EFFICACY OF ARSENIC EXPOSURE REDUCTION VIA DRINKING WATER TREATMENT SYSTEMS

by

STEVEN E. SPAYD

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ABSTRACT OF THE DISSERTATION
Efficacy of Arsenic Exposure Reduction via Drinking Water Treatment Systems

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Arsenic, a known human carcinogen, exceeds the maximum contaminant level in New Jersey private wells at a higher percentage than any other contaminant with a primary drinking water standard. New Jersey's drinking water standard for arsenic at 5 µg/L is currently the most protective in the world. Water treatment systems can remove arsenic from drinking water, either from the entire home (point-of-entry) or just at a single tap (point-of-use) for drinking and cooking. The goal of this research was to compare human exposure to arsenic between point-of-entry and point-of-use water treatment, by biomonitoring, to determine which level of treatment most effectively reduced arsenic exposure and dose from water at home to acceptable risk levels. The study recruited 53 subjects in 22 households obtaining arsenic water treatment, and five control subjects with little or no measurable arsenic in their water supply. The mean arsenic concentration in untreated water was 44 µg/L. Biomonitoring started before initiation of water treatment and continued for up to three years with samples analyzed at the Environmental and Occupational Health Sciences Institute. The study determined that: 1) dietary arsenic can be a major confounder in arsenic biomonitoring studies; 2) arsenic speciation techniques are extremely valuable for arsenic biomonitoring studies; 3) sampling protocols and reference values for

arsenic in urine and blood should be recommended; 4) arsenic water treatment systems are effective in reducing arsenic exposure from well water; 5) there is a measurable arsenic body burden after chronic exposure to arsenic in drinking water; 6) there is a two-compartment clearance of arsenic from urine, after cessation of ingesting the arsenic contaminated water; and 7) after nine months of water treatment, the adjusted mean inorganic-related arsenic concentrations in urine were significantly lower in the point-of-entry treatment group with a mean ± standard error of 2.7 ± 0.6 μg/g creatinine than in the point-of-use treatment group at 6.1 ± 0.7 μg/g creatinine. In conclusion, point-of-entry treatment of arsenic-contaminated well water should be recommended in preference to point-of-use.

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Carlene Radix, MD, MPH and Rosalind Julius Mickel, RN of the Environmental and Occupational Health Sciences Institute made house calls with me to help make sample collection for the subjects convenient and painless. Willie Johnson Jr. provided the support and training needed for much of the analytical work. Ruimin Xie, PhD provided arsenic speciation analytical methods and practical advice. Special thanks are due to Pamela Ohman-Strickland, PhD of UMDNJ – School of Public Health for assistance with the statistical analyses; Mike Serfes,

PhD of the New Jersey Geological Survey for working tirelessly with me on arsenic in ground water and drinking water since the first hint it was a regional water quality and public health issue; and Gail Carter of the NJDEP, Division of Science, Research, and Technology for her expert map making skills. Many others at the UMDNJ - School of Public Health, the Environmental and Occupational Health Sciences Institute, and the NJ Department of Environmental Protection have provided support.

The research was fully reliant on the subjects who gave up their time and the privacy of their homes to participate. Many of the subjects wanted answers to the questions we were studying and knew their participation would help protect the health of many people in New Jersey and elsewhere who are living with an arsenic contaminated water supply.

My parents, Dolly and Elwood Spayd, provided the foundation on which my interest in science, health, and the environment was built. Last, but most important, my wife, Debi, and my four daughters, Stefanie, Nicole, Jessica, and Rachel, provided the support, encouragement, assistance, and patience that was needed to help me see this project through to the end.

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Chapter 1: Arsenic Introduction

Background

Arsenic of natural geologic origin is commonly found in well water in many parts of the world including Bangladesh (Chowdhury et al., 2000), India (Chakraborti et al., 2002; Mazumder et al., 1998), Taiwan (Hsueh et al., 1998), China (Ning et al., 2007), Vietnam (Berg et al., 2001), Cambodia (Gault et al., 2008; Polizzotto et al., 2008), Argentina (Concha et al., 1998; Hopenhayn-Rich et al., 1998), Brazil (de Figueiredo et al., 2007), Mexico (Armienta and Segovia, 2008; Wyatt et al., 1998a; Wyatt et al., 1998b), and in the United States. In the United States, several regions have high arsenic levels including the West (Lewis et al., 1999; O'Rourke et al., 1999; Seiler, 2004), Alaska (Harrington et al., 1978), Midwest (Erickson and Barnes, 2005; Meliker et al., 2006), New England (Ayotte et al., 2003; Peters et al., 1999), and New Jersey (Serfes et al., 2005; Spayd, 2007).

In 2002, the US Environmental Protection Agency (EPA) reduced the Maximum Contaminant Level (MCL) for arsenic in drinking water from 50 µg/L to 10 µg/L with an effective date of January 2006 (USEPA, 2001). The MCL is the maximum permissible level of a contaminant in drinking water. The New Jersey Department of Environmental Protection (NJDEP) has set the New Jersey MCL for arsenic at 5 µg/L with the same effective date as EPA (NJDEP, 2004). The New Jersey MCL for arsenic is currently the most protective in the world.

Arsenic exceeds the maximum contaminant level in New Jersey private wells at a higher percentage (11.8%) than all other contaminants with primary drinking water standards (e.g., bacteria, nitrate, lead, 26 volatile organic chemicals, mercury, and gross alpha particle activity). In certain parts of the state, up to 15% of the private wells tested have arsenic concentrations exceeding 10 µg/L and 30% have concentrations exceeding 5 µg/L (Serfes et al., 2005). In one New Jersey community surveyed in 2004, 45% of the 114 residential wells tested exceeded 5 µg/L. As shown in Figure 1-1, between September 2002 and April 2007, New Jersey's Private Well Testing Act Program identified 1,445 out of 12,263 private wells tested exceeding the New Jersey MCL in the northern counties of the state (New Jersey Department of Environmental Protection, 2008). A substantial number of public community and public non community wells also have arsenic exceeding the MCL (Figure 1-2). Concentrations of arsenic in well water in this area can be as high as 250 µg/L. In parts of South Asia, levels exceeding 1,000 µg/L have been documented in up to 2% of the wells tested (Chowdhury et al., 2000).

Research by the New Jersey Geological Survey (NJGS) indicates the arsenic in New Jersey well water is predominantly naturally occurring in specific geologic settings (Serfes et al., 2005).

Figure 1-1: Private Wells Exceeding 5 µg/L Arsenic in Northern NJ

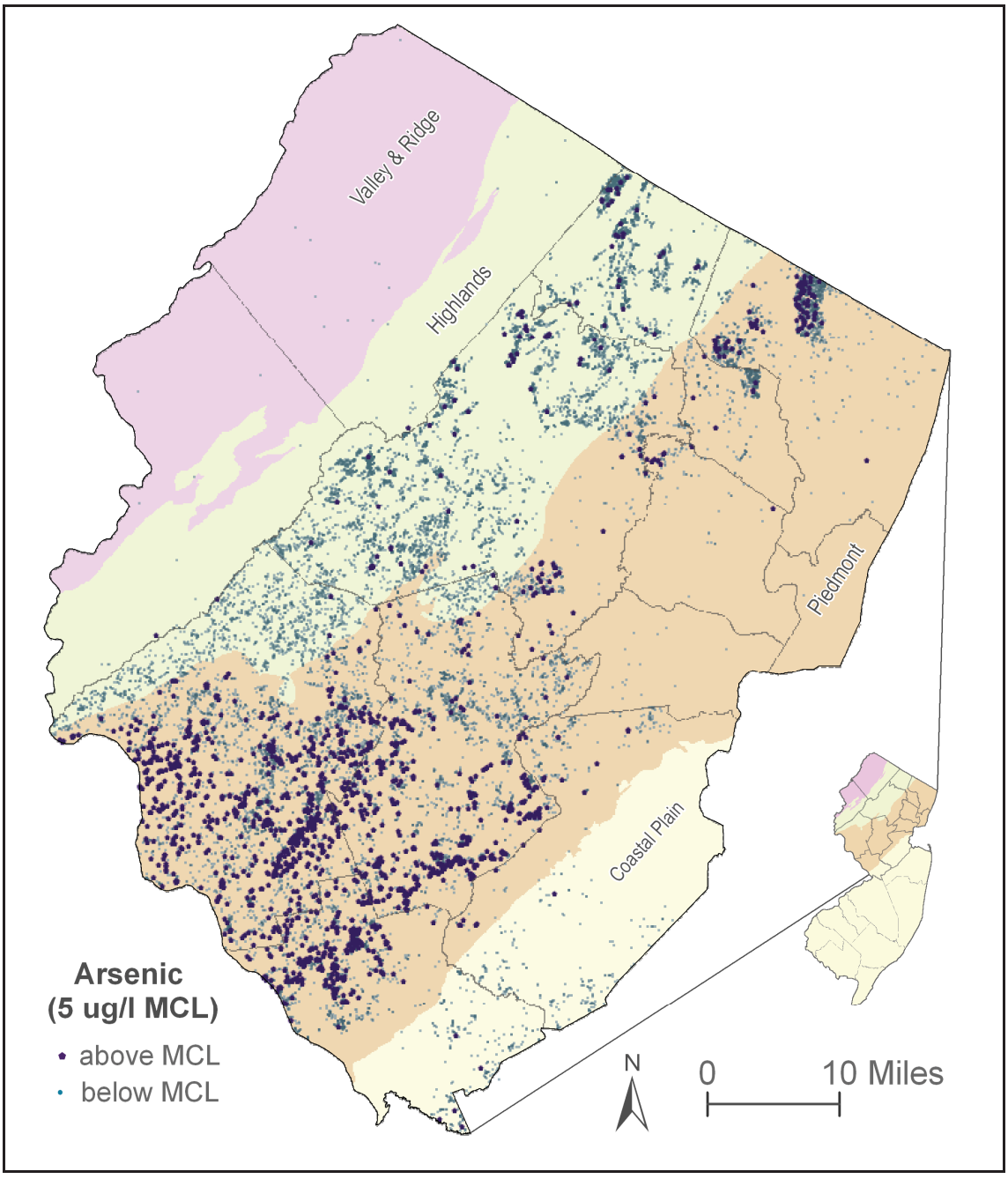
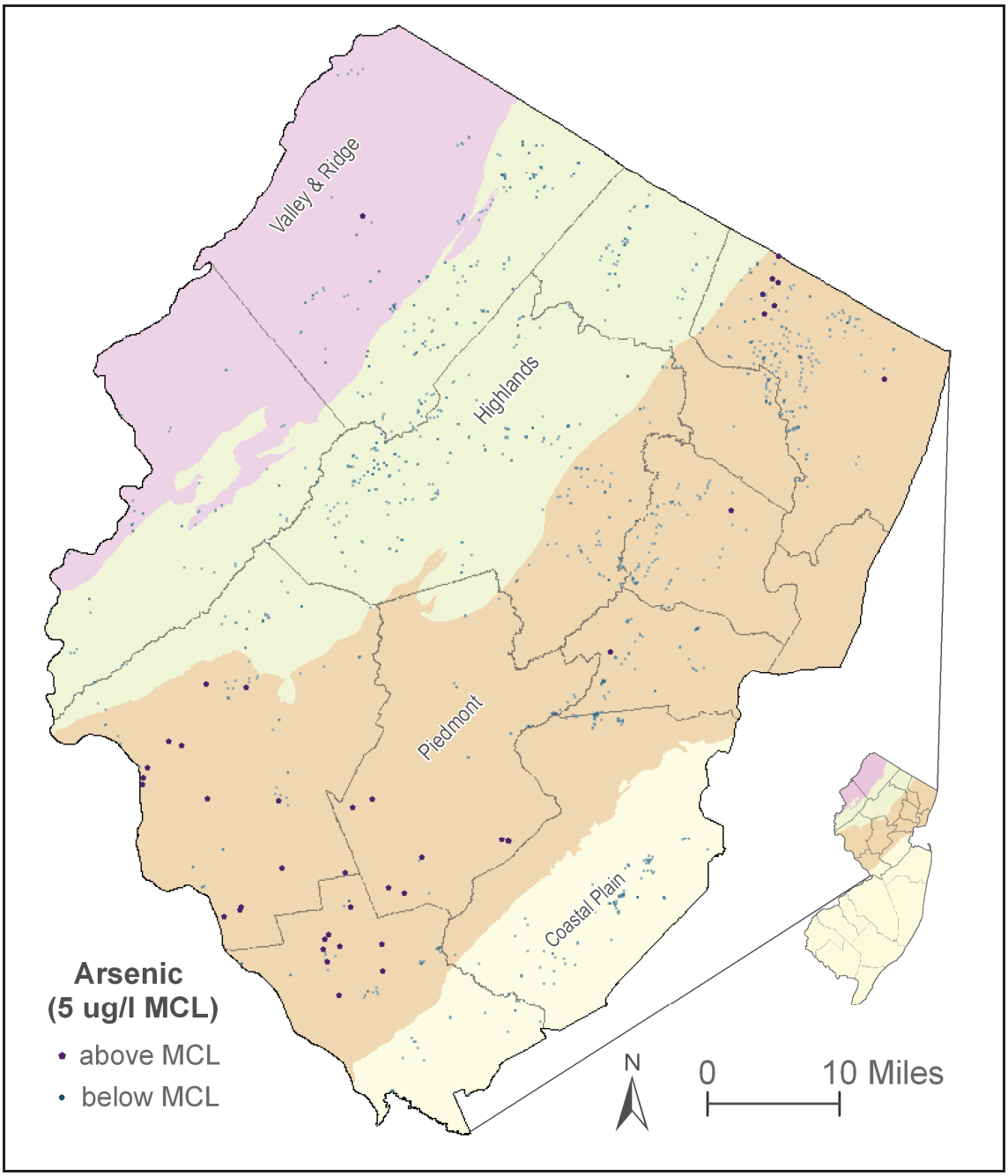


Figure 1-2: Public Wells Exceeding 5 µg/L Arsenic in Northern NJ



Arsenic Exposures

Arsenic is a common element in the environment and occurs naturally in certain foods and drinking water (National Research Council, 1977). Occupations involving wood treatment, pesticide application, farming, and metal smelting may involve a risk of arsenic exposure (Chou and De Rosa, 2003; Cocker et al., 2006; Frost et al., 1987; Popper et al., 1978), and arsenic as arsine gas is widely used in electronics (Liao et al., 2004; Sheehy and Jones, 1993). Exposures can also occur at home from cutting, sanding or burning arsenic treated wood, or from use of out-of-date rat and ant poisons, weed killers, and certain types of medication (Bates et al., 1992; Brender et al., 2006; Centeno et al., 2002; Robson and Jelliffe, 1963; Stephanopoulos et al., 2002; Waxman and Anderson, 2001).

Arsenic Health Effects

Arsenic is toxic to the basic energetic mechanism of cells, interfering with and uncoupling oxidative phosphorylation (IARC, 2004; Shi et al., 2004). It therefore poisons many functions in many types of cells and organisms. Arsenic has no essential function in humans, that is no deficiency state has been identified (National Research Council, 2001). EPA classifies arsenic as a Group A known human carcinogen (USEPA, 1984; USEPA, 2001). In fact, arsenic is one of the few contaminants, along with certain radioactive isotopes, with sufficient evidence to conclude that they cause cancer in humans via the drinking water exposure route. As a Group A carcinogen, arsenic has a Maximum Contaminant Level Goal (MCLG) of zero (USEPA, 2001). Epidemiological studies have

documented health effects from exposure to arsenic in drinking water including cancer of the bladder (Chen and Wang, 1990; Hopenhayn-Rich et al., 1996; Smith et al., 1998; Steinmaus et al., 2000), lung (Chiu et al., 2004; Guo, 2004; Marshall et al., 2007; Wu et al., 1989), skin (Tseng, 1977; Yu et al., 2006), kidney (Guo et al., 1997; Hopenhayn-Rich et al., 1998; Yang et al., 2004) and the liver (Liaw et al., 2008; Liu and Waalkes, 2008). The limited data on cancer latency periods indicate that it may exceed 20 years in some cases prior to cancer detection (Bates et al., 2004; Chiou et al., 2001; Haque R, 2003; Luchtrath, 1983; Marshall et al., 2007).

Non-cancer health effects include: cardiovascular-disease mortality and hypertension (Chen et al., 1995; Yuan et al., 2007); diabetes mellitus (Chiu et al., 2006; Rahman et al., 1998; Tseng et al., 2000); respiratory effects categorized as chronic bronchitis (Guha Mazumder, 2007; Islam et al., 2007; Milton et al., 2003); hepatotoxic effects including portal fibrosis, perturbed porphyrin metabolism and irreversible non-cirrhotic portal hypertension (Garcia-Vargas et al., 1994; Nevens et al., 1990; Santra et al., 1999); dermal effects including hyperpigmentation and keratoses, particularly on palms and soles of feet (Guha Mazumder et al., 1998; Saha, 2003); peripheral vascular disease (Pi et al., 2005; Yang, 2006); possible reproductive effects including impaired fetal growth, increased infant mortality, and increased cancers in children and adults after prenatal exposure (Ahmad et al., 2001; Borzsonyi et al., 1992; Vahter, 2008); increased risk of erectile dysfunction (Hsieh et al., 2008); neurological effects (Calderon et al., 2001;

Vahidnia et al., 2007; Wasserman et al., 2004; Yoshida et al., 2004); and hematological effects including anemia (Biswas et al., 2008; Heck et al., 2008; Rezuke et al., 1991). These have been summarized in several reviews by the International Agency for Research on Cancer (IARC, 2004), Agency for Toxic Substances and Disease Registry (ATSDR, 2007b), and the National Research Council (National Research Council, 2001).

Arsenic Dose Response, Species, and Toxicology

An evaluation of Table 6-1 in the National Academy of Sciences 2001 Update on Arsenic in Drinking Water demonstrates that a dose of approximately 0.003 micrograms per day (µg/d), of inorganic arsenic via drinking water in the United States, could result in a combined lifetime excess risk of bladder and lung cancer incidence of one in a million ($1X10^{-6}$) (National Research Council, 2001).

Arsenic exists in both organic and inorganic forms. Pure elemental arsenic is rarely seen in nature. Arsenic is a metalloid and can form positive, negative or neutral ions depending on the pH and oxidation/reduction conditions. Arsenic is found in a variety of chemical forms in water, food, and living organisms. In well water in New Jersey, arsenic has been found to occur in two inorganic species: arsenate (As^{V}) and arsenite (As^{III}) as shown in Table 1-1. The predominant species is As^{V}. The relative distribution of the inorganic arsenic species is important as it affects toxicity, analytical testing, and water treatment considerations. As^{III} is more toxic than As^{V}, but when As^{V} is ingested by

humans, it is reduced to As^{III} in the first step of the arsenic biotransformation pathway (Figure 1-3). Significant concentrations of As^{III} are found in about 20% of the wells with total arsenic above 5 µg/L in New Jersey (Serfes et al., 2005; Spayd, 2007). Well water in New Jersey with arsenic and any one of the following characteristics is likely to contain a significant percentage of As^{III}: 1) concentrations of iron greater than 0.1 milligrams per liter (mg/L), 2) concentrations of manganese greater than 0.05 mg/L, 3) a negative oxidation reduction potential, or 4) a hydrogen sulfide odor.

Table 1-1: Common Arsenic Species

Arsenic Species	Name	Chemical Formula	Where Found
As^{III}	Arsenite	H_3AsO_3	Water
As^{V}	Arsenate	H_3AsO_4	Water
MMA^{III}	Monomethylarsonous Acid	$CH_3As(OH)_2[CH_3AsO]_n$	Metabolite
DMA^{III}	Dimethylarsinous Acid	$(CH_3)_2AsOH[((CH_3)_2As)_2O]$	Metabolite
MMA^{V}	Monomethylarsonate	$CH_3AsO(OH)_2$	Metabolite
DMA^{V}	Dimethylarsinate	$(CH_3)_2AsO(OH)$	Metabolite, Mushrooms
AsB	Arsenobetaine	$(CH_3)_3As^+CH_2COO^-$	Seafood & Fish
AsC	Arsenocholine	$(CH_3)_3As^+CH_2CH_2OH$	Seafood & Fish
Arsenosugars I-XV	Dimethylarsinoylribosides		Seaweed & Scallops

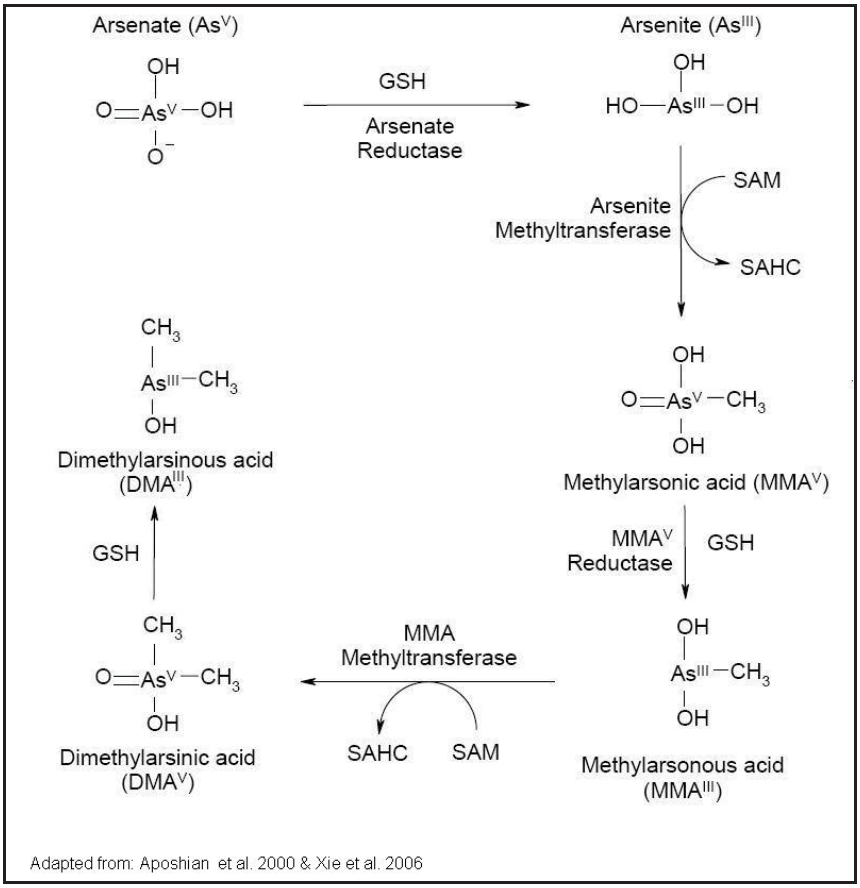
In food, a wide variety of arsenic species are found, including some inorganic arsenic (As^{III} and As^{V}) is present in food. In the United States, the average total inorganic arsenic intake from food, based on the US Food and Drug Administration (FDA) Total Diet Study, is estimated at 9 micrograms per day (µg/d) for adults, aged 25 and over, 5 µg/d for children aged 2-16 years, and 1.3 µg/d for children aged 6-11 months (National Research Council, 1999; Tao and Bolger, 1999).

Organic arsenic species are found at higher concentrations in food, especially in

seafood, fish, seaweed, and mushrooms. In these foods total arsenic is generally around 1 milligram per kilogram (mg/Kg) and this arsenic is generally composed of more than 90% organic species (Buchet et al., 1994; Tao and Bolger, 1999). Table 1-1 contains a list of common arsenic species. Arsenobetaine (AsB) and arsenocholine (AsC) are the organic arsenic species most common in fish and shellfish (National Research Council, 1999). Arsenosugars are the main form of arsenic in seaweed (kelp) and scallops (Le et al., 1994). Arsenic in mushrooms can be in a variety of species depending on the variety of mushroom. They can include As^{III}, As^{V}, AsB, AsC, monomethylarsenate (MMA^{V}), and dimethylarsinate (DMA^{V}) (National Research Council, 1999). Rice and poultry also contain significant concentrations of arsenic (Silbergeld, 2004; Tao and Bolger, 1999; Williams et al., 2005; Williams et al., 2007; Zavala and Duxbury, 2008; Zavala et al., 2008). Some nutritional supplements (i.e., herbal kelp) can also contain arsenic (Amster et al., 2007).

Each arsenic species is metabolized differently in the human body after ingestion. AsB and AsC are thought to pass through the human body very quickly and unchanged (Le et al., 1994; Vahter, 1994). As shown in Figure 1-3 and 4, As^{III} and As^{V} are metabolized in humans by reduction and methylation to MMA^{V}, MMA^{III}, DMA^{V}, and DMA^{III} (Aposhian et al., 2000a; Loffredo et al., 2003; Vahidnia et al., 2007; Vahter, 2002). Arsenosugars are metabolized mainly to DMA^{V}, but also to MMA^{V} and other yet unidentified arsenic species (Francesconi et al., 2002; Le et al., 1994; Le et al., 1996).

Figure 1-3: Inorganic Arsenic Biotransformation Pathway



GSH, Glutathione; SAM, S-adenosylmethionine;
SAHC, S-adenosylhomocysteine after: (Aposhian et al., 2000b; Xie et al., 2006)

The metabolism and toxicology of the various arsenic species are important factors when studying arsenic exposure via well water. Some level of food-source arsenic will always be present and may be significant. The species ingested is often not the same species present in body tissues or urine. Human metabolism of arsenic was thought to be a detoxification process (Goyer and Clarkson, 2001; National Research Council, 1999). However, recent studies are indicating that in many cases it may be a bio-activation process generating more toxic species of arsenic than what was ingested (Le et al., 2000; Petrick et al.,

2000; Petrick et al., 2001; Styblo et al., 2000; Wildfang et al., 2001). The toxicology of arsenic species varies greatly. MMA^{III} is now thought to be the most toxic species of arsenic related to water and food ingestion (National Research Council, 2001). MMA^{III} is followed in toxicity by As^{III}, As^{V}, DMA^{III}, MMA^{V}, DMA^{V}, AsB, AsC, and the arsenosugars (Kligerman et al., 2003; National Research Council, 2001).

During pregnancy, inorganic arsenic and it's methylated metabolites readily cross the placenta (Vahter, 2008).

The ingestion, metabolism, and toxicology of arsenic can be quite difficult to unravel in a biomonitoring study. A few examples will demonstrate the issues. Consider an individual found to have a very high level of total arsenic in their urine. Is this arsenic from exposure to arsenic in their water or is it from a recent seafood dinner? If the arsenic is from the water, it is likely to be in the more toxic inorganic or methylated forms in the urine. If the arsenic is from a seafood dinner, it may be in a low toxicity form like AsB or AsC. However, if the dinner included seaweed or scallops, the arsenic may be in the more toxic DMA species because the arsenosugars in seaweed or scallops are metabolized in humans to DMA (Le et al., 1994). Another plausible scenario is an individual with a high level of arsenic in their water but an effective treatment system to remove arsenic from drinking and cooking water. The arsenic species measured in urine can be non-specific. If their urine has a high level of arsenic and speciation shows that it

is in the DMA form, this could originate from the metabolism of As^{III} or As^{V} and be related to past water exposure body burden, a current water exposure other than drinking or cooking (bathing or brushing teeth), or it could be from a meal including seaweed, scallops, or mushrooms (Le et al., 1994; National Research Council, 1999).

Arsenic Water Treatment

Special water treatment systems can remove arsenic from drinking water, and can be configured to treat all the water in the home (point-of-entry) or just water at a single tap (point-of-use) for drinking and cooking (Spayd, 2007).

The goal of treating arsenic-contaminated water is to reduce arsenic levels in the water below the MCL and as close to the MCLG as possible and thus reduce the risk of cancer and the many other health problems associated with arsenic exposure. The NJDEP is conducting a study of the effectiveness of various arsenic water treatment systems. The NJDEP study is evaluating both whole-house water treatment systems, commonly referred to as point-of-entry (POE) treatment, and single faucet treatment options for treating only drinking and cooking water, commonly referred to as point-of-use (POU) treatment. This study has been very successful with most treatment systems reducing arsenic to levels below three µg/L, and many systems reducing the arsenic level to below one µg/L.

Arsenic Biomonitoring

Human exposure to arsenic in drinking water occurs mainly via ingestion (National Research Council, 1999; USEPA, 2001). However, secondary routes could arise from inhalation of aerosols during showering or cooking, and dermal absorption during showering, bathing, or brushing of teeth. The contribution of these secondary routes in household exposure is uncertain.

The NJDEP arsenic water treatment study afforded the opportunity to evaluate biological levels of arsenic in humans before and after reduction of arsenic exposure via drinking water treatment systems. Biomonitoring data for humans with exposure to chronic moderate levels of arsenic in their household water supply similar to those found in New Jersey, is greatly lacking in the published literature. Most human biomonitoring data for arsenic has been collected from subjects with acute arsenic exposure or very high chronic arsenic exposures.

POE water treatment for arsenic in a typical New Jersey home costs about $3,000 while POU treatment costs about $400 per POU tap (Spayd, 2007). Because the POE treatment costs about eight times more than a single POU treatment system, there is a need to determine if POU treatment for drinking and cooking water is sufficient, or if POE treatment is required to reduce overall water arsenic exposures to acceptable cancer risk levels.

When EPA issued the proposed arsenic MCL rule in June 2000, they noted that the Centers for Disease Control and Prevention (CDC) is initiating a study of arsenic intake from bathing, and asked for comment on "whether available data on skin absorption and inhalation indicate that these are significant exposure routes that should be considered in the risk assessment" (USEPA, 2000). The CDC study, which is taking place in Maine, has not yet been completed and therefore was not included in the risk assessment that EPA conducted in determination of the final MCL. In the final arsenic MCL rule, published in January 2001, EPA was not able to assess the inhalation or dermal pathway. At the time of adoption of the arsenic MCL rule, EPA stated that exposure by modes other than consumption were not a concern (USEPA, 2001). Hence, the final rule allows POU treatment as an acceptable technology for arsenic exposure reduction.

The 1999 National Academy of Sciences Report on arsenic in drinking water states that "no controlled studies have been conducted on the rate of absorption of inorganic arsenic through intact human skin" (National Research Council, 1999). There is a high likelihood that skin contact with waters containing arsenic above drinking water standards will result in some arsenic absorption. The highly keratinized epidermis provides ample sulfhydryl binding sites for arsenic. Rahman found up to 62% absorption of sodium arsenate when applying 100 µL of an aqueous solution containing arsenic concentrations as low as 50 µg/L to the skin (0.64 cm^2) of mice in vitro (flow through diffusion cell) for 24 hours

(Rahman et al., 1994). About half of the absorbed arsenic passed through the skin and half remained in the skin after 24 hours of exposure. They also found that absorption increased linearly with the applied dose with a constant fraction of the dose being absorbed (Rahman et al., 1994). Wester identified arsenic absorption through the skin of live monkeys, and demonstrated that up to 6.4% of an applied dose of sodium arsenate heptahydrate at 4.8 µg/L arsenic, applied at a rate of 5 µL/cm^2, was absorbed through 12 cm^2 of skin and excreted via urine after 24 hours of exposure (Wester et al., 1993). In a follow-up study, they found up to 4.4% of a much higher applied dose of sodium arsenate heptahydrate at 2860 mg/L arsenic, applied at a rate of 5 µL/cm^2, was absorbed through 100 cm^2 of skin and excreted via urine after eight hours of exposure (Wester et al., 2004). Another recent study using artificial skin found absorption of both As^V and As^{III} at up to 8% of the applied dose (10 µg/L to 1000 µg/L) per hour, applied at 1250 µL/cm^2 after 6 hours (Bernstam et al., 2002; Nriagu and Bernstam, 2004). They found that of the absorbed arsenic, about 30% of the As^V and 90% of As^{III} was being retained in the artificial human skin after the 6-hour exposure, and inferred from this that a higher percentage of As^V applied to the skin may reach the systemic circulation and internal organs, compared to As^{III} (Bernstam et al., 2002). Based on the above studies, humans with elevated arsenic in their well water and a chronic daily exposure via showering or bathing could potentially incur a significant arsenic exposure without POE treatment.

Though drinking and cooking with arsenic contaminated water is obviously the

main exposure pathway in the home, the lack of data on exposure to arsenic via a household water supply from uses other than drinking and cooking (e.g., bathing, brushing teeth, etc.) is a major data gap. The above studies indicate that a POU system is inadequate to block all routes of exposure to arsenic in water. There is no assurance that a POU treatment system for reduction of arsenic in drinking and cooking water is sufficient to reduce the user's overall drinking water arsenic exposure and intake dose to levels with a typically acceptable increased lifetime cancer risk of one in a million. Because even low levels of arsenic exposure and dose (e.g., < 0.1 µg/d) are estimated to result in unacceptable cancer risks, these other exposures (e.g., bathing, brushing teeth, etc.) may represent a significant risk when arsenic water concentrations are above some currently undetermined level. The Agency for Toxic Substances and Disease Registry (ATSDR) states that one should not shower or bath in water with arsenic above 500 ppb (ATSDR, 2007a).

Because the NJDEP study is evaluating the water treatment capabilities of both POE and POU systems, an opportunity was made available to evaluate and compare overall exposure reduction via the two types of treatment systems.

As discussed in the previous sections, arsenic exposure can result not only from arsenic in drinking water, but also from a variety of occupational and at-home sources, especially from eating certain foods high in arsenic. This fact complicates human biomonitoring studies as researchers and clinicians prefer to

know the source of the arsenic they detect in their subjects/patients urine, blood, hair, or nails.

Research Questions

The following chapters address these research questions:

Chapter 2:

- What are the effects of dietary arsenic and sampling protocol on arsenic concentrations in human urine and blood?
- What analytical methods and sampling protocols can be used to improve arsenic biomonitoring studies?
- What are appropriate reference ranges for arsenic in human urine and blood?

Chapter 3:

- Are available arsenic water treatment systems effective in reducing arsenic exposure from well water?
- What are the effects of arsenic water treatment on arsenic concentrations in human urine and blood?
- Is there an arsenic body burden developed during chronic exposure to arsenic in well water and what are the arsenic elimination rates (arsenic half-life in the body)?

Chapter 4:

- When treating water in a home to remove arsenic, should we treat all the water (POE treatment) or just drinking and cooking water (POU treatment)?

Chapter 5:

- What are the conclusions and recommendations resulting from this research?

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Chapter 2: Biomonitoring Exposure to Arsenic Contaminated Drinking Water

Abstract

BACKGROUND: Arsenic biomonitoring can be used to assess chronic exposure to arsenic contaminated drinking water. Several components of the human diet, especially fish and seafood, contain arsenic at significant concentrations and must be considered in arsenic biomonitoring sample collection and analytical method protocols.

OBJECTIVE: To assess the effects of dietary arsenic and sample collection protocol on arsenic concentrations in human urine and blood, describe useful analytical methods, and summarize existing data for urine and blood concentrations to propose appropriate reference ranges.

METHODS: An arsenic biomonitoring study was conducted with 53 subjects having elevated arsenic (8 - 119 µg/L) in their residential home well water. A series of urine samples were collected from the subjects, some starting before water treatment was installed. Several subjects provided additional urine samples while closely tracking intake of high arsenic foods. Urine samples were analyzed for total arsenic, inorganic-related arsenic, and other arsenic species. Blood samples were collected at the start and end of the water treatment study and analyzed for total arsenic. A literature review was carried out for available urine and blood arsenic data.

