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LONG-TERM, LOW-DOSE EXPOSURE OF PEOPLE RESIDING IN ARSENIC AFFECTED AREAS OF IOWA: A CROSS-SECTIONAL ANALYTICAL STUDY

An Abstract of a Thesis

Submitted

in Partial Fulfillment

of the Requirement for the Degree

Master of Science

Junu Shrestha

University of Northern Iowa

August 2013

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ABSTRACT

Arsenic contamination in the ground water source is a major problem throughout the world. Epidemiological studies show that long-term arsenic exposure in drinking water, even at low concentrations, can lead to chronic toxicity problems and negative health outcomes. In the United States, private wells are not mandated to comply with the drinking water standards that piped water is, putting many people at risk. This study focused on monitoring of arsenic contamination of private well water, hair samples, and questionnaire based surveys. Of the 260 private well owners selected from 13 counties in Iowa who were invited to participate in the study, 50 agreed to participate in the research. Analysis showed that 58% of the water samples tested positive for arsenic and 12% had arsenic concentrations more than the federal drinking water standard of 0.01 mg/L. The mean water arsenic concentration was 0.007 $\pm$ 0.001 mg/L (range, 0.001- 0.027 mg/L). The hair arsenic analysis showed that 14% of the hair samples exceeded the normal arsenic range (0.08 to 0.25 mg/kg). The mean hair arsenic concentration was 0.108 $\pm$ 0.024 mg/kg (range, 0 - 0.54 mg/kg). The bivariate analysis between hair arsenic and water arsenic concentration showed a positive correlation (R-square = 0.25, p = 0.0047). The result showed that the hair arsenic content was higher among the participants who consumed water contaminated with arsenic more than 0.01mg/L arsenic (p = 0.02).

The result also found that 76% of participants did not know about arsenic, indicating that an awareness program should be provided to them about the toxicological effects of arsenic. The hair and water arsenic concentrations were correlated with different health parameters and a statistically significant correlation was found between

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hair loss and hair/water arsenic concentration ($p < 0.05$). Health conditions such as kidney, liver, and lung as well skin problems also had a correlation with arsenic in hair and water, but the result was not statistically significant ($p > 0.05$). Other health problems such as stomach pain, diabetes, heart problem, numbness on hands and feet, tiredness, depression, anxiety, and confusion of mind were not correlated with hair arsenic concentration ($p > 0.05$). There was a significant relationship ($p < 0.05$) between multiple factors such as water arsenic concentration, age, gender, occupation, education, years of residence, and drinking water sources that might increase arsenic concentration in hair.

Arsenic levels are present in a significant number of wells in the study area, and that the ingestion of arsenic contaminated water leads to an increase in arsenic deposition in the hair. In addition environmental health education program on arsenic and its health impacts is necessary, especially for private well owners who are unknowingly consuming contaminated water.

Keywords: Arsenic, Iowa, human hair, private wells

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This Study By: Junu Shrestha

Entitled: LONG-TERM, LOW-DOSE EXPOSURE OF PEOPLE RESIDING IN ARSENIC AFFECTED AREAS OF IOWA: A CROSS-SECTIONAL ANALYTICAL STUDY

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DEDICATION

I lovingly dedicate this thesis to my husband, and my parents who supported and encouraged me in each step of the way.

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ACKNOWLEDGEMENTS

Foremost, I would like to express my sincere gratitude to my advisor Dr. Catherine Zeman for her continuous support during my master's study and research. I acknowledge her patience, enthusiasm, and guidance that helped me to motivate and focus more on my research. I am sincerely thankful to Dr. Maureen Clayton and Dr. Susan Dobie for their feedback as committee members. I would like acknowledge the Environmental Program at the University of Northern Iowa (UNI) for providing funding and opportunity to be a part of this institution.

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ABBREVIATIONS

As: Arsenic

As (III): Trivalent arsenic

As (V): Pentavalent arsenic

ASTDR: Agency for Toxic Substances and Disease Registry

CCA: Chromated Copper Arsenate

DDT: Dichlorodiphenyltrichloroethane

DMA: Dimethylarsinic acid

EPA: Environmental Protection Agency

ICP-MS: Inductively Coupled Plasma-Mass Spectrometry

IRB: Institutional Review Board

kg: kilogram

kt: kiloton

MCL: Maximum Contaminant Level

mg/L: milligram per liter

mg/g: milligram per gram

mg/kg: milligram per kilogram

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ml: milliliter

MMA: Monomethylarsinic acid

NPL: National Priority List

NTU: nephelometric turbidity units

PPB: Parts per billion

WHO: World Health Organization

%: Percentage

µg/L: microgram per liter

µg/g: microgram per gram

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CHAPTER 1

INTRODUCTION

Background

In 2005, the United States Geological Survey (USGS) estimated that about 20% of total national water came from ground water. The majority of ground water was used for irrigation and about 19% was used for public drinking water supply, to meet the need of much of the Nation's population. The majority of households that have self-supplied private water rely on ground water that accounts for 98% of total private water supply (Kenny et al., 2009). The monitoring of private water supplies is not done by government agencies and it is the responsibility of well owner's to understand the quality of their water supply. Homeowners have full responsibility for their own water supply and should test their water periodically to provide a safe, secure supply of potable water to their household.

In the United States, widespread arsenic concentration in ground water is mainly due to arsenic released from rocks containing iron oxide. This is due to geochemical conditions which include the reaction of iron oxides with natural or anthropogenic organic carbon and alkaline ground water. In contrast, in the western United States, iron oxide and sulfide minerals are the main sources of arsenic contaminated ground water (Welch, Westjohn, Helsel, & Wanty, 2000).

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In 2011, it was estimated that 90.3% (2.75 million) of Iowans were served by community, public water supplies; the remaining 9.7% of Iowans were served by private water supplies (Iowa Department of Natural Resources, 2012). Approximately 67% of Iowa residents depend on ground water from private and public wells to meet daily water needs. In addition it has been estimated that about 450,000 Iowans solely depend on private wells for their daily household needs. Federal and state laws only require that public water supplies be tested regularly for quality, so there is no mandatory requirement for water testing of private wells. Therefore these private well owners could be consuming water that is of uncertain safety and a significant percentage of individuals may be currently drinking water that is not safe to drink (Center for Health Effects of Environmental Contamination [CHEEC], 2009).

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Problem Statement

Arsenic and its congeners such as the sulfides or oxides have been used for a wide variety of purpose in the United States including, agricultural application, wood preservation, and glass production. Additionally, in the past 60 years inorganic arsenic was used as a wood preservative and it currently represents the single greatest use of arsenic compounds in the United States (Welch et al., 2000).

In the United States, over 15 million households rely on private, household wells for drinking water. The EPA regulation on arsenic contamination in ground water applies only for the public water system in the United States. Therefore the owners of private wells are entirely responsible for the safety of their water supply (CDC, 2011).

It is estimated that 8% of public water supplies and 10% of all drinking water sources in the United States have arsenic concentrations greater than 0.01 mg/L and until relatively recently arsenic was not considered a serious threat to public health in the United States (Ravenscroft, Brammer, & Richards, 2009). Inorganic arsenic is toxic and a well-known human carcinogen, and is frequently found in the ground water supplies of the United States (Knobeloch, Zierold, & Anderson, 2006). Since arsenic has been recognized as an increasing threat and now is considered very toxic to humans, USEPA has progressively lowered the Maximum Contaminant Level (MCL) guideline under the Safe Drinking Water Act (SDWA) for arsenic in drinking water. In 2001 the EPA decreased the maximum allowable level of arsenic from 0.05 mg/L to 0.01 mg/L (Ayotte, Montgomery, Flanagan, & Robinson 2003). This decrease in the regulatory level required

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that many public water supply utilities institute tertiary control measures above and beyond standard water treatment that would remove arsenic before the water could be used for human consumption. Concerns about the potential human health effects caused by arsenic exposure from drinking water were the most important factor considered when the regulatory level was lowered, resulting in increased cost to public and private water utilities, again the private well-owner is expected to monitor and treat their drinking water without regulatory oversight.

Health effects of arsenic are dependent on the dose ingested and the duration of exposure-the higher the dose and the longer the exposure the more adverse the long-term health impacts are thought to be. Epidemiological studies have confirmed that the chronic effects of inorganic arsenic exposure via drinking water include: skin lesions, respiratory problem, cancer of the bladder, colon/liver, and lung, high blood pressure, diabetes and many more (Kapaj, Peterson, Liber, & Bhattacharya, 2006). The skin lesions are some of the most common nonmalignant effects of chronic arsenic exposure. Even at low concentrations in the range of 0.005-0.01 mg/l, there has been an increased prevalence of skin lesions (Yoshida, Yamauchi, & Sun, 2004). The study done at Yatenga Province, Bukina Faso indicated that the frequency of melanosis and keratosis distribution was 20.63% among the population drinking arsenic contaminated water of less than 0.01 mg/L (Somé et al., 2012).

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CHAPTER 2

LITERATURE REVIEW

Arsenic is a recognized poison that occurs naturally and is acknowledged by many toxicologists and environmental health researchers as one of the world's great environmental hazards, threatening millions of people's lives (Ravenscroft et al., 2009). This toxic element is unevenly distributed in the Earth's crust and is ranked the twentieth most abundant element (Luong, Majid, & Male, 2007). It is also considered "the king of poisons" and probably the single most important element having influenced human history due to its toxicological properties (Nriagu, 2002). It also has a history of use as a poison applied for political purposes, especially during the age of monarchies and hereditary royalty, where movement up the ruling ranks required the death of another reigning ruler (Ravenscroft et al., 2009).

Mineral arsenicals have been very well known for centuries among human cultures due to their use in low-doses for traditional medicines in the treatment of various diseases such as leukemia, Hodgkin's disease, pernicious anemia, psoriasis, pemphigus, eczema, and asthma (Evens, Tallman, & Gartenhaus, 2004; Miller, Schipper, Lee, Sinfer & Waxma, 2002). In traditional medicine, it is used in its mineral forms to include: orpiment (As_2S_3), realgar (As_4S_4), and arsenolite (As_2O_3; Liu, Wu, Goyer, & Waalkes, 2008). Even in modern times, arsenic has been used to poison others for revenge or financial gain. Arsenic is an attractive poison because it is colorless, odorless, and tasteless. It is toxic at very low levels if the exposure is continuous for many years

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(Ravenscroft et al., 2009). Long term exposure to arsenic even below the maximum contaminant level of 0.01mg/L is still considered to impact human health but research is needed to access health effects using a biologically based mechanical model (Liao et al., 2009).

Arsenic species are classified as either inorganic or organic depending on the nature of the carbon present in the compound. The most dangerous forms are the valance states of arsenic giving the element the most opportunity for movement in the environment and into the human body. This includes the trivalent (III) and pentavalent (V) states. Elemental arsenic has a valance state of (0) and arsenic in the form of arsine gas and arsenide has a valance state of (III). Inorganic arsenic in the trivalent form is more toxicologically potent than organic arsenic in the pentavalent (V) state (Hughes, Beck, Chen, Lewis & Thomas, 2011; Jain & Ali, 2000).

Sources of Arsenic

Arsenic is widely distributed in the Earth's crust and, thus, the environment. The total arsenic amount in the Earth's crust is estimated to be $4.01*10^{16}$ kg based on concentration in rock material (Matschullat, 2000). In the global arsenic cycle, oceans contain $3.7*10^{6}$ kt arsenic, earth (land) has $9.97*10^{5}$ kt , sediments have $25*10^{9}$ kt arsenic and the atmosphere has 8.12 kt arsenic (Bissen & Frimmel, 2003). Arsenic is naturally found in over 200 different mineral forms, of which approximately 60% are arsenates, 20% sulfides and sulfosalts and remaining 20% are arsenides, arsenites, oxides, silicates, and elemental arsenic (Baur & Onishi, 1978).

