, bacterial, and organic contaminants. If you prefer to have the state or a private company test your well I would be happy to provide you with contact information and will send you this information in the mail.

Information collected from your house will be stored at Duke in a place where only the research team can access it. Any personal identifying information such as your name and address will only be stored until completion of the study. Your name and address will not be used in any presentations or publications from this study.

The benefits of participating in the study are that you will learn more about the levels of arsenic and other inorganic contaminants in your water and in your nails. You will also learn if you may have been exposed to arsenic.

While testing and information will be provided free of charge, participation in this study does create some potential risks of which you should be aware. In particular, the State of North Carolina requires the landowner to make known if there are “any known problems (malfunction or defect) in the water supply system upon sale or lease of the property. Depending on the results of the test, you may be required to tell about the problem if you sell or lease your property.

Each adult member of your household interested in participating will need to sign his or her own copy of this consent form. If there are any household members under the age of 18 a parent or guardian’s permission and signature is needed on the parent permission form before the minor can participate in this study; also, minors between 10 and 18 years old will be asked to sign an assent form if they agree. If there is a minor who is under ten years old and would like to participate s/he will need to be present when the researchers come to collect the samples so s/he can say orally, instead of signing a form, that s/he agrees to participate in the study.

Do you have any questions at this time?

You may contact Brittany Merola if you have any questions about what will be done on your property or in your home, if you have any questions about the survey, or what will be done with your nail clippings. You can contact her at 919-681-8228 or toll free at 866-788-2558, or by email at rose.merola@duke.edu.

If you have questions about your rights as a research participant, you may call (919) 684-3030 to talk to the chair of the Committee for the Protection of Human Subjects at Duke University.

Do you wish to participate?

A copy of this information will be sent to you so that you have it for your records.

Consent
Yes (1) or **NO (0)**

Phone: Best number to call during the day to schedule appointment?

If you would like, we can complete the survey at this time. The survey will take approximately 10-15 minutes. If you do not have time now, we can schedule a time for me to call you back.

Would you like to complete the initial survey now
If no-When should I call you back

Email address:____

Housetype: How would you classify your home?
(4) Townhouse/duplex
(3) Apartment/condo
(2) Mobile home
(1)House
(5)Other____________________________________

Houseyear: What year was your home built? ____

Residancetime: How many years have you lived at this residence? __________________________
Please round to the nearest whole number. For example, if you have lived there for 1 year and 8 months, please enter the number two (2). If you have lived in your home for less than 6 months, please enter zero (0).

Wellage: What is the age of your well in years?______________________________

Please round to the nearest whole number. If your well is less than 6 months old, please enter zero (0).

Wellestimate: If you do not know the exact age of your well, please select your best estimate below.
(1) Less than 5
(2) 5-10
(3) 10-20
(4) More than 20

Contamtest: Have you ever had your well tested for any contamination? **Yes (1) No (0)**

Contamtestyear: When was the most recent test done?________________________________

Please mark all contaminants that were part of the test
Bacteria, lead, as, radioactive materials (Ra and Rd), nitrates, other, don't know
Contambact Yes (1) No (0)
Contamlead Yes (1) No (0)
Contamas Yes (1) No (0)
Contamradio Yes (1) No (0)
Contamnit Yes (1) No (0)
Contamother Yes (1) No (0)
Contamnotsure Yes (1) No (0)

Contampass: Did your well pass the most recent test? **Yes (1) No (0)**

What problems were detected?