RESULTS: Dietary arsenic has the potential to be a major confounder in arsenic biomonitoring studies. In this study, on average, approximately 75 % of total arsenic in urine was attributed to fish and seafood in the diet. Appropriate analytical methods are described, such as hydride generation ICP-MS, which separates organic arsenic species found in fish and seafood from the sample, and quantifies the inorganic-related arsenic species. Reference ranges for arsenic in urine and blood are recommended.

CONCLUSIONS: The importance of designing arsenic biomonitoring sampling and analytical methodology protocols that account for the potential confounding of dietary arsenic is demonstrated, and reference ranges for inorganic-related arsenic in urine are proposed for studies conducted with these protocols. A sampling protocol requiring avoidance of high arsenic foods for one week prior to biomonitoring sample collection is proposed. The recommended reference range for inorganic-related arsenic in urine is < 8 µg/L and < 5 µg/g creatinine. For total arsenic in whole blood, the recommended reference range is < 2.5 µg/L.

KEY WORDS: arsenic, biomonitoring, blood arsenic, dietary arsenic, drinking water, exposure, hydride generation, New Jersey, reference range, urinary arsenic, urine, water treatment, well water.

Introduction

Arsenic is found in a variety of chemical forms in water, food, and living organisms. In well water in New Jersey, arsenic has been found to occur in two inorganic species (chemical form and/or oxidation state): arsenate (As^{V}) and arsenite (As^{III}) as shown in Table 1-1. The predominant species is As^{V}. The relative distribution of the inorganic arsenic species is important as it affects toxicity, analytical testing, and water treatment considerations. As^{III} is more toxic than As^{V}, but when As^{V} is ingested by humans, it is reduced to As^{III} in the first step of the arsenic biotransformation pathway (Figure 1-3). Significant concentrations of As^{III} are found in about 20% of the wells with total arsenic above 5 µg/L in New Jersey (Serfes et al., 2005; Spayd, 2007).

In food, a wide variety of arsenic species are found, including some inorganic arsenic (As^{III} and As^{V}). In the United States, the average total inorganic arsenic intake from food, based on the US Food and Drug Administration (FDA) Total Diet Study, is estimated at 9 micrograms per day (µg/d) for adults, aged 25 and over, 5 µg/d for children aged 2-16 years, and 1.3 µg/d for children aged 6-11 months (National Research Council, 1999; Tao and Bolger, 1999).

Arsenic in the diet can be a great confounder of arsenic biomonitoring data. Foods high in total arsenic, as determined by the FDA Total Diet Study, are shown in Table 2-1 (USFDA, 2007).

Table 2-1: Arsenic in Food per FDA Total Diet Study	
Food	**Total Arsenic Mean Concentration (mg/Kg)**
haddock, pan cooked	5.540
tuna, canned in oil	0.929
tuna, canned in water	0.878
fish sticks, frozen	0.736
shrimp, boiled	0.678
fish sandwich on bun	0.501
Salmon	0.469
clam chowder	0.141
crisped rice cereal	0.139
tuna noodle casserole	0.112
mushrooms, raw	0.081
white rice, cooked	0.071
rice, infant cereal	0.042
chicken, fried fast food	0.024

Organic arsenic species are found at higher concentrations in food, especially in seafood, fish, seaweed, and mushrooms. In these foods total arsenic is generally around 1 milligram per kilogram (mg/Kg) and this arsenic is generally composed of more than 90% organic species (Buchet et al., 1994; Tao and Bolger, 1999). Arsenobetaine (AsB) and Arsenocholine (AsC) are the organic arsenic species most common in fish and shellfish (National Research Council, 1999). Arsenosugars are the main form of arsenic in seaweed (kelp) and scallops (Le et al., 1994). Arsenic in mushrooms can be in a variety of species depending on the variety of mushroom. They can include As^{III}, As^{V}, AsB, AsC, Monomethylarsenate (MMA^{V}), and Dimethylarsinate (DMA^{V}) (National Research Council, 1999). Rice and poultry also contain significant concentrations of arsenic (Silbergeld, 2004; Tao and Bolger, 1999; Williams et al., 2005; Williams et al., 2007; Zavala and Duxbury, 2008; Zavala et al., 2008). Some nutritional supplements (i.e., herbal kelp) can also contain arsenic (Amster et al., 2007).

Each arsenic species is metabolized differently in the human body after ingestion. AsB and AsC are thought to pass through the human body very quickly and unchanged (Le et al., 1994; Vahter, 1994). As shown in Figure 1-3 and 4, As^{III} and As^{V} are metabolized in humans by reduction and methylation to MMA^{V}, MMA^{III}, DMA^{V}, and DMA^{III} (Aposhian et al., 2000; Loffredo et al., 2003; Vahidnia et al., 2007; Vahter, 2002). Arsenosugars are metabolized mainly to DMA^{V}, but also to MMA^{V} and other yet unidentified arsenic species (Francesconi et al., 2002; Le et al., 1994; Le et al., 1996).

The metabolism and toxicology of the various arsenic species are important factors when studying arsenic exposure via well water. Some level of food-source arsenic will always be present and may be significant. The species ingested is often not the same species present in body tissues or urine. Human metabolism of arsenic was thought to be a detoxification process (Goyer and Clarkson, 2001; National Research Council, 1999). However, recent studies are indicating that in many cases it may be a bio-activation process generating more toxic species of arsenic than what was ingested (Le et al., 2000; Petrick et al., 2000; Petrick et al., 2001; Styblo et al., 2000; Wildfang et al., 2001). The toxicology of arsenic species varies greatly. MMA^{III} is now thought to be the most toxic species of arsenic related to water and food ingestion (National Research Council, 2001). MMA^{III} is followed in toxicity by As^{III}, As^{V}, DMA^{III}, MMA^{V}, DMA^{V}, AsB, AsC, and the arsenosugars (Kligerman et al., 2003; National Research Council, 2001).

The ingestion, metabolism, and toxicology of arsenic can be quite difficult to unravel in a biomonitoring study. A few examples will demonstrate the issues. Consider an individual found to have a very high level of total arsenic in their urine. Is this arsenic from exposure to arsenic in their water or is it from a recent seafood dinner? If the arsenic is from the water, it is likely to be in the more toxic inorganic or methylated forms in the urine. If the arsenic is from a seafood dinner, it may be in a low toxicity form like AsB or AsC. However, if the dinner included seaweed or scallops, the arsenic may be in the more toxic DMA species because the arsenosugars in seaweed or scallops are metabolized in humans to DMA (Le et al., 1994). Another plausible scenario is an individual with a high level of arsenic in their water but an effective treatment system to remove arsenic from drinking and cooking water. The arsenic species measured in urine can be non-specific. If their urine has a high level of arsenic and speciation shows that it is in the DMA form, this could originate from the metabolism of As^{III} or As^{V} and be related to past water exposure body burden, a current water exposure other than drinking or cooking (bathing or brushing teeth), or it could be from a meal including seaweed, scallops, or mushrooms (Le et al., 1994; National Research Council, 1999).

Arsenic Water Treatment

Special water treatment systems can remove arsenic from drinking water (Spayd, 2007). The goal of treating arsenic-contaminated water is to reduce arsenic

levels in the water below the maximum contaminant level (MCL) and as close to the MCL Goal (0 µg/L) as possible and thus reduce the risk of cancer and the many other health problems associated with arsenic exposure. The NJDEP is conducting a study of the effectiveness of various arsenic water treatment systems. The NJDEP study has been very successful with most treatment systems reducing arsenic to levels below three µg/L, and many systems reducing the arsenic level to below one µg/L. The NJDEP arsenic water treatment study afforded the opportunity to evaluate biological levels of arsenic in humans before and after reduction of arsenic exposure via drinking water treatment systems and to evaluate the contribution of arsenic from diet.

Arsenic Biomonitoring

Arsenic biomonitoring is often conducted in cases of suspected acute arsenic poisoning related to attempted homicide or suicide, when arsenic is used as a chemotherapy agent, for measuring potential occupational exposures, and occasionally as a result of chronic exposure to arsenic contaminated drinking water in biomonitoring studies like the present one, or by a family physician when arsenic contaminated well water is suspected. Several components of the human diet contain arsenic at significant concentrations and must be considered in arsenic biomonitoring sample collection and analytical method protocols.

Biomonitoring data for humans with exposure to chronic moderate levels of arsenic in their household water supply similar to those found in New Jersey, is

greatly lacking in the published literature. Most human biomonitoring data for arsenic has been collected from subjects with acute arsenic exposure or very high chronic arsenic exposures. Only a limited number of representative population biomonitoring studies have included arsenic, such as the National Health and Nutrition Examination Survey (NHANES) conducted by the Centers for Disease Control (Caldwell et al., 2008) in the United States, and the German Environmental Survey conducted in 1998 (Wilhelm et al., 2004).

As discussed in Chapter 1, arsenic exposure can result not only from arsenic in drinking water, but also from a variety of occupational and at-home sources, especially from eating certain foods high in arsenic. This fact complicates human biomonitoring studies as researchers and clinicians need to know the source of the arsenic they detect in their subjects/patients urine or blood. Therefore, the protocol used for sample collection and analytical methodology is very important.

Arsenic Reference Ranges for Urine and Blood

An evaluation of arsenic biomonitoring data must include a comparison of the observed data to “normal levels”. These normal levels, also known as “Reference Range” or “Reference Interval” are typically determined by using the endpoints of the 95% confidence interval of a sufficiently large sample of appropriate (having the same age, sex, and ethnicity) healthy or non-exposed people (Marshall and Bangert, 2008). Similar sampling protocols and analytical methods must be used to provide the reference range data and the data that is to

be compared to the reference range (Marshall and Bangert, 2008). Because very few non-exposed people are tested for arsenic, and sampled populations and analytical methods vary widely, it is very difficult to calculate a typical reference range for arsenic. Rather than using a calculated reference range, many labs use a “text book” range. For example, LabCorp, a commercial laboratory, uses ranges of 0-50 µg/L for total arsenic in urine, < 20 µg/L for inorganic arsenic in urine (LabCorp, 2008b), and 2-23 µg/L for total arsenic in whole blood (LabCorp, 2008a). These ranges are reportedly based on a 1990 publication (Tietz, 1990) which was based on (Iyengar and Woittiez, 1988) who gathered older data from all over the world and likely included many people who were exposed to both drinking water and dietary sources of arsenic. LabCorp also uses < 35 µg/g creatinine of inorganic arsenic plus methylated metabolites in urine, for end of week occupational exposure based on the recommendations of the American Conference of Governmental Industrial Hygienists (ACGIH), Biological Exposure Indices (ACGIH, 2001; LabCorp, 2008b). Quest Diagnostics, another commercial laboratory, uses ≤ 80 µg/L total arsenic in urine for their reference range. Table 2-2 summarizes these commercial lab reference values. Due to the currently large variation in published reference range values for arsenic in urine and blood, there is a need to look at the available data that may contribute to more appropriate reference ranges.

Table 2-2: Selected Commercial Laboratory Reference Ranges				
Lab/Arsenic Type	**Total As Urine**	**Inorganic As Urine**	**Inorganic As Urine –Occupational**	**Total As Blood**
LabCorp	0-50 µg/L	< 20 µg/L	< 35 µg/g creatinine	2-23 µg/L
Quest	< 80 µg/L			

Materials and Methods

Selection of Wells and Subjects

As the NJDEP study of arsenic in ground water and the effectiveness of various arsenic water treatment systems proceeded, owners of wells with elevated arsenic concentrations were asked to participate in the present study of arsenic water treatment and biomonitoring. Fifty three subjects, in 22 families, with elevated arsenic concentrations (8 - 119 µg/L) in their residential well water were recruited between August 2002 and September 2004. Five control subjects with drinking water arsenic concentrations below 3 µg/L, which is below the New Jersey MCL of 5 µg/L, also participated.

Recruitment and study procedures were reviewed and approved by the Institutional Review Board of the University of Medicine and Dentistry of New Jersey. Participation was voluntary and written informed consent was obtained. There were no financial costs to subjects and no payments or compensation for participating. Subjects could refuse to participate, or discontinue participation at any time during the project. Only subjects between the ages of 6 months and 75 years were asked to participate. Children under 17 years of age had consent

provided by their parent or legal guardian. Children between the ages of 12 and 17 years also had a special assent form that explained the project in language appropriate to their age and allowed them to refuse to participate even if their parents wanted them to be in the study. A 100% participation rate was achieved as all families who were asked to participate in the study agreed to participate.

Water Treatment Monitoring and Analysis

Throughout the project, arsenic levels were regularly measured in both the raw water entering the home from the well and the treated water. Water samples were routinely collected by NJDEP, at approximately the same schedule as urine sample collection. These water samples were analyzed by an NJDEP lab certified to analyze drinking water for arsenic via EPA Method 200.8 - Inductively Coupled Plasma – Mass Spectrometry (ICP-MS). Water samples were occasionally split from NJDEP as a quality control procedure and analyzed by ICP-MS at the Environmental and Occupational Health Sciences Institute (EOHSI) Chemical Analysis laboratory in Piscataway, New Jersey.

Biomonitoring Protocol

The goal was to collect an initial urine and blood sample before subjects obtained water treatment or stopped drinking the water with elevated arsenic, or as close to this date as possible. During the NJDEP arsenic in well water study, when arsenic concentrations above the MCL were identified in a given well, the well

owners were notified by telephone. Upon notification of elevated arsenic levels in home well water, many subjects stopped using the water for drinking and cooking before they were enrolled in the biomonitoring study and collection of their initial urine and blood samples could begin. As a result, only 24 of the subjects were able to provide samples that allowed us to measure urine arsenic levels while they were still drinking and cooking with the arsenic-contaminated water. In addition, some subjects chose not to provide an initial or final blood sample and this resulted in only 13 of the subjects providing both an initial and final blood sample.

After initiation of water treatment, subsequent samples were collected to determine if any change of the arsenic level in the urine or blood occurred. The planned biomonitoring schedule included urine samples on days 3, 7, 14, 30, 60, 90, 120, and 180 after installation of water treatment, and a final blood sample at 180 days. Due to scheduling difficulties, some families were unable to comply with the exact planned biomonitoring schedule. As a result, urine sample results have been grouped into time periods. All time periods are based on the number of days after the subject ceased to drink the arsenic contaminated water or had an arsenic water treatment system installed in the home, whichever occurred first. Some of the time periods are longer than planned because many families stopped drinking the arsenic contaminated water before the biomonitoring was initiated. The time periods are 0, 1-14, 15-30, 31-90, 91-140, 141-395, and 396-897 days. Time periods are identified by the median number of days for each

period (0, 7, 23, 61, 116, 268, and 647). If more than one sample was collected for a given subject within a time period, the sample results were averaged.

There were no mandated restrictions on diet during this study, but subjects were asked to try and avoid high arsenic foods, such as fish and seafood for four days before any scheduled sample collection date.

Sample Collection

Urine samples were collected as first morning voids in sterile 4.5 ounce, graduated, wide mouth, specimen containers with polyethylene screw caps (Kendall Health Care Products). Samples were refrigerated at the subjects' home until picked up by the researcher, typically on the same day as the collection. Blood samples were collected at the subjects' homes by a nurse or physician. Blood samples consisted of two tubes totaling approximately 20 ml for adults and 15 ml for children. One tube (Becton Dickinson - Green Top), containing sodium heparin to prevent clotting, was collected for whole blood analysis. The other tube (Becton Dickinson – Red-Gray Top), containing silica clot activator and polymer gel, was centrifuged with a serum separator at 3210 RPM for 10 minutes and the serum was manually removed from the tube with a pipette. Both serum and red blood cells were saved for analysis. Urine and blood samples were transported in coolers to EOHSI and stored at 4-degrees Celsius until analysis.

Three subjects provided additional urine samples on a more frequent schedule over a time course while keeping track of their dietary intake of high arsenic foods. These subjects were evaluated for elevations in total urine arsenic concentration related to dietary intake.

Questionnaire

Based on in-person interviews at the time of subject enrollment, a household water use and exposure history questionnaire was completed for each subject by the investigator (the questionnaire is included in Appendix 1). Determining how much arsenic contaminated water was consumed per day, how many years each subject was exposed to arsenic in drinking water, and knowing the arsenic concentration of the water was sufficient background data to estimate each subject's cumulative arsenic ingestion dose from drinking and cooking with the water prior to obtaining arsenic water treatment. The cumulative arsenic ingestion dose in milligrams (mg) was calculated by multiplying the arsenic concentration of the well water in mg/L (shown by repeated measurements to be fairly constant) by daily amount of drinking water from the home water supply in l/d (assumed to be constant), by the years of exposure to the home water supply, and by 365 d/year. An ingestion dose per body weight in mg/Kg was then calculated by dividing the cumulative arsenic ingestion dose by the weight of the subject (self reported). Information on the subject's bathing and dietary habits were also tabulated on the questionnaire as they may affect arsenic biomonitoring results. The questionnaire relied on the subject's recall, which has

its limitations, but subject recall is better than estimating a value based on population averages.

For diet, the questionnaire asked how many times per week the subject had a meal including shrimp, lobster, clams, oysters, tuna, other seafood or fish, mushrooms, rice, chicken, or turkey. The questionnaire relied on the subject's recall, which has its limitations.

Laboratory Analysis

All biomonitoring samples were analyzed at the EOHSI Chemical Analysis laboratory.

Total Arsenic in Urine

Urine samples were analyzed at the EOHSI Chemical Analysis laboratory. Total arsenic analysis was performed on an ICP-MS (Thermo Fischer Scientific X5). Urine samples were prepared for analysis by pipetting a 1 ml aliquot from the middle of the sample collection container after lightly swirling the container. The 1 ml urine aliquot was diluted 10:1 with 8.8 ml of 18.2 mega-ohm deionized water (MilliQ ultra pure deionized, Millipore Corp) and 0.2 ml of Ultra Pure nitric acid (EMD Omni Trace Ultra, VWR). Initial calibration standards were prepared using multi element standards (High Purity Standards, Charleston, SC) in 18.2 mega-ohm deionized water with 2% nitric acid. Quality control (QC) samples were analyzed every 10 samples to verify continued calibration. A National Institute of

Standards and Technology (NIST) standard, NIST SRM 2670a (Toxic Elements in Urine, National Institute of Standards and Technology, Gaithersberg, MD, USA) was used as a QC sample. SRM 2670a was reconstituted by adding 18.2 mega-ohm deionized water (MilliQ ultra pure deionized, Millipore Corp) according to NIST instructions and stored at 4 degrees Celsius in the dark until used. If the arsenic concentrations for the QC samples were not within 20%, the data were rejected and the samples re-analyzed. Total urine arsenic concentrations were determined using this method.

The measurement of arsenic via ICP-MS depends on detection of its ion, which has a mono-isotopic mass-to-charge ratio (m/z) of 75. Because chloride in samples combines with argon in the plasma gas to create argon chloride ($^{40}Ar^{35}Cl$), there is a potential isobaric interference from the polyatomic ion of argon chloride, which has the same m/z of 75 (Amarasiriwardena et al., 1998). This would result in inaccurate results unless a correction is applied. The Thermo Elemental Plasma Lab software was set up to apply a mathematical correction for argon chloride interference by measuring the signals at m/z 75, 77, 82, and 83. Based on the natural isotopic abundance of chloride, for a given amount of $^{40}ArCl$ formed, 75% should be $^{40}Ar^{35}Cl$, and 25% should be $^{40}Ar^{37}Cl$. The signal at m/z 75 from $^{40}Ar^{35}Cl$ can be estimated from the signal at m/z 77 due to $^{40}Ar^{37}Cl$. Many of the urine samples also contained Se which has a stable isotope at m/z 77 which will interfere with the signal for $^{40}Ar^{37}Cl$. Therefore a similar procedure, using the natural abundances of Se isotopes at m/z 77 and 82

was also used. Finally, the argon gas may contain some krypton which has an isotope at m/z 82, and which can be corrected using the natural abundances of Kr isotopes at m/z 82 and 83.

Urinary Creatinine

Urine arsenic concentrations were adjusted for hydration using urinary creatinine as is typical in urinary biomonitoring studies (Barr et al., 2005). Creatinine measurements were conducted using a standard assay kit (Sigma Chemicals, St Louis, MO, USA). Briefly, for each sample, 20 µl of urine was diluted approximately 50-fold with 18.2 mega-ohm deionized water (MilliQ ultra pure deionized, Millipore Corp). A 0.5 ml aliquot of each diluted urine sample was transferred into 5 ml cuvettes. Then, 3 ml of alkaline picrate solution (Sigma-Aldrich; picric acid, approximately 0.6%, sodium borate and surfactant) was added to each cuvette. The cuvettes were covered with lids, mixed well, and allowed to react for 10 to 12minutes. The cuvettes were inserted into the autosampler of a spectrophotometer (Genesys 10; Thermo Fisher Scientific Inc., Waltham, MA, USA) set at a wavelength of 500 nm (Han et al., 2008). The absorbance of each sample was recorded and the creatinine concentrations were calculated in g/L using standard calibration curves made with standards provided with the kit. Urinary arsenic measurements are presented as micrograms arsenic per gram of creatinine (µg/g creatinine).

Inorganic-Related Arsenic in Urine

In addition to total arsenic, a speciation technique was employed to determine the sum of six inorganic-related arsenic species. The details of this method were previously described (Xie et al., 2007). The technique employed was Hydride Generation ICP-MS (HG-ICP-MS). The goal of this method is to separate the inorganic arsenic species (see Table 1-1) found in water (As^{III} and As^{V}) and their metabolites (MMA^{V}, MMA^{III}, DMA^{V}, and DMA^{III}) from arsenic species commonly found in seafood and fish (AsB, AsC, and arsenosugars). Briefly, in HG-ICP-MS, samples are first reduced with concentrated HCl and L-cysteine, and then mixed with a sodium tetraborohydride ($Na(BH)_4$) solution in sodium hydroxide (NaOH). In this method, As^{V}, As^{III}, MMA^{V}, MMA^{III}, DMA^{V}, and DMA^{III} are all converted with very high efficiency to arsenic hydrides, and are separated from the sample matrix solution in a gas-liquid separator. Theoretically, any AsB, AsC, or arsenosugars in the sample will not be converted to hydrides, or are converted with very low efficiency (< 5%) (Xie et al., 2007), and will remain in the sample solution and go to waste while the gas with the arsenic hydrides goes on to the ICP-MS torch and the detector.

Employing this method in combination with a total arsenic analysis allows quantification of the total arsenic in the sample; quantification of the total of As^{V} + As^{III} + MMA^{III} + MMA^{V} + DMA^{V} + DMA^{III} in the sample, which should closely represent the total arsenic from water exposure and its resulting metabolites

(inorganic-related arsenic); and then quantification of the total of AsB + AsC + arsenosugars by subtraction (total arsenic minus inorganic-related arsenic), which should represent the total "seafood/fish" arsenic in the sample to a first approximation. Urine samples (Control Number S0506) for quality control purposes, with known total arsenic (306 µg/L) and "non-dietary" (inorganic-related) arsenic (8.2 µg/L) concentrations, were obtained from the Institut de Sante Publique du Quebec, Interlaboratory Comparison Program (Quebec, Canada). QC samples were analyzed every 10 samples to verify continued calibration. If QC samples were not within 20%, the data were rejected and the samples re-analyzed.

Arsenic Speciation by IC-ICP-MS

Individual arsenic species may also be quantified by separating the species via ion chromatography and then analyzing each species by ICP-MS (IC-ICP-MS). A subset of urine samples in this study were analyzed by this method for comparison purposes (Xie et al., 2006). Briefly, As^{V}, As^{III}, MMA^{V}, and DMA^{V} carry negative charges at high pH, and were separated by an anion exchange column from other arsenic species in urine, while MMA^{III}, DMA^{III}, and AsB, which carry positive charges at low pH, were separated by a cation exchange column. The separated arsenic species were detected by ICP-MS with a sub µg/L detection level. The method was partially validated by analyzing standard reference material NIST SRM 2670a and a spiked urine sample. The concentration of the arsenic species determined with this method were close to

those reported in the reference, and the sum of all species was in agreement with the reference value given by NIST for total arsenic.

Total Arsenic in Blood

Whole blood samples were analyzed at EOHSI for total arsenic by ICP-MS using the standard addition method. Standard addition was used to avoid matrix effect problems. For each blood sample, four analysis tubes were prepared by mixing the blood samples with a vortex mixer, pipetting and weighing approximately 0.2 grams (g) of blood into 7-ml Teflon tubes. Each Teflon tube was prepared for microwave digestion by adding 0.2 ml of 18.2 mega-ohm deionized water (MilliQ ultra pure deionized, Millipore Corp) and 0.25 ml of Ultra Pure nitric acid (EMD Omni Trace Ultra, VWR). The first tube had no arsenic added to it. The other three tubes had increasingly higher arsenic concentrations added to them. Based on the final dilution, equivalent spiked concentrations of 4, 8, and 12 µg/L were added. Samples were digested using the microwave method shown in Table 2-3. After digestion, samples were cooled at 4-degrees Celsius for 30 minutes, and diluted to 5 ml, approximately a 25:1 final dilution factor. Samples were vortexed for 20 seconds before being placed in the sample rack. Whole blood samples for quality control purposes, with known total arsenic concentrations, were obtained from the Institut de Sante Publique du Quebec, Interlaboratory Comparison Program (Quebec, Canada).

Table 2-3: Microwave Digestion Protocol for Blood Analysis		
Step	**Time (Minutes)**	**Power (Watts)**
1	5	360
2	5	240
3	5	300
4	5	360
5	5	0
6	10	420
7	10	480

Whole blood samples were only analyzed for total arsenic rather than inorganic-related arsenic. Efforts were made to overcome this limitation, but for whole blood samples the combination of matrix effect problems, digestion methods, and equipment limitations precluded the separation of inorganic-related arsenic and organic arsenic from consumption of seafood/fish.

Reference Range Literature Review

To determine appropriate reference ranges for arsenic in human urine and blood a literature review was conducted of large scale regional biomonitoring studies of representative populations such as the National Health and Nutrition Examination Survey (NHANES) conducted by the Centers for Disease Control (Caldwell et al., 2008), as well as biomonitoring studies where control groups, with little or no arsenic exposure via drinking water or diet, were tested along with those exposed to arsenic.

Data Analysis

Geometric means, percentiles, Pearson correlations, and T- Tests were calculated using SPSS 15.0 for Windows.

Results

Wells and Subjects

Characteristics of the study population are presented in Table 2-4. Fifty three subjects, within 22 families, with elevated arsenic concentrations in their residential well water were recruited. A total of four subjects were lost to follow-up or provided insufficient samples to be included in the analyses. Therefore, sufficient data was collected on 49 subjects, in 19 families (Exposed Group), to be included in at least part of the analysis. Five control subjects, from five different families, who were required to have home water arsenic concentrations less than 5 µg/L to participate as a control subject, were also recruited as the Control Group.

A subset of the Exposed Group, called the "Pre-Post Group" was established for data analyses and includes subjects who provided both pre-treatment and post-treatment biomonitoring samples. To be included in the Pre-Post Group, the pre-treatment urine samples had to be collected before obtaining water treatment or ceasing to drink the water with elevated arsenic, and a Nine-Month post-treatment urine sample had to be collected. As shown in Table 2-4, the Pre-Post Group includes 24 subjects (49%) of the Exposed Group who met the criteria for urine analyses.

Table 2-4: Characteristics of Study Subjects

Subject Groups	Exposed	Pre-Post[a]	Control
General			
Number of Subjects Per Water Treatment Group	49	24	5
Race (% Caucasian)	94	100	80
Sex (% Male)	49	58	100
Age in Years, (Mean ± SE)	37.4 ± 3.1[†]	41.3 ± 4.6	54.3 ± 4.2
Children < 18 Years Old (%)	31	25	0
Weight in Kg, (Mean ± SE)	60 ± 3.6	62 ± 5.5	83 ± 7.2
Any Tobacco Use During Study (%)	2	4	0
Prior Water Ingestion Exposure (Mean ± SE)			
Well Water As (µg/L)	45 ± 4.5[†]	42 ± 6.0[†]	1.1 ± 0.5
Home Water Ingestion Reported (L/d)	1.0 ± 0.1	1.0 ± 0.2	1.3 ± 0.6
Years of Exposure	11.2 ± 1.6	13.5 ± 2.9	7.0 ± 4.9
Cumulative As Ingestion Dose (mg)	165 ± 33[†]	204 ± 58[†]	3 ± 2
Ingestion Dose per Body Weight (mg/Kg)	2.7 ± 0.5[†]	3.2 ± 0.9[†]	0.05 ± 0.03
Dermal Exposure (Mean ± SE)			
Showers per Week at Home	5.2 ± 0.4	5.5 ± 0.5	6.6 ± 0.5
Baths per Week at Home	0.6 ± 0.2[†]	0.5 ± 0.2	0 ± 0
Teeth Brushing per Week at Home	11.4 ± 0.7[†]	11.9 ± 0.9[†]	14.4 ± 0.4
Pool Use per Week During Season	0.3 ± 0.1	0.4 ± 0.2	1.9 ± 1.4
Dietary Exposure (Mean ± SE)			
Seafood and Fish Meals per Week	1.4 ± 0.2	1.3 ± 0.2	1.4 ± 0.4
Mushrooms with Meals per Week	0.5 ± 0.1	0.5 ± 0.2	0.3 ± 0.1
Rice with Meals per Week	1.4 ± 0.1	1.2 ± 0.2	2.0 ± 1.3
Poultry with Meals per Week	2.7 ± 0.2	2.3 ± 0.3	2.8 ± 0.6
Urine Biomonitoring (Mean ± SE)			
Creatinine (g/L)	1.5 ± 0.1	1.6 ± 0.1	1.8 ± 0.2

[a] Subset of exposed subjects still drinking arsenic contaminated water at time of pre-treatment urine and/or blood sample collection and providing a Nine-Month post treatment sample.

[†] $p < 0.05$, significant difference from Control Group by Independent Sample T-Test.

Most subjects (92%) were Caucasian, three were African American, and one was Asian. Subjects in the Exposed and Pre-Post Groups were almost evenly divided between male and female, but all five of the control subjects were male. The mean ± standard error (SE) age of the Control Group (54.3 ± 4.2 years) was significantly ($p = 0.010$) higher than the Exposed Group (37.4 ± 3.1 years), but not significantly different than the Pre-Post Group (41.3 ± 4.6 years). Children, under 18 years old, made up 31% of the Exposed Group and 25% of the Pre-Post Group. No children were in the Control Group. The mean weight of the

Exposed Group at 60 ± 3.6 Kilograms (Kg) and the Pre-Post Group at 62 ± 5.5 Kg was lower than in the Control Group. Due to the relatively high percentage of children in the Exposed and Pre-Post Groups, and the fact that the Control Group was made up of only adult males, the Control Group was older and heavier (83 ± 7.2 Kg). There was only one smoker in the overall study population, and this person was one of the cases.

The mean untreated water arsenic concentration was 45 ± 4.5 µg/L for the Exposed Group and 42 ± 6.0 µg/L for the Pre-Post Group, and were not significantly different from each other. All subjects in the Exposed and Pre-Post Groups had water arsenic concentrations exceeding the New Jersey arsenic drinking water standard of 5 µg/L, and 94% of these subject's water arsenic levels exceeded the USEPA federal standard and the World Health Organization guideline level of 10 µg/L. All of the control subjects had water arsenic concentrations less than 3 µg/L which was significantly lower than the Exposed and Pre-Post Groups ($p < 0.0005$). The mean rate of drinking water at home was 1.0 ± 0.1 liter per day (L/d) for the Exposed Group and 1.0 ± 0.2 for the Pre-Post Group and 1.3 ± 0.6 L/d for the Control Group. The mean years of exposure to the home water supply were 11.2 ± 1.6 and 13.5 ± 2.9 years in the Exposed and Pre-Post Groups. The cumulative arsenic ingestion dose was significantly greater in the Exposed Group (165 ± 33 mg) and Pre-Post Group (204 ± 58 mg) than in the Control Group (3 ± 2 mg) with respective p-values of < 0.0005 and 0.002. The cumulative arsenic ingestion dose per body weight was significantly

greater in both the Exposed (2.7 ± 0.5 mg/Kg) and the Pre-Post Groups (3.2 ± 0.9 mg/Kg) than in the Control Group (0.05 ± 0.03 mg/Kg) with respective p-values of < 0.0005 and 0.001.

Water Treatment Effectiveness

All of the arsenic water treatment systems in the study, except one POE system, consistently and effectively reduced the arsenic concentrations in water to below 3 µg/L. At one home, the arsenic water treatment system had a temporary arsenic breakthrough during the biomonitoring program that was identified by the NJDEP water treatment system monitoring program. The homeowners were notified by NJDEP to switch to bottled water until the problem with the treatment system was corrected.

Potential Dermal Exposure Pathways

The mean rate of showering at home for the Exposed Group (5.2 ± 0.4 showers per week) and the Pre-Post Group (5.5 ± 0.5 showers per week) was not significantly different from the Control Group at 6.6 ± 0.5 showers per week. The mean number of baths was 0.6 ± 0.2 per week for the Exposed Group and 0.5 ± 0.2 per week for the Pre-Post Group. The Control Group did not take baths and the Exposed Group had a significantly higher number of baths ($p = 0.003$) than the Control Group. The mean rate of teeth brushing at home was 11.4 ± 0.7 times per week for the Exposed Group, 11.9 ± 0.9 times per week for the Pre-

Post Group, and 14.4 ± 0.4 times per week for the Control Group, and the Exposed and Pre-Post Groups had significantly less teeth brushing than the Control Group with respective p-values of 0.001 and 0.017. Some homes had swimming pools and/or hot tubs and the mean use of the pool and/or hot tubs during the swimming season was 0.3 ± 0.1 days per week for the Exposed Group and 0.4 ± 0.2 days per week for the Pre-Post Group, and 1.9 ± 1.4 days per week for the Control Group.

Dietary Arsenic Exposure

Fish, seafood, mushrooms, poultry, and rice were frequently consumed by the participants and these foods are thought to contribute more arsenic to the diet than other foods. The mean meals per week with seafood or fish were 1.4 ± 0.2 meals per week for the Exposed Group, 1.3 ± 0.2 meals per week for the Pre-Post Groups, and 1.4 ± 0.4 meals per week for the Control Group. The mean meals per week with mushrooms were 0.5 ± 0.1 meals per week for the Exposed Group, 0.5 ± 0.2 for the Pre-Post Group, and 0.3 ± 0.1 meals per week for the Control Group. The mean meals per week with rice was 1.4 ± 0.1 meals per week for the Exposed Group, 1.2 ± 0.2 meals per week for the Pre-Post Group, and 2.0 ± 1.3 meals per week for the Control Group. The mean meals per week with poultry were 2.7 ± 0.2 meals per week for the Exposed Group, 2.3 ± 0.3 meals per week for the Pre-Post Group, and 2.8 ± 0.6 meals per week for the Control Group. There were no significant differences between the three groups for these dietary exposures.

Creatinine

The urine arsenic results were corrected for creatinine and are presented as µg/g creatinine. Many of the creatinine results in this study had a correction applied to them because samples in storage can lose creatinine over time (Schneider et al., 2002; Schober et al., 2002). Whether or not samples are frozen or stored at 4-degrees Celsius, creatinine losses of up to 24% were seen within two weeks to four months of storage (Schneider et al., 2002; Schober et al., 2002). Other studies, have shown creatinine to be stable in storage at 4-degress Celsius for up to four weeks (d'Eril et al., 1994; Spierto et al., 1997). Many of the samples in the present study were in storage more than a year before the creatinine analysis was performed. However, early in the study, portions of 18 samples were sent to a commercial laboratory for urinary creatinine analysis while the remainder of the sample was placed into storage at 4-degrees Celsius. Portions of six of these samples were also frozen at this time. After five years in storage, the urinary creatinine was reanalyzed on these samples and correlated with the original results. A significant loss in creatinine was seen. The slope and R^2 values for the trend lines of the refrigerated samples were 1.37 and 0.70 and for the frozen samples were 1.20 and 0.99. These correlations compare favorably with the urinary creatinine losses seen by the Schneider and Schober studies. The equations for the trend lines were used to correct urinary creatinine concentrations for samples in this study that were stored for more than three weeks before creatinine analysis.