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Consequently, both natural and anthropogenic activities can result in significant input of arsenic to the hydrologic cycle. Further, the rate of arsenic accumulation in the soil surface environment depends on the retention and mobility of host materials such as soils and rocks (Bhattacharya et al., 2002).

Natural Sources

Arsenic is naturally found in two distinct mineral associations, sulfides and oxides (Ravenscroft et al., 2009). In sulfides, arsenic occurs in reduced form while arsenic occurs in oxidized form in the mineral arsenolite (As_2O_3). The natural sources of arsenic include windblown dust from weathered continental crust, forest fires, volcanoes, sea spray, hot springs, and geysers. Natural processes such as weathering and volcanic eruptions also release arsenic into the environment and transport it over long distances through water and air. Weathering of rocks containing arsenic converts arsenic sulfides to arsenic trioxide which then enters the arsenic cycle as dust or by dissolution in rain, river, or groundwater. It can also enter the food chain causing wide spread distribution throughout the plant and animal kingdoms (Mandal & Suzuki, 2002). Arsenic is present in high concentration in soils as compared to rocks, with estimated concentrations in soil of an average of 5 mg/kg. Arsenic release to the hydrologic cycle in the natural environment is mainly dependent on the organic/inorganic component and redox potential states of soil (Shih, 2005). Table 1 shows the arsenic abundance in crustal materials.

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Table 1: *Abundance of arsenic (mg/kg) in crustal materials*

(Source: Bhattacharya et al., 2002)

Rock type	Range
Igneous rocks	
Ultrabasics	0.3–16
Basalts	0.06–113
Andesites	0.5–5.8
Granites/silicic volcanics	0.2–13.8
Sedimentary rocks	
Shales and clays	0.3–490
Phosphorites	0.4–188
Sandstones	0.6–120
Limestones	0.1–20
Coals	0.5–80

Anthropogenic Sources

The human health impact of the arsenic level in the environment depends on the amount and frequency of human contact, which is a correlate of the distance from environmental sources, dispersal rate and the transport fate of the arsenic released (EPA, 2000). Arsenic is released into the environment from natural and anthropogenic sources. Anthropogenic sources of arsenic include things such as miscellaneous industrial releases, metal ore smelting, mining, agricultural uses, wood preservation, and to a lesser degree medicinal use. Anthropogenic sources of arsenic are ranked first on the ATSDR/EPA priority list of hazardous substances. Arsenic has been found in at least 1,014 current or former National Priority List (NPL) sites (Selene & De Rosa, 2003).

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Historically, inorganic arsenic was used as a constituent in numerous varieties of pesticides, insecticides, herbicides, and fungicides (EPA, 2000). In 1867 arsenic was used for the insecticide commonly known as “Paris Green” to control the Colorado potato beetle. After it was found to be toxic to pest species, lead arsenate was rapidly adopted for insecticidal use throughout the world. It was used as an insecticide in Washington fruit orchards for the purpose of controlling codling moth from 1905 to 1947 before the introduction of DDT and organophosphorus insecticides (Peryea, 1998). In 1947, it was estimated that the average annual application rate was as high as 125 kg of lead and 45 kg of arsenic per acre (Wolz, Fenske, Simcox, Palcisko & Kissel, 2003). This use was not restricted to the United States; lead arsenate was also used extensively in Australia, Canada, New Zealand, England, and France particularly to control codling moth (Peryea, 1998). Exposure to environmental conditions and soils with certain redox potentials increases the risk that biochemical transformations of the lead arsenate can occur such that some of the most toxic tri- and pentavalent forms of arsenic are liberated to biogeochemical and hydrologic cycling.

Another form of anthropogenic arsenic, CCA (Chromated Copper Arsenate) was widely used as a wood preservative due to its excellent fungicidal and pesticidal properties. The large-scale use of CCA treated wood has been one of the main causes of environmental contamination from anthropogenic arsenic, posing a major potential toxic risk to humans, animals, and plants. In 1986 it was estimated that 10.6 million cubic meters of wood treated with CCA preservatives was produced in the United States

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(Stilwell & Gorny, 1997). This wood was widely used for construction of decks, fences, walkways, piers, restraining walls, and bridges prior to the mid-1990s (Lebow, 1996).

Anthropogenically sourced arsenic is also emitted into the atmosphere from metal smelting. High concentrations of arsenic occur in areas with current or historical mining activities (EPA, 2000). Occupational exposure studies done on workers at copper smelting industries indicates a strong correlation between arsenic exposure (a byproduct of copper smelting) and lung cancer. The main route of exposure for these workers is inhalation of arsenic dust and arsenic trioxide vapors (Wicks, Archer, Auerbach, & Kuschner, 1981; Yager, Hicks, & Fabianova, 1997).

Additionally, arsenic and antimony oxides have been used in the glass manufacturing industry as fining agents to remove bubbles in glass generated when melting batch ingredients. Eventually disadvantages to the manufacturing process from crystallization were recognized and the glass industry stopped using arsenic and antimony oxides (Demarest, 1976). However, the release of these arsenical, compounds occurred for decades prior to this recognition. As the foregoing examples illustrate, arsenic and its compounds have been used in many different types of industry: semiconductor, glass, timber treatment, and chemical manufacturing firms, all contributing to the anthropogenically sourced loads of environmental arsenic (Farmer & Johnson, 1990).

A final, interesting historical example of anthropogenically sourced arsenic was its use as an embalming agent in the Civil War period from 1860 to 1910. It was

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considered a sanitary practice to prevent decomposition and a practical means to preserve the body for transport and until burial. Arsenic used in these past embalming procedures is now considered a significant danger to forensic archeologists, cemetery workers, and individuals that may be utilizing potentially contaminated ground water supplies as a result of proximity to known and forgotten cemetery sites (Konefes & McGee, 2000; Langley & Abbott, 2000)

Global Distribution of Arsenic

Arsenic contamination in natural water is a worldwide problem and has been reported in the USA, China, Chile, Bangladesh, Taiwan, Mexico, Argentina, Poland, Canada, Hungary, Japan, and India (Jain & Ali, 2000). The presence and mobility of arsenic in groundwater mainly depends on the local geology, hydrogeology, and geochemistry of the sediments as well as several other anthropogenic factors such as the land use pattern (Bhattacharya et al., 2002). In a global scale environment, arsenic is mainly transported through water because it dissolves quite well in both stream water and seawater (Shih, 2005). Arsenic contamination in the ground water was first documented at a larger, regional scale about a half century ago, correlating deep water wells and Black Foot Disease in Taiwan (Wang & Wai, 2004). It has been reported that nine districts in West Bengal, India and 42 districts in Bangladesh have arsenic contamination above the World Health Organization, WHO guideline of 50 ppb (0.05 mg/L). Millions of individuals living in these areas are directly affected by drinking ground water contaminated with arsenic (Chowdhury et al., 2000). Again, anthropogenic sources of

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arsenic deposition are often suspect in the contamination, especially at regional scales. Table 2 shows the arsenic concentration in ground water used for drinking purposes and from burning coal in areas around the world, indicating a strong correlation between these two phenomena.

Table 2: *Arsenic and population at risk around the world*

(Source: Ng, Wang, & Shraim, 2003)

Arsenic contaminations in groundwater and coal and population at risk around the world

Country or area	Population at risk	Groundwater concentration (μg As l^{-1})	Guidelines (μg As l^{-1})	Discovery date	References
Argentina	2 000 000	100–1000	50	1981	Sancha and Castro (2001)
Bangladesh	50 000 000	<1–4700	50	1980s	Ahmad (2001)
Bolivia	20 000		50	1997	Sancha and Castro (2001)
Chile	437 000	900–1040	50	1971	Sancha and Castro (2001)
China, Guizhou[a]	20 000	100–10 000 (mg kg^{-1})	8 mg kg^{-1}	1950s	An et al. (1997)
China, Inner Mongolia	600 000	1–2400	50	1990s	Guo et al. (2001)
China, Xinjiang Province	100 000	1–8000	50	1980s	Wang (1997)
Hungary	220 000	10–176	10	1974	Sancha and Castro (2001)
India, West Bengal	1 000 000	<10–3900	50	1980s	Chakraborti et al. (2001)
Mexico	400 000	10–4100	50	1983	Sancha and Castro (2001)
Nepal	Unknown	Up to 456	50	2002	Tandukar and Neku (2002)
Peru	250 000	500	50	1984	Sancha and Castro (2001)
Romania	36 000	10–176	10	2001	Gurzau and Gurzau (2001)
Taiwan	200 000	10–1820	10	1950s	Tseng (1977)
Thailand, Ronpibool	1000	1–5000	50	1980s	Choprapwon and Porapakkham (2001)
USA	Unknown	10–48 000	10	1988	Welch et al. (1988)
Vietnam	Millions	1–3050	10	2001	Berg et al. (2001)

[a] Guizhou is the only known arsenicosis endemic area caused by arsenic contamination in coal.

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Arsenic in the United States

Arsenic is a common trace element found in varying amounts in the United States originating from both natural and anthropogenic sources. The element was used in the past for industrial purposes, especially for the production of insecticides, wood preservatives, chemicals, and for medicinal uses. Natural sources of arsenic in the United States are mainly of geologic origin and find their way into ground water. The arsenic concentration in ground water varies regionally due to both climatic and geologic factors. Areas of high concentration in the United States are the Interior Plains and the Rocky Mountain System whereas the concentration in the Appalachian Highlands and the Atlantic Plain is generally very low (Welch et al., 2000).

Along the Eastern Seaboard, some areas of high arsenic concentration can be found. The New Jersey Private Well Testing Act program indicated that 3.4% of the wells tested in ten counties had arsenic concentrations above the EPA standard with maximum concentration of 254 ppb (0.25 mg/L; New Jersey Department of Environmental Protection, 2008).

In eastern New England, arsenic is also found in ground water with a concentration that is a concern for human health. The highest concentrations in New England states, contaminating nearly 30% of wells, are found in Maine and New Hampshire (Ayotte et al., 2003).

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In the mid-west state of Wisconsin about 39% of well water samples contained detectable arsenic concentrations less than the EPA standard (MCL) but 20% contained equal to or more than the EPA standard of 0.01 mg/L. The arsenic contamination ranged from less than 0.001 mg/L to 3.1 mg/L (Knobeloch et al., 2006). High arsenic concentration up to 12 mg/L has been recorded in the confined aquifer in Eastern Wisconsin and the main source of arsenic is a sulfide bearing secondary cement horizon (SCH) having variable thickness, morphology, and arsenic concentration (Schreiber, Simo, & Freiberg, 2000).

Arsenic Contamination in Iowa

Arsenic contamination in the drinking water sources of Iowa has recently been the subject of more investigation when the study done by CHEEC at the University of Iowa found that nearly half of the 475 private wells checked between 2006 and 2008 tested positive for arsenic, with 8 percent above the drinking water standard of 0.01 mg/L (CHEEC, 2009). The study also revealed that the ground water of Cerro Gordo County has a persistently high level of arsenic more than 0.01 mg/L. In addition, a follow up study done by the Cerro Gordo County Health Department also indicated that arsenic was present in 70% of the water samples with 38% measuring above 0.01 mg/L (University of Iowa News Release, 2011).