Bacteria, lead, as, radioactive materials (Ra and Rd), nitrates, other, don't know
Contfoundbact Yes (1) No (0)
Contfoundlead Yes (1) No (0)

Contfoundas Yes (1)   No (0)
Contfoundradio Yes (1)   No (0)
Contfoundnit Yes (1)   No (0)
Contfoundother Yes (1)   No (0)
Contfoundnotsure Yes (1)   No (0)

Treatment: Do you presently use any type of water treatment system or employ a water treatment service for your home? **Yes (1)   No (0)**
Common water treatment systems include water filters (such as brita filters or refrigerator filters), water softerners, and reverse osmosis systems. A common water treatment services is the "Culligan Man."
Treattype:
Which of the following treatment systems do you currently use in your home? Please check all that apply.
(1) Carbon filter(brita, refrigerator)
(2) Water softner (culligan man)
(3) Reverse osmosis
(4) Ultra violet
(5) Distillation
(6) Aeration

Please answer the following questions for yourself:
Name1
ID1:
Gender1 Male (1)   Female (2)
Ethnicity1 Cacucasian (1)   African American (2) Hispanic(3) Hispanic/Caucasian(4)
Nail1 yes (1)   no (0)
Age1
Pregnant1_0 (preg1_0) yes (1)   no (0)
Pregnant in the last year1_1 (preg1_1) yes (1)   no (0)
Smoke1_0 yes (1)   no (0)
Smoke1_1 yes (1)   no (0)
Smoke1_2 yes (1)   no (0)

Housesize: How many people live in your household? _______________________

Please answer the following questions for the rest of your household:

NAME			
ID2			
GENDER			
ETHNIC			
NAIL POLISH			
AGE			
PREGNANG			
PREG 6MON			
SMOKE			
SMOKE 6			
SMOK EVER			

We will send you a ziploc bag for each member of your household. It is their decision whether or not they would like to participate. While we encourage everyone in your household to participate it is not necessary. There will be one ziploc bag for each member of the household and there will be a label on it with his or her name. Once the researchers come to collect the samples they will remove the label and replace it with a code so that at no part of the analysis will your identity be known.

For this question, note that an 8 oz glass is the size of a typical juice glass or half the size of a soda can. For this questions do not include water used in cooking, but do include water used in making other beverages such as coffee, iced tea, lemonade, etc.

Totwatcons1: Approximately, how many 8 ounce glasses of water do you drink on a typical day? Please include water consumed in your home and elsewhere. Please answer only for yourself.

Houswatcons1: How many of these 8 ounce glasses do you consume in your home? Again, please answer only for yourself.

Housesource: What is the primary source of the water you drink in your home? Please answer only for yourself.
(1) Tap water unfiltered
(2) Tap water filtered
(3) Bottled water

Do your water drinking habits reflect, on average, the amount of water consumed by other adult members of your household? (Does any one drink considerably more or less)

Who drinks more or less?
Toth20cons2-X
Housh2ocons2-X

the questions on this page pertain to children living in your home.

Minortotwat: How many 8 oz. glasses of water do your children drink during the day? Please include an estimate of water consumed both at home and at school or daycare. Please include water used to make formula for infants.

Minorhouswat: How many of these 8 ounce glasses are consumed at home?

Minorhousesource: What is the primary source of the water that your children drink in your home?
(1) Tap water unfiltered
(2) Tap water filtered
(3) Bottled water

Bottled: Does anyone in your household consume bottled water in your home at least once per week?
Yes (1)   No (0)

Who:

Bottledwho:

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

Mealsource: What is the primary source of water used for cooking in your home?
(1) Tap water unfiltered
(2) Tap water filtered
(3) Bottled water

Appendix C: Adult Consent Form

Thank you for considering to be in this study on private well water quality run by researcher Brittany Merola with the supervision of Dr. Avner Vengosh from Duke University. Please read the following information and decide if you would like to be in the study.

The research will improve the understanding of risks of several inorganic elements, particularly arsenic, that could be present in private well water in North Carolina.

If you decide to participate, we will test your toenail clippings and your well water for several inorganic elements and arsenic which should take approximately 45 minutes of your time. A report of the level of all inorganic contaminants in your well water, sink water, and toenails will be mailed to you. You will also receive information on how to understand these results. You will also be asked to complete a short survey that will take approximately 10 minutes and will ask you questions such as: How old is your well?