The mean creatinine levels were highest in the Control Group (1.8 ± 0.2 g/L), but not significantly higher than the Exposed Group (1.5 ± 0.1 g/L) or the Pre-Post Group (1.6 ± 0.1 g/L).

Total and Inorganic-Related Arsenic Levels in Urine

Table 2-5 provides the mean total arsenic and the inorganic-related arsenic in urine for the Pre-Post Group and the Control Group at seven sampling time periods, based on the median number of days in each time period after the subject ceased to drink the arsenic contaminated water or had an arsenic water treatment system installed in the home. Figure 2-1 shows the data graphically, and the rapid drop in the concentration of inorganic-related arsenic in urine during the first week following the time when subjects stopped drinking the contaminated water is evident. Arsenic body burden and clearance from the human body after cessation of chronic exposure via well water will be discussed in Chapter 3.

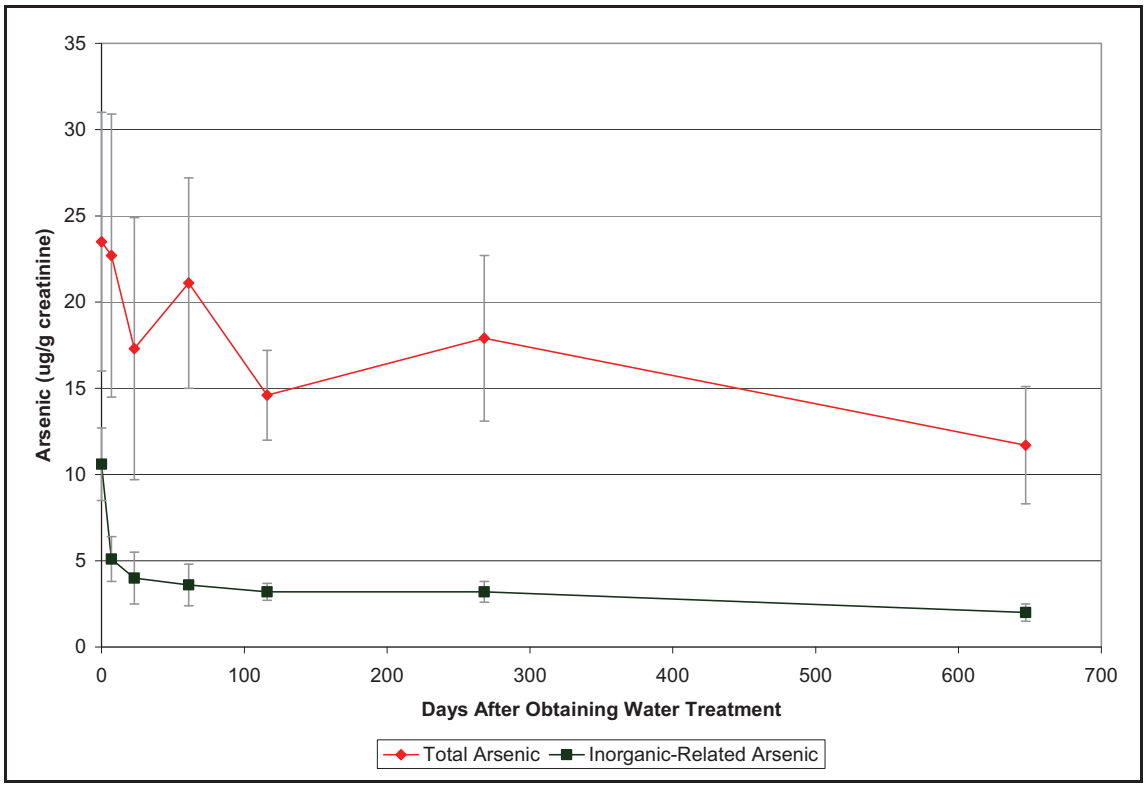
Table 2-5: Total and Inorganic-Related Arsenic in Urine of Pre-Post Group [a]

		Days After Obtaining Water Treatment [b]						
Treatment Group	**Analysis**	**0**	**7**	**23**	**61**	**116**	**268**	**647**
Pre-Post Group	Total	23.5 ± 7.5	22.7 ± 8.2	17.3 ± 7.6	21.1 ± 6.1	14.6 ± 2.6	17.9 ± 4.8	11.7 ± 3.4
	Inorganic [c]	10.6 ± 2.1	5.1 ± 1.3	4.0 ± 1.5	3.6 ± 1.2	3.2 ± 0.5	3.2 ± 0.6	2.0 ± 0.5
Control Group [d]	Total	13.8 ± 2.8						
	Inorganic [c]	1.5 ± 0.4						

[a] Results are geometric mean ± SE (µg/g creatinine).
[b] Time periods are identified by the median number of days, for each period, after the subject ceased to drink the arsenic contaminated water or had an arsenic water treatment system installed in the home. If more than one sample was collected for a given subject within a time period, the sample results were averaged.
[c] Inorganic-related arsenic.
[d] The Control Subjects only provided one urine sample each.

Figure 2-1: Total and Inorganic-Related Arsenic in Urine of Pre-Post Group

Geometric Means +/- SE Error Bars for the Group at the Median of Each Time Period



Correlation results using the Pre-Post and Control Groups (total n = 29) found good correlation for initial total arsenic in urine with concentration of arsenic in well water (R^2 = 0.183, p = 0.020) as shown in Figure 2-2a. With the three high urinary total arsenic outliers removed, the correlation was stronger (R^2 = 0.336, p = 0.002). However, a significant correlation with the initial total arsenic in urine was also seen with the number of seafood/fish meals per week (R^2 = 0.419, p < 0.0005) as shown in Figure 2-3a. This correlation also remained significant with the three high urinary total arsenic outliers removed (R^2 = 0.259, p = 0.008) as shown in Figure 2-3b.

The concentration of arsenic in well water correlated with the initial inorganic-related arsenic in urine (R^2 = 0.224, p = 0.010) as seen in Figure 2-4a, and with the one high urinary inorganic-related arsenic outlier removed (R^2 = 0.292, p = 0.003), as shown in Figure 2-4b. However, the number of seafood/fish meals per week did not correlate with the initial inorganic-related arsenic in urine as shown in Figure 2-5a and Figure 2-5b.

The concentration of inorganic-related arsenic in urine correlated with total arsenic in urine (R^2 = 0.281, p = 0.003) as shown in Figure 2-6a, and a similar correlation with the three high urinary total arsenic outliers removed (R^2 = 0.285, p = 0.005) is shown in Figure 2-6b.

Figure 2-2a: Total Arsenic in Water vs. Total Arsenic in Urine

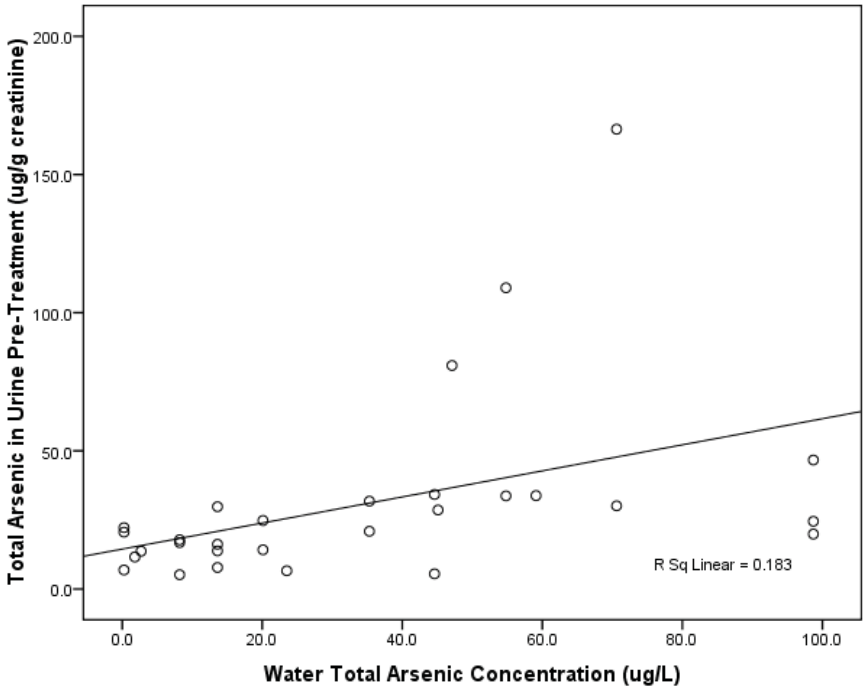


Figure 2-2b: Total Arsenic in Water vs. Total Arsenic in Urine Without Three High Urine Arsenic Outliers

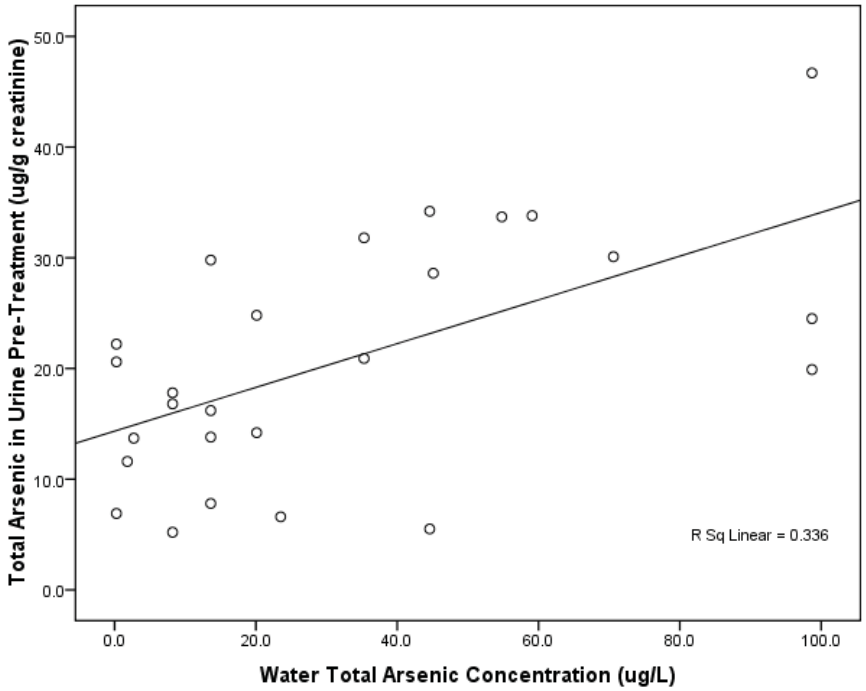


Figure 2-3a: Fish/Seafood Meals per Week vs. Total Arsenic in Urine

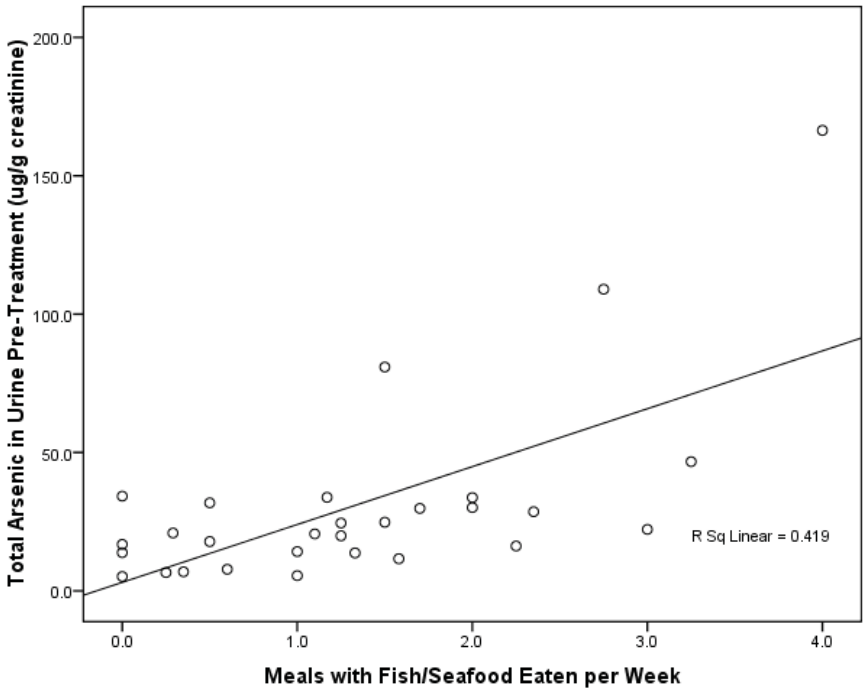


Figure 2-3b: Fish/Seafood Meals per Week vs. Total Arsenic in Urine Without Three High Urine Arsenic Outliers

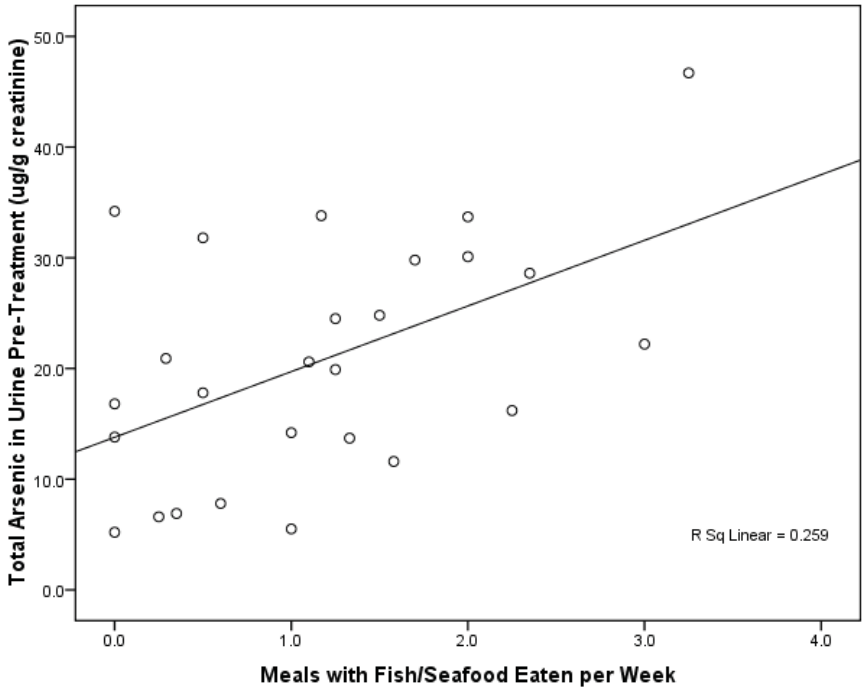


Figure 2-4a: Total Arsenic in Water vs. Inorganic-Related Arsenic in Urine

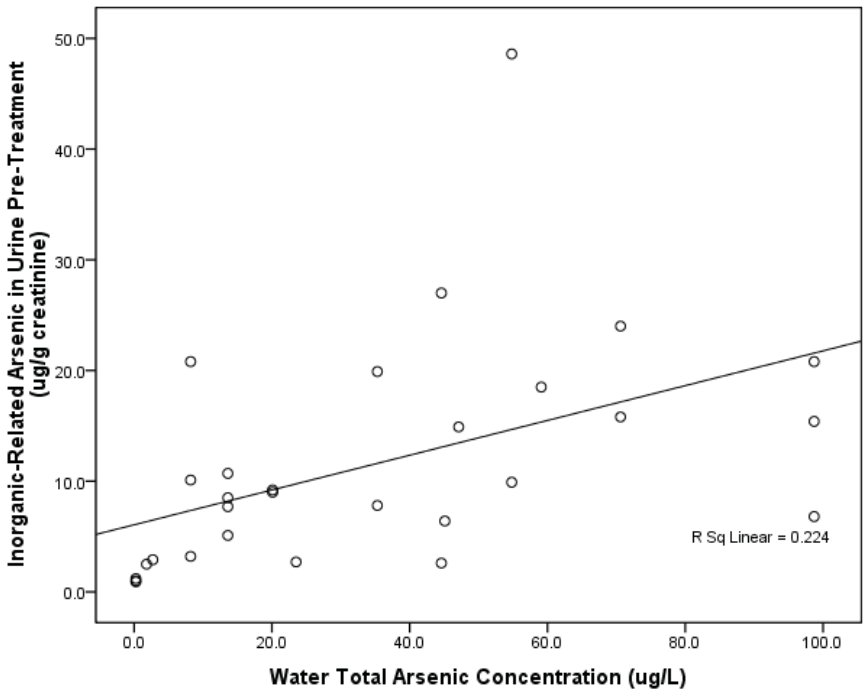


Figure 2-4b: Total Arsenic in Water vs. Inorganic-Related Arsenic in Urine Without One High Urine Inorganic-Related Arsenic Outlier

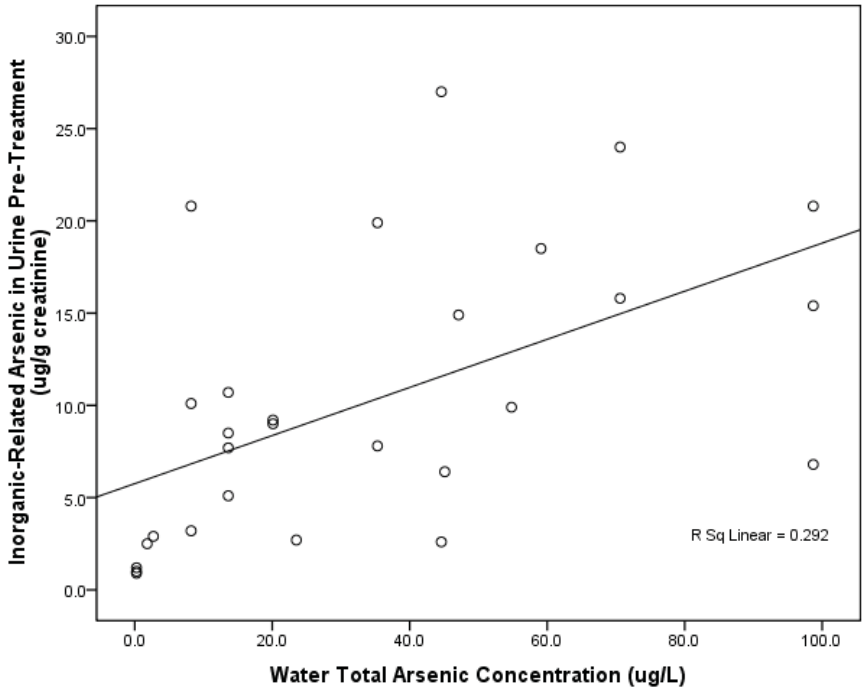


Figure 2-5a: Fish/Seafood Meals per Week vs. Inorganic-Related Arsenic in Urine

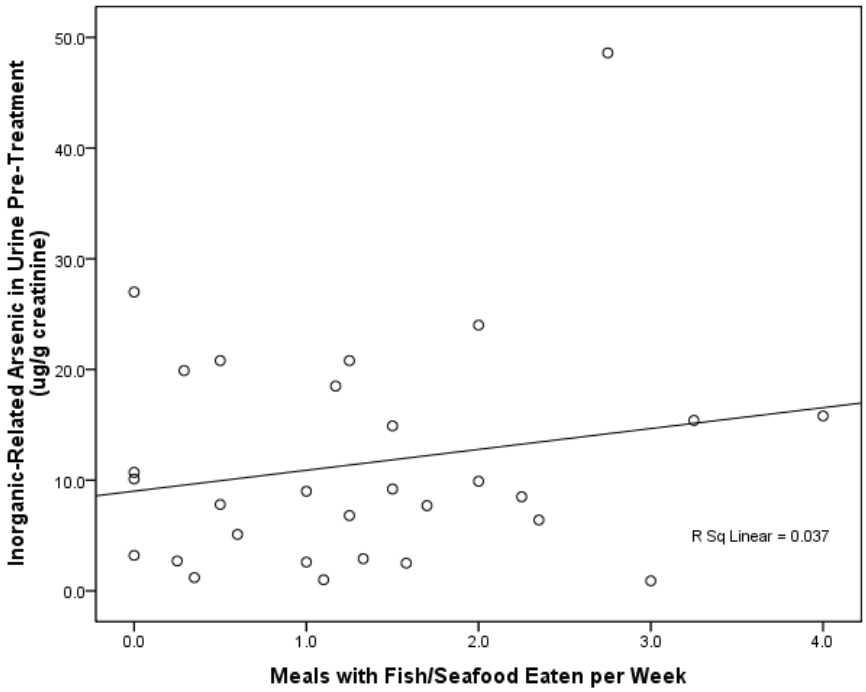


Figure 2-5b: Fish/Seafood Meals per Week vs. Inorganic-Related Arsenic in Urine Without One High Urine Inorganic-Related Arsenic Outlier

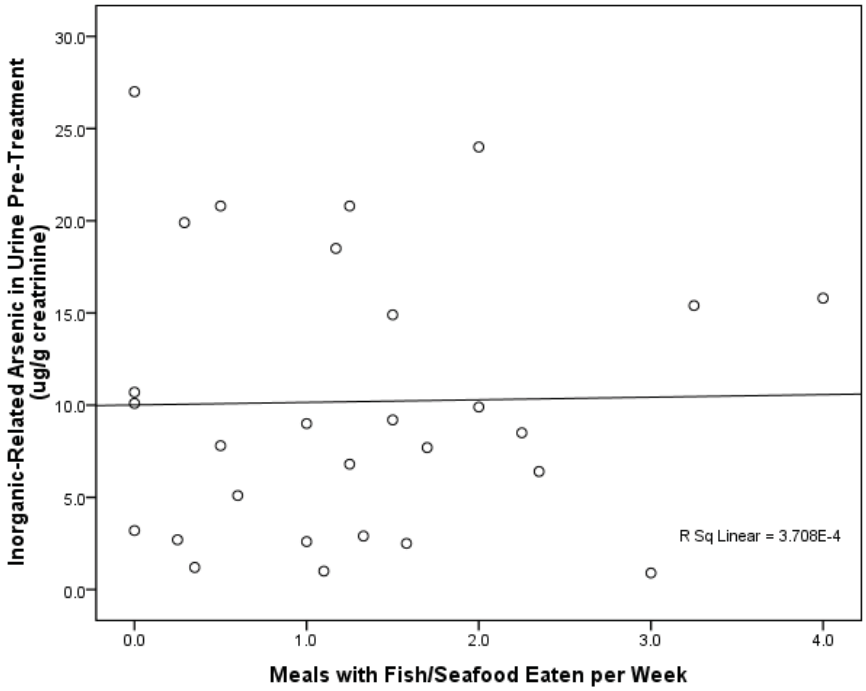


Figure 2-6a: Inorganic-Related Arsenic in Urine vs. Total Arsenic in Urine

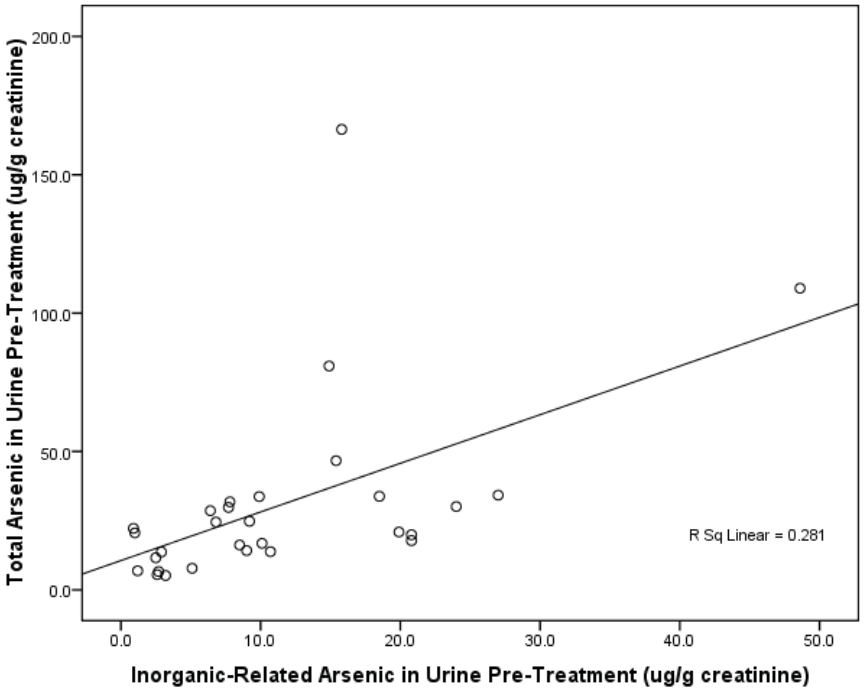
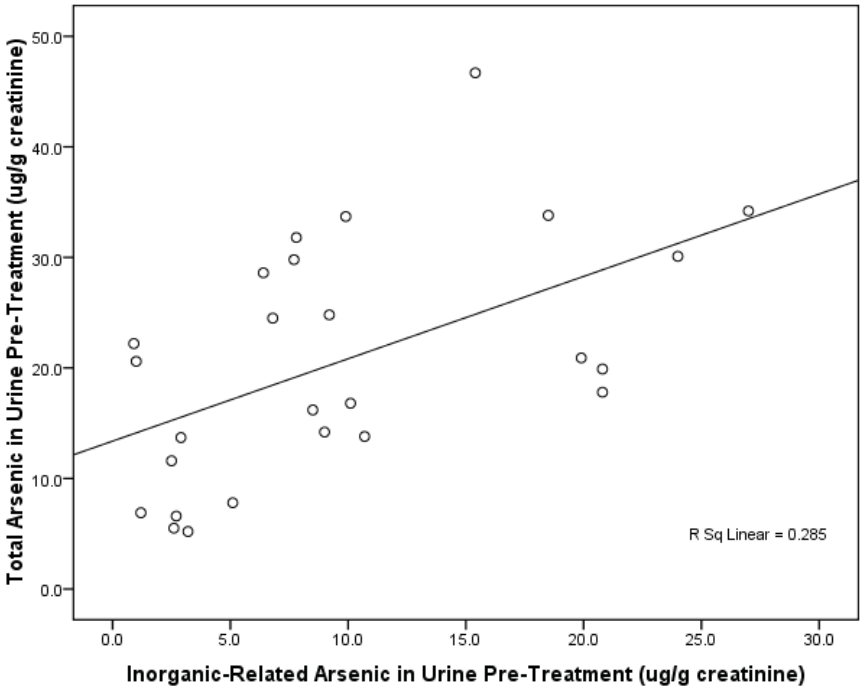


Figure 2-6b: Inorganic-Related Arsenic in Urine vs. Total Arsenic in Urine Without Three High Total Urinary Arsenic Outliers



IC-ICP-MS Compared with Hydride Generated Arsenic

IC-ICP-MS analyses were run on 10 urine samples to compare the results with both the HG-ICP-MS inorganic-related arsenic and the ICP-MS total arsenic results. The results were previously published (Xie et al., 2007) and compared very well. The difference between the sum of inorganic-related arsenic species, As^{V} + As^{III} + MMA^{III} + MMA^{V} + DMA^{V} + DMA^{III}, as measured by IC-ICP-MS was not significantly different than the inorganic-related arsenic measured by HG-ICP-MS.

Blood Arsenic Levels

The results for total arsenic in blood determined by the standard addition method using ICP-MS are given in Table 2-6. Whole blood samples were analyzed for total arsenic only. Forty subjects in the Exposed Group provided both an initial and final blood sample; however, only 16 of these subjects met the criteria to be in the Pre-Post Group such that they were still drinking the arsenic-contaminated water at the time the initial blood sample was provided. The initial mean arsenic blood concentrations for the Exposed Group (11.1 ± 0.9 µg/L) and the 16 subjects with sufficient blood data in the Pre-Post Group (12.8 ± 2.0 µg/L) were not significantly greater than the Control Group (9.7 ± 1.6 µg/L). The mean final blood arsenic level in the Exposure Group (7.2 ± 0.5 µg/L) and the mean of the Pre-Post Group (6.0 ± 0.8 µg/L) were both lower than in the Control Group (9.7 ± 1.6 µg/L), but only the Pre-Post Group was significantly lower than the Control Group ($p = 0.034$).

Table 2-6: Blood Arsenic Levels

Subject Groups	Exposed	Pre-Post [a]	Control
Blood Biomonitoring (Mean ± SE)			
Subjects with Initial and Final Blood Samples (n)	40	16	N/A [b]
Total Arsenic Initial Blood (µg/L)	11.1 ± 0.9	12.8 ± 2.0	9.7 ± 1.6
Total Arsenic Final Blood (µg/L)	7.2 ± 0.5	6.0 ± 0.8 [†]	N/A [b]

[a] Subset of Exposed Group subjects still drinking arsenic contaminated water at time of initial blood sampling,
[†] $p < 0.05$, significant difference from Control Group.
N/A [b] = Not Applicable because control subjects provided only one blood and urine sample.

The concentration of total arsenic in water did not correlate with the initial total arsenic in blood (Figure 2-7). The total urinary arsenic concentrations also did not correlate with the initial blood arsenic concentrations, with the three high urinary total arsenic outliers (Figure 2-8a), or without (Figure 2-8b).

Figure 2-7: Arsenic in Well water vs. Total Arsenic in Blood

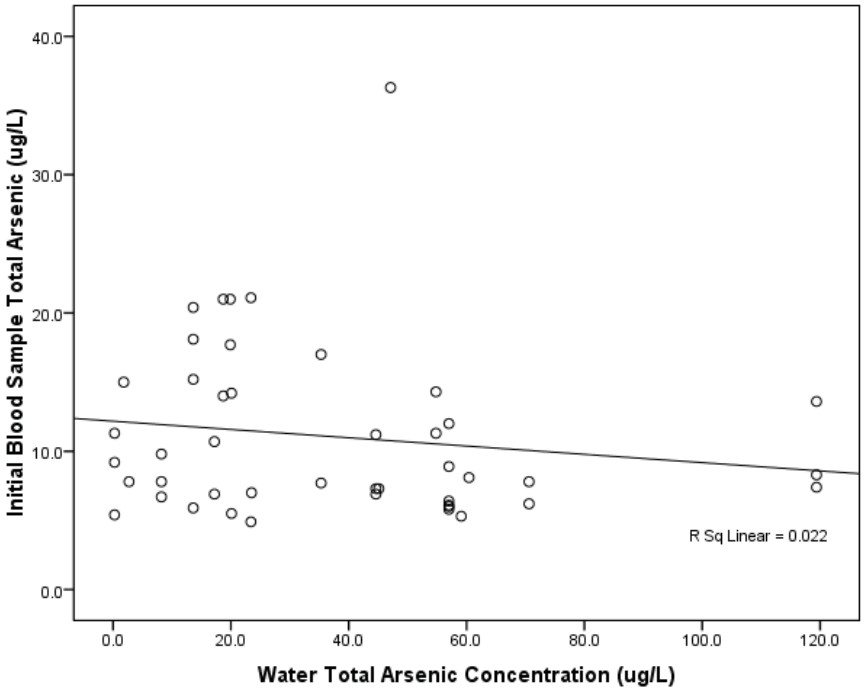


Figure 2-8a: Total Arsenic in Urine vs. Total Arsenic in Blood

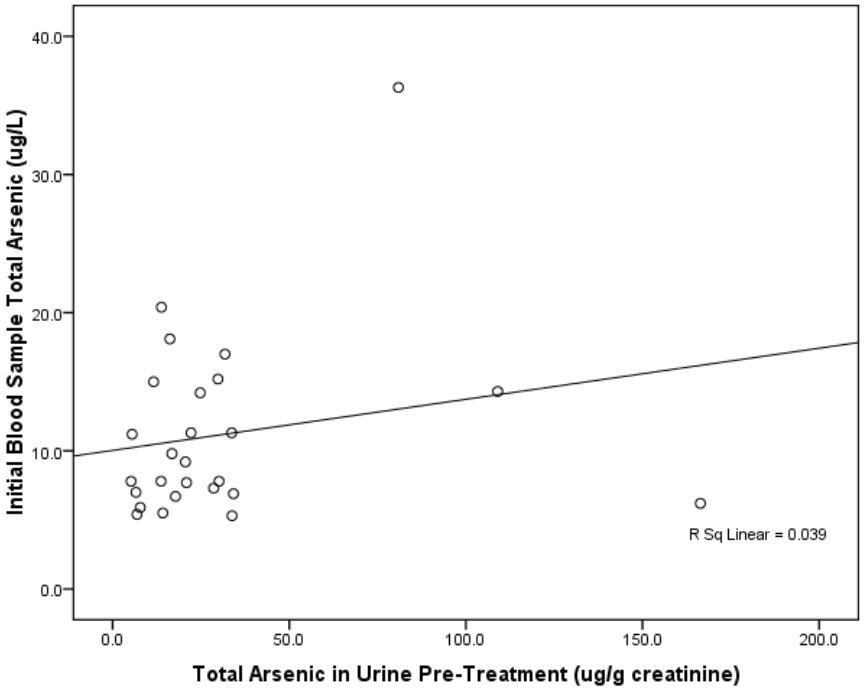
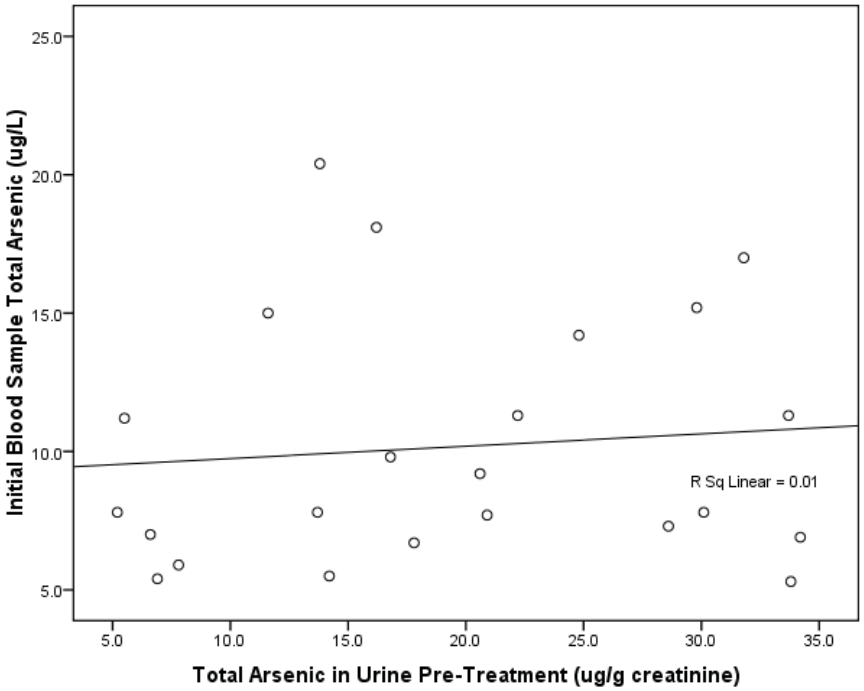