According to the CHEEC (2009) study, arsenic was most common in the southwest, north-central, and northwest region of Iowa but high arsenic >0.01 mg/L was most prevalent in the north-central region. The prevalence of arsenic in Iowa is mainly

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due to the Des Moines lobe (University of Iowa News Release, 2011) which includes other states such as Minnesota, North Dakota, and South Dakota. The Des Moines lobe was deposited by glaciers that traveled across north-north west and central Canada during the late Wisconsin period, from 16,000 to 12,000 years ago (Erickson & Barnes, 2005). The public water systems within and outside the footprint of late Wisconsin till were compared. The results found that 10.7% of public water exceeded te MCL limit inside footprint of late Wisconsin till whereas only 2.4% of public water exceeded the MCL limit outside the footprint. The statistically significant relationship confirmed that high naturally occurring arsenic concentration in the groundwaters of Minnesota,North Dakota, South Dakota, and Iowa is directly linked the presence of Des Moines lobe till (Erickson & Barnes, 2004).

Figure 1 indicates that arsenic is dispersed in most regions of Iowa with high concentration found at the north central regions. Red dots in the figure show arsenic concentrations ranging above the national standard of 0.01 mg/L and the blue dots indicates measurable arsenic concentration found, but below the maximum contaminant level.

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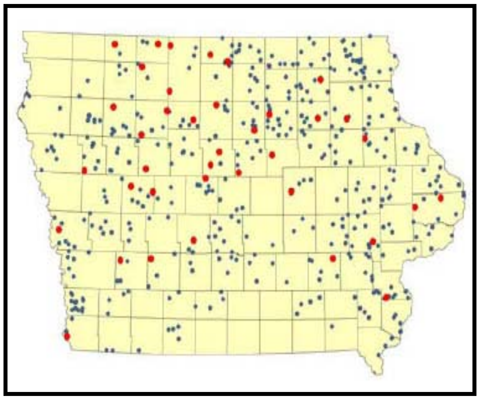

Sampling Region	Arsenic Concentration (mg/L)			Total
	< 0.001	0.001 - < 0.01	≥ 0.01	
East-central	89	56	9	154
North-central	39	43	19	101
Northeast	58	20	3	81
Northwest	13	17	1	31
South-central	23	16	1	40
Southwest	25	35	6	66
Total	247	187	39	473

Figure 1: Spatial arsenic distribution of private wells of Iowa

(Source: CHEEC, 2009)

Route of Entry and Arsenic Metabolism

Long-term exposure to arsenic in humans results in chronic arsenic poisoning called arsenicosis. This condition has been reported among people living in areas with high endemic arsenic concentrations in drinking water or from the burning of coal (Ng et al., 2003). This can occur because the routes of entry into the body for arsenic are multiple and include ingestion, inhalation, and skin absorption (Saha, Dikshit, Bandyopadhyay, & Saha, 1999). It should be noted, however, that the majority of past studies indicate that ingesting arsenic contaminated water and food is the most common route of arsenic entry into the human body. However, a study done in Egypt indicates that the participants were exposed to arsenic through exposure routes by smoking, consumption of fish and animal protein rather that arsenic content in domestic tap water (Saad & Hassanien, 2001). Another study done in Taiwan also shows that there was a

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significant dose-response relationship present between ingested arsenic and lung cancer, which is more prominent among cigarette smokers (Chen, Wu, & Chen, 2004).

In certain situations, the inhalation route of exposure may be of primary importance; for example, occupational health studies of miners and smelter workers indicates that inhaling high levels of inorganic arsenic in airborne dust is a primary cause of respiratory tract cancer (Lubin, Moore, Fraumen, & Cantor, 2008).

Arsenic partitions to a large number of organs once it enters the body and it is very difficult to diagnose the early symptoms of arsenicosis because they are subtle (Saha et al., 1999). The few studies on dermal absorption of arsenic which have been done indicate that the absorption rate is generally low but certain forms of arsenic have higher absorption rates (Mandal & Suzuki, 2002).

Arsenic metabolism in human is characterized by two types of reactions; 1) reduction of pentavalent arsenate As (V) to trivalent arsenite As (III) in blood, and 2) Oxidative methylation of trivalent arsenite As (III) to monomethyl arsonic acid (MMA) and then to dimethyl arsenic acid (DMA; Loffredo, Aposhian, Cebrian, Yamauchi & Silbergeld, 2003). Around 60-70% of the inorganic arsenic ingested by the average individual is excreted through urine in the form of MMA and DMA due to the methylation of inorganic arsenic or trivalent arsenic. The rate of methylation (and clearance) depends on dose, age, gender, and smoking habits (WHO, 2001; Ya´n˜ez et al., 2005).

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Biomarkers of Arsenic Exposure

Humans are exposed to different forms of organic and inorganic arsenic from food, water, air, or other environmental media (Mandal & Suzuki, 2002). The individuals exposed transform, accumulate, and eliminate ingested inorganic arsenic from the body (Ya´n˜ez et al., 2005). Once ingested through drinking water, arsenic is absorbed and then distributed in the bloodstream (Luong et al., 2007).

To understand the accumulation of arsenic in the human body three types of biomarkers can be used; total arsenic in hair and/or nails, blood arsenic, and metabolites of arsenic in urine (Mandal & Suzuki, 2002). Blood and urine samples are used as biomarkers for recent arsenic exposure, whereas hair and nail samples indicate arsenic exposure over the longer term, mainly several months (Ratnaike, 2003).

Arsenic has an affinity to sulfhydryl groups in keratin. Since hair and nail are rich in keratin tissues, arsenic accumulates in these tissues (Gault et al., 2008). Once arsenic accumulates in these tissues, it remains for a longer time due to low mobility inside the tissue. Therefore hair and nail analysis results are used as a biological indicator of longer term arsenic exposure over several months (Mandal & Suzuki, 2002; Hinwood et al., 2003). Some studies reveal that hair and nails do not justify arsenic concentration in the body due to exogenous contamination but still they are considered as an important indicator for long term (several months to year) arsenic toxicity (Hindmarsh, 2000; Ya´n˜ez et al, 2005). Background or "normal" arsenic levels in hair is about 0.008 to 0.025 mg/100g (0.08 to 0.25 mg/kg) and a concentration above 0.1 mg/100g (1 mg/kg) is

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considered an indication of excess arsenic exposure (Arnold, Odom, & James, 1990; Saad & Hassanien, 2001).

Toenail and fingernail clippings are also considered excellent biomarkers of exposure because they are less susceptible to external arsenic contamination, for example, from washing hair in arsenic containing water. These samples are easy to collect and maintain as well and, again, represent long term exposure (3 to 12 months; Freeman, Dennis, Lynch, Thorne & Just, 2004).

Arsenic is excreted through urine with a half-life of approximately four days in the human (NRC, 1999). Normal arsenic in urine samples is found in the range of 0.005 to 0.04 mg/l and analyzed as total arsenic or speciation arsenic (Arnold et al., 1990; Hughes, 2006). Analysis of arsenic in blood is best utilized to determine recent high-dose arsenic exposure. Blood levels of arsenic as a biomarker of exposure are more difficult to work with in population based studies as participants generally do not want to consent to the blood draw and as these samples represent only a narrow window of exposure. The typical, background arsenic concentration for blood is 0.0005 to 0.02 mg/l (Hughes, 2006; NRC, 1999).

Health Impacts of Arsenic

The toxicity of arsenic exposure is dependent on the chemical form of arsenic one is exposed to. This is mainly a function of the arsenic's oxidation state, with trivalent arsenic compounds (As (III)) being more toxic than pentavalent arsenic compounds (As

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(V) ; Hughes, 2006). Compound specific exposure is, of course, also complicated by dose, frequency and duration of exposure variables. If a large dose of arsenic is experienced for a shorter duration, acute toxicity is a likely outcome, whereas if a small dose of arsenic is experienced for a prolonged time period chronic toxicity and/or detrimental chronic disease (such as cancer) may result.

Acute arsenic poisoning can create chronic debilitating illness and is sometimes deadly, but its origins are usually anthropogenic and rarely results from environmental exposure. On the other hand, chronic poisoning is related to environmental exposure that results in such problems as dermatological and carcinogenic effects (Ravenscroft et al., 2009).

Acute Arsenic Poisoning

Acute poisoning is commonly associated with accidental or deliberate ingestion of arsenic. An acute lethal dose of arsenic is 0.6 mg/kg/day (Ratnaike, 2003). Acute poisoning has two main manifestations; gastrointestinal syndrome and paralytic syndrome (Ravenscroft et al., 2009). The gastrointestinal syndrome is the most common effect of acute arsenic poisoning and manifests as dry mouth, burning lips, and dysphagia (difficulty swallowing). It's root etiology lies in a paralysis of capillary control of the intestinal tract due to enteric nervous system damage. The net result is decreased blood volume, low blood pressure, and electrolyte imbalance due to dysfunction in the intestinal tract which finally leads to multi-organ failure (WHO, 2001). Acute paralytic syndrome on the other hand results in cardiovascular collapse (due to impairment of the cardiac nerves) or depression of the central nervous system which can cause death within several

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hours (Ravenscroft et al., 2009). Survivors of acute poisonings may develop long-term irreversible sequel such as bone-marrow suppression, hepatomegaly, melanosis, and damage to the peripheral nervous system (WHO, 2001).

Chronic Arsenic Poisoning

Long-term arsenic toxicity has a more subtle presentation but can lead to multi-organ system damage (Ratnaike, 2003) resulting in organ failure or cancer. Indication of chronic arsenic exposure may be seen in disordered function of the melanocytes (pigment cells) of the skin but this is not always present in chronic exposure cases. Organ systems that may be involved in chronic exposures include the cardiovascular, neurological, gastrointestinal, respiratory system, and endocrine system (Ratnaike, 2003; Ravenscroft et al., 2009).

Dermatological effect: Pigmentation changes to the skin may be indicative of chronic arsenic exposure and may be associated with hyperkeratosis (thickening of the skin), and skin cancer (WHO, 2001). The pigmentation can appear in a 'raindrop' pattern widely dispersed across the body, or it may appear as diffused, localized patchy pigmentation, giving a spotty white appearance to the skin. Keratosis refers to the diffuse thickening of skin mainly on the palms of the hands and soles of the feet; alternatively nodules may form that are symmetrically distributed. Keratosis is also further graded into mild, moderate, and severe (Mazumder, 2008). Skin cancer has mainly been associated with the advanced stages of arsenic poisoning wherein chronic low-dose exposures (below the MCL level) have been on-going for many years (Ravenscroft et al., 2009).

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Respiratory diseases: Several epidemiological studies indicate that the risk of lung cancer is elevated in chronic exposure populations and is more prominent in individuals with skin lesions associated with chronic arsenic poisoning (Ratnaike, 2003; Ravenscroft et al., 2009). Studies have also illustrated that the risk of lung cancer is potentiated among individuals drinking arsenic contaminated water who also smoke (Ravenscroft et al., 2009).

Vascular disease: Vascular and cardiovascular disease is a real risk to populations experiencing chronic arsenic exposure through drinking water. Cardiovascular diseases associated with arsenic exposures through drinking water include: Black Foot Disease (BFD), hypertension, diabetes, and hyperlipidemia (WHO, 2001). Arsenic exposure is considered as a risk factor for arteriosclerosis that causes cardiovascular disease and coronary artery disease (Ravenscroft et al., 2009). Epidemiological studies also reported an increased prevalence of hypertension among residents having BFD and a dose-response relationship between ingested inorganic arsenic and hypertension occurrence (Mazumder, 2008).