You do not have to be in the study if you don't want to and you may stop being in the study at any time. You do not need to participate in this study in order to have your well tested. The State Department of Public Health can test your well for arsenic. If you would like to have your well tested by the State, please contact Dr. Ken Rudo at (919) 707-5911. In addition, many laboratories are certified by the State to test your well water for a variety of inorganic, bacterial, and organic contaminants. You can find a list of certified laboratories by calling the North Carolina State Laboratory of Public Health at (919) 733-7834.

The benefits of being in the study are that you will learn more about the levels of arsenic and other inorganic contaminants in your well water. From the results of the testing on your toenail clippings, you will also learn if you may have been exposed to arsenic through your well water. If we find that there is arsenic in your well, we will give you information about what you can do about it.

There is a risk associated with the discovery that your property has environmental hazards such as arsenic in your well water. The State of North Carolina requires the landowner to make known if there are "any known problems (malfunction or defect) in the water supply system upon sale or lease of the property." Depending on the results of the test, you may be required to tell about these problems if you sell or lease your property.

Some medical conditions may affect the rate at which nails grow. If you have any of the following medical conditions, please do not agree to be in the study:

A-V shunts
Beau's lines
Hyperthyroidism
Hypothyroidism
Onycholysis- nail lifting
Pityriasis rubra pilaris
Psoriasis
Yellow nail syndrome

If you do not have any of the medical conditions listed above and you wish to participate, you will be ask to sign this form below and then to clip your toenails into the bag provided. A separate plastic bag is to be used for each person's nail clippings. Please mark the label on each bag with a person's name so the researcher will know whose toenail clippings belong to whom. The name label will be removed later by the researcher and replaced by a code. If you have any questions concerning clipping collection please call Brittany Merola at (919) 681-8228 or email her at rose.merola@duke.edu. You may also contact her advisor about the water testing or the nail clippings: Dr. Vengosh at (919) 681-6831 or by email at vengosh@duke.edu.

Each eligible person of your household, who is participating in this study and is at least 18 years old, must also sign a consent form. Those in your household who are younger than 18 will need a parent's permission in order to be able to participate in this study.

Information collected from your house will be stored at Duke in a place where only the research team can access it. Also, your name and address will not be used in any presentations or publications from this study.

Please ask any questions you have about this research and what will be done in your home before you sign this form. If you have any questions about your rights as a research participant, please call 919-684-3030 to talk to the chair of the Committee for the Protection of Human Subjects at Duke University.

If you are willing to participate in the study, please complete the lines below. You will receive a copy of this consent form for your records.

____

____
Name, printed

____ ____________________
Signature Date

Appendix D1: Parental Permission Form for minors:

Thank you for agreeing to be in this study on private well water quality run by researcher Brittany Merola with the supervision of Dr. Avner Vengosh from Duke University.

We would like to ask your permission for your child to also participate in this study. This means that we are asking your permission to collect toenail clippings from your child and to test the clippings. For any children between the ages of 10 years and less than 18 years old we will ask them, with your permission, to be in this study. If s/he agrees to participate, s/he will then sign an assent form. If your child is younger than 10, and you have given your permission, we will ask the child for his or her agreement orally. Whatever each child decides is fine. S/he can decide not to participate even if you have given permission.

A report of the level of all inorganic contaminants in your child's toenails will be mailed to you. You will also receive information on how to understand these results.

You do not have to allow your child to be in the study if you don't want your child to participate. Your child does not need to participate in this study in order for you to be in the study.

The benefit of allowing your child to be in the study is that you will learn from the results of the testing on your child's toenail clippings, if your child may have been exposed to arsenic through your well water.