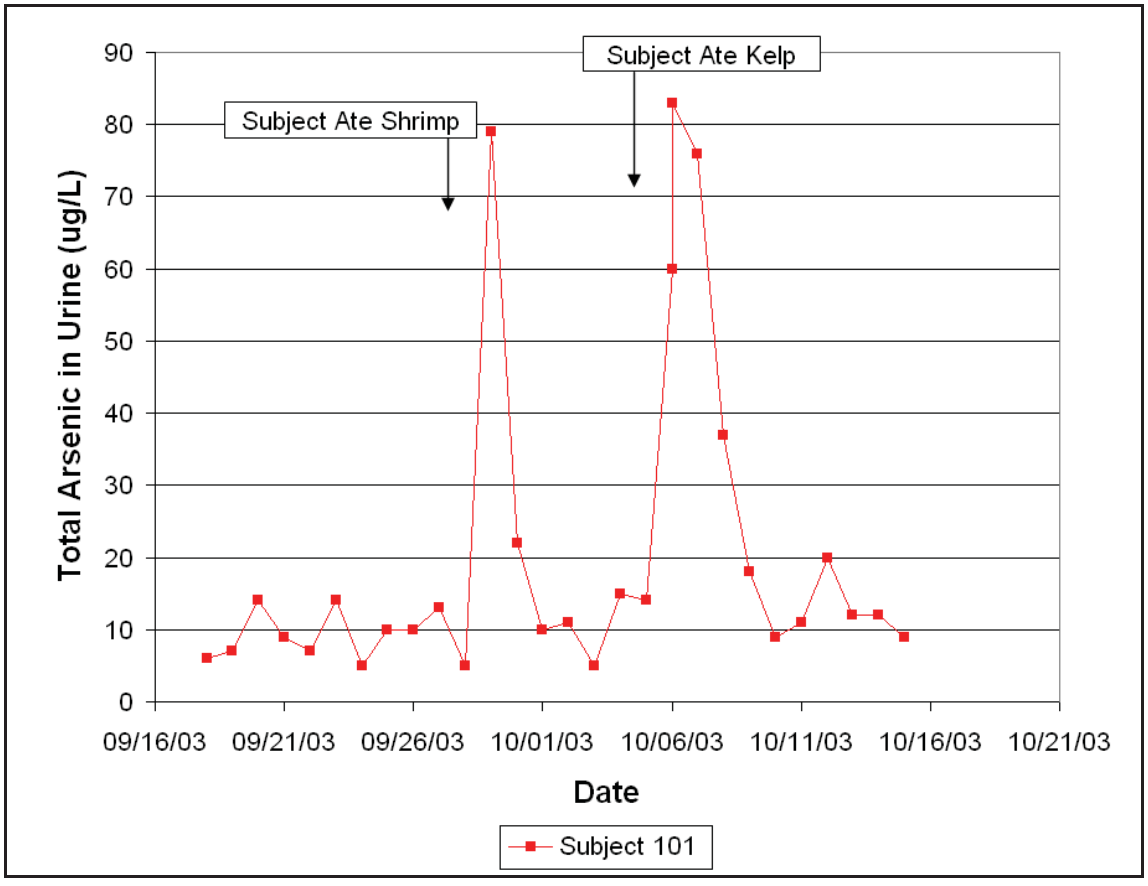
Figure 2-8b: Total Arsenic in Urine vs. Total Arsenic in Blood Without Three High Urinary Total Arsenic Outliers



Dietary Spikes

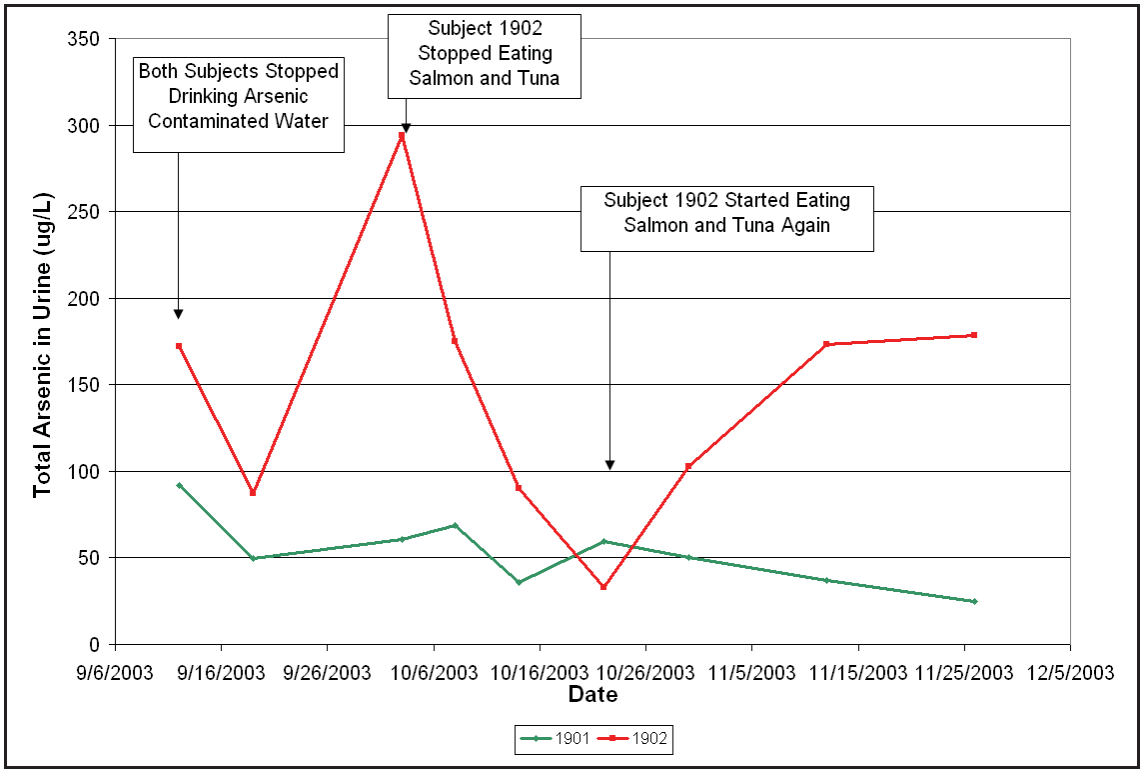
To see how great an effect dietary sources might have on a biomonitoring study, three subjects were asked to collect additional urine samples and keep track of ingestion of high arsenic foods. Figure 2-9 shows daily first morning void urine samples and documents a very large spike in the urine total arsenic concentrations within 24 hours of consumption of high arsenic foods (Spayd, 2004). Total arsenic concentrations remained elevated for two days after eating shrimp and for five days after eating kelp (seaweed).

Figure 2-9: Longitudinal Series of Total Urinary Arsenic with Dietary Arsenic Inputs of Shrimp and Seaweed - After: (Spayd, 2004)



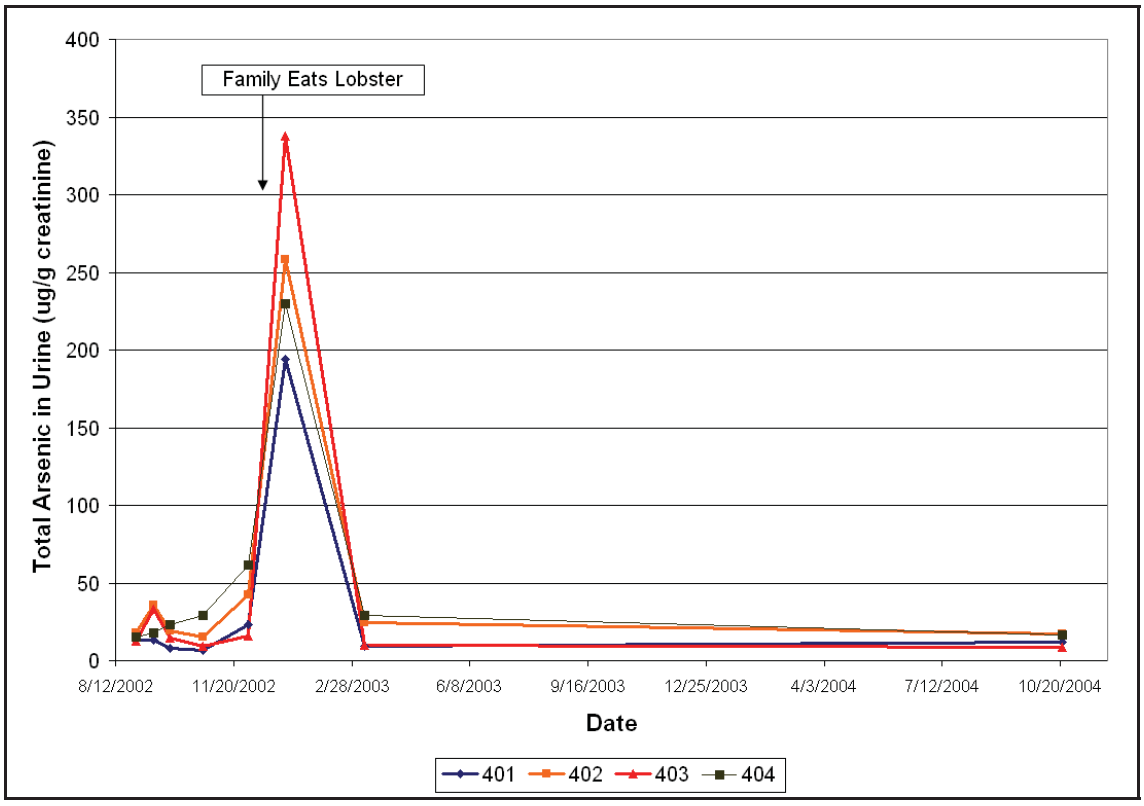
In Figure 2-10, subject 1902's diet regularly included salmon and tuna while the spouse's diet (subject 1901) did not include any salmon or tuna (Spayd, 2004). Subject 1902 agreed not to eat any salmon and tuna for at least three weeks, and the data show that it took almost three weeks for subject 1902's urine total arsenic level to normalize with that of subject 1901. Then subject 1902 started eating salmon and tuna again, and the total urinary arsenic dramatically increased again.

Figure 2-10: Longitudinal Series of Total Urinary Arsenic after 80 ug/L Arsenic in Water and Dietary Arsenic Inputs of Salmon and Tuna



In Figure 2-11, the data for Family 4 shows a large spike in urine total arsenic from a lobster meal eaten by all four family members (Spayd, 2004).

Figure 2-11: Effect on Total Uriary Arsenic After Lobster Meal (Spayd, 2004)



Discussion

In this study, the effect of dietary arsenic was evident. As shown in Figure 2-1, approximately 75% of total arsenic in urine was attributed to organic arsenic species which are mainly found in fish and seafood in the diet. As seen in Table 2-4, on average, subjects routinely had meals with high arsenic foods including 1.3 - 1.5 meals per week with fish or seafood, 0.3 - 0.5 meals per week with mushrooms, 1.2 - 2.0 meals per week with rice, and 2.3 -2.8 meals per week with poultry. The results presented in Table 2-5 and Figure 2-1 clearly show that the total arsenic measure in urine was, on average 13.9 µg/g of creatinine higher

than the inorganic-related arsenic concentration in urine. Although total arsenic in urine levels did decline over the time course of the study, the pattern of arsenic clearance from the human body after the arsenic contaminated water ingestion exposure had ceased was significantly clouded by the organic arsenic from dietary sources. The inorganic-related arsenic, which is mainly attributed to the arsenic in well water, has a very clear pattern of reduction.

Figures 2-2 and 2-3 show how the pre-treatment total arsenic concentrations in urine were better correlated with the number of fish and seafood meals per week than with the total arsenic in well water. However, as expected, the number of fish and seafood meals per week did not correlate with pre-treatment inorganic-related arsenic concentrations in urine while total arsenic in well water correlated nicely (Figure 2-4 and 2-5). These findings demonstrate how organic arsenic from diet can confound an investigation of arsenic biomonitoring from well water unless the organic arsenic is dealt with either by restricting seafood, fish, and seaweed from the diet in the sampling protocol or by speciating the arsenic in the analytical protocol.

Figures 2-9, 2-10, and 2-11 specifically demonstrate how a meal with shrimp, seaweed, salmon, tuna, mushrooms, or lobster can greatly increase the concentration of total arsenic in urine. These findings are consistent with published reports (National Research Council, 1999). A single meal can significantly raise total arsenic in urine for up to five days. In Figure 2-9, the extra

time required to clear the arsenosugars and their metabolites from urine compares favorably with prior studies (Le et al., 1994). In Figure 2-10, subject 1902's diet included salmon or tuna at a frequency of four meals per week. The slow clearance (more than two weeks) of total arsenic from this subject's urine may be related to the regular diet of fish. This implies that people who regularly eat fish and seafood may need a longer period of time before the organic arsenic is cleared from the body. It has been demonstrated that eating fish or seafood on a regular basis causes an extension in the length of time for urinary arsenic excretion to reach base line levels (Foa et al., 1984).

Rice and rice-based foods (e.g., crisped rice cereals) have been shown to contain elevated concentrations (typically 0.1-0.2 mg/Kg) of inorganic-related arsenic (Sun et al., 2008; USFDA, 2007; Zavala and Duxbury, 2008; Zavala et al., 2008). In the present study, the mean number of meals per week with rice was 1.2 ± 0.2 meals per week for the Pre-Post Group and 2.0 ± 1.3 meals per week for the Control Group. However, no significant correlations between the number of meals with rice per week and urine or blood concentrations were identified in this study.

Analytical Methods

Even in occupational biomonitoring, measuring total arsenic in urine can often cause a false alarm regarding arsenic exposure. In a recent study of workers occupationally exposed to copper chromate arsenic wood preservatives, the

exposed workers could not be distinguished from the control subjects when looking at total arsenic in urine data (Cocker et al., 2006). Only when a hydride generation (HG) arsenic analysis technique was used could the influence of dietary organic arsenic and occupational inorganic-related arsenic exposure be identified. Urine samples collected up to six days after a fish or seafood meal can have total arsenic concentrations as high as several hundred µg/L or more.

A German study of 101 male employees, living in a harbor city (thought to be associated with higher seafood consumption), and without occupational exposure, found that urinary DMA remained elevated for up to six days after ingestion of fish, and that DMA concentrations became significantly lower, when collecting urine samples more than six days after eating fish (Heinrich-Ramm et al., 2001). The major arsenic species resulting from fish and seafood consumption are the relatively non-toxic organic arsenic species. Therefore, an analysis of total arsenic in urine is only useful for detecting arsenic from contaminated drinking water if no seafood, fish, scallops, seaweed (kelp), or seaweed wrapped sushi has been eaten within the previous six days.

Analyses for inorganic-related arsenic in urine by HG-ICP-MS, and other HG methods, are best for assessing exposure to arsenic from contaminated water. The organic arsenic related to fish and seafood is separated and only the inorganic-related arsenic is detected. Although there is some inorganic-related arsenic in the diet, it is only estimated at up to 9 micrograms per day (µg/d) for

adults, aged 25 and over in the United States, and less for children (National Research Council, 1999; Tao and Bolger, 1999). Therefore, a significant arsenic water exposure should still be evident. It is not possible to separate inorganic arsenic found in food consumed by subjects from inorganic arsenic related to the water exposure.

IC-ICP-MS can quantify the individual arsenic species present in the urine sample, but since IC-ICP-MS is more expensive than HG-ICP-MS, this method is generally not needed unless a specific research goal of the analysis involves arsenic metabolism.

Sample Collection Protocol

The sample collection protocol should depend on the analytical methods that will be used to analyze the samples. If total arsenic in urine will be used to analyze the samples, fish, seafood, scallops, seaweed (kelp), sushi with a seaweed wrap, and mushrooms should be completely avoided for one week prior to sample collection.

If HG-ICP-MS or IC-ICP-MS will be used to analyze the samples, eating fish can be allowed, but scallops, seaweed (kelp), and sushi wrapped in seaweed must still be avoided for one week prior to sample collection. The problem with scallops and seaweed is that a large percentage of the arsenosugars found in them are metabolized in humans to DMA (Le et al., 1994), and DMA is one of the

inorganic-related arsenic species detected by HG-ICP-MS. Therefore, foods containing arsenosugars remain a confounder when HG-ICP-MS or IC-ICP-MS methods are used, and should be restricted from the diet for one week prior to sample collection.

Reduction of Arsenic Water Ingestion Before Treatment Installation

A comparison of the first urine samples collected from subjects who had already stopped drinking the arsenic contaminated water can not be made with the first urine samples from subjects who were still drinking the arsenic contaminated water. This was demonstrated by the rapid 50% reduction of inorganic-related urine arsenic concentrations in the first week after the chronic ingestion exposure to the arsenic-contaminated water ended as shown in Figure 2-1. Therefore, whenever biomonitoring for exposure to arsenic contaminated well water is conducted, it must be determined if and when the subjects stopped drinking the arsenic-contaminated well water.

Arsenic Reference Ranges for Urine

In the ATSDR Arsenic Toxicity Case Study in Environmental Medicine, normal total urinary arsenic is given as < 50 µg/L, in the absence of eating seafood in the past 48 hours, and that values > 200 µg/L are considered abnormal (ATSDR, 2000). The following data review will show that these numbers appear to be too high and that new reference ranges for arsenic biomonitoring would be valuable.

Table 2-7 presents the mean data collected from selected arsenic biomonitoring studies around the world. The table clearly shows that urine and blood arsenic concentrations are correlated with drinking water arsenic concentrations. Based on this data, if we consider the populations who were drinking water containing arsenic at less than 10 µg/L, the USEPA MCL level, we can see that their total arsenic in urine was ≤ 20 µg/L, their inorganic-related arsenic in urine was ≤ 13 µg/L, and their total arsenic in blood was <1.5 µg/L. These values represent a preliminary view of what we can expect in people who are drinking water that contains <10 µg/L.

Table 2-7
Relationship of Drinking Water, Blood, and Urine Arsenic Levels (µg/L)

Drinking Water	Blood	Urine Total	Urine Inorganic-Related	Location	Data Source
600	-	-	583	San Pedro, Chile	(Biggs et al., 1997)
500	-	100	-	Millard County, Utah, USA	(Calderon et al., 1999)
401	-	178	-	Fairbanks, Alaska, USA	(Harrington et al., 1978)
393	13.3	-	-	Edison, California, USA	(Valentine et al., 1979)
215	9.0	-	320	S.A. Cobres, Argentina	(Concha et al., 1998a;
200	8.0	274	261	S.A. Cobres, Argentina	(Vahter et al., 1995)
123	4.2	84	-	Hidden Valley, Nevada, USA	(Valentine et al., 1979)
100	-	70	-	Millard County, Utah, USA	(Calderon et al., 1999)
98	4.3	-	-	Fallon, Nevada, USA	(Valentine et al., 1979)
91	11.9	128	-	Matlab, Bangladesh	(Hall et al., 2007)
75	-	45	-	Fairbanks, Alaska, USA	(Harrington et al., 1978)
51	5.1	40	-	Virginia Foothills, Nevada, USA	(Valentine et al., 1979)
37	1.5	55	45	Santa Rosa de los P.G.,	(Vahter et al., 1995)
31	-	41	-	Fairbanks, Alaska, USA	(Harrington et al., 1978)
30	-	28	-	Hermosillo, Sonora, Mexico	(Wyatt et al., 1998)
15	-	-	59	Toconao, Chile	(Biggs et al., 1997)
14	1.5	34	24	Olacapato, Argentina	(Vahter et al., 1995)
11	-	38	-	Fairbanks, Alaska, USA	(Harrington et al., 1978)
10	-	10	-	Millard County, Utah, USA	(Calderon et al., 1999)
9	-	14	-	Hermosillo, Sonora, Mexico	(Wyatt et al., 1998)
2.5	1.2	20	13	Tolar Grande, Argentina	(Vahter et al., 1995)
1.9	-	19	8.6	Anaconda, Montana, USA	(Hwang et al., 1997)
0.7	0.9	10	-	Rosario de Lerma, Argentina	(Concha et al., 1998a;

The NHANES 2003-2004 survey has provided urinary arsenic data for a representative sample of the US population aged 6 years and older (Caldwell et al., 2008). In this NHANES survey, the geometric mean of total urinary arsenic for 2,557 participants was 8.3 µg/L and 8.2 µg/g creatinine. For inorganic-related urinary arsenic the geometric mean was not published, but the 50th percentile was 6.0 µg/L.

A comparison of the NHANES data with the urinary arsenic data from the present study is presented in Tables 2-8, 2-9, and 2-10 with geometric means and the 25th, 50th, 75th, and 95th percentiles. The 95th percentiles for total urinary arsenic concentrations from NHANES were 65.4 µg/L and 50.2 µg/g creatinine. For inorganic-related arsenic, the 95th percentile was 18.9 µg/L. When evaluating the NHANES data, it must be remembered that this is a representative sample, not an unexposed sample. Some unknown percentage of the NHANES sample was no doubt exposed to elevated arsenic concentrations in drinking water from both residential and public well water as the samples were collected in 2003-2004, which was before the January 2006 effective date of the new US drinking water standard for arsenic being reduced from 50 µg/L to 10 µg/L.

The comparison of the present study's urinary arsenic concentrations with those found in NHANES shows that the overall Pre-Post Group's initial geometric mean total arsenic in urine at 23.5 µg/g creatinine is between the 75th and 95th

percentile of the NHANES sample of 2,557 subjects (Table 2-9). This present study's Control Group's total arsenic in urine with a geometric mean of 13.8 µg/g creatinine is between the 50th and 75th percentile of the NHANES sample.

The inorganic-related arsenic in urine concentrations in the present study can be compared to the NHANES sum of urinary inorganic-related arsenic species (Table 2-10). The overall Pre-Post Group's geometric mean of initial water-related arsenic in urine at 17.5 µg/L is within the 95% confidence interval of the 95th percentile (18.9 µg/L) of the NHANES sample sum of urinary inorganic-related arsenic in urine. The geometric mean of the 268-day time period inorganic-related arsenic in urine, at 6.7 µg/L, is near, but above, the 50th percentile (6.0 µg/L) of the NHANES sample sum of urinary inorganic-related arsenic in urine.

The present study's Control Group geometric mean of inorganic-related arsenic in urine at 3.9 µg/L is well below the 95% confidence interval of the 50th percentile (6.0 µg/L) of the NHANES sample sum of urinary inorganic-related arsenic in urine. This comparison shows that compared to a representative sample of the US population, the Pre-Post Group initial arsenic concentrations were quite elevated (near the 95th percentile of the NHANES sample), and after having effective arsenic water treatment for approximately nine months, the concentrations had dropped to near the 50th percentile of the NHANES sample. This means that an average exposed subject in the present study had an

inorganic-related urinary arsenic concentration comparable to an NHANES subject at near the 95th percentile. Furthermore, an average Control Group subject in the present study, who did not drink arsenic contaminated water, had an inorganic-related urinary arsenic concentration comparable to an NHANES subject well below the 50th percentile for inorganic-related urinary arsenic.

Table 2-8: Comparison of Study Data to NHANES 2003-2004

Total Arsenic Data (µg/L)				Selected Percentiles			
Groups		**Sample Size**	**Geometric Mean**	**25**	**50**	**75**	**95**
NHANES		2557	8.3	4.1	7.7	16.0	65.4
This Study	Controls	5	24.6	16.6	27.6	37.1	43.3
	Pre-Post Time Period 0	24	35.0	21.3	35.3	56.1	160.7
	Pre-Post Time Period 268	24	27.4	14.8	24.8	37.4	179.6

Table 2-9: Comparison of Study Data to NHANES 2003-2004

Total Arsenic Data (µg/g creatinine)				Selected Percentiles			
Groups		**Sample Size**	**Geometric Mean**	**25**	**50**	**75**	**95**
NHANES		2557	8.2	4.2	7.0	14.1	50.2
This Study	Controls	5	13.8	9.3	13.7	21.4	22.2
	Pre-Post Time Period 0	24	23.5	14.7	24.7	33.8	152.1
	Pre-Post Time Period 268	24	17.9	11.9	14.7	25.7	102.5

Table 2-10: Comparison of Study Data to NHANES 2003-2004							
Inorganic-Related Arsenic Data (µg/L)				Selected Percentiles			
Groups		Sample Size	Geometric Mean	25	50	75	95
NHANES		2557	N/A	<LOD	6.0	N/A	18.9
This Study	Controls	5	3.9	2.5	3.6	6.6	7.6
	Pre-Post Time Period 0	24	17.5	12.0	15.5	28.8	138.8
	Pre-Post Time Period 268	24	6.7	3.5	7.0	13.4	28.7

Table 2-11: Present Study Data Inorganic-Related Arsenic							
Inorganic-Related Arsenic Data (µg/g creatinine)				Selected Percentiles			
Groups		Sample Size	Geometric Mean	25	50	75	95
This Study	Controls	5	1.5	1.0	1.2	2.7	2.9
	Pre-Post Time Period 0	24	10.6	7.0	10.0	19.6	43.2
	Pre-Post Time Period 268	24	3.2	1.6	3.8	5.8	10.2

Another major regional biomonitoring study was the German Environmental Survey conducted in 1998 (Wilhelm et al., 2004). The data from this study is summarized in Table 2-12. In this study, the effect of fish on total urinary arsenic was demonstrated as the more often fish was eaten, the higher was the total urinary arsenic. Even those subjects who had not consumed any fish in the 48

hours prior to the sample collection, had higher total urinary arsenic 13.1 µg/L than those subjects who never consumed fish (10.3 µg/L).

Table 2-12: German Environmental Survey 1998
Total Arsenic in Urine (µg/L)

	n	n < LOQ	50th Percentile	95th Percentile
All Subjects	4741	208	4.1	18.9
Fish in Diet				
Never	476	31	3.1	10.3
1/Month	719	32	3.8	15.2
2-3/Month	1155	47	3.8	17.2
1/Week	1842	80	4.3	19.9
None in 48 hr	3924	199	3.7	13.1
Within 48 hr	788	6	7.5	48.1

Adapted from (Wilhelm et al., 2004).
LOQ, Limit of Quantification; hr, hours.

The data from the German Environmental Survey, for those who never ate fish, with 50th and 95th percentiles of 3.1 µg/L and 10.3 µg/L more closely matched the inorganic-related urinary arsenic concentrations of the Control Group in the present study, with 50th and 95th percentiles of 3.6 µg/L and 7.6 µg/L than did the NHANES subjects with 50th and 95th percentiles of 6.0 µg/L and 18.9 µg/L.

Therefore, based on the data discussed above, with an emphasis on the inorganic-related urinary arsenic concentrations of the Control Group in the present study (Table 2-10 and Table 2-11), the total urinary arsenic of subjects who never ate fish in the German Environmental Survey (Table 2-12), the

NHANES inorganic-related urinary arsenic data (Table 2-10), and urinary arsenic data from subjects with known low exposures (Table 2-7), that inorganic-related arsenic in first-morning urine samples should be at or below 8 ug/L or 5 ug/g creatinine in subjects when their drinking water has less than 5 ug/L arsenic and their diet has avoided fish, seafood, and seaweed for one week. Inorganic-related urinary arsenic concentrations higher than these would indicate a break from the protocol's dietary restrictions or an exposure to inorganic arsenic.

Reference Range Data for Total Arsenic in Blood

According to ATSDR, typical values for total arsenic in blood of "nonexposed individuals are < 1 ug/L" (ATSDR, 2007). Background blood arsenic values reported by the National Research Council range from 0.5 to 2.0 ug/L (Hughes, 2006; National Research Council, 1999). However, due to the matrix difficulties in analyzing blood, it is generally only analyzed for total arsenic, and it appears to be a less sensitive biomarker compared to urine and thus may be an unreliable means of biomonitoring for arsenic exposure (ATSDR, 2007; National Research Council, 1999).

There are very few studies where blood samples were collected for arsenic analysis from subjects without a significant drinking water or fish/seafood arsenic exposure. The most pertinent studies were conducted in Argentina. Rosario de Lerma, Argentina is a village known to have a low concentration of arsenic (0.7 ug/L) in drinking water (Concha et al., 1998a). In Rosario de Lerma. 20 children

and 11 women provided blood samples that were dry ashed and then analyzed by hydride generation atomic absorption spectrophotometry (HG-AAS). The blood arsenic concentrations ranged from 0.3 to 1.8 ug/L with a mean of 0.9 ug/L (Concha et al., 1998a) (Table 2-7).

In another Argentinian village, Tolar Grande, with arsenic in tap water at 2.5 ug/L, five subjects provided blood samples analyzed by HG-AAS that ranged from 1.0 to 1.3 ug/L with a mean of 1.2 ug/L (Vahter et al., 1995) (Table 2-7).

In Fairfax, California, with arsenic in drinking water reported at < 6.0 ug/L, 17 subjects provided blood samples analyzed by HG-AAS that ranged from 2.5 to 7.4 ug/L with a mean of 4.9 ug/L (Valentine et al., 1979).

As arsenic concentrations in drinking water increase, generally the blood arsenic levels also increase. For example, in San Antonio de los Cobres, Argentina, where drinking water arsenic averaged 200 ug/L, 15 subjects provided blood samples analyzed by HG-AAS that ranged from 2.7 to 18.3 ug/L with a mean of 8.0 ug/L (Vahter et al., 1995). At Taco Pozo, Argentina where drinking water arsenic averaged 215 ug/L, 24 subjects provided blood samples analyzed by HG-AAS that ranged from 4.7 to 17.0 with a mean of 10.0 ug/L (Concha et al., 1998a). In a study of 101 pregnant women who gave birth in Bangladesh, where water arsenic concentrations had a mean and (range) of 90.5 ug/L (0.1-661.0 ug/L), the mother's blood arsenic concentrations were 11.9 ug/L (3.1-76.5 ug/L),

and the cord blood from newborns had a mean total arsenic concentration of 15.7 ug/L (Hall et al., 2007).

Arsenic concentrations in blood are not always higher at locations with higher drinking water arsenic concentrations. For example, in Olacapato and Santa Rosa de los Pastos Grandes, Argentina, where drinking water concentrations were 14 and 37 ug/L respectively, blood samples analyzed by HG-AAS found a range of 1.1 to 2.4 ug/L with both villages averaging only 1.5 ug/L (Vahter et al., 1995).

In other studies, healthy control subjects in the general population volunteered blood samples for arsenic analysis, but for these subjects there is no data available regarding the level of arsenic in their drinking water or the amount of arsenic in their diet. A study of 50 US women smokers and 49 nonsmokers found mean whole blood total arsenic values of 2.3 and 1.5 ug/L respectively (Kagey et al., 1977). Seven Danish control subjects had a mean total blood arsenic concentration of 2.5 ug/L by neutron activation analysis (Heydorn, 1970), 8 healthy volunteers in Japan had a mean total blood arsenic concentration of 2.5 ug/L (Tanaka et al., 1996), 8 healthy workers in Sweden had a mean total blood arsenic concentration of 4.0 ug/L by ICP-MS after dry ashing (Brune et al., 1966), 100 healthy volunteers in France had a range of arsenic concentrations in blood of 2.6 to 17.8 ug/L with a mean of 5.0 ug/L (Goulle et al., 2005), and 148 people with a “normal environmental exposure” in Italy had a range of total

arsenic concentrations in blood of 0.5 to 32.0 ug/L with a mean of 5.1 ug/L (Foa et al., 1984).

A summary of eight sets of data found a range of arsenic concentrations in blood of 2.0 to 23.0 ug/L with a mean of 5.0 ug/L (Iyengar and Woittiez, 1988; Tietz, 1990). However, water arsenic concentrations and dietary information for this data set is not available. These values are still used by LabCorp as the reference range for arsenic in whole blood (LabCorp, 2008a).

In these general population studies it is probable that those subjects with the higher blood arsenic concentrations had either a significant drinking water or dietary source of arsenic exposure. The same conclusion was reached in a study of blood arsenic data of inhabitants from the European Community that proposed a tentative reference concentration of < 3 ug/L total arsenic in whole blood from individuals not exposed to marine foods and mining areas (Hamilton et al., 1994).

In the present study, water arsenic concentrations ranged from 8.2 to 119.4 ug/L and blood arsenic concentrations ranged from 1.4 to 36.3 ug/L (Table 2-6). Two blood samples were collected from most subjects, one at the start of the study, and the other at the end of the study. The mean ± SE initial blood total arsenic level in the Pre-Post Group was 12.8 ± 2.0 ug/L and the final blood total arsenic level was 6.0 ± 0.8 ug/L (Table 2-6). Dietary arsenic from fish and seafood was

likely present in some of the blood samples as it was found in some of the urine samples collected on the same day as the blood samples.

The importance of dietary exposure was very obvious in the control subjects of our study. These control subjects had arsenic in drinking water concentrations ranging from < 0.5 to 2.7 ug/L, but had blood total arsenic concentrations ranging from 5.4 to 15.0 ug/L with a mean of 9.7 ± 1.6 ug/L. Dietary arsenic from fish and seafood was very likely present in many of the blood samples from the control subjects as it was found in most of their urine samples collected on the same day.

The water arsenic concentrations did not correlate with the total arsenic in blood concentrations as shown in Figure 2-7, further supporting the importance of dietary arsenic on total arsenic in blood concentrations.

Therefore, based on the available data discussed above, with an emphasis on the ATSDR and NRC conclusions and blood data from subjects with known low exposures, total arsenic in whole blood should be at or below 2.5 ug/L in subjects when their drinking water has less than 5 ug/L and their diets have not contained fish, seafood, and seaweed. Total arsenic concentrations in whole blood higher than 2.5 ug/L would indicate a break from the protocol's dietary restrictions or an exposure to inorganic arsenic.

Recommendations for Family Physicians

If a physician suspects their patient may be exposed to arsenic from contaminated well water, the following course of action should be considered.

1. Inquire about the quality of the well water, and if the well water has been tested for arsenic.

2. Inquire about the presence of foods in the diet that are high in organic arsenic (e.g., fish, seafood, scallops, seaweed (kelp), or sushi wrapped in seaweed).

3. If organic-arsenic rich foods have been completely absent from the diet for one week, a urine sample could be analyzed for total arsenic.

4. If the diet has included organic-arsenic rich foods, an HG method of analysis (e.g., HG-ICP-MS) should be used to determine inorganic-related arsenic concentrations in urine. HG methods are commercially available and are often described as “arsenic-inorganic”.

5. If the diet included scallops, seaweed, or sushi wrapped in seaweed within the last week, even an inorganic-related arsenic test may show an elevated arsenic related to diet. In this case, the diet should be restricted from these foods for one week before collecting a urine sample.

6. Blood samples are typically not helpful in the case of exposure to arsenic via contaminated well water.

7. Results based on the sample collection protocol and analytical methodology described here should be compared to the reference ranges suggested for inorganic-related arsenic in urine of < 8 ug/L or < 5 ug/g creatinine.

Conclusions

The importance of designing arsenic biomonitoring sampling and analytical methodology protocols that account for the potential confounding of dietary arsenic has been demonstrated, and reference ranges for inorganic-related arsenic in urine are proposed for studies conducted with these protocols.

The analytical methodology should be one that will determine the inorganic-related arsenic, which is the sum of the inorganic arsenic species found in water (As^{III} and As^{V}) and their metabolites (MMA^{V}, MMA^{III}, DMA^{V}, and DMA^{III}). The best methods for this analysis include a hydride generation step that separates the inorganic-related arsenic from the relatively non-toxic organic arsenic species commonly found in seafood, fish, and some other foods.