Liver disease: Portal hypertension associated with portal fibrosis has also been reported among people drinking arsenic contaminated water at greater than 0.01 mg/L. Additionally, liver complications such as hepatomegaly, portal zone expansion, hepatic fibrosis, and splenomegaly have been related with arsenic exposure in drinking water for decades (Mazumder, 2008). The cross-sectional epidemiological study conducted in West Bengal with arsenic level below and above 0.05 mg/L showed prevalence of

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hepatomegaly was significantly high in arsenic exposed people (10.2%) as compared to the control group (2.99%, $p < 0.001$; Mazumder, 2005).

Diseases of the nervous system: Epidemiological studies also indicate that long term, low level exposure to arsenic leads to neurotoxicity (WHO, 2001). Chronic exposure to arsenic results in peripheral neuropathy with such symptoms as tingling, numbness, and limb weakness (Mazumder, 2008). Additional nervous system impacts include: polyneuropathy, EEG abnormalities, and in extreme cases behavior changes such as hallucinations, disorientation, and agitation. It has been noted that arsenic has an impact on the nervous system and behavior but very few studies has been done to address this issue (Rodr´ıguez, Jiménez, & Giordano, 2003).

Pregnancy outcomes: Investigations on pregnant women reveal that arsenic concentrations above 50 ppb (0.05 mg/L) in drinking water increase the risk of spontaneous abortion, stillbirth, preterm birth, and infant mortality (Ehrenstein et al., 2005). Arsenic readily crosses the placental barrier and, thus, affects fetal development (Ahmad et al., 2001). Further, similar arsenic concentrations were found in cord blood and maternal blood (˜9 µg/l) of maternal/infant pairs exposed to high arsenic containing drinking water at approximately 200 µg/L (0.2 mg/L; Concha,Vogler,Lezcano, Nermell & Vahter, 1998).

Epidemiological Studies on Health Effects of Arsenic

Numerous epidemiological studies have documented the health impact of arsenic exposure via drinking water at different concentrations. In highly arsenic affected areas

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such as Bangladesh, West Bengal, India, Taiwan and other parts of the world, adverse health impacts resulting in different forms of cancer have been recorded. Even exposure to low-dose arsenic level for longer time periods (years or decades) can have subtle impacts to humans making exposure related health problems very difficult to diagnose.

Epidemiological Study on Non-Cancerous Disease

Epidemiological studies such as cross-sectional, case-control, prospective-cohort, and retrospective cohort studies have all been conducted to identify the chronic impacts of arsenic to human health. Most of these have been conducted on drinking water exposures as this is one of the most common forms of exposure. These studies have confirmed the cancerous, gastrointestinal and neurological impacts of chronic arsenic ingestion above the maximum contaminant level. Less well characterized have been subtle effects below the MCL threshold. According to Yoshida et al. (2004) nonmalignant skin lesions have been observed among the population drinking low arsenic concentration in drinking water (0.005-0.01 mg/L).

An interesting study in this category would include a cross-sectional study done in Mexico indicated that chronic environmental or occupational exposure to inorganic arsenic increased the risk of diabetes. The study showed increase prevalence of diabetes among the population exposed to inorganic arsenic when compared to unexposed populations with individuals exposed to 0.01 mg/L arsenic in drinking water having 1.13 times the chance of having diabetes than an unexposed population. Diabetes was also positively associated with cumulative arsenic exposure of the preceding five years (Razo et al., 2011).

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Another epidemiological study examining the prevalence of type 2 diabetes and arsenic exposure also indicated that total urine arsenic was associated with increased prevalence of type 2 diabetes. The study indicated that the participants with type 2 diabetes had 26% higher concentration of total arsenic in their urine samples as compared to the participants without type 2 diabetes (Navas-Acien, Silbergeld, Pastor, & Guallar, 2008). In addition to both type 1 and type 2 diabetes, cardiovascular system disease including hypertension, ischemic heart disease, cerebral infraction, and mortality from cardiovascular disease are highly associated with arsenic endemic areas by correlational analysis (Tseng, 2008).

Epidemiological Study on Cancerous Disease

A population based case-control study of lung cancer conducted in New Hampshire and Vermont indicated that arsenic exposure was associated with small cell and squamous cell carcinoma of the lung when correlated with toenail arsenic concentration ≥ 0.114 µg/g (Heck et al., 2009).

Chronic arsenic ingestion from drinking water is also associated with the skin related disorders. An epidemiological case-control study done in the south western region of Taiwan indicated that individuals having skin lesion cases had a higher percentage of inorganic arsenic, MMA, a lower percentage of DMA, and a higher ratio of MMA to DMA as compared to control groups with similar exposure of arsenic in drinking water. The result also indicated that individuals with a higher percentage of MMA had an odds ratio of developing skin disorders 5.5 times than the individuals having a lower

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percentage of MMA. The methylation capacity of the individual may have a role in the development of skin disorders related to arsenic exposures (Yu, Hsu, Chen, & Froines, 2000).

Finally, in a case-control study done in the Western United States the odds of developing bladder cancer was 3.67 times greater among a population sample of individuals with 40 years or more smoking history who were also consuming arsenic at rates of greater than 80 µg /day. The data also indicated that smokers consuming arsenic levels near 200 µg/day may be at increased risk of bladder cancer, as arsenic can often be found as a contaminant in cigarette smoke (Steinmaus, Yuan, Bates, & Smith, 2003).

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CHAPTER 3

HYPOTHESIS AND OBJECTIVES

This study had two null hypotheses and two alternate hypotheses:

Null Hypothesis

H_{01}: There is no relationship between drinking water arsenic levels and the levels of arsenic detected in hair samples.

H_{02}: There are no relationships between current arsenic exposure levels in the sample and any reported health effects.

Alternate Hypothesis

Ha_1: There is a relationship between drinking water arsenic levels and the levels of arsenic detected in hair samples.

Ha_2: There are relationships between current arsenic exposure levels in the sample and any reported health effects.

Objectives

- To identify residents with arsenic contamination in private wells through mass monitoring.
- To confirm correlations between water arsenic and hair arsenic levels
- To explore subtle health impacts of populations exposed to arsenic through questionnaire survey, water arsenic analysis, and hair arsenic analysis

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CHAPTER 4

METHODS

This cross-sectional, analytical study examined the relationships between long-term (at least one year), low-dose arsenic exposure through drinking water and a variety of self-reported health parameters as well as the concentration of arsenic in hair samples of participants. The analytical cross-sectional design measures exposure and outcomes simultaneously. The main purpose of this study was to quantify the exposure source as drinking water and monitor for any subtle health impacts of drinking arsenic contaminated water to the people residing in north-central and north-eastern Iowa. Importantly, the majority of exposures were below the Safe Drinking Water Act, Maximum Contaminant Level (SDWA-MCL) of 0.01 mg/L. The study used data gathered from sampling of well-water for arsenic concentration, a general health questionnaire survey, and analysis of hair samples for arsenic concentration. Potential participants were located through the Iowa "Grants to County" database.

Assumptions of the Study

- The study assumed that the information provided by the participants was correct and not biased on any of the question asked to them.
- The water sample provided by the participants was from the private well.
- The hair sample provided by the participant was from the same person who filled out the questionnaire survey.

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Limitations

- The sample size for the study was dramatically reduced from the anticipated number due to lack of response from the invited participants and lack of arsenic detection in the water samples.
- We concentrated the study to few counties after arsenic was widely detected in those counties.
- The information provided by the participants may not be accurate for each question.

Research Design

The study was divided into six main parts:

1. Selection of study area
2. Selection of participants and recruitment to the study
3. Water arsenic testing
4. Questionnaire survey and hair sampling
5. Hair arsenic analysis
6. Statistical analysis

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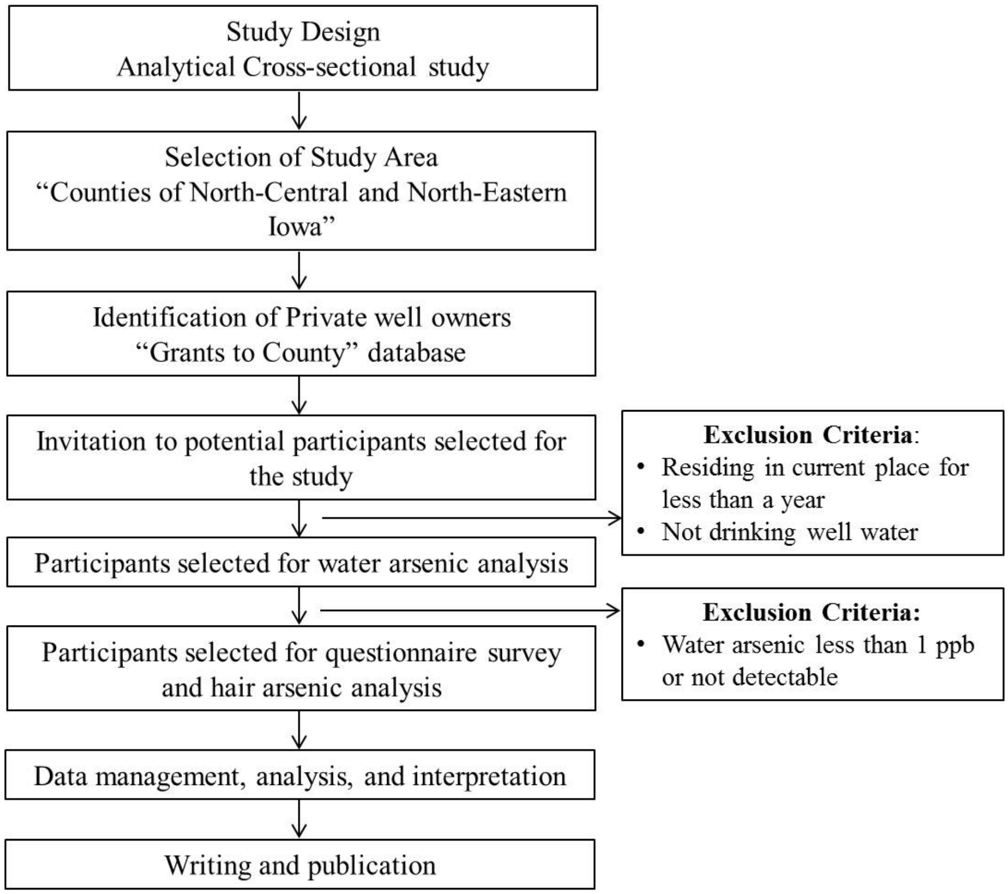

Figure 2: Flowchart of the study design

Professionals from the Iowa Department of Natural Resources and the State Hygienic Laboratory at the University of Iowa were contacted for access to the Grants to County data base used in locating potential participants, water analysis for arsenic levels in drinking water, and hair arsenic analysis. A proposal was then submitted to the University of Northern Iowa Institutional Review Board (IRB). After IRB approved the recruitment plan, private well owners were recruited by sending an invitation letter (Appendix B).

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The active data collection phase ran from January, 2012 to February, 2013 and was divided into four main parts. The first part consisted of sending an invitation letter to potential well-owners. The second part was collecting and analyzing water for arsenic contamination levels. The third part was the questionnaire survey and the hair sample analysis for arsenic contamination. Finally, an analyses report of water arsenic levels and hair arsenic levels was sent to the participants.

The participants were selected as a convenience sample identified through the Iowa Department of Natural Resources records of private well owners and came from 11 counties in north-central Iowa (with the exception of Cerro Gordo county) and 2 counties from north-eastern Iowa (Figure 2). Participants were excluded if they had resided at their current address for less than a year and/or if they were not currently drinking well-water. They were also excluded from the study after water analysis if they were drinking well-water that contained less than 0.01 mg/L of arsenic.