Some medical conditions may affect the rate at which nails grow, If your child has any of the following medical conditions, please do not allow your child to be in the study:

A-V shunts
Beau's lines
Hyperthyroidism
Hypothyroidism
Onycholysis- nail lifting
Pityriasis rubra pilaris
Psoriasis
Yellow nail syndrome

If your child does not have any of the medical conditions listed above and you wish to allow your child to participate, you will be ask to sign this form below and then to collect your child's toenails into the bag provided. A separate plastic bag is to be used for each person's nail clippings. Please mark the label on each bag with a person's name so the researcher will know whose toenail clippings belong to whom. The name label will be removed later by the researcher and replaced by a code. If you have any questions concerning clipping collection please call Brittany Merola at (919) 681-8228 or email her at rose.merola@duke.edu. You may also contact her advisor about the water testing or the nail clippings: Dr. Vengosh at (919) 681-6831 or by email at vengosh@duke.edu.

Information collected from your house will be stored at Duke in a place where only the research team can access it. Also, your child's name and address will not be used in any presentations or publications from this study.

Please ask any questions you have about this research and what will be done in your home before you sign this form. If you have any questions about your child's rights as a research participant, please call 919-684-3030 to talk to the chair of the Committee for the Protection of Human Subjects at Duke University.

If you are willing to give your permission for your child to participate in the study, please complete the lines below. You will receive a copy of this permission form for your records.
I, ____, give my permission for my

(PARENT'S NAME)

for my child ____
(CHILD'S NAME)

to be in this study, if he or she wishes, and to have her/his toenail clippings tested for arsenic.

Signature of parent Date

____ _____________________

Appendix D2: Assent Form for Minors (between 10 and less than 18 years old)

Your parents have agreed that we could ask you to be a part of our research study about your family's well water.

We are looking at what could be in your well water. Your parents have said we can test your water. Now we are asking you if we can have some of your toenail clippings. From testing toenail clippings we can also find out what is in your well water.

You do not have to be in the study if you don't want to. Being in the study means giving us some toenail clippings in a small bag.

Do you have any questions that you would like to ask about this study?
____

If you would like to give us some of your toenail clippings and be in our study, please sign here.

____ ____________________
Signature Date

____ ____________________
Signature (Parent or Guardian) Date

Appendix D3: Assent Script for Minors (younger than age 10)

The script will be the same as the text above. If necessary, we will tailor it to very young children's comprehension level, and will conduct the process in a very conversational way, ensuring understanding. We will make sure the child knows that even if his/her parent has said OK, it is now completely the child's choice. (No one will be mad if you say no, etc.)

Appendix F: Sample Results Letter
(all letters will be on Duke Letterhead)

<Insert Date>

Name
Address
City, State Zip

Dear <Insert Name>,

We have completed the analysis of the samples we obtained from your well water, tap water, and from your nails. This letter details the findings of the test for **arsenic**.

Arsenic in Water

Amount Detected in Your Well:	**7.2 ppb (parts per billion)**
EPA Standard	**10 ppb**
North Carolina Recommendation	**0.0016 ppb**

Arsenic in Nail

Amount Detected in Your Nails	**0.5 µg Arsenic/ g nail (microgram* Arsenic/ gram nail)**
Elevated Level	**Above 1.08 µg Arsenic/ g nail**

*1 microgram is 1/1000000 of a gram

There are several health risks from exposure to arsenic in drinking water. The primary health risk is that long-term exposure to arsenic increases the risk of developing certain types of cancer (particularly bladder and lung cancer).

The risk from arsenic in your drinking water depends on many factors including:

- The level of arsenic in the water
- The amount of water you consume
- The number of years you consume the water
- Your personal health history and risk factors.

Information on the attached page will help you better understand your results. **If you have any questions regarding your water or nail results you may call Brittany Merola toll free at 866-788-2558 or email her at rose.merola@duke.edu. You can also contact Dr. Vengosh at (919) 681-6831 or by email at vengosh@duke.edu. In addition we will contact you in approximately one week to ask if you have any questions or concerns.**

Sincerely,

Frequently Asked Questions About Arsenic Test Results

1. What are the health risks from arsenic?

Short-term (5 years) and long term (20-30 years) exposure to arsenic in drinking water has been linked to certain types of cancer, particularly, cancer of the bladder, lungs, skin, kidney, nasal passages, liver, and prostate. These cancer risks are the primary risks examined by government agencies such as the Environmental Protection Agency and the North Carolina Department of Public Health in making recommendations about arsenic in drinking water.