The biomonitoring sample collection protocol must be based on the analytical methodology. If only a total urinary arsenic test is available, the diet must be

restricted from high arsenic foods for one week prior to sample collection. If a hydride generation method will be used, most fish and seafood will not cause a problem, but foods containing arsenosugars, such as scallops, seaweed, and seaweed-wrapped sushi must still be restricted from the diet for one week prior to sample collection because arsenosugars are metabolized into DMA (an inorganic-related arsenic) in the human body.

Based on the recommended protocols and the data collected and reviewed in this study, the recommended reference range for inorganic-related arsenic in urine is < 8 µg/L and < 5 µg/g creatinine. For total arsenic in whole blood, the recommended reference range is < 2.5 µg/L. However, testing urine for arsenic is the preferred biomonitoring approach for exposure to arsenic contaminated well water.

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Chapter 3: Efficacy of Water Treatment Systems to Reduce Arsenic Exposures and Biomonitoring Levels

Abstract

BACKGROUND: Arsenic exceeds the maximum contaminant level in New Jersey private wells at a higher percentage than any other contaminant with a primary drinking water standard (e.g., bacteria, nitrate, lead, 26 volatile organic chemicals, mercury, and gross alpha particle activity). Special water treatment systems can remove arsenic from drinking water. The goal of treating arsenic-contaminated water is to reduce arsenic levels in the water below the MCL and as close to the MCLG as possible and thus reduce the risk of cancer and the many other health problems associated with arsenic exposure.

OBJECTIVE: To determine the efficacy of arsenic water treatment systems at reducing the exposure from arsenic contaminated well water and hence reducing arsenic concentrations in urine and blood.

METHODS: A non-random observational study was conducted with 49 subjects in 19 families having elevated arsenic in their residential home well water in New Jersey. The subjects obtained arsenic water treatment systems in their homes. Prior ingestion exposure to arsenic in well water was determined by testing arsenic concentrations in the well water and obtaining water-use histories for each subject, including years of residence with the current well and amount of

water consumed from the well per day. A series of urine samples were collected from the subjects, some starting before water treatment was installed, and continuing for at least nine months. Urine samples were analyzed for inorganic-related arsenic concentrations. Organic arsenic from seafood, fish and other foods in the diet were controlled for in the laboratory.

RESULTS: Urine and blood arsenic concentrations were significantly reduced after subjects stopped drinking the arsenic-contaminated water and had effective arsenic water treatment systems installed in their home. A two-phase clearance of inorganic-related arsenic from urine was identified. After nine months of water treatment, the previously exposed subjects' geometric mean inorganic-related arsenic concentrations in urine were significantly reduced from 10.6 ± 2.1 μg/g creatinine to 3.2 ± 0.6 μg/g creatinine, but remained significantly higher than the mean in the control subjects (1.5 ± 0.4 μg/g creatinine).

CONCLUSIONS: Effective arsenic exposure reduction, via water treatment, was confirmed by biomonitoring. The multi-phase clearance of arsenic from the human body after chronic exposure, and the failure of the previously exposed subjects' urine concentrations to decline to the level in the controls, indicates the presence of an excess arsenic body burden after chronic exposure to arsenic in drinking water.

KEY WORDS: arsenic, biomonitoring, clearance, drinking water, exposure, half life, New Jersey, urinary arsenic, urine, water treatment, well water.

Introduction

The New Jersey Department of Environmental Protection (NJDEP) has set the New Jersey Maximum Contaminant Level (MCL) for arsenic at 5 µg/L. The New Jersey MCL for arsenic is currently the most protective in the world.

In New Jersey, NJDEP research has shown that up to 15% of the private wells tested in certain parts of the state have arsenic concentrations that exceed 10 µg/L and 30% of the private wells exceed 5 µg/L (Serfes et al., 2005). In one New Jersey community surveyed in 2004, 45% of the 114 residential wells tested exceeded 5 µg/L. As shown in Figure 1-1, between September 2002 and April 2007, New Jersey's Private Well Testing Act Program identified 1,445 out of 12,263 private wells tested exceeding the New Jersey MCL in the northern counties of the state (New Jersey Department of Environmental Protection, 2008). Arsenic exceeded the maximum contaminant level in New Jersey private wells at a higher percentage (11.8%) than any other contaminant with a primary drinking water standard. A substantial number of public community and public non community wells also have arsenic exceeding the MCL (Figure 1-2). The southwestern and central portions of the Piedmont Physiographic Province (which includes portions of Mercer, Hunterdon, and Somerset Counties) appear to be the area most impacted with many residential wells supplying water

containing arsenic in the 10 – 200 µg/L range (Figure 1-1). Research by the New Jersey Geological Survey (NJGS) indicates the arsenic is predominantly naturally occurring in specific geologic settings (Serfes et al., 2005).

Arsenic Health Effects and Exposures

Arsenic is a known human carcinogen and also increases the risk of many non-cancer health effects (ATSDR, 2007). As a Group A carcinogen, arsenic has a Maximum Contaminant Level Goal (MCLG) of zero (USEPA, 2001).

Arsenic is found in a variety of chemical forms in water, food, and living organisms. In well water in New Jersey, arsenic has been found to occur in two inorganic species: arsenate (As^{V}) and arsenite (As^{III}) as shown in Table 1-1. In about 80% of the wells with total arsenic above 5 µg/L, the predominant species is As^{V}, while in the other 20% of the wells, As^{III} is the predominant species (Serfes et al., 2005; Spayd, 2007). Well water in New Jersey with arsenic and any one of the following characteristics is likely to contain a significant percentage of As^{III}: 1) concentrations of iron greater than 0.1 milligrams per liter (mg/l), 2) concentrations of manganese greater than 0.05 mg/l, 3) a negative oxidation reduction potential, or 4) a hydrogen sulfide odor.

Arsenic Water Treatment

Special water treatment systems can remove arsenic from drinking water, and can be configured to treat all the water in the home (point-of-entry) or water at only a single tap (point-of-use) for drinking and cooking (Spayd, 2007).

The goal of treating arsenic-contaminated water is to reduce arsenic levels in the water below the MCL and as close to the MCLG as possible and thus reduce the risk of cancer and the many other health problems associated with arsenic exposure. The NJDEP is conducting a study of the effectiveness of various arsenic water treatment systems. The NJDEP study is evaluating both whole-house water treatment systems, commonly referred to as point-of-entry (POE) treatment, and single faucet treatment options for treating only drinking and cooking water, commonly referred to as point-of-use (POU) treatment. The NJDEP study (Spayd, 2007) has been very successful with most treatment systems reducing arsenic to levels below 3 µg/L, and many systems reducing the arsenic level to below 1 µg/L.

Arsenic Biomonitoring

The NJDEP arsenic water treatment study afforded the opportunity to evaluate and compare overall exposure reduction via arsenic drinking water treatment systems. The objective was to determine if available arsenic water treatment systems are effective in reducing arsenic exposure from well water and what reductive effects arsenic water treatment would have on arsenic concentrations

in human urine and blood.

Methods

Selection of Wells and Subjects

As wells with elevated arsenic concentrations were identified as part of the NJDEP arsenic in ground water study, well owners with the highest arsenic concentrations were asked to participate in an arsenic water treatment and biomonitoring study. Fifty three subjects, in 22 families, with elevated arsenic concentrations (8 - 119 µg/l) in their residential well water were recruited. Five control subjects with drinking water arsenic concentrations below 3 µg/L also participated.

Recruitment and study procedures were reviewed and approved by the Institutional Review Board of the University of Medicine and Dentistry of New Jersey, as described in Chapter 2.

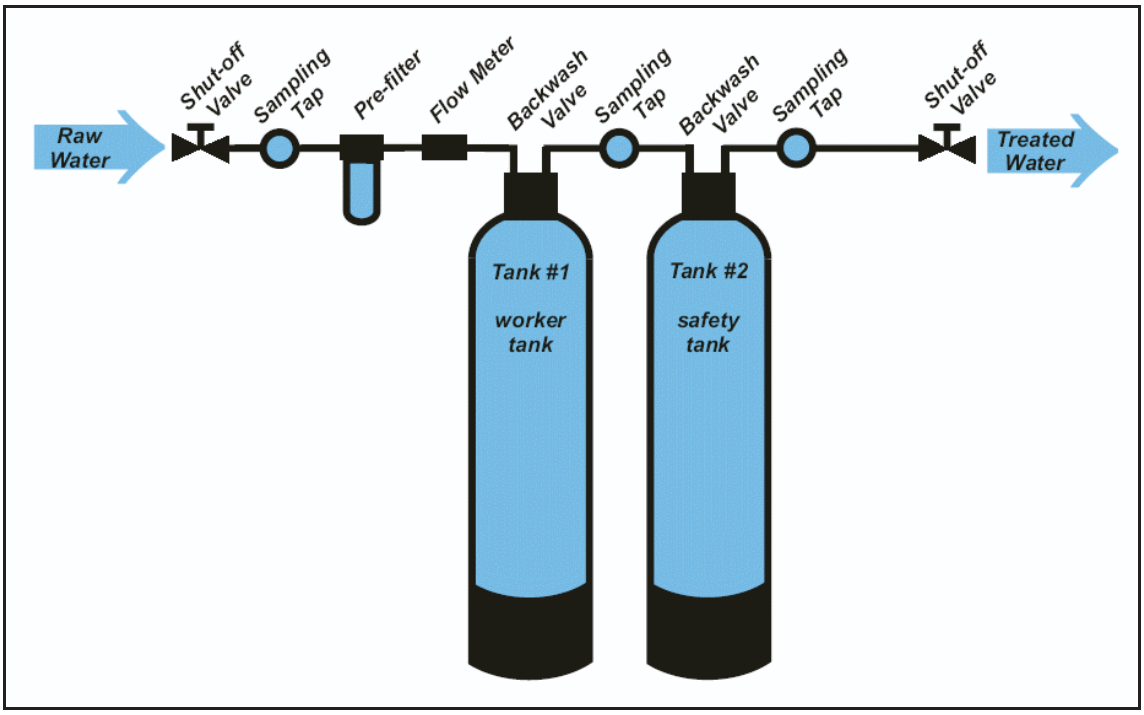
Water Treatment Technologies Used

POE water treatment systems were installed in 12 homes where 31 subjects resided. The POE systems were predominantly adsorption media based systems and included seven using Adedge AD33 granular ferric oxide media, one with Apyron Aqua-Bind MP granular metal oxide composite media, and one

with Aquatic Treatment System proprietary granular adsorption media. Two strong-base anion exchange systems were also used.

The typical POE adsorption system installation was a lead/lag or worker tank/safety tank system including a minimum of two 10-inch by 44-inch fiberglass tanks with one-cubic foot of adsorption media in each as a shown in Figure 3-1 (Spayd, 2007).

Figure 3-1: Typical POE Adsorption System Design – After (Spayd, 2007)



This type of POE system consists of a shut-off valve, raw water sampling tap, 5-micron sediment pre-filter, flow meter, the two adsorption tanks, backwash control valves on each tank, a sampling tap between and after the tanks, and a shut-

off valve after the system. This is the preferred arsenic water treatment system design in New Jersey (Spayd, 2007). This type of system is thoroughly backwashed before being placed into service, and the backwash valves are set to backwash the media at least once per month, each tank on a separate day. The backwash line is piped to a suitable disposal location according to local plumbing codes; however, the backwash water from an adsorption system runs through the treatment media and therefore contains very low concentrations of arsenic.

The POE anion exchange treatment systems included one cubic foot of strong-base anion exchange resin in 10-inch by 44-inch fiberglass tanks. These tanks were set to regenerate with salt brine based on the sulfate content in the water. If the system is not regenerated on the proper schedule or if the salt level is not maintained, the system will dump arsenic into the treated water at concentrations higher than in the raw water. The regeneration discharge water from anion exchange systems contains high levels of arsenic and a proper disposal location is critical. In addition, anion exchange systems only remove As^{V}. When these problems with anion exchange became apparent, NJDEP began to discourage the use of anion exchange for arsenic removal in New Jersey. This was based on the high level of maintenance required to be conducted by the homeowner for anion exchange systems to prevent arsenic dumping into the treated water at concentrations higher than in the untreated water, the limitation of the system in

removing only As^{V}, and the problem of extracted arsenic disposal to the environment near the home (Spayd, 2007).

POU water treatment systems were installed in 9 homes where 20 subjects resided. The POU systems were predominantly adsorption media based systems and included two using Adedge AD33 granular ferric oxide media, two using Multi-Pure granular ferric oxide impregnated carbon block media, two using Isolux zirconium media, and one using Hydroglobe titanium based media. One home used a POU reverse osmosis system. One final home with two subjects did not install a treatment system, but used bottled water for all drinking and cooking as a surrogate for a POU treatment system.

At a few homes, pre-treatment of the water was required for iron, manganese, and/or hardness.

Water Treatment Monitoring and Analysis

Throughout the project, arsenic levels were regularly measured in both the raw water entering the home from the well and the treated water as described in detail in Chapter 2.

Biomonitoring Sample Collection

A series of urine samples were collected from the subjects, some starting before water treatment was installed, and continuing for at least nine months. Blood

samples were collected at the start and end of the water treatment study. The biomonitoring protocol is described in detail in Chapter 2.

Questionnaire

Based on in-person interviews, a household water use and exposure history questionnaire (Appendix 1) was completed for each subject by the investigator. A detailed description of the questionnaire is provided in Chapter 2. Briefly, the purpose of the questionnaire was to determine the arsenic exposure history for each subject and to estimate each subject's cumulative arsenic ingestion dose from drinking and cooking with the water prior to obtaining arsenic water treatment.

Laboratory Analysis

All biomonitoring samples were analyzed at the EOHSI Chemical Analysis laboratory, as described in detail in Chapter 2. Briefly, analysis of total arsenic in urine was conducted by ICP-MS, and a speciation technique (hydride generation) was employed to determine the sum of six arsenic species in urine related to arsenic exposure via drinking water, and termed inorganic-related arsenic. Creatinine measurements were conducted using a standard assay kit, and urinary arsenic measurements are presented as micrograms As per gram of creatinine (μg/g creatinine). Whole blood samples were also analyzed at EOHSI for total arsenic by ICP-MS using the standard addition method.

Data Analysis

Statistical analyses were run using SPSS Version 15.0 for Windows. Tests for normality were run and several data sets displayed a log-normal distribution. These data sets were transformed using the natural log function to make their distributions closer to a normal distribution. The transformed data sets were: well water arsenic concentration, home water ingestion rate, years of exposure to home water supply, cumulative arsenic ingestion dose, ingestion dose per body weight, number of meals with seafood/fish per week, number of meals with mushrooms per week, number of meals with rice eaten per week, total arsenic in blood, total arsenic in urine, inorganic-related arsenic in urine, and urine creatinine. Scatter plots and bivariate Pearson correlation coefficients were used to examine relationships between variables. Paired t-tests were used to compare means of initial and final biomonitoring data within groups and independent t-tests were used to compare means between the groups. A visual inspection of the log concentration verses time graph was used to calculate arsenic clearance from the human body and associated half-lives (Klaassen, 2001).

Results

Wells and Subjects

Characteristics of the study population are presented in Table 2-4 and described in detail in Chapter 2. Briefly, 53 subjects, within 22 families, with elevated arsenic concentrations in their residential well water were recruited. A total of four subjects were lost to follow-up or provided insufficient samples to be included in the analyses. Therefore, sufficient data was collected on 49 subjects, in 19 families (Exposed Group), to be included in at least part of the analysis. Five control subjects, from five different families, with arsenic drinking water concentrations below 3 µg/L also participated as the Control Group.

A subset of the Exposed Group, called the "Pre-Post Group" was established for data analyses and includes subjects who provided both pre-treatment and post-treatment biomonitoring samples. To be included in the Pre-Post Group, the pre-treatment urine samples had to be collected before obtaining water treatment or ceasing to drink the water with elevated arsenic, and a Nine-Month post-treatment urine sample had to be collected. As shown in Table 2-4, the Pre-Post Group includes 24 subjects (49%) of the Exposed Group who met the criteria for urine analyses.

The Exposed Group and the Pre-Post Group were not significantly different from each other, but were significantly different from the Control Group regarding prior arsenic-contaminated water ingestion exposure. In addition, in the potential

dermal exposure category, the Control Group had significantly higher numbers of teeth brushing per week than both the Exposed Group and the Pre-Post Group, and significantly less baths per week than the Exposed Group. There were no significant differences between the three groups for potential arsenic dietary exposures.

Water Treatment Effectiveness

All but one of the arsenic water treatment systems in the study consistently and effectively reduced the arsenic concentrations in water to below 3 µg/L. At one home with a POE treatment system, the arsenic water treatment system had a temporary arsenic breakthrough during the biomonitoring program that was identified by the NJDEP water treatment system monitoring. The homeowners were notified by NJDEP to switch to bottled water until the problem with the treatment system was corrected.

Urine Biomonitoring

Urine samples were analyzed for both total arsenic and inorganic-related arsenic as described in the Methods section of Chapter 2. The urine results were corrected for creatinine and are presented as µg/g creatinine. As shown in Table-2-3, the mean ± standard error for creatinine levels were highest in the Control Group (1.8 ± 0.2 g/L), but not significantly higher than in the Exposed Group (1.5 ± 0.1 g/L) or the Pre-Post Group (1.6 ± 0.1 g/L).

Table 3-1: Biomonitoring Characteristics of Study Subjects

Subject Groups	Exposed	Pre-Post[a]	Control
Number of Subjects Per Water Treatment Group	49	24	5
Total As in Urine (Geometric Mean ± SE)			
Initial (µg/g creatinine)	23.9 ± 6.9	23.5 ± 7.5	13.8 ± 2.8
Final (µg/g creatinine)	17.7 ± 4.4	17.9 ± 4.8	N/A[b]
Inorganic-Related As in Urine (Geo. Mean ± SE)			
Initial (µg/g creatinine)	5.4 ± 1.4 †#	10.6 ± 2.1 †#	1.5 ± 0.4
Final (µg/g creatinine)	3.3 ± 0.5 #	3.2 ± 0.6 †#	N/A[b]
Inorganic-Related As Reduction (Mean µg/g cre)	8.8 ± 2.1	9.5 ± 2.2	N/A[b]
Blood Biomonitoring (Mean ± SE)			
Subjects with Initial and Final Blood Samples (n)	40	16	N/A [b]
Total Arsenic Initial Blood (µg/L)	11.1 ± 0.9 #	12.8 ± 2.0 #	9.7 ± 1.6
Total Arsenic Final Blood (µg/L)	7.2 ± 0.5 #	6.0 ± 0.8 †#	N/A [b]
Total Arsenic Reduction in Blood (µg/L)	4.6 ± 1.2	6.8 ± 1.9	N/A [b]

[a] Subset of exposed subjects still drinking arsenic contaminated water at time of initial urine and/or blood sampling, and providing a final sample within 6-12 months after ceasing to drink the arsenic contaminated water.

† $p < 0.05$, significant difference from control.

# $p < 0.05$, significant difference within group between initial and final concentrations, by paired T-Test.

N/A[b] = Not Applicable because control subjects provided only one blood and urine sample.

Forty two of the 49 Exposed Group subjects provided both an initial and final urine specimen. For total arsenic, the geometric mean initial urine sample concentrations for the Exposed Group (23.9 ± 6.9 µg/g creatinine) and the Pre-Post Group (23.5 ± 7.5 µg/g creatinine) were greater than in the Control Group (13.8 ± 2.8 µg/g creatinine), but not significantly. The geometric mean final urine total arsenic concentrations for the Exposed Group (17.7 ± 4.4 µg/g creatinine) and the Pre-Post Group (17.9 ± 4.8 µg/g creatinine) were both greater than the Control Group (13.8 ± 2.8 µg/g creatinine), but neither was significantly greater than the Control Group. The within-group differences in the geometric means of the initial and final total arsenic in urine were not significant for the Exposed Group or the Pre-Post Group.

For inorganic-related arsenic, the geometric mean initial urine sample concentrations for the Exposed Group of 5.4 ± 1.4 µg/g creatinine and the Pre-Post Group of 10.6 ± 2.1 µg/g creatinine were both significantly higher ($p < 0.0005$) than the Control Group at 1.5 ± 0.4 µg/g creatinine. The mean final urine inorganic-related arsenic concentrations for the Exposed Group (3.3 ± 0.5 µg/g creatinine) and the Pre-Post Group (3.2 ± 0.6 µg/g creatinine) both were greatly reduced, but remained higher than the Control Group at 1.5 ± 0.4 µg/g creatinine. The mean of the reduction in inorganic-related arsenic in urine from the initial to the final samples was calculated by subtracting the mean of the final from the mean of the initial for each subject and then calculating the mean of the reduction for each group. The mean of the reduction in inorganic-related arsenic in urine from the initial to the final samples was greater in the Pre-Post Group (9.5 ± 2.2 µg/g creatinine) than in the Exposure Group (8.8 ± 2.1 µg/g creatinine). The within-group difference in the geometric mean initial and mean final inorganic-related arsenic concentrations in urine was significant for both the Exposure Group ($p < 0.0005$) and the Pre-Post Group ($p < 0.0005$).

Inorganic-Related Arsenic Clearance from Urine

Graphs of the Pre-Post Group's clearance of inorganic-related arsenic from urine after these subjects stopped drinking the arsenic contaminated water are shown in Figures 3-2a and 3-2b. A two-phase clearance is apparent. Based on a visual inspection of the log concentration verses time graph (Klaassen, 2001) in Figure 3-2b, the first clearance phase had a half-life of approximately 7 days. The

second phase had a half-life of approximately 605 days. The mean inorganic-related arsenic concentrations in the urine of the exposed Pre-Post Group, although greatly reduced, remained significantly higher than in the Control Group, even 600 days after ceasing the drinking water exposure. Both the two-phase clearance of arsenic from urine and the failure of the exposed subjects to reach the geometric mean of the inorganic-related arsenic in urine concentrations found in the Control Group (1.5 ± 0.4 µg/g creatinine) indicate that a body burden of arsenic was present in the exposed subjects.

Figure 3-2a: Clearance of Inorganic-Related Arsenic in Urine

Data Points are Geometric Means ± SE for the Group at the Median of Each Time Period

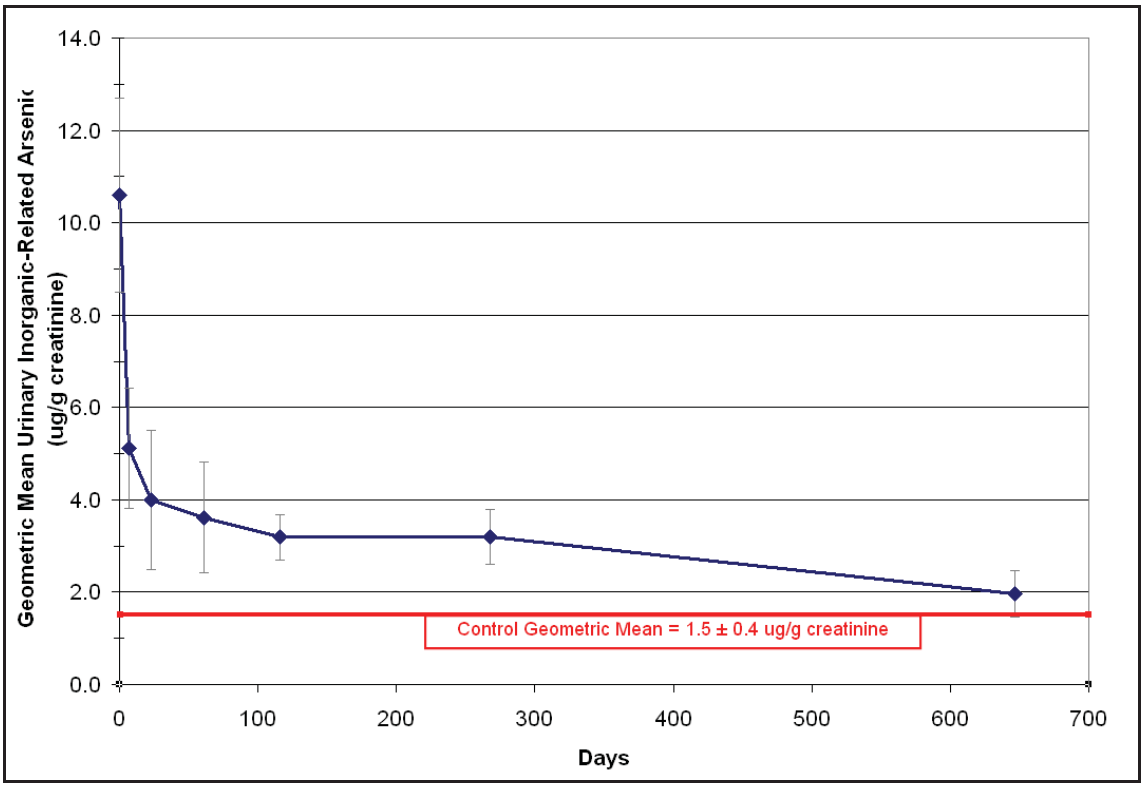
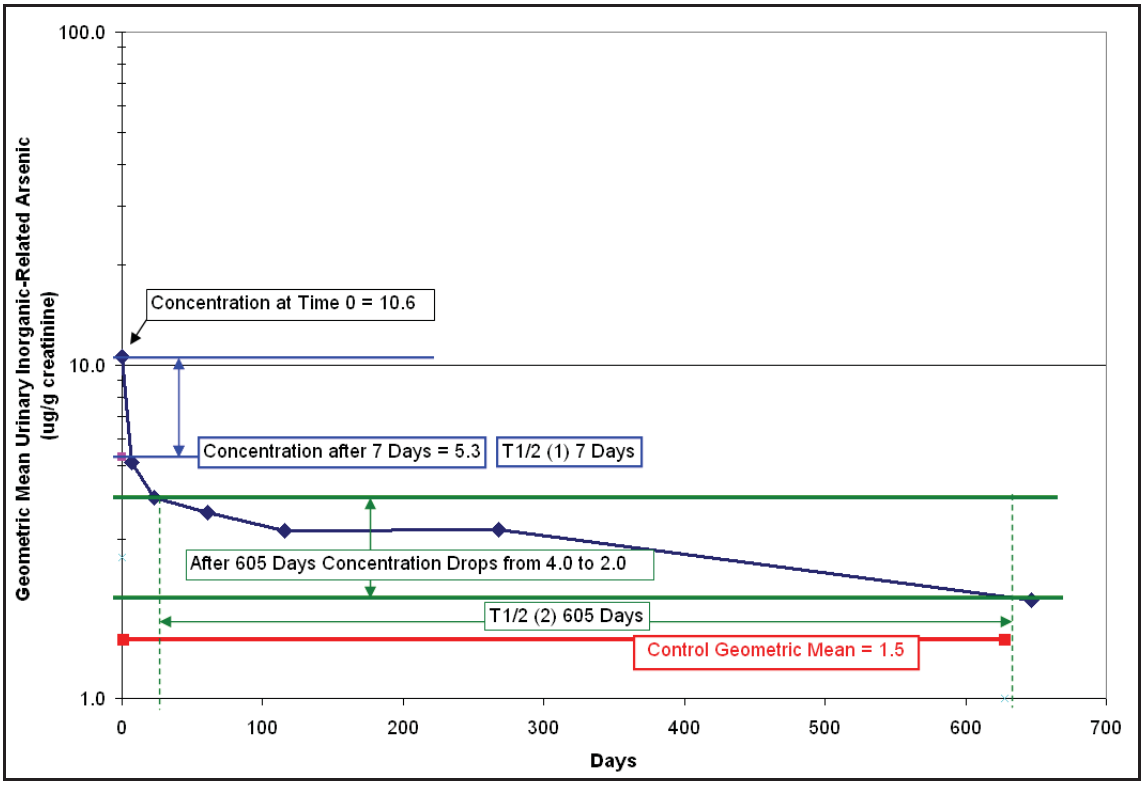


Figure 3-2b: Clearance and Half Life of Inorganic-Related Arsenic in Urine

Data Points are Geometric Means for the Group at the Median of Each Time Period



Urine and Blood Arsenic Reduction Correlations

The reduction in concentrations of inorganic-related arsenic in urine was found to be strongly correlated with the initial urine arsenic concentration ($R^2 = 0.936$, $p < 0.0005$) as shown in Figure 3-3. As a result, the greatest overall reduction in concentrations of inorganic-related arsenic in urine occurred in the subjects with the highest initial concentrations of inorganic-related arsenic in urine. As shown in Figure 3-4, a similar correlation was found between the initial total arsenic in blood with the overall reduction of arsenic in blood by the time the final blood sample was collected ($R^2 = 0.850$, $p < 0.0005$).

Figure 3-3: Correlation between Initial Urinary Inorganic-Related Arsenic and the Arsenic Reduction Nine Months after Water Treatment Began

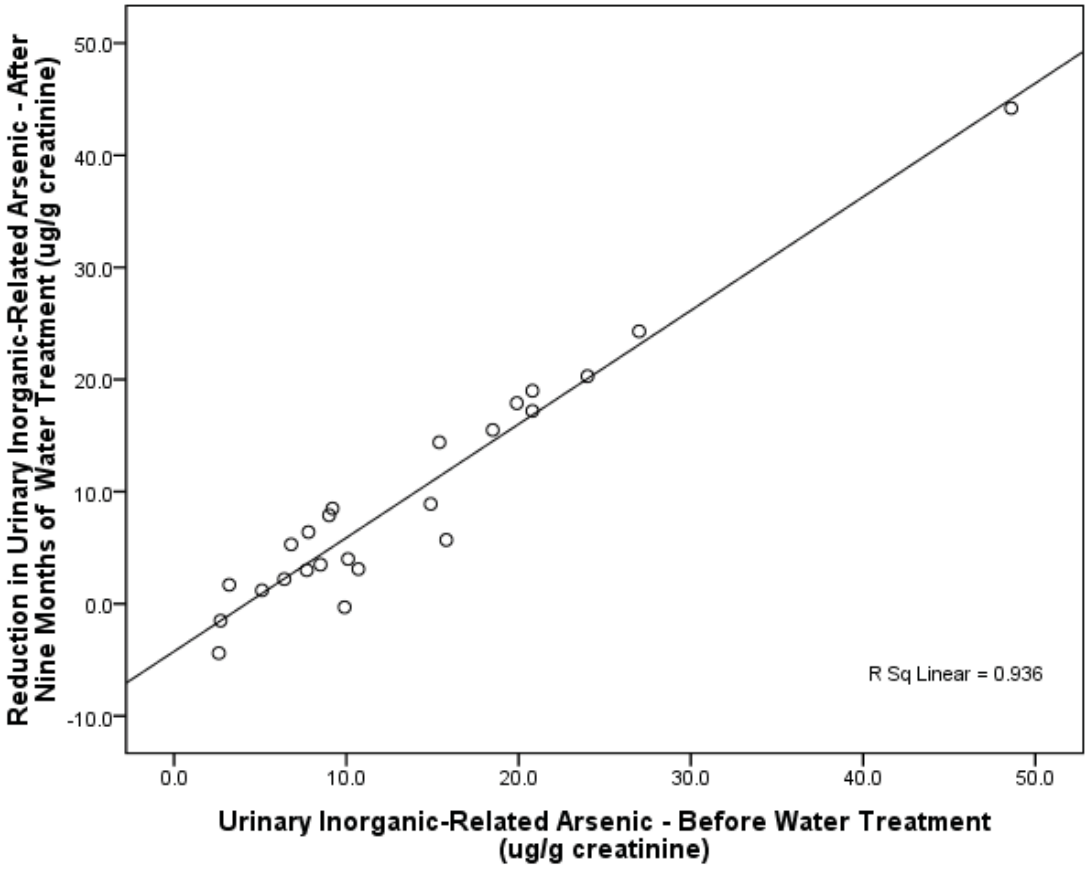
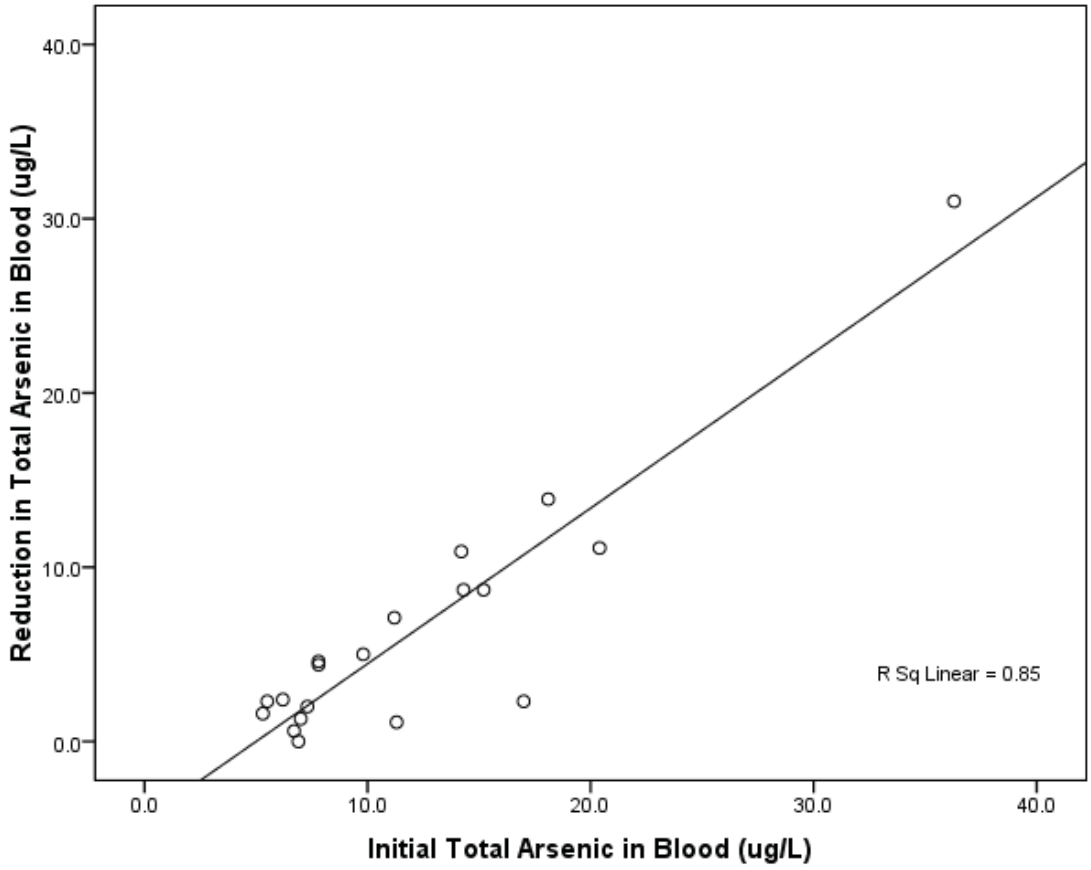


Figure 3-4: Correlation between Initial Total Arsenic in Blood and the Reduction in Total Arsenic in Blood at Final Blood Sample Collection



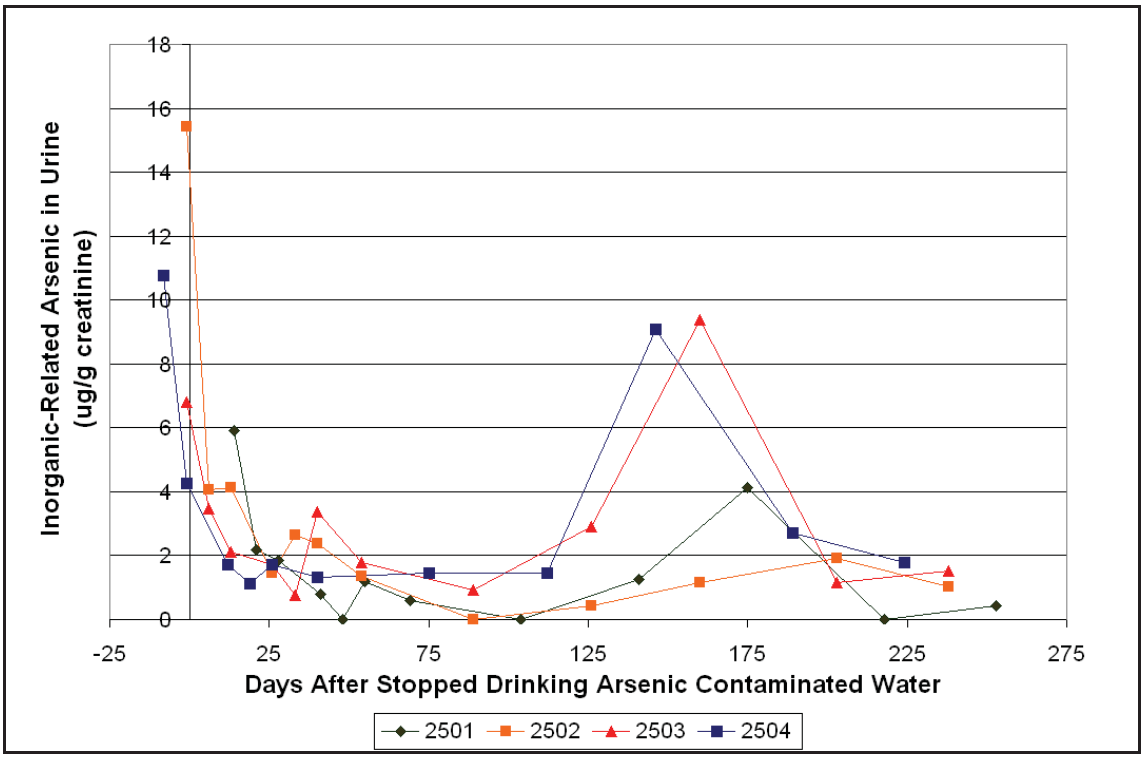
Treatment System Breakthrough Increased Urine Arsenic Levels

At the home where the arsenic water treatment system had a temporary arsenic breakthrough, the raw water averaged 99 µg/L arsenic, and was 96% As^{III}. During the breakthrough period, the arsenic concentration in the treated water at the kitchen sink reached as high as 41 µg/L. The cause of the arsenic breakthrough at this home was determined to be iron bacteria fouling the granular ferric oxide adsorption media. In addition to the high concentration of arsenic, the well water at this home also contained iron at 0.8 mg/l, manganese at 0.25 mg/l, a pH of 7.1, a strongly negative oxidation-reduction potential (-200

millivolts), and a strong sulfur odor. This type of water often contains iron-reducing bacteria which can thrive in a granular ferric oxide media environment. Backwashing the media with a strong chlorine solution removed the fouling. Later, a pulse-feed chlorinator was installed as pre-treatment and prevented any further fouling of the media.