Selection of Study Area

Arsenic contamination in private wells has recently been an increasing concern of environmental health authorities in Iowa. According to Iowa Statewide Rural Well Water Survey, Phase 2 Study, measurable amounts of arsenic are mostly found in the north central part of Iowa accounting for 60.38% of the total samples analyzed for arsenic (CHEEC, 2009). Therefore the study mainly focused on north-central and north-eastern Iowa to find possible arsenic contamination in the private wells. All together 13 counties in the north-central and north–eastern part of Iowa were considered for the study. Since

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there was another project going on at Cerro Gordo county, it was not selected in our research. The list of counties except Cerro Gordo county selected for the study included (Figure 2):

North-Central: Winnebago, Hancock, Wright, Franklin, Hamilton, Hardin, Grundy, Butler, Mitchell, Floyd, and Worth

North-Eastern: Bremer, and Chickasaw

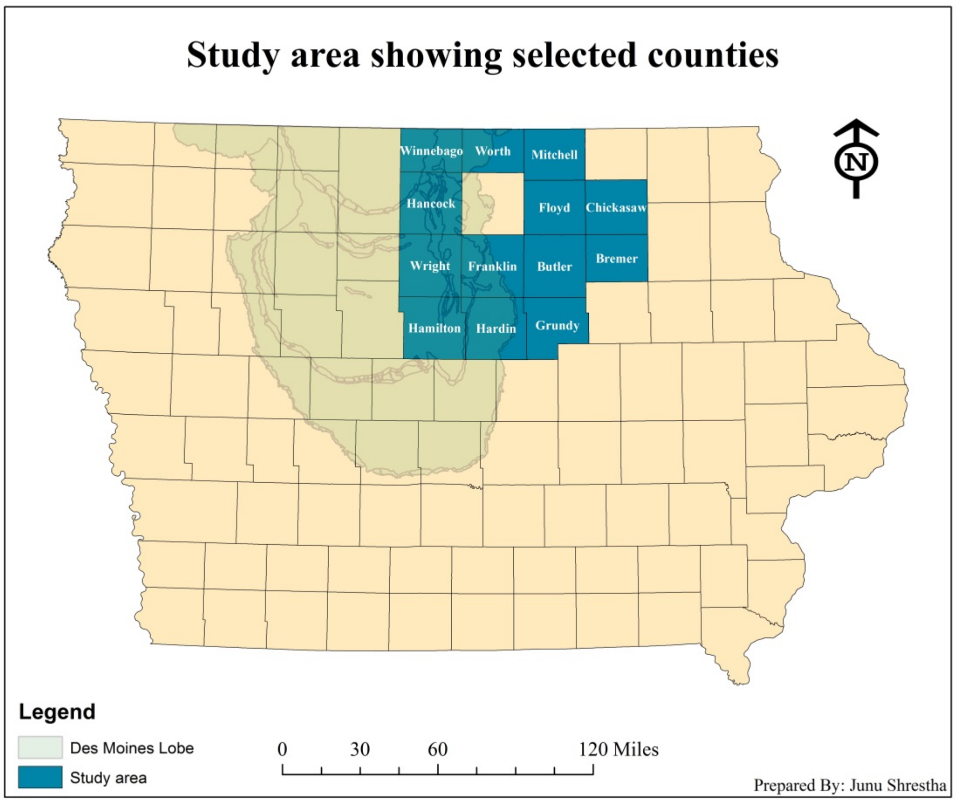

Figure 3: Counties selected for the study

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Invitation and Recruitment of Participants

Participants were first identified from the private well owner information database called the "Grants to County" program maintained and supported by the Iowa Department of Natural Resources. From this database the names and addresses of private well owners from the 13 identified counties were taken and invitations to participate were sent (Appendix B). In the first phase of the study, twenty-five potential participants were randomly selected from each county and send the invitation letter to. The invitation packet, containing the invitation letter, consent form, and survey (see Appendix B, C, and D) involving personal information indicating the total years of residence at the mailing address was sent. Participation was on a totally voluntarily basis. If the participants did not agree to participate in the study or did not responded to the invitation letter, additional participants were recruited. In order to participate in the study the contacted parties had to be living at the current residence for more than one year and be at least 18 years of age. Once the contacted individuals provided full-informed consent, they responded to all queries through a separate identification number to prevent any violation of the participant's privacy.

Water Arsenic Analysis

Participants in the study provided a water sample which was sent to the State Hygienic Laboratory (SHL) in Ankeny, IA for water arsenic testing. During the water collection, participants were provided sampling instructions and supplies by the SHL. The laboratory sent a water sample bottle to the potential participants that contained a

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small amount of 50% of nitric acid for stabilization of biological activity that could affect the arsenic analysis. After collecting the water sample, the laboratory analyzed the sample for arsenic concentration using EPA Method 200.8: "Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma Mass Spectrometry (ICP-MS)" procedure. This procedure is appropriate for the determination of dissolved elements in ground water, drinking water, and surface water (Creed, Brockhoff, & Martin, 1994).

Water Sample Analysis Procedure Provided by the Laboratory

The water analysis procedure was provided by Dr. Brian Wels of SHL, Ankeny, IA. The water samples were analyzed directly if the turbidity was <1 NTU. For water samples with turbidity > 1 NTU, 1 mL of nitric acid (HNO_3) and 0.5 mL of hydrochloric acid (HCL) were added to a 50 mL aliquot of the sample in a screw-top polypropylene digestion tube and then heated to approximately 85°C in a hot block to digest the substance causing turbidity and reduce the volume to 10 mL. After cooling, the digested sample was diluted with 25 mL of deionized water and 2% HNO_3 and 1% HCL to make a final volume of 50 mL. The standard solutions of 1.0, 5.0, 50, and 100 mgAs/L were prepared for calibration standards and a response curve was established. Finally the arsenic concentration of the water sample was compared with the response curve and then the arsenic concentration was recorded from the instrument software. The instrument used for analyzing the arsenic concentration was Agilent 7500 ICPMS (B. Wels, Personal Communication, February 4 and 11, 2013).

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Questionnaire Survey

In the third phase of the study, only the participants exposed to measurable arsenic concentration ($\geq$0.001 mg/L) were invited to continue with the questionnaire survey. The water analysis report, questionnaire survey, arsenic factsheet published by ASTDR, hair sampling manual, and a zip lock bag to put the hair sample in were sent to those participants (see Appendix E, F, and G). The hair sample was given a separate identification in order to maintain privacy of the participants.

The questionnaire survey was designed to evaluate subtle health impacts of arsenic contamination to the people exposed for more than one year. The survey was mainly divided into three parts: General Profile, Exposure Evaluation, and Health Profile (see Appendix E). The general profile section included information about age, gender, education, occupation, and number of family members. This section was designed to find the social and demographic status of the participants.

The exposure evaluation section included information about the participant's water consumption and eating habits. In addition, questions about knowledge of arsenic and number of years living in the current residence were also included.

In the health profile section, various questions on different health conditions of the participants were recorded to explore possible relationships between the on-going low-dose exposures and health concerns. The general health survey included questions on diabetes, skin problems, stomach, lung, kidney, liver problems, hair loss, and neurological disorders. We assumed that the information provided by the participants was

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true. Each of the questions was compared with water arsenic and hair arsenic concentration to find if there was any relationship between them.

Hair Analysis

The hair collection manual with illustrated step by step procedures was provided to the participants. The hair sample was taken by the participant according to a standardized procedure (see Appendix F). The hair sample that was placed inside the sealed zip lock bag was sent to the laboratory to find the arsenic concentration. After hair sample collection the analysis was done by the State Hygienic Laboratory (SHL) at Ankeny, IA.

Hair Analysis Procedure Provided by the Laboratory

Detailed hair analysis procedure was provided by Dr. Brian Wels of SHL, Ankeny, IA. According to the procedure, the hair sample was weighed and then digested with 5 mL of 50% HNO_3 by heating at approximately 85°C in a hot block and reduced to 2 mL in volume. 0.5 mL hydrogen peroxide (H_2O_2) was added to the digested sample and then heated to reduce the volume to 1 mL. After cooling the sample the final volume of 10 mL was attained by adding deionized water. The standard arsenic solutions of 1.0, 5.0, 50, and 100 mg/L were prepared for calibration standards and used to establish the response curve. Finally the arsenic concentration of the digested hair sample was compared with the response curve and then the output was recorded from the instrument software. The final concentration of arsenic in hair is calculated by dividing the output recorded in the instrument with the weight of the hair sample (B. Wels, Personal

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Communication, February 4 and 11, 2013). For example, if the output on the instrument is recorded as 1.652 mg and the weight of the hair sample is 0.116 g then total arsenic in the hair sample is calculated as: 1.652/0.116=14.24 mg/g. Converting in kilograms, the final total arsenic in hair sample is 14.24/1000=0.0142 mg/kg.

Quality Control of the Analysis

The quality control of water and hair sample was maintained by analyzing the calibration standards as samples to verify the precision of output obtained. In addition blank samples were also analyzed to monitor the efficiency of the output. The reference standard for hair was also analyzed to determine the precision and accuracy of the output (B. Wels, Personal Communication, February 4 and 11, 2013).

Arsenic Concentration Reporting to the Participants

The participants were informed about arsenic concentrations in both the water and hair samples. The letter indicating arsenic concentration status and maximum contaminant level was sent to each participant for their records (see Appendix H).

Statistical Analysis and Data Interpretation

The data collected from questionnaire survey, water arsenic concentration, hair arsenic concentration, and the “Grants to County” database were first entered into MS Excel program and then imported to JMP10, SAS Institute software.

Data were analyzed by a standard statistical procedure that was mainly divided into three sections. The first section was univariate analysis where the distribution

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statistics of data collected during the study were calculated to find mean, standard deviation, standard error, range, minimum, and maximum in order to describe the complete data set. The second section was bivariate analysis where the data collected were compared to examine the relationships between variables. This analysis includes the chi-square test, correlation, and one-way ANOVA test. The third section was multivariate analyses that include the whole model test.

ArcGIS 10 software was used to find the spatial distribution of arsenic in the selected counties. The latitude and longitude of individual well-owners were taken from the "Grants to County" database and then imported to ArcGIS 10 software. The spatial mapping of each well-owner was done to find arsenic distribution in the counties and is not associated with a specific address in the database.

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CHAPTER 5

RESULTS

Arsenic Concentration in Water

Monitoring of private wells showed that arsenic is widely distributed in the wells of the study area. Among the private well water samples collected from thirteen counties of Iowa, nine counties had measurable arsenic contamination. Figure 4 shows that water samples from eight of eleven counties in north-central and one of two counties in north-eastern Iowa had measurable arsenic contamination whereas the arsenic concentration in the water samples in four counties were not detected and, thus, those counties were eliminated from the study (Figure 4).