Studies have also linked long-term exposure to arsenic in drinking water to non-cancer heath effects including effects on the heart, lungs, immune system, nervous system, and endocrine system (such as diabetes).

There is no level of arsenic exposure that is completely free from risk. However, exposure to small levels of arsenic, even over long periods, may present only minimal risks.

What do the EPA standards mean?

EPA sets standards for contaminants in **public** drinking water systems. These standards do not apply to homeowners with private wells such as yourself. However, they are provided as a reference point. EPA actually sets two standards for each contaminant. The first standard is called the Maximum Contaminant Level Goal (MCLG). The MCLG is the level of the contaminant at which no known health effects occur. The second standard is called the Maximum Contaminant Limit (MCL). The MCL is the level that is protective of public health taking into account costs and technical feasibility.

The MCLG for arsenic is zero, meaning that there is known level of arsenic exposure that is completely safe. However, after taking into account the cost and technological feasibility of reducing arsenic in **public** water systems EPA set the MCL at 10 parts per billion (ppb). If you received your drinking water from a public source (such as a city water system) and the level of arsenic in the water was less than 10 ppb, the water system would be in full compliance with the law.

What does the North Carolina recommendation mean?

North Carolina does not have additional water quality standards. Public water providers in North Carolina are required to meet EPA's standard of 10 ppb. However, the North Carolina Department of Public Health has developed a health-based recommendation for arsenic in drinking water. This recommendation is based on the level of arsenic exposure that poses a lifetime excess cancer risk of 1 in 1 million. That is the risk level that the Department of Health frequently uses as a "safe" risk level. Based on analysis by a national blue-ribbon panel of experts, the North Carolina Department of Health estimated that exposure to 0.0002 ppb of arsenic over your lifetime increases your cancer risk by 1 in 1 million. Thus the health-based recommendation is that arsenic levels in drinking water be below 0.0002 ppb.

Why do the EPA and North Carolina standards differ?

The North Carolina recommendation is based only on health considerations. It is the level of exposure to arsenic that limits excess cancer risk to 1 in 1 million. The EPA standard (MCL) takes into account both the health benefits of reduced arsenic exposure and the cost of attaining the standard. Both EPA and the State of North Carolina agree that no level of exposure to arsenic is completely safe.

My arsenic level is below EPA's standard, but the letter says that drinking my water still puts me at risk of developing cancer. How can this be?

In the answer to question #2 we talked about the difference between EPA's maximum contaminant level goal (MCLG) and its maximum contaminant level (MCL). The MCLG for arsenic is zero, which means that consumption of any amount of arsenic poses some risk. The level of risk is directly proportional to the amount of arsenic consumed. So if you consume very low amounts of arsenic, your risk is very low. The more arsenic is in your drinking water the higher the risk. At EPA's MCL of 10 ppb, the excess risk of developing cancer over a lifetime for a person who drinks 1 liter of water a day (just over 4 8-ounce glasses of water) is roughly 1 in 300.

What does "excess lifetime cancer risk" mean?

The excess lifetime cancer risk is the additional increase in the likelihood that the average person will develop cancer due to exposure to arsenic. Your **total** lifetime cancer risk depends on many things including family history of cancer, smoking habits, nutrition, physical fitness, and exposures to cancer causing agents in food, your home, your workplace, and the environment. The excess cancer risk is the amount you could expect your total lifetime cancer risk to increase because of your exposure to arsenic.

How were the excess lifetime cancer risks presented in my letter determined?

In 1999 and 2001, a blue-ribbon panel of experts was brought together to evaluate scientific studies of the health effects of arsenic in drinking water. The panel of experts was organized by the National Research Council, which is a private, nonprofit institution that provides science, technology and health policy advice to the federal government. The panel issued two reports that have been widely used to estimate excess cancer risks from arsenic exposure.