A rebound in the urinary arsenic concentrations was seen during the time period between treatment system breakthrough, its detection, and the families return to use of bottled water until the system was again working effectively (Figure 3-5).

Figure 3-5: Inorganic-related Urinary Arsenic Rebound During Treatment System Breakthrough at Approximately Day 104 Through Day 167



Blood Biomonitoring

The results for total arsenic in blood determined by the standard addition method using ICP-MS are given in Table 3-1. Whole blood samples were analyzed for total arsenic only. Forty subjects in the Exposed Group provided both an initial and final blood sample; however, only 16 of these subjects met the criteria to be in the Pre-Post Group such that they were still drinking the arsenic-contaminated water at the time the initial blood sample was provided. The initial mean arsenic blood concentrations for the Exposed Group (11.1 ± 0.9 μg/L) and the 16 subjects with sufficient blood data in the Pre-Post Group (12.8 ± 2.0 μg/L) were not significantly greater than the Control Group (9.7 ± 1.6 μg/L). The mean final blood arsenic level in the Exposure Group (7.2 ± 0.5 μg/L) and the mean of the Pre-Post Group (6.0 ± 0.8 μg/L) were both lower than in the Control Group (9.7 ± 1.6 μg/L), but only the Pre-Post Group was significantly lower than the Control Group ($p = 0.034$). The mean of the reduction total arsenic in blood from the initial to the final samples was calculated by subtracting the mean of the final from the mean of the initial for each subject and then calculating the mean of the reduction for each group. The mean reduction in total arsenic in blood from the initial to the final samples was greater in the Pre-Post Group (6.8 ± 1.9 μg/L) than in the Exposure Group (4.6 ± 1.2 μg/L). The within-group difference in the mean initial and mean final total arsenic concentrations in whole blood was significant for the Exposure Group ($p < 0.0005$) and the Pre-Post Group ($p = 0.003$).

Discussion

Urine and blood arsenic concentrations were significantly reduced after subjects stopped drinking the arsenic contaminated water and had effective arsenic water treatment systems installed in their home. In this study, the most informative data set is the Pre-Post Group of exposed subjects because they were the only subjects who were still drinking the arsenic contaminated water at the time they provided the initial urine and blood samples, and they also provided a post sample collected at the nine month time period after obtaining water treatment or ceasing to drink the water with elevated arsenic. Therefore, in this group the contrast was stronger which provided more power to the statistical analysis. Therefore, in these subjects the change from initial to final concentrations can be compared in light of removal of the arsenic drinking water exposure. The general characteristics of the Pre-Post Group were not very different from the overall Exposed Group (Table 2-4). The differences of note between the Exposed and Pre-Post Groups were: a higher cumulative arsenic ingestion dose, ingestion dose per body weight, initial inorganic-related arsenic in urine, and initial total arsenic in urine and blood. The higher initial urine and blood concentrations were expected due to these subjects continued exposure up to the date of the initial biomonitoring samples. The significant differences between the characteristics of the Pre-Post Group and the Control Group were the arsenic well water concentrations, cumulative arsenic ingestion dose, and dose per body weight. There were no significant differences between the three groups for potential dermal or dietary exposures.

The fact that seafood/fish ingestion was the best predictor of total arsenic in urine (Figure 2-3), even in the Pre-Post Group, confirmed the need to control for dietary confounding of urinary arsenic concentrations by using the HG-ICP-MS analysis method. Dietary confounding is evaluated in more detail in Chapter 2 and the potential impact of dermal exposure in this study is evaluated in more detail in Chapter 4.

The within-group difference in the geometric means of the initial and final inorganic-related arsenic concentrations in urine was significant for both the Exposure Group ($p < 0.001$) and the Pre-Post Group ($p < 0.001$), and was greatest in the Pre-Post Group at 9.5 ± 2.2 µg/g creatinine. The within-group difference in the mean initial and mean final total arsenic in whole blood was significant for the Exposure Group ($p < 0.0005$) and the Pre-Post Group ($p = 0.003$), and was again greater in the Pre-Post Group at 6.8 ± 1.9 µg/L.

The mean final total arsenic in blood of the Pre-Post Group (6.0 ± 0.8µg/L) was lower than in the Control Group (9.7 ± 1.6 µg/L). However, the mean final inorganic-related arsenic in urine of the Pre-Post Group (3.2 ± 0.6 µg/g creatinine) remained significantly higher than in the Control Group (1.5 ± 0.4 µg/g creatinine). The difference in the total arsenic in blood values may be attributed to a dietary influence, but the difference in the final inorganic-related urinary arsenic concentrations is cause for concern and could be related to a remaining

body burden of arsenic in the previously exposed subjects even after having the arsenic water ingestion exposure removed for approximately nine months.

The rebound in the inorganic-related urinary arsenic concentrations in the subjects at the home with the arsenic water treatment system breakthrough (Figure 3-5), shows how quickly a failure in a treatment system can result in the return of elevated inorganic-related arsenic in urine. The children in this family reported drinking home water at a rate of 0.56 L/d whereas the parents reported drinking home water at a rate of 0.35 L/d, and this difference may have contributed to the rebound in the children, Subjects 2503 and 2504, being greater than in the adults.

The best predictors of reduction in inorganic-related arsenic in urine and total arsenic in blood were their initial concentrations (Figure 3-3 and Figure 3-4).

The clearance of inorganic-related arsenic from urine appeared to have two phases in this study. This two-phase clearance of arsenic from urine is another indication that an excess body burden of arsenic was present in the exposed subjects even nine months after ceasing the drinking water exposure.

Most studies on arsenic clearance and half-life have been completed as acute dose experiments. A typical example is a 1981 study in which four subjects were given different arsenic doses for 5 days, and then followed for 14 days of urinary

monitoring when levels went down to near background concentrations (Buchet et al., 1981). Buchet reported a very rapid clearance in these acutely exposed subjects with a half life of one to two days. In another single dose arsenic retention study conducted with ^{74}As, a three compartment exponential model best fit the retention/excretion of the ^{74}As, with half lives of 2.1, 9.5, and 38.4 days (Pomroy et al., 1980). However, urinary excretion has only rarely been monitored consistently over time among chronically exposed persons after their exposure was reduced. Chronically exposed subjects may have a different pharmacokinetic profile than acute exposure subjects due to potential body burden in internal tissues. Storage of arsenic in the human body, including reaction with sulfhydryl groups in proteins, is known to occur in many organs including especially the skin (Bernstam et al., 2002; Nriagu and Bernstam, 2004), liver, kidney, muscle, and heart (Benramdane et al., 1999).

In one study, subjects were given 22 µg of As^{V} at regular 8-hour intervals for 10 days. This resulted in a significant proportion (about 50%) of the ingested arsenic to be retained in the body after 18 days of monitoring (Johnson and Farmer, 1991).

In a study in West Bengal, India, five families with 17 members (8 with arsenical skin lesions) were provided a new drinking water source with < 2 µg/L arsenic for two years. Although their arsenic biomonitoring levels decreased, even after two

years of using the new water source, the arsenic concentrations in urine, hair, and nails did not reach background concentrations (Mandal et al., 1998).

Investigations of the use of a chelation agent, dimercaptopropane sulfonate (DMPS), has shown that subjects, in both Chile and India, exposed to arsenic in drinking water will have a greatly increased arsenic concentration in their urine after administration of DMPS (Aposhian et al., 1997; Guha Mazumder et al., 2001). This suggests that arsenic is stored in the human body. The increased urinary excretion of arsenic after DMPS administration confirmed body retention and that the body burden was greater than indicated by the urinary arsenic level found without the DMPS challenge.

Data are reported for a population in northern Chile, chronically exposed to 600 µg/L arsenic in their drinking water, that were provided an alternate drinking water supply for two months containing only 45 µg/L (Hopenhayn-Rich et al., 1996). A substantial decrease in total urine arsenic was observed; however, the final urinary levels of arsenic were higher than what would be expected from consumption of drinking water at 45 µg/L. Inorganic-related urine arsenic levels determined by hydride generation atomic absorption spectroscopy dropped from 696 µg/L to only 185 µg/L at the end of the two-month study. This suggests that these subjects may still have been releasing arsenic from their body burden after the two month period. Other explanations could be that they were still being exposed to arsenic via water uses other than drinking and cooking, or non-

compliance with drinking the lower arsenic water that was provided by the investigators.

In another study, Hsueh, reported on people who had stopped consuming arsenic contaminated drinking water (700+ µg/L) 30 years prior to testing. The subjects were using water with less than 50 µg/L for 30-years, yet they still had inorganic-related urinary arsenic concentrations of 70-100 µg/L (Hsueh et al., 1998). Again, this suggests either continued release from body burden or continued exposure.

These studies give a strong indication that after chronic exposure to arsenic has ceased and an initial phase of arsenic clearance with a short half-life has ended, the body will only slowly release the remaining accumulated arsenic. The arsenic body burden could be a confounder in biomonitoring studies because the urinary arsenic concentrations may remain elevated above background levels for a significant length of time after the drinking water exposure has ended.

Limitations

One limitation of this study was the limited number of exposed subjects (24 for urine and 13 for blood) that were still being exposed to arsenic contaminated water at the time they provided their initial biomonitoring samples (Table 2-4). Another limitation was that blood samples were only analyzed for total arsenic rather than inorganic-related arsenic. Efforts were made to overcome this

limitation, but for whole blood samples the combination of matrix effect problems, digestion methods, and equipment limitations precluded the separation of inorganic-related arsenic and seafood/fish arsenic in whole blood.

The questionnaires depended on subject recall rather than measurement to determine each subject's exposure history. Subject characteristics such as rate of drinking water from the home water supply, the years of exposure to the home water supply, the weight of the subject, as well as the subject's bathing and dietary habits all depended on recall which has its limitations.

Some inorganic arsenic (As^{III} and As^{V}) is present in food and this inorganic arsenic and its metabolites, upon reaching the urine, would be included in the component we labeled "inorganic-related" arsenic in urine. There is no analytical method capable of separating the inorganic arsenic originating in food from the inorganic arsenic originating in ingested water. The average total inorganic arsenic intake from food in the United States, based on the FDA Total Diet Study, is estimated at 9 μg/d for adults, aged 25 and over, 5 μg/d for children aged 2-16 years, and 1.3 μg/d for children aged 6-11 months (National Research Council, 1999; Tao and Bolger, 1999). These intake amounts will have an effect on the final "inorganic-related" arsenic concentrations in urine. However, the effect should be similar in the Exposed, Pre-Post, and Control Groups in our study because there were no significant differences found between these groups in their potential arsenic exposure from dietary sources (Table 2-4).

Conclusions

Effective arsenic water treatment is available for residential well remediation. Effective arsenic water treatment reduces arsenic exposure as evidenced by statistically significant reductions in inorganic-related arsenic concentrations in urine and total arsenic concentrations in blood after initiation of water treatment.

A two-phase clearance of inorganic-related arsenic from urine was identified. At the end of the study, the previously exposed subject's geometric mean inorganic-related arsenic concentrations in urine were greatly reduced, but remained significantly higher than the geometric mean in the control subjects. The two-phase clearance combined with the failure of the previously exposed subjects' urine concentrations to decline to the level in the Control Group subjects, even approximately nine months after ceasing the drinking water exposure, indicates that an arsenic body burden is present in humans after chronic exposure to arsenic in drinking water.

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Chapter 4: Point-of-Entry Arsenic Water Treatment Provided Greater Urinary Arsenic Reduction than Point-of-Use Water Treatment

Abstract

BACKGROUND: Thousands of people in New Jersey and millions worldwide have been exposed to unacceptably high levels of arsenic by drinking water from contaminated wells. Special water treatment systems can remove arsenic from drinking water and can be configured to treat all the water in the home (point-of-entry) or water at only a single tap for drinking and cooking (point-of-use).

OBJECTIVE: To compare the effectiveness of point-of-entry and point-of-use arsenic water treatment systems in reducing arsenic exposure from well water.

METHODS: A non-random observational study was conducted with 49 subjects having elevated arsenic in their residential well water in New Jersey. The subjects obtained either point-of-entry or point-of-use arsenic water treatment. Prior ingestion exposure to arsenic in well water was determined by testing arsenic concentrations in the well water and obtaining water-use histories for each subject, including years of residence with the current well and amount of water consumed from the well per day. A series of urine samples were collected from the subjects, some starting before water treatment was installed, and continuing for at least nine months. Urine samples were analyzed for inorganic-related arsenic concentrations. Propensity scores were calculated to reduce bias

resulting from the non-random assignment of water treatment systems. Generalized estimating equations were used to examine the association between urinary arsenic and urinary arsenic reduction, by treatment group, at nine months after subjects stopped drinking the water or obtained water treatment, while adjusting for correlation among family members by using the propensity score as a covariate.

RESULTS: After nine months of water treatment, the adjusted mean urinary inorganic-related arsenic concentrations (± standard error) were significantly lower in the point-of-entry treatment group (2.5 ± 0.6 μg/g creatinine) than in the point-of-use treatment group (7.2 ± 0.8 μg/g creatinine). The adjusted mean urinary inorganic-related arsenic reduction was significantly greater in the point-of-entry group (10.2 ± 0.4 μg/g creatinine) than in the point-of-use group (8.1 ± 0.9 μg/g creatinine).

CONCLUSIONS: These results suggest that point-of-entry arsenic water treatment systems provide a more effective reduction of arsenic exposure from well water than that obtained by point-of-use treatment.

KEY WORDS: arsenic, biomonitoring, drinking water, exposure, New Jersey, point-of-entry, point-of-use, urinary arsenic, urine, water treatment, well water.

Introduction

Arsenic is a known human carcinogen and also increases the risk of many non-cancer health effects (ATSDR, 2007b). As a Group A carcinogen, arsenic has a Maximum Contaminant Level Goal (MCLG) of zero µg/L (USEPA, 2001). The New Jersey Department of Environmental Protection (NJDEP) has set the New Jersey Maximum Contaminant Level (MCL) for arsenic at 5 µg/L. The New Jersey MCL for arsenic is currently the most protective in the world.

Arsenic is widely distributed in New Jersey well water, reaching to just over 200 µg/L, with up to 30% of wells in some localities exceeding the New Jersey standard of 5 µg/L (see Chapter 1).

Arsenic is found in a variety of chemical forms in water, food, and living organisms. In well water in New Jersey, arsenic has been found to occur in two inorganic species: arsenate (As^{V}) and arsenite (As^{III}), mainly the former (Serfes et al., 2005; Spayd, 2007).

Arsenic Water Treatment

Special water treatment systems can remove arsenic from drinking water, and can be configured to treat all the water in the home or water at only a single tap for drinking and cooking (Spayd, 2007), thereby reducing arsenic concentrations as close to the MCLG of 0 µg/L as possible and thus reducing the risk of cancer and the many other health problems associated with arsenic exposure. The

NJDEP is conducting a study of the effectiveness of various arsenic water treatment systems, comparing both whole-house water treatment systems, commonly referred to as point-of-entry (POE) treatment, and single faucet treatment options for treating only drinking and cooking water, commonly referred to as point-of-use (POU) treatment. This study has been very successful with most treatment systems reducing arsenic in the treated water to levels below three µg/L, and many systems reducing the arsenic level to below one µg/L.

Arsenic Exposure From Well Water

Human exposure to arsenic in drinking water occurs mainly via ingestion (National Research Council, 1999; USEPA, 2001). However, secondary routes could arise from inhalation of aerosols during showering or cooking, and dermal absorption (Bernstam et al., 2002; Nriagu and Bernstam, 2004; Rahman et al., 1994; Wester et al., 2004; Wester et al., 1993) during showering, bathing (Weisel and Jo, 1996), or brushing of teeth. The contribution of these secondary routes in household exposure is uncertain (National Research Council, 1999). Other contaminants in drinking water have, in addition to the ingestion pathway, been found to be absorbed through the skin and inhaled while showering (Weisel and Jo, 1996).

The 1999 National Academy of Sciences Report on arsenic in drinking water states that “no controlled studies have been conducted on the rate of absorption of inorganic arsenic through intact human skin” (National Research Council,

1999). When EPA issued the proposed arsenic MCL rule in June 2000, they asked for comment on "whether available data on skin absorption and inhalation indicate that these are significant exposure routes that should be considered in the risk assessment" (USEPA, 2000). In the final arsenic MCL rule, published in January 2001, EPA was not able to assess any other potential arsenic exposures from drinking water sources except that from consumption via drinking and cooking. At the time of adoption of the arsenic MCL rule, EPA stated that exposure by modes other than consumption were not a concern (USEPA, 2001). Hence, the final rule allows POU treatment as an acceptable technology for arsenic exposure reduction.

Due to the chemical properties of arsenic, there is a high likelihood that skin contact with waters containing arsenic above drinking water standards will result in some arsenic absorption. The highly keratinized epidermis provides ample sulfhydryl binding sites for arsenic. Several studies have demonstrated the absorption of arsenic by mammalian skin including the skin of mice and monkeys (Rahman et al., 1994; Wester et al., 2004; Wester et al., 1993). Another recent study using artificial skin found absorption of both As^{V} and As^{III} at up to 8% of the applied dose per hour (Bernstam et al., 2002; Nriagu and Bernstam, 2004).

Though drinking and cooking with arsenic contaminated water is obviously the main exposure pathway in the home, the lack of data on exposure to arsenic via a household water supply from uses other than drinking and cooking (e.g.,

bathing, brushing teeth, etc.) is a major data gap. The above studies indicate that a POU system is inadequate to block all routes of exposure to arsenic in water. There is no assurance that a POU treatment system for reduction of arsenic in drinking and cooking water is sufficient to reduce the user's overall drinking water arsenic exposure and intake dose to levels with a typically acceptable increased lifetime cancer risk of one in a million. Because even low levels of arsenic exposure and dose (e.g., < 0.1 µg/d) are estimated to result in unacceptable cancer risks, these other exposures (e.g., bathing, brushing teeth, etc.) may represent a significant risk when arsenic water concentrations are above some currently undetermined level. The Agency for Toxic Substances and Disease Registry (ATSDR) states that one should not shower or bath in water with arsenic above 500 ppb (ATSDR, 2007a).

In homes with POE water treatment, all water taps in the home provide treated water. In homes with POU water treatment, typically only one water tap in the home, usually at the kitchen sink, provides treated water. In the POU homes, the opportunity for family members to ingest water from untreated taps in the home is very high.

Cost is another factor that must be considered. Arsenic water treatment systems are expensive in New Jersey with the average cost of installing a POE treatment system at $2,740 and a POU treatment system at $365 based on a cost survey conducted in 2003 (Spayd, 2007). Maintenance costs are also higher for POE

systems, and are just under $1.00 per day whereas the POU system maintenance is about $0.33 per day for each tap treated. Due to this cost difference, many families faced with the need to treat their water for arsenic opt for the less expensive POU treatment system.

The NJDEP study is evaluating both POE and POU water treatment systems, and this provided an opportunity to compare overall exposure reduction via the two types of treatment systems. Based on a literature review, it appears this is the first published study to compare the effectiveness of POE and POU water treatment systems in reducing exposure from arsenic or any other contaminant in residential well water.

Methods

Selection of Wells and Subjects

As the NJDEP study of arsenic in ground water proceeded, owners of wells with elevated arsenic concentrations were asked to participate in the present study of arsenic water treatment and biomonitoring. Fifty three subjects, in 22 families, with elevated arsenic concentrations in their residential well water, ranging from 8 to120 µg/L, were recruited. Five control subjects with arsenic drinking water concentrations below 3 µg/L also participated.

Recruitment and study procedures were reviewed and approved by the Institutional Review Board of the University of Medicine and Dentistry of New Jersey as described in Chapter 2.

Water Treatment Technologies Used

POE water treatment systems were installed in 12 homes where 31 subjects resided. The POE systems were predominantly adsorption media based systems.

POU water treatment systems were installed in 9 homes where 20 subjects resided. The POU systems were predominantly adsorption media based systems. One home used a POU reverse osmosis system. One home with two subjects did not install a treatment system, but used bottled water for all drinking and cooking as a surrogate for a POU treatment system. At a few homes, pre-treatment of the water was required for iron, manganese, and/or hardness.

Details of the treatment system designs are presented in Chapter 3.

Water Treatment Monitoring and Analysis

Throughout the project, arsenic levels were regularly measured in both the raw water entering the home from the well and the treated water, as described in detail in Chapter 2.

Biomonitoring Sample Collection

A series of urine samples were collected from the subjects, some starting before water treatment was installed, and continuing for at least nine months. Blood samples were collected at the start and end of the water treatment study. The biomonitoring protocol is described in detail in Chapter 2.

Questionnaire

Based on in-person interviews, a household water use and exposure history questionnaire (Appendix 1) was completed for each subject by the investigator. A detailed description of the questionnaire is provided in Chapter 2. Briefly, the purpose of the questionnaire was to determine the arsenic exposure history for each subject and to estimate each subject's cumulative arsenic ingestion dose from drinking and cooking with the water prior to obtaining arsenic water treatment.

Laboratory Analysis

All biomonitoring samples were analyzed at the EOHSI Chemical Analysis laboratory, as described in detail in Chapter 2. Briefly, analysis of total arsenic in urine was conducted by ICP-MS, and a speciation technique (hydride generation) was employed to determine the sum of six arsenic species in urine related to arsenic exposure via drinking water, and termed inorganic-related arsenic. Creatinine measurements were conducted using a standard assay kit, and

urinary arsenic measurements are presented as micrograms As per gram of creatinine (µg/g creatinine). Whole blood samples were also analyzed at EOHSI for total arsenic by ICP-MS using the standard addition method.

Data Analysis

Statistical analyses were run using SPSS Version 15.0 for Windows. Tests for normality were run and several data sets displayed a log-normal distribution. These data sets were transformed using the natural log function to make their distributions closer to a normal distribution. The transformed data sets were: well water arsenic concentration, home water ingestion rate, years of exposure to home water supply, cumulative arsenic ingestion dose, ingestion dose per body weight, total arsenic in blood, total arsenic in urine, inorganic-related arsenic in urine, and urine creatinine. The data analyses were run with the dependent variables, inorganic-related arsenic in urine and reduction in inorganic-related arsenic in urine, transformed and non-transformed, and the significance of the results were of the same order of magnitude. Due to the difficulty interpreting results from transformed variables, only the non-transformed results are presented here. Scatter plots and bivariate Pearson correlation coefficients were used to examine relationships between variables. Paired t-tests were used to compare means of initial and final biomonitoring data within groups and independent t-tests were used to compare means between the groups.

In a non-randomized observational study like this one, there was little or no control over the assignment of treatment group. Therefore, the resulting POE and POU treatment groups may have large biases on some of the observed covariates (Table 4-1). The propensity score, in this case defined as the conditional probability of being in one treatment group or the other given the covariates, can be used to balance the covariates, reduce bias, and create a quasi-randomized experiment (D'Agostino, 1998). Propensity scores were calculated using predicted probabilities from the results of a logistic regression model with the binary dependent variable being treatment group and covariates including prior arsenic ingestion dose per body weight, age, and showers per week at home. These covariates were chosen using stepwise selection methods such that prior arsenic ingestion dose per body weight, age, and showers per week at home were significant at the 0.10 level. See Appendices 2 and 3 for the logistic regression results and the resulting propensity scores for each subject. These propensity scores are entered as adjustment covariates in the analyses examining the effects of treatment group on urinary arsenic.

In observational studies like this one where some of the subjects were family members sharing the same well and water treatment system, the analysis must account for the potential correlation of data within families. If only a standard statistical analysis was used (e.g., analysis of covariance), which assumes all observations are independent, the results may be misleading (Ghisletta and Spini, 2004; Hanley et al., 2003). Generalized estimating equations (GEE) are

an extension of the basic generalized linear model, and were developed to accommodate the analysis of correlated data (Ghisletta and Spini, 2004; Hanley et al., 2003). GEE provides population-averaged estimates of regression coefficients. Therefore, GEE was used to examine the association between urinary arsenic and urinary arsenic reduction, by treatment group, at nine months after subjects stopped drinking the water or obtained water treatment, while controlling for correlation among family members and including propensity scores as a covariate.

Results

Wells and Subjects of the Overall Study Population

Characteristics of the study population by treatment group are presented in Table 4-1. Fifty three subjects with elevated arsenic concentrations in their residential well water were recruited. A total of four subjects were lost to follow-up or provided insufficient samples to be included in the analyses. Therefore, sufficient data was collected on 49 subjects, in 19 families (Exposed Group), to be included in at least part of the analysis. Five control subjects, from five different families, with arsenic drinking water concentrations below 3 µg/L also participated as the Control Group. Most subjects (92%) were Caucasian, three were African American, and one was Asian. Subjects were evenly divided between male and female, though all five of the control subjects were male. The mean and standard error (SE) of the age of the overall study population was 39 ± 2.9 years. The POE water treatment group had the youngest mean age and was

significantly younger than the POU Group ($p = 0.003$) and the Control Group ($p = 0.001$). Children, under 18 years old, made up 28% of the overall study population, and had the highest percentage (40%) in the POE Group. The mean weight of the study population was 62 ± 3.5 kilograms (Kg). Due to the relatively high percentage of children in the POE Group, it had the lowest mean weight and was significantly lower than the Control Group ($p = 0.027$). The Control Group, made up of only male adults, was the oldest and heaviest group. There was only one smoker in the overall study population, and this person was one of the cases.

The mean of the untreated water arsenic concentrations were very similar in the POE and POU groups at 44 ± 5 µg/L and 47 ± 8 µg/L. All POE and POU subjects had water arsenic concentrations exceeding the New Jersey arsenic drinking water standard of 5 µg/L, and 94% of these subjects' water arsenic concentrations exceeded the USEPA federal standard and the World Health Organization guideline level of 10 µg/L. All of the control subjects had water arsenic concentrations less than 3 µg/L. The arsenic well water concentrations were significantly lower in the Control Group than in the POE and POU Groups ($p < 0.0005$). The mean rate of drinking water at home for the overall study population was 1.0 ± 0.1 liter per day (l/d). At a mean of 1.3 ± 0.2 l/d, the POU Group ingested significantly ($p = 0.038$) more water at home than the POE Group which only drank a mean of 0.7 ± 0.1 l/d. The years of exposure to the home water supply was significantly greater ($p = 0.012$) in the POU Group (17.5 ± 3.6

years) than in the POE Group (7.2 ± 0.9 years). The cumulative arsenic ingestion dose in mg was calculated by multiplying the arsenic concentration of the well water in mg/l by the rate of drinking water from the home water supply in l/d by the years of exposure to the home water supply by 365 d/year. The mean cumulative arsenic ingestion dose was significantly greater in the POE Group ($p < 0.0005$) and in the POU Group ($p = 0.001$) than in the Control Group. The mean cumulative arsenic ingestion dose was significantly ($p = 0.017$) greater in the POU Group (284 ± 73 mg) than in the POE Group (89 ± 16 mg). The arsenic ingestion dose per body weight was significantly greater in the POE Group ($p < 0.0005$) and in the POU Group ($p < 0.0005$) than in the Control Group. The arsenic ingestion dose per body weight was greater in the POU Group (4.1 ± 1.1 mg/Kg) than in the POE Group (1.8 ± 0.4 mg/Kg) but the difference was only borderline significant ($p = 0.052$).

Water Treatment Effectiveness

All but one of the arsenic water treatment systems in the study consistently and effectively reduced the arsenic concentrations in water to below 3 µg/L. At one home, the arsenic water treatment system (POE) had a temporary arsenic breakthrough during the biomonitoring program that was identified by the NJDEP water treatment system monitoring program. The homeowners were notified by NJDEP to switch to bottled water until the problem with the treatment system was corrected. The urine data collected from the breakthrough time period was excluded from the statistical analyses.

Potential Dermal Exposure Pathways

The mean rate of showering at home for the overall study population was 5.4 ± 0.3 showers per week and there was no significant difference among the groups. The mean number of baths per week was 0.6 ± 0.2 and the POE Group was significantly higher ($p = 0.017$) than the Control Group that took 0 baths per week. The mean rate of teeth brushing at home for the overall study population was 11.7 ± 0.6 times per week and the POE Group at 10.6 ± 0.9 teeth brushings per week was significantly lower ($p < 0.0005$) than the Control Group at 14.4 ± 0.4 teeth brushings per week. Some homes had swimming pools and/or hot tubs and the mean number of days using the pool and/or hot tubs for the overall study population was 0.4 ± 0.2 times per week during the swimming season. There were no significant differences between the groups for swimming pool and hot tub use.

Dietary Arsenic Exposure

The overall study population had a mean of 1.4 ± 0.2 meals per week with seafood or fish, 0.5 ± 0.1 meals per week with mushrooms, 1.4 ± 0.2 meals per week with rice, and 2.7 ± 0.2 meals per week with poultry. There were no significant between-group differences for dietary exposures in the overall study population.

Table 4-1: Characteristics of all Study Subjects by Treatment Group

Water Treatment Groups	POE	POU	Control
General			
Subjects Per Water Treatment Group (n)	30	19	5
Families Per Water Treatment Group (n)	11	8	5
Race (% Caucasian)	100	84	80
Sex (% Male)	47	53	100
Age in Years, (Mean ± SE)	30 ± 3 *†	49 ± 5 *	54 ± 4
Children < 18 Years Old (%)	40	16	0
Weight in Kg, (Mean ± SE)	55 ± 5 †	68 ± 5	83 ± 7
Any Tobacco Use During Study (%)	3	0	0
Prior Water Ingestion Exposure (Mean ± SE)			
Well Water As (µg/L)	44 ± 5 †	47 ± 8 †	1.1 ± 0.5
As Water Ingestion Reported (L/d)	0.7 ± 0.1 *	1.3 ± 0.2 *	1.3 ± 0.6
Years of Exposure	7.2 ± 0.9 *	17.5 ± 3.6 *	7 ± 5
Cumulative As Ingestion Dose (mg)	89 ± 16 †	284 ± 73 †	3 ± 2
Ingestion Dose per Body Weight (mg/Kg)	1.8 ± 0.4 †	4.1 ± 1.1 †	0.05 ± 0.03
Dermal Exposure (Mean ± SE)			
Showers per Week at Home	5.4 ± 0.5	5.0 ± 0.6	6.6 ± 0.5
Baths per Week at Home	0.6 ± 0.2 †	0.7 ± 0.4	0 ± 0
Teeth Brushing per Week at Home	10.6 ± 0.9 †	12.7 ± 1.1	14.4 ± 0.4
Pool Use per Week During Season	0.3 ± 0.2	0.2 ± 0.1	1.9 ± 1.4
Dietary Exposure (Mean ± SE)			
Seafood and Fish Meals per Week	1.2 ± 0.2	1.8 ± 0.3	1.5 ± 0.4
Mushrooms with Meals per Week	0.5 ± 0.2	0.4 ± 0.1	0.3 ± 0.1
Rice with Meals per Week	1.5 ± 0.1	1.2 ± 0.2	2.0 ± 1.3
Poultry with Meals per Week	2.7 ± 0.2	2.6 ± 0.4	2.8 ± 0.6

* $p < 0.05$, significant difference between POE and POU, using independent T-Test.
† $p < 0.05$, significant difference from Control Group, using independent T-Test.

Grouping Subjects by Sample Collection Dates to Analyze Biomonitoring Results

To compare the effectiveness of POE and POU arsenic water treatment systems in reducing arsenic exposure from well water, the subjects had to be grouped into two subsets.

The first subset, called the “Post-Only” Group, includes 36 subjects. This subset includes all subjects who provided a nine-month urine sample.

The second subset, called the “Pre-Post” Group, includes 24 subjects. This subset includes all subjects who provided both an initial urine sample collected before obtaining water treatment or ceasing to drink the water with elevated arsenic, and a nine-month urine sample.

Post-Only Group Results

The characteristics of the Post-Only Group POE and POU Groups are given in Table 4-2 along with the Control Group for comparison. Thirty-six subjects (68% of the study population) met the criteria to be included in the Post-Only Group. In this subset, there are 20 POE subjects in nine families and 16 POU subjects in seven families. When comparing the Post-Only Group subjects’ characteristics in Table 4-2 to the overall study subjects’ characteristics in Table 4-1, the main differences are in the prior water ingestion exposure area. The differences between the POE and POU group became greater for all five variables in this category. Most importantly, the cumulative arsenic ingestion dose and ingestion dose per body weight remained significantly different between the two groups with p-values of 0.004 and 0.009 respectively. The difference in age between the two groups also remained significant with a p-value of 0.006. The POE, POU, and Control Groups remained very similar on dermal and dietary exposure variables.