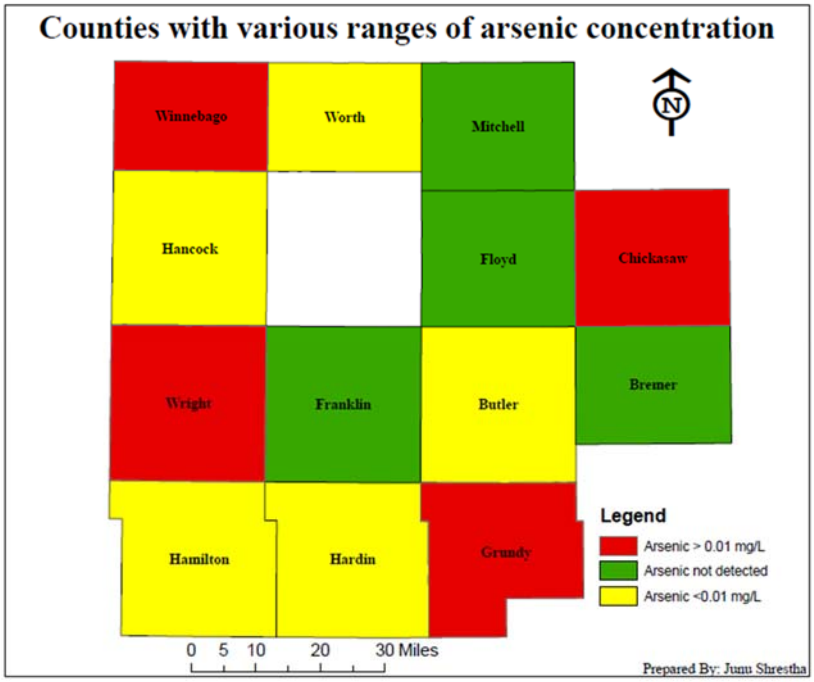

Figure 4: Arsenic detected in different counties of Iowa

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Of the 260 private well owners selected from 13 counties in Iowa and recruited for the study, 50 of them agreed to participate in the research and provided the water sample for arsenic testing of which 29 (58%) tested positive (Table 3). The water analyses also indicated that arsenic is widely distributed and mainly found in Winnebago, Wright, and Chickasaw counties (Table 3 and Figure 5). These counties have highest arsenic detection level as compared to the other counties. Five of six sample with arsenic concentrations exceeding the maximum contaminant level were observed in these counties except Grundy County. The analysis also found that 6 (12%) water samples had arsenic concentrations above the maximum contaminant level of 0.01 mg/L whereas 11 (22%) and 12 (24%) water samples had arsenic concentration of 0.005-0.0099 and 0.0001-0.0049 mg/L respectively.

Table 3: *Arsenic concentration status of study area*

		Arsenic concentration (mg/L)			**Total number**
	<0.001	**0.0001-0.0049**	**0.005-0.0099**	**>0.01**	
Bremer	7				7
Butler	3		1		4
Chickasaw	2	3	2	1	8
Floyd	1				1
Franklin	1				1
Grundy	0			1	1
Hamilton	1		1		2
Hancock	0	1			1
Hardin	0	1			1
Mitchell	1				1
Winnebago	4	3	6	2	15
Worth	1		1		2
Wright	0	4	0	2	6
Total number	21 (42%)	12 (24%)	11 (22%)	6 (12%)	50 (100%)

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Geospatial mapping also indicated that arsenic concentration was widely distributed in Winnebago County, with two private wells exceeding the maximum contaminant level. Similarly, the arsenic concentration was high in the private wells of Wright and Chickasaw County. The red dots shown in Figure 5 indicate private wells exceeding the maximum contaminant level, yellow dots on the county map indicate arsenic concentration ranging from 0.005 to 0.0099mg/L and green dots indicate private wells with arsenic concentration ranging from 0.001 to 0.0049 mg/L.

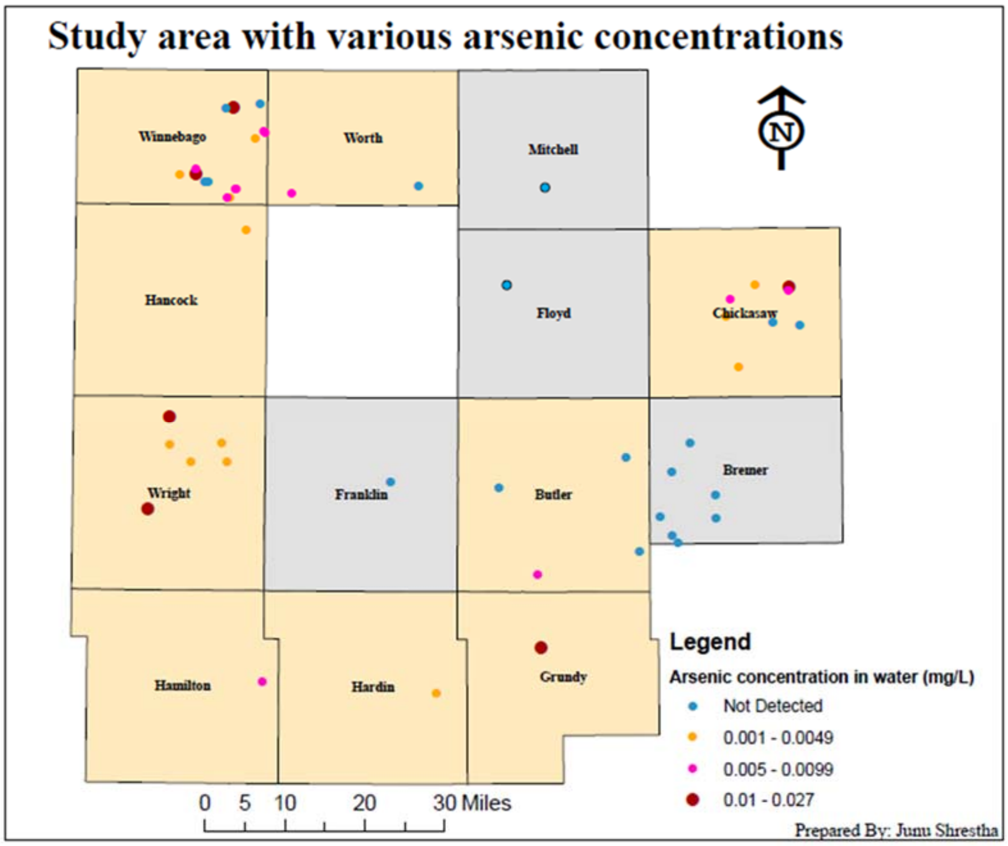

Figure 5: Varying arsenic concentrations in private wells

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Arsenic concentrations were also found in other counties such as Butler, Hardin, Grundy, Worth, Hancock, and Hamilton but not widely distributed. Mean arsenic concentration in water was 0.006 ± 0.001 mg/L (Mean ± SE). The maximum arsenic was 0.027 mg/L and the minimum was 0.001 mg/L (Table 4 and Figure 6). Detailed distribution statistics of arsenic in water are available in the Appendix A1.

Table 4: *Summary statistics of arsenic in water*

	Arsenic in water MCL= 0.01mg/L
Mean	0.007
Standard Deviation	0.006
Standard Error Mean	0.001
Maximum	0.027
Minimum	0.001
N	29

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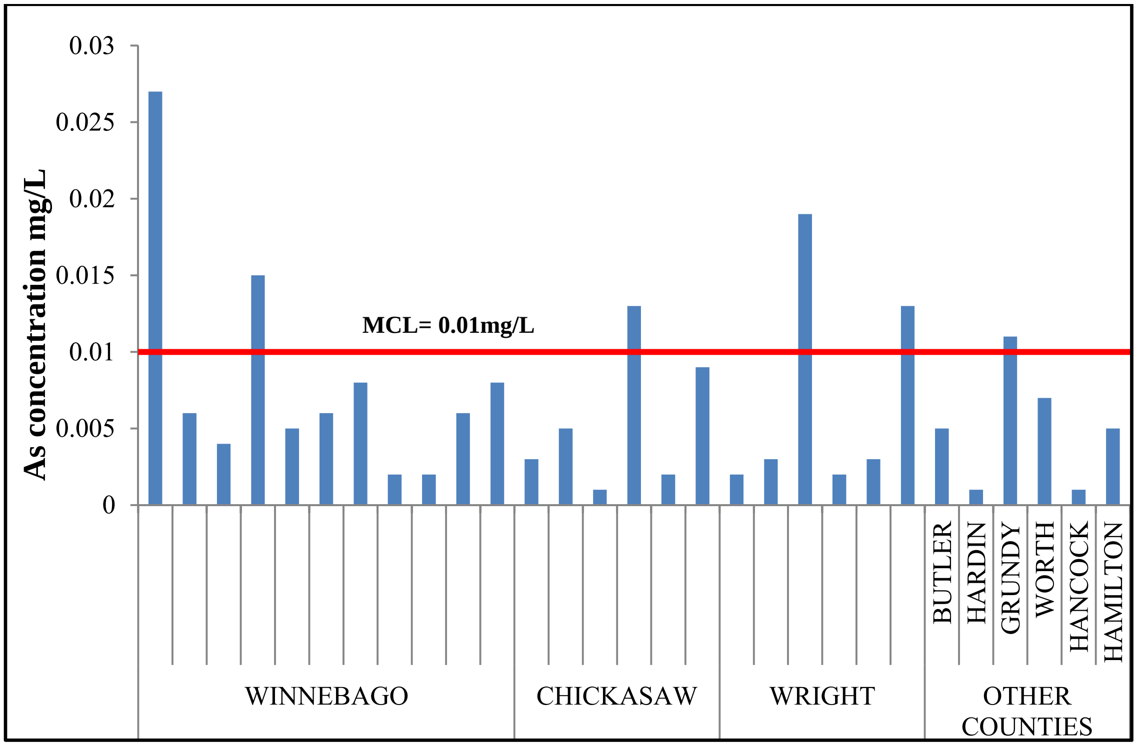

Figure 6: Arsenic concentration status in the study area

The analysis indicated that arsenic was distributed mainly between well depths of 100 to 200 feet. Two water samples showed less arsenic concentration at a depth of 300 and 375 feet. The correlation analysis between water arsenic with well depth was not statistically significant.

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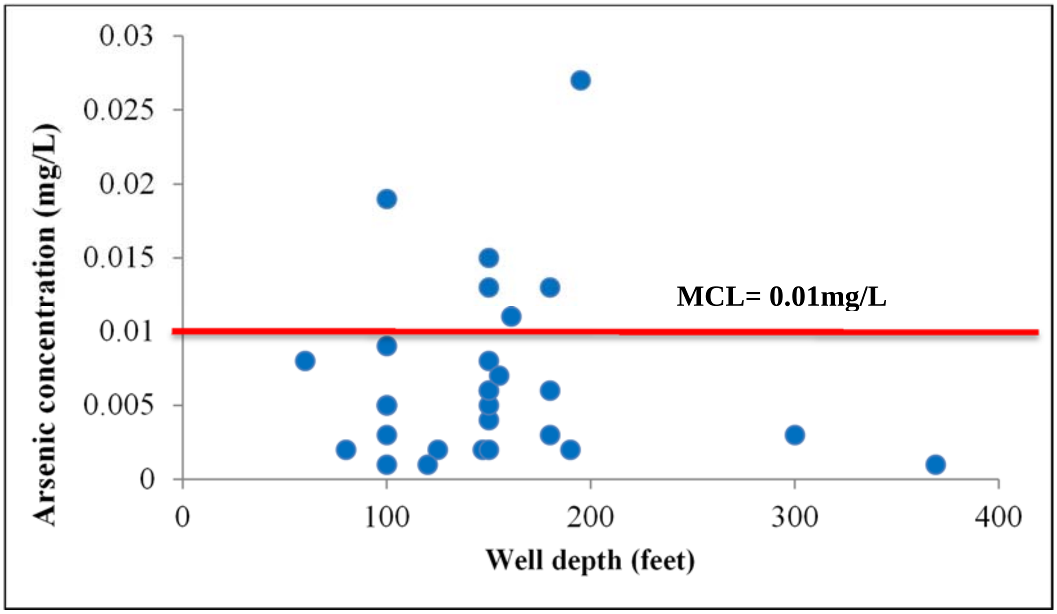

Figure 7: Relationship between well depth and arsenic concentration

Arsenic Concentration in Hair

The mean arsenic concentration in hair was 0.11 $\pm$ 0.02 mg/kg (Mean $\pm$ SE) (Table 5). Two hair samples from Chickasaw County and one hair sample each from Wright and Winnebago counties each had arsenic concentrations above the normal range. Nine hair samples had arsenic concentrations between the normal ranges. Four hair samples did not have any measurable arsenic concentration, and twelve hair samples had detectable arsenic concentrations but they were below the normal range (Figure 8). The detailed distribution statistics of hair arsenic concentration are available in the Appendix A2.