One of the key features of the panel's report is that health effects for arsenic are largely based on human health studies in countries with naturally high levels of arsenic. The health effects are not extrapolated from studies on rats. The bulk of the scientific evidence on the effects of arsenic in drinking water come from several studies of human response to high levels of arsenic exposure in Tawain, Chile, and ??? where there are naturally high levels of arsenic. One of the key tasks the blue-ribbon panel had was to determine how best to use information from these studies to determine health effects at much lower levels of arsenic that are common in the United States.

The excess cancer risks are for lifetime exposures. If I am only going to live in my home for a few years, am I safe?

The excess cancer risk number that you have been given is based on calculations done by EPA and the North Carolina Department of Public Health and these calculations assume that you will be exposed to arsenic over your whole lifetime. If you are exposed for fewer years, your risk of developing cancer is reduced. This is similar to the risk posed by smoking cigarettes. Smoking cigarettes for any amount of time increases your risk of developing lung cancer, but if you smoked for 5 years your risk of developing lung cancer will be less than if smoked for 25 years.

How does my lifetime excess cancer risk from exposure to arsenic in drinking water compare to other risks I face in my life?

The National Safety Council (http://www.nsc.org) publishes statistics on the likelihood of dying from a variety of causes. The table below presents the **lifetime** risk of dying from each of the listed causes. Your lifetime excess cancer risk from arsenic is also included.

Cause of Death	Lifetime Risk
Car accident	1 in 228
Falling	1 in 229
Cancer from arsenic exposure	1 in 500
Complications of medical treatment or surgery	1 in 1,310
Airplane accident	1 in 5,700
Struck by lightening	1 in 56,439

I understand what my risk level is, but what should I do about it?

Different people with the same level of arsenic in their drinking water may make different decisions about what to do. Each person has to weigh the risk posed by arsenic exposure and the cost of reducing this exposure. Reasonable people may arrive at different conclusions and no decision is right or wrong for everyone. There are basically three options to consider.

Option 1: Do Nothing

It may be perfectly reasonable to decide that the risk presented by arsenic in your drinking water is a risk you can live with. People may reach this decision because they feel that money they would have to spend to reduce their arsenic levels could be better spent on other things that improve their (or their family's) health or welfare. Alternatively, people might reach this decision if they will not be exposed to arsenic for a very long time period because they intend to move within a few years. And people may reach this decision because they feel that the level of risk presented by arsenic in their drinking water is quite small.

Option 2: Reduce Water Consumption

You can reduce your lifetime excess cancer risk by drinking less of the water that contains arsenic. This can be accomplished by simply drinking less water (not necessarily recommended) or by substituting clean water sources for more of your drinking water needs. This may mean drinking more bottled water or drinking more water at work or other locations outside of your home.

Option 3: Treat Your Drinking Water

You can reduce your lifetime excess cancer risk by reducing the level of arsenic in your drinking water. There are several treatment systems that can reduce arsenic in your drinking water. The National Sanitation Foundation (http://www.nsf.org) certifies treatment systems for different contaminants. Currently the National Sanitation Foundation has certified over 40 suppliers and over 150 treatment systems for arsenic removal in drinking water. The most frequent treatment system used for arsenic removal is a reverse osmosis filter. A reverse osmosis filter is normally installed on a single faucet in a home (usually the kitchen sink). In order to be protected from arsenic, drinking and cooking water should be taken from that faucet.

If you choose to install a water treatment system, we highly recommend that you specifically install a system certified by the National Sanitation Foundation. This will insure that your system actually removes arsenic. It is a good idea to test your water after installation to ensure that the arsenic level has been reduced and hire an installation service that will guarantee their work. Certified reverse osmosis systems start around $300 plus installation. Replacement filters cost around $100 dollars per year. These costs are just estimates, your costs may be more or less.

If you have questions about how to locate certified products or reputable installation services please contact us on our toll-free information line at 1-866-788-2558.