The urine results were corrected for creatinine and are presented as µg/g creatinine. The mean creatinine level was highest in the Control Group (1.8 ± 0.2 g/L), but was not significantly higher than in the POE Group (1.5 ± 0.1 g/L) or the POU Group (1.4 ± 0.1 g/L).

For inorganic-related arsenic, the geometric mean nine-month urine sample concentrations for the POE Group of 3.0 ± 0.5 µg/g creatinine and the POU Group of 3.7 ± 0.8 µg/g creatinine were not significantly different from each other, but both were significantly higher than the Control Group at 1.5 ± 0.4 µg/g creatinine (with respective p-values of < 0.0005 and 0.024).

A one-way between groups analysis of covariance (ANCOVA) was conducted to compare the effectiveness of treatment type (POE vs POU) on the inorganic-related urinary arsenic concentrations after nine months of treatment (Appendix 4). The independent variable was treatment type, and the dependent variable was the inorganic-related urinary arsenic concentrations after nine months of treatment. The subject's propensity scores were used as a covariate in this analysis. Preliminary checks were conducted to ensure that there was no violation of the assumptions of normality, linearity, homogeneity of variances, homogeneity of regression slopes, and reliable measurements of the variables. After adjusting for the propensity scores, there was a significant difference between the means of the inorganic-related urinary arsenic concentrations after

nine months of treatment with the POE group (2.7 ± 0.6 µg/g creatinine) significantly ($p = 0.005$) lower than the POU Group (6.1 ± 0.7 µg/g creatinine).

When GEE was used to examine the association between inorganic-related urinary arsenic concentrations at nine months and treatment group, for the Post-Only Group, while controlling for correlation among family members, and the propensity score as a covariate, a significant difference between the POE and POU groups remained strong ($p = 0.002$) (Appendix 5). The GEE estimated means ± the SE of the nine-month inorganic-related urine arsenic concentrations were 2.7 ± 0.6 µg/g creatinine for the POE Group and 6.1 ± 0.7 µg/g creatinine for the POU Group, the same as when ANCOVA was run.

Whole blood samples were analyzed for total arsenic only. The geometric mean of the initial blood arsenic concentrations for the POE Group (9.8 ± 2.1 µg/L) and the POU Group (10.6 ± 1.5 µg/L) were not significantly different from each other or the Control Group (9.2 ± 1.6 µg/L). The geometric mean of the final blood arsenic concentrations for the POE Group (6.9 ± 1.0 µg/L) and the POU Group (6.3 ± 1.1 µg/L) were also not significantly different from each other or the Control Group.

Table 4-2: Characteristics of Subjects in the Post-Only Group by Treatment Group

Water Treatment Groups	POE	POU	Control
General			
Subjects Per Water Treatment Group (n)	20	16	5
Families Per Water Treatment Group (n)	9	7	5
Race (% Caucasian)	100	100	80
Sex (% Male)	55	50	100
Age in Years, (Mean ± SE)	33 ± 4 *†	52 ± 5 *	54 ± 4
Children < 18 Years Old (%)	30	13	0
Weight in Kg, (Mean ± SD)	58 ± 6	70 ± 5	83 ± 7
Any Tobacco Use During Study (%)	5	0	0
Prior Water Ingestion Exposure (Mean ± SE)			
Well Water As (µg/L)	45 ± 7 †	33 ± 5 †	1.1 ± 0.5
As Water Ingestion Reported (L/d)	0.6 ± 0.1 *	1.6 ± 0.2 *	1.3 ± 0.6
Years of Exposure	6.6 ± 1.2 *	20.3 ± 3.9 *	7 ± 5
Cumulative As Ingestion Dose (mg)	67 ± 19 *†	338 ± 80 *†	3 ± 2
Ingestion Dose per Body Weight (mg/Kg)	1.3 ± 0.4 *†	4.9 ± 1.2 *†	0.05 ± 0.03
Dermal Exposure (Mean ± SE)			
Showers per Week at Home	5.4 ± 0.6	5.0 ± 0.6	6.6 ± 0.5
Baths per Week at Home	0.6 ± 0.3	0.6 ± 0.4	0 ± 0
Teeth Brushing per Week at Home	10.3 ± 1.2 †	12.4 ± 1.2	14.4 ± 0.4
Pool Use per Week During Season	0.5 ± 0.2	0.2 ± 0.1	1.9 ± 1.4
Dietary Exposure (Mean ± SE)			
Seafood and Fish Meals per Week	1.3 ± 0.2	1.7 ± 0.3	1.5 ± 0.4
Mushrooms with Meals per Week	0.5 ± 0.3	0.4 ± 0.1	0.3 ± 0.1
Rice with Meals per Week	1.4 ± 0.2	1.3 ± 0.3	2.0 ± 1.3
Poultry with Meals per Week	2.5 ± 0.3	2.3 ± 0.4	2.8 ± 0.6
Urine Biomonitoring (Mean ± SE)			
Creatinine (g/L)	1.5 ± 0.1	1.4 ± 0.1	1.8 ± 0.2
Arsenic in Urine (Geometric Mean ± SE)			
Inorganic-related As at Nine-Months (µg/g creatinine)	3.0 ± 0.5 †	3.7 ± 0.8 †	1.5 ± 0.4
Adjusted Arsenic in Urine[a] (Estimated Mean ± SE)			
Inorganic-related As at Nine-Months (µg/g creatinine)	2.7 ± 0.6 *	6.1 ± 0.7 *	
Blood Biomonitoring (Geometric Mean ± SE)			
Total Arsenic Initial Blood (µg/L)	9.8 ± 2.1	10.6 ± 1.5	9.2 ± 1.6
Total Arsenic Final Blood (µg/L)	6.9 ± 1.0	6.3 ± 1.1	

* $p < 0.05$, significant difference between POE and POU.

† $p < 0.05$, significant difference from Control.

[a] Inorganic-related arsenic in final urine adjusted for propensity score and family correlation in GEE. Control subjects provided only one blood and urine sample.

Pre-Post Group Results

Characteristics of the subjects who were still drinking their arsenic contaminated well water at the time of their initial urine sample collection and who provided a nine-month urine sample are presented in Table 4-3. Twenty four of the exposed subjects (45% of the study population) met this criterion. For comparison purposes, the five control subjects' data are also presented in Table 4-3.

In the Pre-Post Group, there are 16 POE subjects in eight families and eight POU subjects in four families. When comparing the Pre-Post Group subjects' characteristics in Table 4-3 to the Post-Only Group of subjects' characteristics in Table 4-2, the main observation is that the prior water ingestion exposure variables continue to have the major differences between the POE and POU groups. The difference in age between the two groups also remained significant with a p-value < 0.0005. In this subset, meals with rice per week were significantly greater ($p = 0.005$) in the POE group (1.5 ± 0.2 meals per week) than in the POU group (0.5 ± 0.2 meals per week).

The unadjusted geometric mean inorganic-related arsenic in the initial urine sample concentrations for the POE Group (9.0 ± 1.9 µg/g creatinine) and the POU Group (14.8 ± 4.8 µg/g creatinine) were not significantly different from each other, but both were significantly higher than in the Control Group at 1.5 ± 0.4 µg/g creatinine (with respective p-values of < 0.0005 and 0.024). The unadjusted

geometric mean inorganic-related arsenic concentration in the nine-month urine samples for the POE Group (3.4 ± 0.5 µg/g creatinine) and the POU Group (2.8 ± 1.4 µg/g creatinine) were not significantly different from each other, but the POE Group was significantly higher than in the Control Group ($p = 0.026$). The unadjusted mean reduction in inorganic-related urinary arsenic from the initial sample to the nine-month sample was not significantly different between the POE (7.3 ± 2.1 µg/g creatinine) and POU (13.8 ± 4.9 µg/g creatinine) Groups when analyzed with an independent T-Test.

The within-group differences in the mean initial and mean nine-month inorganic-related arsenic concentrations in urine were significant for both the POE Group ($p = 0.003$) and the POU Group ($p = 0.027$).

A one-way between groups analysis of covariance (ANCOVA) was conducted to compare the effectiveness of treatment type (POE vs POU) on the inorganic-related urinary arsenic concentrations after nine months of treatment in the Pre-Post Group (Appendix 6). The independent variable was treatment type, and the dependent variable was the inorganic-related urinary arsenic concentrations after nine months of treatment. The subject's propensity scores, age, and natural log of cumulative arsenic ingestion dose per body weight, were used as covariates in this analysis. Preliminary checks were conducted to ensure that there was no violation of the assumptions of normality, linearity, homogeneity of variances, homogeneity of regression slopes, and reliable measurements of the variables.

After adjusting for the covariates, there was a significant difference between the means of the inorganic-related urinary arsenic concentrations after nine months of treatment with the POE group (2.6 ± 0.7 µg/g creatinine) significantly lower ($p = 0.016$) than the POU Group (7.0 ± 1.2 µg/g creatinine).

When GEE was used to examine the association between inorganic-related urinary arsenic concentrations after nine months of treatment in the Pre-Post Group, while controlling for correlation among family members, and the propensity score, age, natural log of cumulative arsenic ingestion dose per body weight, and showers per week as covariates, the significant difference between the POE and POU groups was stronger ($p < 0.0005$) (Appendix 7). The GEE estimated means ± the SE of the nine-month inorganic-related urine arsenic concentrations were 2.5 ± 0.6 µg/g creatinine for the POE Group and 7.2 ± 0.8 µg/g creatinine for the POU Group.

The GEE analysis also found the mean reduction in inorganic-related urinary arsenic from the initial sample to the nine-month sample to be significantly different ($p = 0.04$) between the POE and POU Groups. The GEE estimated means ± the SE of the urinary inorganic-related arsenic reduction were 10.2 ± 0.4 µg/g creatinine for the POE Group and 8.1 ± 0.9 µg/g creatinine for the POU Group.

Table 4-3: Characteristics of Subjects in the Pre-Post Group by Treatment Group

Water Treatment Groups	POE	POU	Control
General			
Subjects Per Water Treatment Group (n)	16	8	5
Families Per Water Treatment Group (n)	8	4	5
Race (% Caucasian)	100	100	80
Sex (% Male)	63	50	100
Age in Years, (Mean ± SE)	31 ± 5 *†	61 ± 3 *	54 ± 4
Children < 18 Years Old (%)	38	0	0
Weight in Kg, (Mean ± SD)	58 ± 8 †	70 ± 5	83 ± 7
Any Tobacco Use During Study (%)	6	0	0
Prior Water Ingestion Exposure (Mean ± SE)			
Well Water As (µg/L)	40 ± 8 †	45 ± 7 †	1.1 ± 0.5
As Water Ingestion Reported (L/d)	0.7 ± 0.1 *	1.5 ± 0.4 *	1.3 ± 0.6
Years of Exposure	6.8 ± 1.2 *	27.0 ± 6.1 *†	7 ± 5
Cumulative As Ingestion Dose (mg)	65 ± 19 *†	482 ± 122 *†	3 ± 2
Ingestion Dose per Body Weight (mg/Kg)	1.3 ± 0.5 *†	7.0 ± 1.8 *†	0.05 ± 0.03
Dermal Exposure (Mean ± SE)			
Showers per Week at Home	5.1 ± 0.7	6.4 ± 0.6	6.6 ± 0.5
Baths in per Week at Home	0.8 ± 0.4	0.03 ± 0.03	0 ± 0
Teeth Brushing per Week at Home	10.8 ± 1.1 †	14.0 ± 1.3	14.4 ± 0.4
Pool Use per Week During Season	0.4 ± 0.3	0.4 ± 0.3	1.9 ± 1.4
Dietary Exposure (Mean ± SE)			
Seafood and Fish Meals per Week	1.1 ± 0.2	1.8 ± 0.4	1.5 ± 0.4
Mushrooms with Meals per Week	0.5 ± 0.3	0.3 ± 0.2	0.3 ± 0.1
Rice with Meals per Week	1.5 ± 0.2 *	0.5 ± 0.2 *	2.0 ± 1.3
Poultry with Meals per Week	2.6 ± 0.4	1.6 ± 0.4	2.8 ± 0.6
Urine Biomonitoring (Mean ± SE)			
Creatinine (g/L)	1.6 ± 0.1	1.5 ± 0.2	1.8 ± 0.2
Arsenic in Urine (Geometric Mean ± SE)			
Initial Inorganic-Related As (µg/g creatinine)	9.0 ± 1.9 †#	14.8 ± 4.8 †#	1.5 ± 0.4
Inorganic-Related As at Nine-Months (µg/g creatinine)	3.4 ± 0.5 †#	2.8 ± 1.4 #	
Inorganic-Related As Reduction (Mean µg/g creatinine)	7.3 ± 2.1	13.8 ± 4.9	
ANCOVA Adjusted As in Urine[a] (Est. Mean ± SE)			
Inorganic-Related As at Nine-Months (µg/g creatinine)	2.6 ± 0.7 *	7.0 ± 1.2 *	
GEE Adjusted As in Urine[a] (Estimated Mean ± SE)			
Inorganic-Related As at Nine-Months (µg/g creatinine)	2.5 ± 0.6 *	7.2 ± 0.8 *	
Inorganic-Related As Reduction (µg/g creatinine)	10.2 ± 0.4 *	8.1 ± 0.9 *	
Blood Biomonitoring (Geometric Mean ± SE)			
Total Arsenic Initial Blood (µg/L)	10.2 ± 2.4	9.7 ± 1.5	9.2 ± 1.6
Total Arsenic Final Blood (µg/L)	6.2 ± 0.9	5.3 ± 1.7	

* $p < 0.05$, significant difference between POE and POU.

† $p < 0.05$, significant difference from Control.

# $p < 0.05$, significant difference within group between initial and final concentration

[a] Inorganic-related arsenic in final urine adjusted for propensity score and family correlation in GEE. Control subjects provided only one blood and urine sample.

Whole blood samples were analyzed for total arsenic only. The geometric mean of the initial blood arsenic concentrations for the POE Group (10.2 ± 2.4 µg/L) and the POU Group (9.7 ± 1.5 µg/L) were not significantly different from each other or the Control Group (9.2 ± 1.6 µg/L). The geometric mean of the final blood arsenic concentrations for the POE Group (6.2 ± 0.9 µg/L) and the POU Group (5.3 ± 1.7 µg/L) were also not significantly different from each other or the Control Group.

Discussion

In this study, the water treatment technologies performed very well. They consistently reduced water arsenic concentrations from as high as 120 µg/L to concentrations less than 3 µg/L. However, the reduction of inorganic-related arsenic in the urine of study subjects was not the same in the POE and POU water treatment groups. The ANCOVA and GEE-adjusted results from this study demonstrate whole-house POE arsenic water treatment reduced inorganic-related arsenic in urine to significantly lower concentrations than single-tap POU arsenic water treatment.

Controlling for Study Design

The non-randomized nature of this observational study resulted in the need for propensity score and GEE analysis. The POE and POU water treatment groups were significantly different on several covariates including age, weight, amount of

water consumed at home per day, years of exposure to the arsenic contaminated well water, and prior cumulative arsenic ingestion dose from the well water (Table 4-1, 4-2, and 4-3). The POE, POU, and Control groups were similar on potential dermal and dietary arsenic exposure variables. Propensity scores were calculated for each subject to balance the covariates, reduce the bias presented by the covariates, and convert the study into a quasi-randomized experiment (D'Agostino, 1998).

Furthermore, because some of the subjects were family members sharing the same well and water treatment system, GEE was needed to allow for analysis of the correlated observations within families. The correlation within families turned out to be low as the GEE analyses accounting for family correlation was very similar to the ANCOVA analyses which did not.

Comparison to NHANES Data

The NHANES 2003-2004 survey has provided urinary arsenic data for a representative sample of the US population (Caldwell et al., 2008). In this NHANES survey, the geometric mean of total urinary arsenic for 2557 participants was 8.3 µg/L and 8.2 µg/g creatinine. For inorganic-related urinary arsenic the geometric mean was not published, but the 50^{th} percentile of the data was 6.0 µg/L.

A comparison of the NHANES data with the urinary arsenic data from the present study is presented in Tables 2-8, 2-9, and 2-10 with geometric means and the 25th, 50th, 75th, and 95th percentiles. The 95th percentiles for total urinary arsenic concentrations from NHANES were 65.4 µg/L and 50.2 µg/g creatinine. For inorganic-related arsenic, the 95th percentile was 18.9 µg/L. When evaluating the NHANES data, it must be remembered that this is a representative sample, not an unexposed sample. Some unknown percentage of the NHANES sample was no doubt exposed to elevated arsenic concentrations in drinking water from both residential and public well water as the samples were collected in 2003-2004, which was before the January 2006 effective date of the new US drinking water standard for arsenic being reduced from 50 µg/L to 10 µg/L.

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The comparison of the present study's urinary arsenic concentrations with those found in NHANES shows that the Pre-Post Group's POE initial geometric mean total arsenic in urine at 18.4 ± 4.8 µg/g creatinine is between the 75th and 90th percentile of the NHANES sample of 2557 subjects. The Pre-Post POU initial geometric mean total arsenic in urine at 38.0 ± 19.2 µg/g creatinine is between the 90th and 95th percentile of the NHANES sample (Table 4-4).

This present study's Control Group's total arsenic in urine with a geometric mean of 13.8 ± 2.8 µg/g creatinine is within the 95% confidence interval of the 75th percentile (14.1 µg/g creatinine) of the NHANES sample (Table 4-4).

Table 4-4: Comparison of Treatment Group Data to NHANES 2003-2004							
Total Arsenic Data (µg/g creatinine)				Selected Percentiles			
Groups		Sample Size	Geometric Mean	25	50	75	95
NHANES		2557	8.2	4.2	7.0	14.1	50.2
This Study	Controls	5	13.8	9.3	13.7	21.4	22.2
	Pre-Post POE Time Period 0	16	18.4	9.3	18.9	32.8	
	Pre-Post POU Time Period 0	8	38.0	21.9	31.0	90.2	

The inorganic-related arsenic in urine concentrations in the present study can be compared to the NHANES sum of urinary inorganic-related arsenic species. The Pre-Post Group's POE initial geometric mean of inorganic-related arsenic in urine at 13.5 ± 4.8 µg/L is between the 50th and 95th percentile of the NHANES sample of 2557 subjects. The Pre-Post POU initial geometric mean of inorganic-related arsenic in urine at 30.0 ± 18.2 µg/L is well above the 95% confidence interval of the 95th percentile of the NHANES sample (Table 4-5).

This present study's Control Group's inorganic-related arsenic in urine with a geometric mean of 3.9 ± 1.0 µg/L is below the 95% confidence interval of the 50th percentile (6.0 µg/L) of the NHANES sample (Table 4-5).

Table 4-5: Comparison of Treatment Group Data to NHANES 2003-2004							
Inorganic-Related Arsenic Data (µg/L)				Selected Percentiles			
Groups		Sample Size	Geometric Mean	25	50	75	95
NHANES		2557	N/A	<LOD	6.0	N/A	18.9
This Study	Controls	5	3.9	2.5	3.6	6.6	7.6
	Pre-Post POE Time Period 0	16	13.5	10.7	14.1	16.7	
	Pre-Post POU Time Period 0	8	30.0	14.2	26.3	50.8	

The Post-Only Group's POE nine-month geometric mean total arsenic in urine at 16.6 ± 5.5 µg/g creatinine is within the 95% confidence interval of the 75th percentile (14.1 µg/g creatinine) of the NHANES sample. The Post-Only POU nine-month geometric mean total arsenic in urine at 19.3 ± 7.1 µg/g creatinine is between the 75th and 90th percentile of the NHANES sample (Table 4-6).

The Pre-Post Group's POE nine-month geometric mean total arsenic in urine at 17.2 ± 6.8 µg/g creatinine is within the 95% confidence interval of the 75th percentile (14.1 µg/g creatinine) of the NHANES sample. The Pre-Post POU nine-month geometric mean total arsenic in urine at 19.4 ± 5.1 µg/g creatinine is between the 75th and 90th percentile of the NHANES sample (Table 4-6).

Table 4-6: Comparison of Treatment Group Data to NHANES 2003-2004							
Total Arsenic Data (µg/g creatinine)				Selected Percentiles			
Groups		Sample Size	Geometric Mean	25	50	75	95
NHANES		2557	8.2	4.2	7.0	14.1	50.2
This Study	Controls	5	13.8	9.3	13.7	21.4	22.2
	Post-Only POE at Nine-Months	20	16.6	10.7	14.9	23.2	115.2
	Post-Only POU at Nine-Months	16	19.3	12.2	15.5	30.9	
	Pre-Post POE at Nine-Months	16	17.2	10.1	14.9	23.6	
	Pre-Post POU at Nine-Months	8	19.4	13.3	14.7	31.0	

The Post-Only Group's POE nine-month geometric mean of inorganic-related arsenic in urine at 5.9 ± 1.1 µg/L is within the 95% confidence interval of the 50th percentile of the NHANES sample. The Post-Only POU nine-month geometric mean of inorganic-related arsenic in urine at 7.0 ± 1.9 µg/L is slightly above the 95% confidence interval of the 50th percentile of the NHANES sample (Table 4-7).

The Pre-Post Group's POE nine-month geometric mean of inorganic-related arsenic in urine at 7.0 ± 1.2 µg/L is slightly above the 95% confidence interval of the 50th percentile of the NHANES sample. The Pre-Post POU nine-month

geometric mean of inorganic-related arsenic in urine at 6.2 ± 3.8 µg/L is also slightly above the 95% confidence interval of the 50th percentile of the NHANES sample (Table 4-7).

Table 4-7: Comparison of Treatment Group Data to NHANES 2003-2004

Inorganic-Related Arsenic Data (µg/L)				Selected Percentiles			
Groups		**Sample Size**	**Geometric Mean**	**25**	**50**	**75**	**95**
NHANES		2557	N/A	<LOD	6.0	N/A	18.9
This Study	Controls	5	3.9	2.5	3.6	6.6	7.6
	Post-Only POE at Nine-Months	20	5.9	3.3	7.0	10.0	17.0
	Post-Only POU at Nine-Months	16	7.0	3.6	7.4	12.1	
	Pre-Post POE at Nine-Months	16	7.0	4.3	7.6	12.3	
	Pre-Post POU at Nine-Months	8	6.2	2.3	5.8	16.5	

The comparison of the NHANES data with the data from the present study shows that compared to a representative sample of the US population, the Post-Only and Pre-Post Group initial arsenic concentrations were quite elevated (between the 75th and 95th percentiles of the NHANES sample), and after having effective arsenic water treatment for approximately nine months, the concentrations had dropped to near the 50th percentile of the NHANES sample inorganic-related

arsenic concentrations. This means that an average exposed subject in the present study had an inorganic-related urinary arsenic concentration comparable to an NHANES subject at near the 75th to 95th percentile. Furthermore, an average Control Group subject in the present study, who did not drink arsenic contaminated water, had an inorganic-related urinary arsenic concentration comparable to an NHANES subject well below the 95% confidence interval of the 50th percentile (6.0 µg/L) of the NHANES sample.

When comparing the GEE-adjusted inorganic-related urinary arsenic data after nine months of water treatment for POE subjects from either the Post-Only (2.7 ± 0.6 µg/g creatinine) (Table-4-2) or the Pre-Post (2.5 ± 0.6 µg/g creatinine) (Table 4-3) Groups, the POE subjects are well below the 50th percentile of the NHANES sample, while the POU subjects at 6.1-7.2 µg/g creatinine (Table 4-2 and Table 4-3) are near the 50th percentile of the NHANES sample.

Arsenic Reduction in Urine Samples

After an average of nine months of water treatment, the adjusted inorganic-related urinary arsenic concentrations were significantly lower in the POE group. In addition, the reduction of inorganic-related urinary arsenic from the initial arsenic concentration to the nine-month concentration was greater in the POE group. The nine-month adjusted inorganic-related urinary arsenic concentrations were significantly lower in the POE group in both subsets of subjects analyzed in this study.

In a home with single-tap POU water treatment, one likely cause of continued exposure to arsenic in water is from subjects ingesting water from untreated taps in the home (e.g., from a bathroom sink when brushing teeth). In this study, subjects with POU water treatment were encouraged by the investigators to only ingest water from the POU treatment system. However, we did not attempt to assess compliance with this request.

A population in northern Chile, chronically exposed to 600 µg/L arsenic in their drinking water, was provided an alternate drinking water supply (with 45 µg/L arsenic) for two months (Hopenhayn-Rich et al., 1996). A substantial decrease in total urine arsenic was observed; however, the final urinary levels of arsenic were higher than what would be expected from consumption of drinking water at 45 µg/L. Inorganic-related urine arsenic levels determined by hydride generation atomic absorption spectroscopy dropped from 696 µg/L to only 185 µg/L at the end of the two-month study. In addition to the possibility that these subjects may still have been mobilizing arsenic from their body burden after the two month period, other explanations include non-compliance with drinking the lower arsenic water that was provided by the investigators, and the potential exposure to arsenic via water uses other than drinking and cooking.

In the present study, another potential cause of higher inorganic-related urinary arsenic concentrations in the POU group are secondary routes of exposure such

as inhalation of aerosols during showering or cooking, and dermal absorption during showering, bathing, or brushing of teeth.

There is a high likelihood that skin contact with waters containing arsenic above drinking water standards will result in some arsenic absorption. The highly keratinized epidermis provides ample sulfhydryl binding sites for arsenic. Rahman found up to 62% absorption of sodium arsenate when applying 100 µL of an aqueous solution containing arsenic concentrations as low as 50 µg/L to the skin (0.64 cm^2) of mice in vitro (flow through diffusion cell) for 24 hours (Rahman et al., 1994). About half of the absorbed arsenic passed through the skin and half remained in the skin after 24 hours of exposure. They also found that absorption increased linearly with the applied dose with a constant fraction of the dose being absorbed (Rahman et al., 1994). Wester identified arsenic absorption through the skin of live monkeys, and demonstrated that up to 6.4% of an applied dose of sodium arsenate heptahydrate at 4.8 µg/L arsenic, applied at a rate of 5 µL/cm^2, was absorbed through 12 cm^2 of skin and excreted via urine after 24 hours of exposure (Wester et al., 1993). In a follow-up study, they found up to 4.4% of a much higher applied dose of sodium arsenate heptahydrate at 2860 mg/L arsenic, applied at a rate of 5 µL/cm^2, was absorbed through 100 cm^2 of skin, and excreted via urine after eight hours of exposure (Wester et al., 2004). Another recent study, using artificial skin, found absorption of both As^V and As^{III} at up to 8% of the applied dose (10 µg/L to 1000 µg/L) per hour, applied at 1250 µL/cm^2 after 6 hours (Bernstam et al., 2002; Nriagu and Bernstam, 2004). They

found that of the absorbed arsenic, about 30% of the As^{V} and 90% of As^{III} was being retained in the artificial human skin after the 6-hour exposure, and inferred from this that a higher percentage of As^{V} applied to the skin may reach the systemic circulation and internal organs, compared to As^{III} (Bernstam et al., 2002). Assuming the thickness of water film on the body during a shower is 100 µm, their data indicate that a 15 minute shower with water containing 100 µg/L arsenic would result in dermal absorption up to 1.9 µg of arsenic in a 28.2 Kg 8-year old child with 10,700 cm^2 of skin, and 3.8 µg of arsenic in a 70 Kg adult with 20,000 cm^2 of skin (Nriagu and Bernstam, 2004). Based on these studies, humans with elevated arsenic in their well water and a chronic daily exposure via showering or bathing could potentially incur a significant arsenic exposure without POE water treatment.

Implications of the Study Findings

There is a significant cost difference between installing and maintaining a POE or POU arsenic water treatment system, with POE treatment costing about eight times more than a single POU treatment device. Considering the fact that arsenic is a known human carcinogen and that the maximum contaminant level goal for arsenic is zero µg/L, the additional cost of a POE treatment system may be warranted. Considering the higher costs associated with POE arsenic water treatment, a larger randomized study should be conducted to confirm the present findings and quantify the contribution of the dermal and inhalation exposure pathways.

Regulatory agencies should consider requiring POE arsenic water treatment when and where they have jurisdiction. POE water treatment is especially important when a home with an arsenic contaminated well is sold to a new owner. Typically, the new owner will be unfamiliar with the water problem and may not even know why the treatment system is in the home. They are often told that the well has a problem, but the problem has been remediated by a water treatment system. Even if a POU water treatment system is installed at only the kitchen sink, the new owner may not realize that other taps in the home do not have treated water. If POE water treatment is installed, all water taps in the home will be treated, and the only remaining problem for a new homeowner will be to educate them about the need to occasionally monitor the quality of the treated water and have maintenance conducted to ensure the continued effectiveness of the system.

Limitations

One limitation of this study was the limited number of subjects. There were only 36 subjects in the Post-Only Group, and 24 subjects in the Pre-Post Group. Another limitation was that blood samples were only analyzed for total arsenic rather than inorganic-related arsenic. Efforts were made to overcome this limitation, but for whole blood samples the combination of matrix effect problems, digestion methods, and equipment limitations precluded the separation of inorganic-related arsenic and seafood/fish arsenic in whole blood.

The questionnaires depended on subject recall rather than measurement to determine each subject's exposure history. Subject characteristics such as rate of drinking water from the home water supply, the years of exposure to the home water supply, the weight of the subject, as well as the subject's bathing and dietary habits all depended on recall which has its limitations.

Some inorganic arsenic (As^{III} and As^{V}) is present in food and this inorganic arsenic and its metabolites, upon reaching the urine, would be included in the component labeled "inorganic-related" arsenic in urine. There is no analytical method capable of separating the inorganic arsenic originating in food from the inorganic arsenic originating in ingested water. The average total inorganic arsenic intake from food in the United States, based on the FDA Total Diet Study, is estimated at 9 µg/d for adults, aged 25 and over, 5 µg/d for children aged 2-16 years, and 1.3 µg/d for children aged 6-11 months (National Research Council, 1999; Tao and Bolger, 1999). These intake amounts can have an effect on the final "inorganic-related" arsenic concentrations in urine. However, the effect should be similar in the POE, POU, and Control groups in the present study because there were little to no significant differences found between these groups in their potential arsenic exposure from dietary sources (Table 4-1, 4-2, and 4-3).

Conclusions

This study compared the effectiveness of point-of-entry and point-of-use arsenic water treatment systems in reducing arsenic exposure from well water. It was a non-randomized observational study conducted with 49 subjects having elevated arsenic in their residential home well water in New Jersey. The subjects obtained either point-of-entry or point-of-use arsenic water treatment. Prior ingestion exposure to arsenic in well water was determined by testing arsenic concentrations in the well water and obtaining water-use histories for each subject, including years of residence with the current well and amount of water consumed from the well per day. A series of urine samples were collected from the subjects, some starting before water treatment was installed, and continuing for at least nine months. Urine samples were analyzed for inorganic-related arsenic concentrations. Propensity scores were calculated to reduce bias resulting from the non-random assignment of water treatment systems. Analysis of covariance and generalized estimating equations were used to examine the association between urinary arsenic and urinary arsenic reduction, by treatment group, at nine months after subjects stopped drinking the water or obtained water treatment, while controlling for correlation among family members, and the propensity score as a covariate.

After nine months of water treatment in the Pre-Post Group, the adjusted mean ± SE of the inorganic-related arsenic concentrations, after controlling for propensity

score, prior cumulative arsenic ingestion exposure per body weight, and family correlations, were significantly lower ($p < 0.0005$) in the POE treatment group (2.5 ± 0.6 µg/g creatinine) than in the POU treatment group (7.2 ± 0.8 µg/g creatinine). The adjusted mean urinary inorganic-related arsenic reduction, after controlling for propensity score, prior cumulative arsenic ingestion exposure per body weight, initial urinary inorganic-related arsenic concentration, and family correlations was significantly greater ($p = 0.040$) in the point-of-entry group (10.2 ± 0.4 µg/g creatinine) than in the point-of-use group (8.1 ± 0.9 µg/g creatinine).

The results from this study suggest that POE arsenic water treatment systems provide a more effective reduction of arsenic exposure from well water than that obtained by POU treatment.

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Chapter 5: Conclusions and Recommendations

Thousands of people in New Jersey and millions worldwide have been exposed to unacceptably high levels of arsenic by drinking water from contaminated wells. Arsenic is a known human carcinogen and also increases the risk of many non-cancer health effects (ATSDR, 2007). As a Group A carcinogen, arsenic has a Maximum Contaminant Level Goal (MCLG) of zero µg/L (USEPA, 2001). The New Jersey Department of Environmental Protection (NJDEP) has set the New Jersey Maximum Contaminant Level (MCL) for arsenic at 5 µg/L, which is currently the most protective in the world. Arsenic is widely distributed in New Jersey well water, exceeds the MCL in New Jersey private wells at a higher percentage than all other contaminants with primary drinking water standards (NJDEP, 2008), and has been found to exceed 200 µg/L in some wells, with some municipalities having 30% of their private wells exceeding the MCL. The arsenic in New Jersey well water is predominantly naturally occurring in specific geologic settings (Serfes et al., 2005). Special water treatment systems can remove arsenic from drinking water and can be configured to treat all the water in the home (point-of-entry) or water at only a single tap for drinking and cooking (point-of-use) (Spayd, 2007).

The main goal of this research was to compare human exposure to arsenic between point-of-entry (POE) and point-of-use (POU) water treatment, by biomonitoring for arsenic, in order to determine which level of treatment most

effectively reduced arsenic exposure and dose from water at home to acceptable risk levels. As part of this study, it was also important to investigate the effects of dietary arsenic, sampling protocol, arsenic analytical methodologies, appropriate arsenic reference ranges, and arsenic body burden and clearance from the human body after chronic exposure to arsenic contaminated well water has ended.

A non-random observational study was conducted with 49 subjects having elevated arsenic (8 - 119 µg/L) in their home well water in New Jersey. The subjects obtained either point-of-entry or point-of-use arsenic water treatment. Prior ingestion exposure to arsenic in well water was determined by testing arsenic concentrations in the well water and obtaining water-use and exposure histories for each subject, including years of residence with the current well and amount of water consumed from the well per day. A series of urine samples were collected from the subjects, some starting before water treatment was installed, and continuing for at least nine months. Urine samples were analyzed for total arsenic and inorganic-related arsenic. Blood samples were collected at the start and end of the water treatment study and analyzed for total arsenic. Propensity scores were calculated to reduce bias resulting from the non-random assignment of water treatment systems. Analysis of covariance and generalized estimating equations were used to examine the association between urinary arsenic and urinary arsenic reduction, by treatment group, at nine months after

subjects stopped drinking the water or obtained water treatment, while controlling for correlation among family members, and the propensity score as a covariate.

The effect on urinary arsenic concentrations from ingestion of high arsenic foods, and the importance of designing arsenic biomonitoring sampling and analytical methodology protocols that account for the potential confounding of dietary arsenic was demonstrated.

The analytical methodology for arsenic in well water biomonitoring studies should be one that will determine the inorganic-related arsenic, which is the sum of the inorganic arsenic species found in water (As^{III} and As^{V}) and their metabolites (MMA^{V}, MMA^{III}, DMA^{V}, and DMA^{III}). The best methods for this analysis include a hydride generation step that separates the inorganic-related arsenic from the relatively non-toxic organic arsenic species commonly found in seafood, fish, and some other foods.