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Table 5: *Summary statistics of hair arsenic concentration*

	Arsenic in hair Normal range (0.08-0.25 mg/kg)
Mean	0.108
Standard Deviation	0.129
Standard Error Mean	0.024
Maximum	0.54
Minimum	ND (= not detected)
N	29

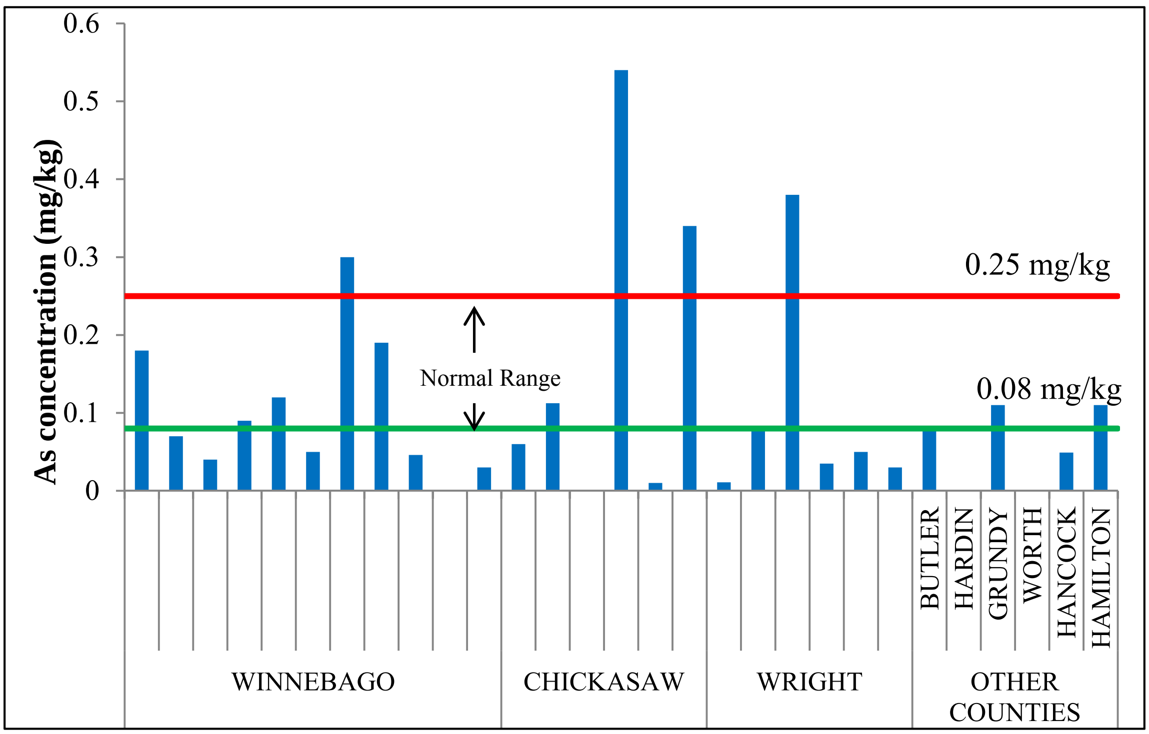

Figure 8: Hair arsenic concentration

<u>Relationship Between Hair and Water Arsenic Concentration</u>

The bivariate analysis indicated that there was a positive relationship between arsenic concentration in water and hair (see Appendix A3). The analysis also showed that

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a one unit increase in water arsenic can result in eleven units increase in the hair arsenic level. Figure 9 shows that the R-square value of this relationship is 0.26 indicating that there is a 26% chance of arsenic accumulation in hair due to exposure of arsenic in water and the relationship was statistically significant ($p<0.05$).

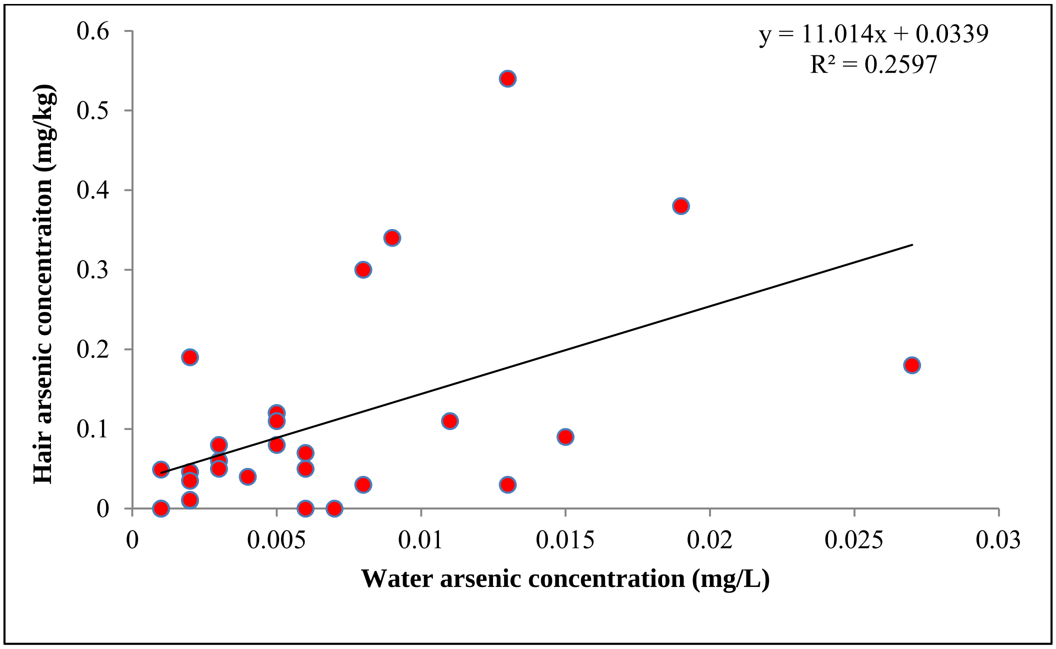

Figure 9: Relationship between water arsenic and hair arsenic

A one-way ANOVA analysis indicated that hair arsenic concentration varies at different water arsenic concentrations, and the hair arsenic was found to be high among the participants drinking water containing arsenic of more than 0.01 mg/L (see Appendix A4). A co-varying and directly proportional relationship between hair arsenic and arsenic concentration in water was statistically significant ($p<0.05$) (Table 6).

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Table 6: *One-way ANOVA*

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Water arsenic category	2	0.121	0.060	4.515	0.0207*
Error	26	0.349	0.0134		
C. Total	28	0.470			

Questionnaire Based Survey (General Profile)

Data presented in Table 7, Table 8, and Figure 10 show demographic information about the participants. The data is presented as univariate data derived from the general information portion of the general health status questionnaire (Appendix B).

Table 7: *Summary statistics (Age and Gender)*

	Female (N=13) 45%	Male (N=16) (55%)
Mean (age)	57.07	60.75
Standard Deviation	6.88	11.91
Standard Error Mean	1.90	2.97
Maximum	70	86
Minimum	42	44

As shown in Table 8, 45% of the participants were female and 55% were male. The mean age of female participants was 57.07 with minimum age of 42 and maximum age of 70. For male participants, the mean age was 60.75 with minimum age of 44 and maximum age of 86 (see Appendix A5).

Educational status results shown in Table 8 indicated that the minimum education of the participants was a high school degree. About 62% of the total participants have a

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high school degree, 14% have an associates or a bachelor's degree, and 10% have a master's degree. Detailed information is provided in Appendix A6.

Table 8*: Education status*

Education	Male (N=16)	Female (N=13)	Total (N=29)
High School	10	8	18 (62%)
Associate	3	1	4 (14%)
Bachelor	1	3	4 (14%)
Masters	2	1	3 (10%)

The bar chart shown on Figure 10 indicates the occupations of participants. Seven participants were retired, three were teachers, two were housewives and the others were distributed across diverse occupations.

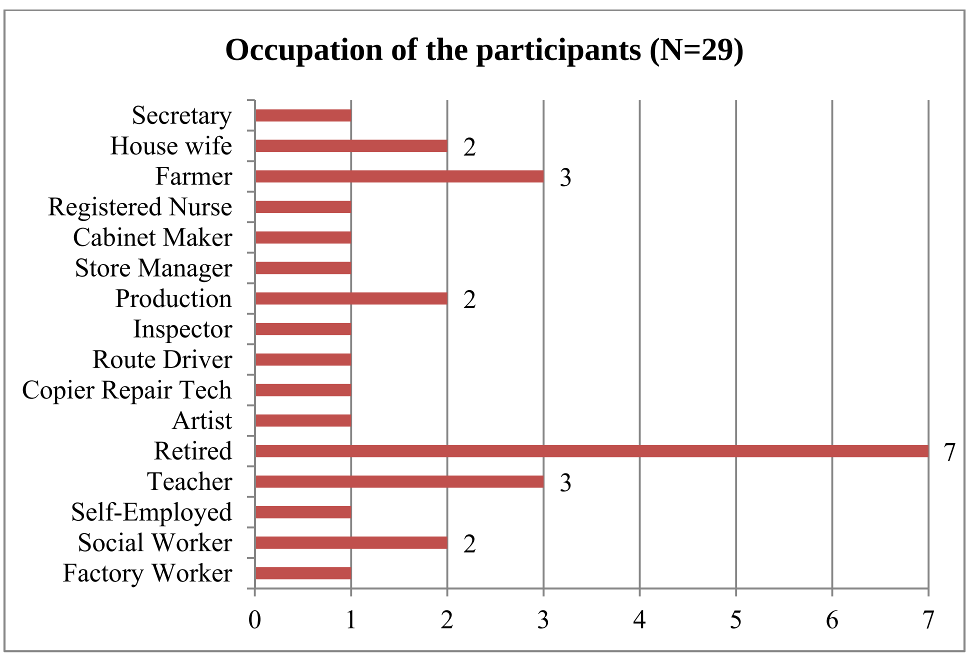

Figure 10: Occupations of participants

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Table 9 illustrates summary statistics of the number of family members and number of years at the current residence. Analyses indicated that the mean number of family members is 3.48 and the mean number of years at the current residence is 22.77.

Table 9: *Summary statistics*

	Family Members	Residence Time (Years)
Mean	4	23
Standard Deviation	2	17
Standard Error Mean	0.3	3
Maximum	10	86
Minimum	1	4

The participants were asked questions about their knowledge concerning arsenic and its health impacts; the analysis indicated that 76% did not know about arsenic whereas only 24% had knowledge about arsenic (Figure 11).