The biomonitoring sample collection protocol must be based on the analytical methodology. If only a total urinary arsenic test is available, the diet must be restricted from high arsenic foods for one week prior to sample collection. If a hydride generation method will be used, most fish and seafood will not cause a problem, but foods containing arsenosugars, such as scallops, seaweed, and

seaweed-wrapped sushi must still be restricted from the diet for one week prior to sample collection because arsenosugars are metabolized by humans into DMA, which is also an inorganic-related arsenic species.

Based on the recommended protocols and the data collected and reviewed in this study, the recommended reference range for inorganic-related arsenic in urine is < 8 µg/L and < 5 µg/g creatinine. Due to the matrix difficulties in analyzing blood, it is generally only analyzed for total arsenic. Blood appears to be a less sensitive biomarker compared to urine and thus may be an unreliable means of biomonitoring for arsenic exposure (ATSDR, 2007; National Research Council, 1999). Furthermore, it is much less difficult to have subjects provide urine rather than blood samples. Therefore, testing urine for inorganic-related arsenic is the preferred biomonitoring approach for exposure to arsenic contaminated well water. However, when total arsenic in whole blood is measured, the recommended reference range is < 2.5 µg/L.

To compare the effectiveness of POE and POU arsenic water treatment systems in reducing arsenic exposure from well water, the focus was on subjects who were still drinking the arsenic-contaminated water when the study began. Upon notification of elevated arsenic levels in home well water, many subjects stopped using the water for drinking and cooking before they were enrolled in the biomonitoring study and collection of their initial urine and blood samples could

begin. As a result, only 24 of the subjects were able to provide samples that allowed us to measure urine arsenic levels while they were still drinking and cooking with the arsenic-contaminated water. This group is called the Pre-Post Subset and includes all subjects who provided both an initial urine sample, collected before obtaining water treatment or ceasing to drink the water with elevated arsenic, and a nine-month urine sample. Another subset, called the "Post-Only" subset, includes 36 subjects who provided a nine-month urine sample.

This study demonstrated that effective arsenic water treatment is available for residential well remediation. Effective arsenic water treatment reduces arsenic exposure as evidenced by statistically significant reductions in inorganic-related arsenic concentrations in urine and total arsenic concentrations in blood after initiation of water treatment.

After nine months of water treatment in the Pre-Post subset, the adjusted mean ± SE of the inorganic-related arsenic concentrations, after adjusting for propensity score, prior cumulative arsenic ingestion exposure per body weight, and family correlations, were significantly lower ($p < 0.0005$) in the POE treatment group (2.5 ± 0.6 µg/g creatinine) than in the POU treatment group (7.2 ± 0.8 µg/g creatinine). The adjusted mean urinary inorganic-related arsenic reduction, after adjusting for propensity score, prior cumulative arsenic ingestion exposure per body weight, initial urinary inorganic-related arsenic concentration, and family

correlations was significantly greater ($p = 0.040$) in the point-of-entry group (10.2 ± 0.4 µg/g creatinine) than in the point-of-use group (8.1 ± 0.9 µg/g creatinine). This difference in exposure outcome between the two treatment methods may, in part, be related to dermal absorption of arsenic, which has been reported in studies of monkeys (Wester et al., 2004; Wester et al., 1993), other mammals (Rahman et al., 1994), and artificial human skin (Bernstam et al., 2002; Nriagu and Bernstam, 2004).

A two-phase clearance of inorganic-related arsenic from urine was identified for the Pre-Post subset of subjects. The first phase of clearance had a half life of about 7 days, and the second phase of clearance had a much longer half life of about 605 days. After nine months of water treatment, the previously exposed subject's geometric mean inorganic-related arsenic concentrations in urine were greatly reduced, but remained significantly higher than the geometric mean in the control subjects. The two-phase clearance combined with the failure of the previously exposed subjects' urine concentrations to decline to the level in the Control Group subjects, even approximately nine months after ceasing the drinking water exposure, indicates that an excess arsenic body burden is present in humans after chronic exposure to arsenic in drinking water. This is an important contribution to the weight of evidence that arsenic bioaccumulates in humans during chronic exposure to arsenic-contaminated water and that its toxicity may linger well beyond the point at which remediation is instituted.

One limitation of this study was the limited number of subjects. There were only 36 subjects in the Post-Only subset, and 24 subjects in the Pre-Post subset. Another limitation was that blood samples were only analyzed for total arsenic rather than inorganic-related arsenic. Efforts were made to overcome this limitation, but for whole blood samples the combination of matrix effect problems, digestion methods, and equipment limitations precluded the separation of inorganic-related arsenic and seafood/fish arsenic in whole blood.

The questionnaires depended on subject recall rather than measurement to determine each subject's exposure history. Subject characteristics such as rate of drinking water from the home water supply, the years of exposure to the home water supply, the weight of the subject, as well as the subject's bathing and dietary habits all depended on recall which has its limitations.

Some inorganic arsenic (As^{III} and As^{V}) is present in food and this inorganic arsenic and its metabolites, upon reaching the urine, would be included in the component labeled "inorganic-related" arsenic in urine. In addition, foods such as scallops, seaweed, and seaweed-wrapped sushi contain arsenosugars which are metabolized by humans into DMA, which is also an inorganic-related arsenic species. There is no analytical method capable of separating the inorganic arsenic originating in food from the inorganic arsenic originating in ingested water. The average total inorganic arsenic intake from food in the United States, based on the FDA Total Diet Study, is estimated at 9 µg/d for adults, aged 25

and over, 5 µg/d for children aged 2-16 years, and 1.3 µg/d for children aged 6-11 months (National Research Council, 1999; Tao and Bolger, 1999). These intake amounts can have an effect on the final "inorganic-related" arsenic concentrations in urine. However, the effect should be similar in the POE, POU, and Control groups in the present study because there were little to no significant differences found between these groups in their potential arsenic exposure from dietary sources (Table 4-1, 4-2, and 4-3).

The results from this study suggest that POE arsenic water treatment systems provide a more effective reduction of arsenic exposure from well water than that obtained by POU treatment. Therefore, regulatory agencies should consider requiring POE arsenic water treatment when and where they have jurisdiction. However, there is a significant cost difference between installing and maintaining a POE or POU arsenic water treatment system, with POE treatment costing about eight times more than a single POU treatment device. Considering the fact that arsenic is a known human carcinogen and that the maximum contaminant level goal for arsenic is zero µg/L, the additional cost of a POE treatment system may be warranted.

A larger randomized study should be conducted to confirm the present findings, especially the presence of an arsenic body burden, a two-phase clearance after chronic exposure to arsenic contaminated water has ended, and the apparent better protection provided by POE arsenic water treatment systems as they

resulted in a more effective reduction of arsenic exposure from well water than that obtained by POU treatment. In addition, further study should be conducted to quantify the contribution of the dermal and inhalation exposure pathways, and to establish reference ranges for arsenic in urine and blood. Finally, new methods for arsenic speciation in blood should be developed, and additional pharmacokinetic modeling should be conducted to further define arsenic clearance from the human body after chronic exposure to arsenic has ended.

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Appendix 1

Water Use and Exposure History Questionnaire

(Copy reduced to fit margins.)

Efficacy of Arsenic Exposure Reduction via Drinking Water Treatment Systems
Sponsored by the Environmental and Occupational Health Sciences Institute

Water Use Questionnaire

Background

Subject Identifier: _____ Date Completing Questionnaire: _____

Years at Current Address: _____ Previous Address: City: _____
State: _____

Year Current Well was Installed: ____ Was There an Earlier Well Used: _____

Date You Were Notified of Elevated Arsenic in Your Well Water: _____

Drinking Water Use

Type of Drinking Water or Food Preparation Water	Ounces Per Day		Comments
	Currently	Before Arsenic Was Found in the Water	
Untreated Tap Water at Home			
Treated Tap Water at Home [1]			
Untreated Tap Water Elsewhere [2]			
Bottled Water [3]			
Coffee or Tea Made with Home Tap Water			
Other Drinks Made with Home Tap Water			
Other Drinking Sources [4]			
Untreated Water for Rinsing Food Items			
Untreated Tap Water for Food Preparation [5]			

Bathing Water Use

Type of Bathing	Times Per Week		Comments
	Currently	Before Arsenic Was Found in the Water	
Shower - Untreated Tap Water at Home			
Bath - Untreated Tap Water at Home			
Shower or Bath Elsewhere [2]			
Teeth Brushing - Untreated Tap Water			
Clothes Washing at Home			
Home Swimming Pool Use			

[1] Identify treatment type (Reverse Osmosis, Activated Carbon, Britta, etc.) in comments section.
[2] Identify location (City & State) in comments section.
[3] Identify brand(s) in comments section.
[4] Identify sources (other water sources or other beverages) in comments section.
[5] For example, making soup or boiling water for macaroni, oatmeal, or potatoes.

Efficacy of Arsenic Exposure Reduction via Drinking Water Treatment Systems
Sponsored by the Environmental and Occupational Health Sciences Institute

Please provide any other water use information that you believe may have impacted your exposure to arsenic:

DIET

Arsenic in the diet may affect the level of arsenic in your biomonitoring results. Fish and seafood contain relatively high arsenic concentrations although the arsenic in fish and seafood is present predominantly in organic forms which are considered to be much less harmful to health than the inorganic forms commonly found in well water. In the below table please indicate how many times per week you consumed the listed food.

Type of Food	Times Per Week Consumed		Comments
	Currently	Before Arsenic Was Found in the Water	
Shrimp or Lobster			
Clams or Oysters			
Tuna			
Other Seafood or Fish			
Mushrooms			
Rice			
Chicken or Turkey			

Appendix 2

Logistic Regression for Calculation of Propensity Scores for Post-Only Group

```
SAVE OUTFILE='F:\SPSS\Master File_48 Post Only 36 Subjects Without
Controls New Urines UG per G.sav'
 /COMPRESSED.
LOGISTIC REGRESSION VARIABLES  TreatmentBinary
  /METHOD = BSTEP(COND) LNPIDBod Age Showers
  /CLASSPLOT /CASEWISE
  /PRINT = GOODFIT CI(95)
  /CRITERIA = PIN(.1) POUT(.25) ITERATE(20) CUT(.5) .
```

Logistic Regression

Case Processing Summary

Unweighted Cases(a)		N	Percent
Selected Cases	Included in Analysis	36	100.0
	Missing Cases	0	.0
	Total	36	100.0
Unselected Cases		0	.0
Total		36	100.0

a If weight is in effect, see classification table for the total number of cases.

Dependent Variable Encoding

Original Value	Internal Value
0	0
1	1

Block 0: Beginning Block

Classification Table(a,b)

Observed			Predicted		
			POE = 1		Percentage Correct
			0	1	
Step 0	POE = 1	0	0	16	.0
		1	0	20	100.0
	Overall Percentage				55.6

a Constant is included in the model.
b The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	.223	.335	.443	1	.506	1.250

Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	LNPIDBod	10.352	1	.001
		Age	7.375	1	.007
		Showers	.234	1	.628
	Overall Statistics		15.552	3	.001

Block 1: Method = Backward Stepwise (Conditional)

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	18.602	3	.000
	Block	18.602	3	.000
	Model	18.602	3	.000

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	30.859(a)	.404	.540

a Estimation terminated at iteration number 5 because parameter estimates changed by less than .001.

Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	9.090	7	.246

Contingency Table for Hosmer and Lemeshow Test

		POE = 1 = 0		POE = 1 = 1		Total
		Observed	Expected	Observed	Expected	
Step 1	1	4	3.897	0	.103	4
	2	4	3.623	0	.377	4
	3	3	2.931	1	1.069	4
	4	2	2.125	2	1.875	4
	5	0	1.176	4	2.824	4
	6	0	.794	4	3.206	4
	7	2	.624	2	3.376	4
	8	0	.522	4	3.478	4
	9	1	.307	3	3.693	4

Classification Table(a)

Observed			Predicted		
			POE = 1		Percentage Correct
			0	1	
Step 1	POE = 1	0	12	4	75.0
		1	3	17	85.0
	Overall Percentage				80.6

a The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)	95.0% C.I.for EXP(B)	
		Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
Step 1(a)	LNPIDBod	-1.538	.683	5.066	1	.024	.215	.056	.820
	Age	-.060	.028	4.537	1	.033	.942	.892	.995
	Showers	.415	.220	3.571	1	.059	1.515	.985	2.330
	Constant	1.953	1.180	2.740	1	.098	7.051		

a Variable(s) entered on step 1: LNPIDBod, Age, Showers.

Model if Term Removed(a)

Variable		Model Log Likelihood	Change in -2 Log Likelihood	df	Sig. of the Change
Step 1	LNPIDBod	-18.754	6.648	1	.010
	Age	-18.435	6.010	1	.014
	Showers	-17.614	4.368	1	.037

a Based on conditional parameter estimates

Casewise List

Case	Selected Status(a)	Observed Treatment Code	Predicted	Predicted Group	Temporary Variable	
					Resid	ZResid
1	S	1	.734	1	.266	.602
2	S	1	.759	1	.241	.563
3	S	1	.826	1	.174	.460
4	S	1	.871	1	.129	.385
5	S	0	.056	0	-.056	-.244
6	S	0	.088	0	-.088	-.310
7	S	0	.139	0	-.139	-.402
8	S	0	.225	0	-.225	-.539
9	S	0**	.933	1	-.933	-3.726
10	S	1	.873	1	.127	.382
11	S	1	.799	1	.201	.502
12	S	1	.849	1	.151	.422
13	S	0	.431	0	-.431	-.871
14	S	0**	.840	1	-.840	-2.292
15	S	0**	.587	1	-.587	-1.193
16	S	0	.021	0	-.021	-.145
17	S	0	.026	0	-.026	-.163
18	S	1**	.336	0	.664	1.407
19	S	1	.823	1	.177	.464
20	S	1	.877	1	.123	.375
21	S	1	.857	1	.143	.408
22	S	1	.842	1	.158	.432
23	S	1	.908	1	.092	.319
24	S	1	.601	1	.399	.816
25	S	1	.931	1	.069	.273
26	S	1	.922	1	.078	.291
27	S	0	.010	0	-.010	-.102
28	S	0	.046	0	-.046	-.220
29	S	0	.189	0	-.189	-.483
30	S	0	.094	0	-.094	-.322
31	S	0**	.844	1	-.844	-2.326
32	S	0	.319	0	-.319	-.684
33	S	1**	.390	0	.610	1.251
34	S	1**	.466	0	.534	1.070
35	S	1	.735	1	.265	.601
36	S	1	.755	1	.245	.570

a S = Selected, U = Unselected cases, and ** = Misclassified cases.

Appendix 3

Logistic Regression for Calculation of Propensity Scores for Pre-Post Group

```
LOGISTIC REGRESSION VARIABLES  TreatmentBinary
  /METHOD = ENTER LNPIDBod
  /SAVE = PRED
  /CLASSPLOT /CASEWISE
  /PRINT = GOODFIT
  /CRITERIA = PIN(.05) POUT(.10) ITERATE(20) CUT(.5) .
```

Logistic Regression

```
[DataSet3] F:\SPSS\Master File_42 Pre-Post Group Without Controls New
Urines UG per G.sav
```

Case Processing Summary

Unweighted Cases(a)		N	Percent
Selected Cases	Included in Analysis	24	100.0
	Missing Cases	0	.0
	Total	24	100.0
Unselected Cases		0	.0
Total		24	100.0

a If weight is in effect, see classification table for the total number of cases.

Dependent Variable Encoding

Original Value	Internal Value
0	0
1	1

Block 0: Beginning Block

Classification Table(a,b)

Observed			Predicted		
			POE = 1		Percentage Correct
			0	1	
Step 0	POE = 1	0	0	8	.0
		1	0	16	100.0
	Overall Percentage				66.7

a Constant is included in the model.
b The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 0	Constant	.693	.433	2.562	1	.109	2.000

Variables not in the Equation

			Score	df	Sig.
Step 0	Variables	LNPIDBod	11.817	1	.001
	Overall Statistics		11.817	1	.001

Block 1: Method = Enter

Omnibus Tests of Model Coefficients

		Chi-square	df	Sig.
Step 1	Step	13.584	1	.000
	Block	13.584	1	.000
	Model	13.584	1	.000

Model Summary

Step	-2 Log likelihood	Cox & Snell R Square	Nagelkerke R Square
1	16.969(a)	.432	.600

a Estimation terminated at iteration number 6 because parameter estimates changed by less than .001.

Hosmer and Lemeshow Test

Step	Chi-square	df	Sig.
1	8.562	8	.381

Contingency Table for Hosmer and Lemeshow Test

		POE = 1 = 0		POE = 1 = 1		Total
		Observed	Expected	Observed	Expected	
Step 1	1	2	1.911	0	.089	2
	2	2	1.549	0	.451	2
	3	0	1.431	2	.569	2
	4	2	1.202	0	.798	2
	5	1	.911	1	1.089	2
	6	1	.438	1	1.562	2
	7	0	.168	2	1.832	2
	8	0	.114	2	1.886	2
	9	0	.094	2	1.906	2
	10	0	.183	6	5.817	6

Classification Table(a)

Observed			Predicted		
			POE = 1		Percentage Correct
			0	1	
Step 1	POE = 1	0	7	1	87.5
		1	2	14	87.5
	Overall Percentage				87.5

a The cut value is .500

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Exp(B)
Step 1(a)	LNPIDBod	-2.494	.957	6.788	1	.009	.083
	Constant	3.788	1.481	6.543	1	.011	44.156

a Variable(s) entered on step 1: LNPIDBod.

Casewise List

Case	Selected Status(a)	Observed POE = 1	Predicted	Predicted Group	Temporary Variable Resid	Temporary Variable ZResid
1	S	1	.924	1	.076	.287
2	S	1	.964	1	.036	.194
3	S	1	.978	1	.022	.150
4	S	0	.429	0	-.429	-.867
5	S	0	.369	0	-.369	-.765
6	S	1	.961	1	.039	.201
7	S	1	.971	1	.029	.171
8	S	1	.978	1	.022	.150
9	S	1	.623	1	.377	.778
10	S	1	.954	1	.046	.219
11	S	1	.908	1	.092	.318
12	S	1	.937	1	.063	.260
13	S	1	.952	1	.048	.226
14	S	1	.965	1	.035	.190
15	S	1	.949	1	.051	.231
16	S	0	.050	0	-.050	-.229
17	S	0	.178	0	-.178	-.465
18	S	0	.274	0	-.274	-.614
19	S	0	.039	0	-.039	-.202
20	S	0**	.896	1	-.896	-2.932
21	S	0	.466	0	-.466	-.934
22	S	1	.666	1	.334	.708
23	S	1**	.285	0	.715	1.582
24	S	1**	.284	0	.716	1.588

a S = Selected, U = Unselected cases, and ** = Misclassified cases.

Appendix 4

Analysis of Covariance for Post-Only Group

```
UNIANOVA
  UHG268d  BY Treatment  WITH PRE_1
  /METHOD = SSTYPE(3)
  /INTERCEPT = INCLUDE
  /EMMEANS = TABLES(OVERALL) WITH(PRE_1=MEAN)
  /EMMEANS = TABLES(Treatment) WITH(PRE_1=MEAN)
  /PRINT = DESCRIPTIVE ETASQ OPOWER PARAMETER HOMOGENEITY
  /CRITERIA = ALPHA(.05)
  /DESIGN = PRE_1 Treatment .
```

Univariate Analysis of Variance

[DataSet1] F:\SPSS\Master File_48 Post Only 36 Subjects Without Controls New Urines UG per G.sav

Between-Subjects Factors

		Value Label	N
Water Treatment Type	POE	Point-of-Entry	20
	POU	Point-of-Use	16

Descriptive Statistics

Dependent Variable: Urine HG Arsenic 141-395 Days

Water Treatment Type	Mean	Std. Deviation	N
Point-of-Entry	3.640	2.0400	20
Point-of-Use	4.875	3.3205	16
Total	4.189	2.7151	36

Levene's Test of Equality of Error Variances(a)

Dependent Variable: Urine HG Arsenic 141-395 Days

F	df1	df2	Sig.
2.575	1	34	.118

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.
a Design: Intercept+PRE_1+Treatment

Tests of Between-Subjects Effects

Dependent Variable: Urine HG Arsenic 141-395 Days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Corrected Model	61.026(b)	2	30.513	5.112	.012	.237	10.223	.786
Intercept	19.827	1	19.827	3.321	.077	.091	3.321	.425
PRE_1	47.469	1	47.469	7.952	.008	.194	7.952	.781
Treatment	54.425	1	54.425	9.117	.005	.216	9.117	.834
Error	196.989	33	5.969					
Total	889.700	36						
Corrected Total	258.016	35						

a Computed using alpha = .05
b R Squared = .237 (Adjusted R Squared = .190)

Parameter Estimates

Dependent Variable: Urine HG Arsenic 141-395 Days

					95% Confidence Interval				
Parameter	B	Std. Error	t	Sig.	Lower Bound	Upper Bound	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Intercept	3.454	.792	4.363	.000	1.844	5.065	.366	4.363	.988
PRE_1	4.688	1.662	2.820	.008	1.306	8.070	.194	2.820	.781
[Treatment=POE]	-3.366	1.115	-3.019	.005	-5.633	-1.098	.216	3.019	.834
[Treatment=POU]	0(b)	.	.	.	.	.	.	.	.

a Computed using alpha = .05
b This parameter is set to zero because it is redundant.

Estimated Marginal Means

1. Grand Mean

Dependent Variable: Urine HG Arsenic 141-395 Days

		95% Confidence Interval	
Mean	Std. Error	Lower Bound	Upper Bound
4.376(a)	.412	3.538	5.214

a Covariates appearing in the model are evaluated at the following values: Predicted probability = .5555556.

2. Water Treatment Type

Dependent Variable: Urine HG Arsenic 141-395 Days

Water Treatment Type	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Point-of-Entry	2.693(a)	.641	1.388	3.998
Point-of-Use	6.059(a)	.741	4.551	7.567

a Covariates appearing in the model are evaluated at the following values: Predicted probability = .5555556.

Appendix 5

Generalized Estimating Equations for Post-Only Group

```
* Generalized Estimating Equations.
GENLIN
  UHG268d
  BY Treatment
  (ORDER=ASCENDING)
  WITH PRE_1
 /MODEL
  Treatment PRE_1
  INTERCEPT=YES
  DISTRIBUTION=NORMAL
  LINK=IDENTITY
 /CRITERIA SCALE=MLE
  PCONVERGE=1E-006(ABSOLUTE)
  SINGULAR=1E-012
  ANALYSISTYPE=3 CILEVEL=95
 /EMMEANS TABLES=Treatment SCALE=ORIGINAL
 /REPEATED
  SUBJECT=Family
  SORT=YES CORRTYPE=INDEPENDENT
  ADJUSTCORR=YES COVB=ROBUST
  MAXITERATIONS=100
  PCONVERGE=1e-006(ABSOLUTE) UPDATECORR=1
 /MISSING CLASSMISSING=EXCLUDE
 /PRINT CPS DESCRIPTIVES MODELINFO FIT SUMMARY SOLUTION.
```

Generalized Linear Models

```
[DataSet8] F:\SPSS\Master File_48 Post Only 36 Subjects Without
Controls New Urines UG per G.sav
```

Model Information

Dependent Variable	Urine HG Arsenic 141-395 Days
Probability Distribution	Normal
Link Function	Identity
Subject Effect 1	Family
Working Correlation Matrix Structure	Independent

Case Processing Summary

	N	Percent
Included	36	100.0%
Excluded	0	.0%
Total	36	100.0%

Correlated Data Summary

Number of Levels	Subject Effect	Family	16
Number of Subjects			16
Number of Measurements per Subject	Minimum		1
	Maximum		4
Correlation Matrix Dimension			4

Categorical Variable Information

			N	Percent
Factor	Water Treatment Type	Point-of-Entry	20	55.6%
		Point-of-Use	16	44.4%
		Total	36	100.0%

Continuous Variable Information

		N	Minimum	Maximum	Mean	Std. Deviation
Dependent Variable	Urine HG Arsenic 141-395 Days	36	.7	11.3	4.189	2.7151
Covariate	Predicted probability	36	.01024	.93281	.5555556	.33792301

Goodness of Fit(a)

	Value
Quasi Likelihood under Independence Model Criterion (QIC)	202.841
Corrected Quasi Likelihood under Independence Model Criterion (QICC)	202.989

Dependent Variable: Urine HG Arsenic 141-395 Days
Model: (Intercept), Treatment, PRE_1
a Information criteria are in small-is-better form.

Tests of Model Effects

	Type III		
Source	Wald Chi-Square	df	Sig.
(Intercept)	2.709	1	.100
Treatment	9.225	1	.002
PRE_1	7.209	1	.007

Dependent Variable: Urine HG Arsenic 141-395 Days
Model: (Intercept), Treatment, PRE_1

Parameter Estimates

Parameter			95% Wald Confidence Interval		Hypothesis Test		
	B	Std. Error	Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	3.454	.8535	1.782	5.127	16.381	1	.000
[Treatment=POE]	-3.366	1.1081	-5.538	-1.194	9.225	1	.002
[Treatment=POU]	0(a)	.	.	.	.	.	.
PRE_1	4.688	1.7459	1.266	8.110	7.209	1	.007
(Scale)	5.969						

Dependent Variable: Urine HG Arsenic 141-395 Days
Model: (Intercept), Treatment, PRE_1
a Set to zero because this parameter is redundant.

Estimated Marginal Means: Water Treatment Type

Estimates

Water Treatment Type	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
Point-of-Entry	2.693	.6104	1.497	3.889
Point-of-Use	6.059	.7088	4.670	7.448

Covariates appearing in the model are fixed at the following values: PRE_1=.5555556

Appendix 6

Analysis of Covariance for Pre-Post Group

```
UNIANOVA
  UHG268d  BY Treatment  WITH PRE_3 LNPIDBod Age
  /METHOD = SSTYPE(3)
  /INTERCEPT = INCLUDE
  /EMMEANS = TABLES(OVERALL) WITH(PRE_3=MEAN LNPIDBod=MEAN Age=MEAN)
  /EMMEANS = TABLES(Treatment) WITH(PRE_3=MEAN LNPIDBod=MEAN Age=MEAN)
  /PRINT = DESCRIPTIVE ETASQ OPOWER PARAMETER HOMOGENEITY
  /CRITERIA = ALPHA(.05)
  /DESIGN = PRE_3 LNPIDBod Age Treatment .
```

Univariate Analysis of Variance

[DataSet7] F:\SPSS\Master File_42 Pre-Post Group Without Controls New Urines UG per G.sav

Between-Subjects Factors

		Value Label	N
Water Treatment Type	POE	Point-of-Entry	16
	POU	Point-of-Use	8

Descriptive Statistics

Dependent Variable: Urine HG Arsenic 141-395 Days

Water Treatment Type	Mean	Std. Deviation	N
Point-of-Entry	3.988	2.0189	16
Point-of-Use	4.200	3.8848	8
Total	4.058	2.6947	24

Levene's Test of Equality of Error Variances(a)

Dependent Variable: Urine HG Arsenic 141-395 Days

F	df1	df2	Sig.
.070	1	22	.794

Tests the null hypothesis that the error variance of the dependent variable is equal across groups.
a Design: Intercept+PRE_3+LNPIDBod+Age+Treatment

Tests of Between-Subjects Effects

Dependent Variable: Urine HG Arsenic 141-395 Days

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power(a)
Corrected Model	72.789(b)	4	18.197	3.669	.022	.436	14.677	.782
Intercept	30.140	1	30.140	6.077	.023	.242	6.077	.648
PRE_3	50.587	1	50.587	10.200	.005	.349	10.200	.857
LNPIDBod	38.373	1	38.373	7.737	.012	.289	7.737	.751
Age	29.491	1	29.491	5.946	.025	.238	5.946	.638
Treatment	34.745	1	34.745	7.006	.016	.269	7.006	.709
Error	94.229	19	4.959					
Total	562.300	24						
Corrected Total	167.018	23						

a Computed using alpha = .05
b R Squared = .436 (Adjusted R Squared = .317)

Parameter Estimates

Dependent Variable: Urine HG Arsenic 141-395 Days

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval		Partial Eta Squared	Noncent. Parameter	Observed Power(a)
					Lower Bound	Upper Bound	Lower Bound	Upper Bound	Lower Bound
Intercept	-22.813	10.026	-2.275	.035	-43.798	-1.829	.214	2.275	.579
PRE_3	32.483	10.171	3.194	.005	11.195	53.770	.349	3.194	.857
LNPIDBod	10.915	3.924	2.782	.012	2.702	19.129	.289	2.782	.751
Age	-.075	.031	-2.439	.025	-.140	-.011	.238	2.439	.638
[Treatment =POE]	-4.389	1.658	-2.647	.016	-7.860	-.918	.269	2.647	.709
[Treatment =POU]	0(b)	.	.	.	.	.	.	.	.

a Computed using alpha = .05
b This parameter is set to zero because it is redundant.

Estimated Marginal Means

1. Grand Mean

Dependent Variable: Urine HG Arsenic 141-395 Days

Mean	Std. Error	95% Confidence Interval	
		Lower Bound	Upper Bound
4.790(a)	.532	3.676	5.903

a Covariates appearing in the model are evaluated at the following values: Predicted probability = .6666667, LN PID per Body Weight = 1.030397, Subject Age at Start of Study (Years) = 41.346.

2. Water Treatment Type

Dependent Variable: Urine HG Arsenic 141-395 Days

Water Treatment Type	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
Point-of-Entry	2.595(a)	.716	1.097	4.093
Point-of-Use	6.984(a)	1.195	4.483	9.486

a Covariates appearing in the model are evaluated at the following values: Predicted probability = .6666667, LN PID per Body Weight = 1.030397, Subject Age at Start of Study (Years) = 41.346.

Appendix 7

Generalized Estimating Equations for Pre-Post Group

```
* Generalized Estimating Equations.
GENLIN
  UHG268d
  BY TreatmentBinary
   (ORDER=ASCENDING)
  WITH LNPIDBod Age PRE_3 Showers
 /MODEL
  TreatmentBinary LNPIDBod Age PRE_3 Showers
  INTERCEPT=YES
  DISTRIBUTION=NORMAL
  LINK=IDENTITY
 /CRITERIA SCALE=MLE
  PCONVERGE=1E-006(ABSOLUTE)
  SINGULAR=1E-012
  ANALYSISTYPE=3 CILEVEL=95
 /EMMEANS TABLES=TreatmentBinary SCALE=ORIGINAL
 /REPEATED
  SUBJECT=FamilyCode
  SORT=YES CORRTYPE=INDEPENDENT
  ADJUSTCORR=YES COVB=ROBUST
  MAXITERATIONS=100
  PCONVERGE=1e-006(ABSOLUTE) UPDATECORR=1
 /MISSING CLASSMISSING=EXCLUDE
 /PRINT CPS DESCRIPTIVES MODELINFO FIT SUMMARY SOLUTION.
```

Generalized Linear Models

```
[DataSet7] F:\SPSS\Master File_42 Pre-Post Group Without Controls New
Urines UG per G.sav
```

Model Information

Dependent Variable	Urine HG Arsenic 141-395 Days
Probability Distribution	Normal
Link Function	Identity
Subject Effect	FamilyCode
Working Correlation Matrix Structure	Independent

Case Processing Summary

	N	Percent
Included	24	100.0%
Excluded	0	.0%
Total	24	100.0%

Correlated Data Summary

Number of Levels	Subject Effect	FamilyCode	12
Number of Subjects			12
Number of Measurements per Subject	Minimum		1
	Maximum		4
Correlation Matrix Dimension			4

Categorical Variable Information

			N	Percent
Factor	POE = 1	0	8	33.3%
		1	16	66.7%
		Total	24	100.0%

Continuous Variable Information

		N	Minimum	Maximum	Mean	Std. Deviation
Dependent Variable	Urine HG Arsenic 141-395 Days	24	.7	10.2	4.058	2.6947
Covariate	LN PID per Body Weight	24	.0000	2.8002	1.030397	.8876344
	Subject Age at Start of Study (Years)	24	.7	74.6	41.346	22.7729
	Predicted probability	24	.03932	.97785	.6666667	.34205207
	Showers per Week at Home	24	.0	10.0	5.542	2.4358

Goodness of Fit(a)

	Value
Quasi Likelihood under Independence Model Criterion (QIC)	92.531
Corrected Quasi Likelihood under Independence Model Criterion (QICC)	97.119

Dependent Variable: Urine HG Arsenic 141-395 Days
Model: (Intercept), TreatmentBinary, LNPIDBod, Age, PRE_3, Showers
a Information criteria are in small-is-better form.

Tests of Model Effects

Source	Type III		
	Wald Chi-Square	df	Sig.
(Intercept)	8.426	1	.004
TreatmentBinary	14.664	1	.000
LNPIDBod	7.577	1	.006
Age	17.587	1	.000
PRE_3	14.681	1	.000
Showers	7.816	1	.005

Dependent Variable: Urine HG Arsenic 141-395 Days
Model: (Intercept), TreatmentBinary, LNPIDBod, Age, PRE_3, Showers

Parameter Estimates

Parameter			95% Wald Confidence Interval		Hypothesis Test		
	B	Std. Error	Lower	Upper	Wald Chi-Square	df	Sig.
(Intercept)	-21.694	6.7353	-34.895	-8.493	10.375	1	.001
[TreatmentBinary=0]	4.749	1.2400	2.318	7.179	14.664	1	.000
[TreatmentBinary=1]	0(a)	.	.	.	.	.	.
LNPIDBod	8.294	3.0133	2.389	14.200	7.577	1	.006
Age	-.094	.0224	-.138	-.050	17.587	1	.000
PRE_3	26.195	6.8364	12.795	39.594	14.681	1	.000
Showers	.369	.1321	.110	.628	7.816	1	.005
(Scale)	4.729						

Dependent Variable: Urine HG Arsenic 141-395 Days
Model: (Intercept), TreatmentBinary, LNPIDBod, Age, PRE_3, Showers
a Set to zero because this parameter is redundant.

Estimated Marginal Means: POE = 1

Estimates

POE = 1	Mean	Std. Error	95% Wald Confidence Interval	
			Lower	Upper
0	7.224	.7780	5.699	8.749
1	2.475	.5804	1.338	3.613

Covariates appearing in the model are fixed at the following values: LNPIDBod=1.030397; Age=41.346; PRE_3=.6666667; Showers=5.542

Curriculum Vita

Steven E. Spayd

Education

May 2009	Ph.D., University of Medicine and Dentistry of New Jersey School of Public Health, Piscataway, NJ Environmental and Occupational Health
May 2004	M.P.H., University of Medicine and Dentistry of New Jersey School of Public Health, Piscataway, NJ Environmental and Occupational Health
May 1979	B.S., Montclair State College, Upper Montclair, NJ Geoscience

Work Experience

1982 – 2009	New Jersey Department of Environmental Protection Trenton, NJ Hydrogeologist, Manager, Research Scientist
1980 – 1982	Converse Ward Davis Dixon Roseland, NJ Staff Geologist
1980	Norheast Geo-Consulting Wyckoff, NJ Chief Geologist, Environmental Consulting
1979 -1980	Ramsey High School Ramsey, NJ Earth Science Teacher
1979	Century Geophysical Corp Casper, WY Field Geologist, Mineral Exploration

Publications

Louis, J, Szabo, Z, Spayd, S, Serfes, M, Carter, G, 2008. Northern New Jersey Radionuclide Investigation: Determination of Uranium, Radium and Radon in Ground Water with High Gross Alpha-Particle Activity, and Implications for Future Monitoring Efforts, N. J. Dept. of Environmental Protection, Division of Science, Research and Technology, Research Project Summary.

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