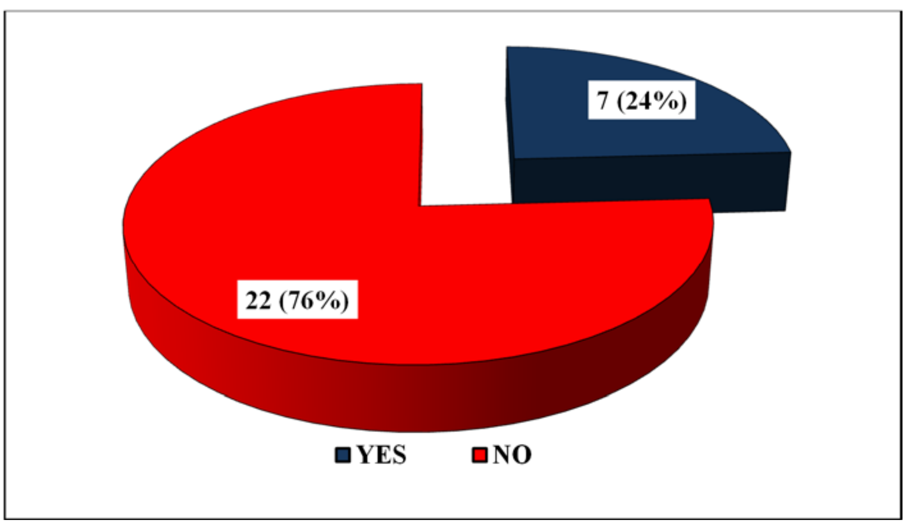

Figure 11: Knowledge about arsenic

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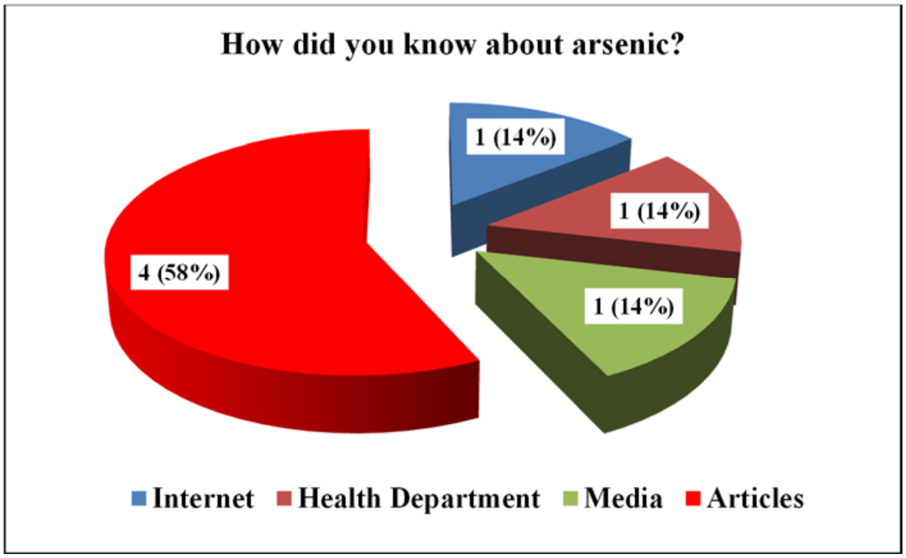

Figure 12: Sources of arsenic information

Participants who were familiar with arsenic issues were asked where they had learned that information. The analysis showed that 58% of the participants knew about arsenic articles they had read and 14% each got knowledge from the internet, the general media, and/or their health department (Figure 12).

Questionnaire Based Survey (Exposure Evaluation)

Data obtained from the exposure evaluation section of questionnaire survey were analyzed using various bivariate tests (see Appendix B). A bivariate relationship between years living in current residence and the hair arsenic concentration showed a very weak positive relationship (Figure 13) indicating that living in an arsenic exposed area could increase the hair arsenic level, but the relationship was not statistically significant ($p>0.05$) (see Appendix A7).

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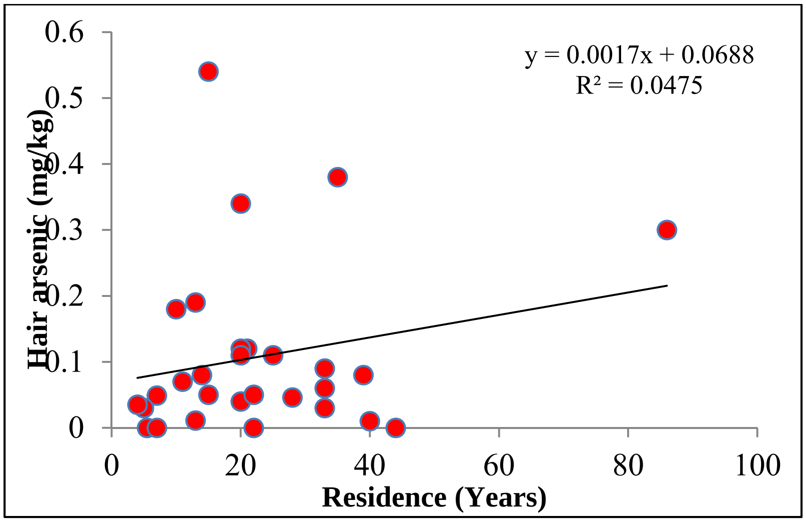

Figure 13: Relation between years of residence and hair arsenic

One way ANOVA analysis was done to explore variation in hair arsenic concentrations in relationship to different drinking water sources (well, public, and bottled water). The analysis indicated that there was no significant co-varying relationship between arsenic level in the hair samples and the water sources ($p>0.05$) (Table 10). The analysis also found that 25 out of 29 of the participants depend on private well as their primary drinking water sources whereas the remaining 4 participants depended on public water and bottled water in addition to private wells (see Appendix A8).

Table 10: *One-way ANOVA (Hair arsenic and drinking water sources)*

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Water Source	2	0.035	0.0178	1.064	0.359
Error	26	0.435	0.0167		
C. Total	28	0.470			

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Questions concerning alcohol consumption and meat consumption were also explored. Analysis done on alcohol consumption and the frequency of meat consumption with hair arsenic concentration did not find any significance relationship ($p>0.05$) (see Appendix A9). Questions were also asked about smoking behaviors, but none of the participants responded 'yes' to questions about smoking.

Questionnaire Based Survey (Health Profile)

Data obtained from the health profile section of the questionnaire survey included questions on history of diabetes, heart problems, stomach ache, skin problems, hair loss, lung, liver, or kidney disease, anxiety, tiredness, depression, and states of mental confusion. All of these questions were analyzed using bivariate fit and/or chi-square tests and compared to the water and hair arsenic level.

Hair Loss Due to Arsenic Exposure

A one-way ANOVA comparing hair loss with hair arsenic concentration showed a significant relationship. However, it should be noted that this was based on two individuals of a small sample of 29. Nevertheless, the variation of mean hair arsenic was high among the participants with daily hair loss. This relationship indicates that there is a significant relationship between hair loss and arsenic concentration in hair samples ($p<0.05$). The R-square value for the relationship was 0.21 indicating that perhaps 21% of the hair-loss in this sample could be explained by increased arsenic concentration in the hair samples (see Appendix A10 and A26).

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Table 11: *One-way ANOVA for hair arsenic concentration by hair loss*

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Frequency of hair loss	2	0.103	0.051	3.6562	0.0399*
Error	26	0.367	0.0141		
C. Total	28	0.470			

On the other hand, the one-way ANOVA analysis between water arsenic and hair loss was not statistically significant indicating that the water arsenic concentration was not responsible for hair loss rate of the participants (see Appendix A11).

For further analysis, water arsenic was categorized into different concentration (0.001-0.049, 0.005-0.0099, $\geq$0.01mg/L). The contingency analysis between water arsenic category and hair loss showed that there is significant difference indicating that water arsenic exposure of more than 0.01 mg/L could result in hair loss ($p<0.05$) (see Appendix A25).

Kidney, Liver, and Lung Conditions

A chi-square test was run to find the logistic fit for kidney, liver, and lung conditions with hair arsenic concentration which indicated a relationship. The analysis indicated that participants who reported having these health conditions also tended to have higher arsenic concentration in hair samples but the relationship was not significant ($p> 0.05$) (see Appendix A12). However, no trends or statistically significant relationships were found with kidney, liver, and lung conditions and the level of arsenic in the drinking water samples ($p>0.05$) (see Appendix A13).

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Skin Problems

Self-reported dermatological issues indicated that 16 participants had skin problems such as warts, dark spots, moles, and melanoma whereas 13 participants did not report any type of skin problem (Figure 14). One-way ANOVA analysis done to find the variation between the mean arsenic concentration in hair and water with different types of skin problems showed no significant relationships ($p>0.05$). This analysis showed that arsenic in hair and water samples did not have any impact to the types of skin problems reported by this study group (see Appendix A14 and A15).

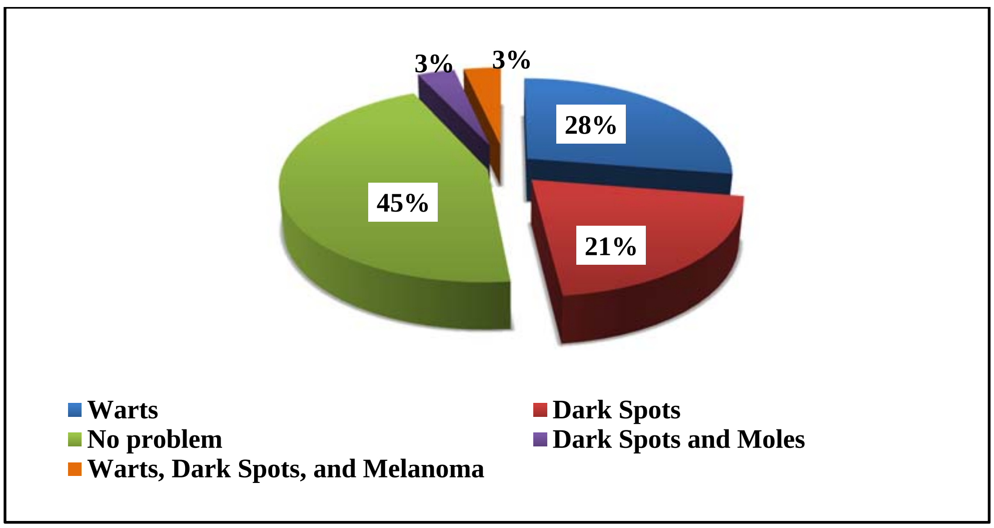

Figure 14: Type of skin problems

Other Health Problems

Information on other health issues such as stomach pain, diabetes, heart problem, numbness on hands or feet, tiredness, depression, anxiety, and confusion of mind were

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also obtained by self-report questionnaire and were compared with hair arsenic concentration. The chi square test done on different health problems and hair arsenic concentration did not show any relationship. Table 12 shows a list of tests run between health problems and hair arsenic and the corresponding p-value. Detailed chi square tests on each health problem by hair arsenic concentration are available in Appendix A16 - A23.

Table 12: *Relationship of health problems with hair arsenic concentration*

Test	DF	Chi Square	P value
Pain in stomach by hair arsenic	1	0.815	0.365
Diabetes by hair arsenic	1	1.035	0.308
Heart problem by hair arsenic	1	2.046	0.152
Numbness on hands or feet by hair arsenic	1	0.169	0.68
Tiredness by hair arsenic	1	0.807	0.36
Depression by hair arsenic	1	0.203	0.651
Anxiety by hair arsenic	1	0.06	0.8
Confusion by hair arsenic	1	1.33	0.24

Multivariate Analysis

A multivariate analysis was performed to find the degree of relationship between multiple factors that might increase arsenic concentration in hair. The variables used for the analysis were water arsenic concentration, age, gender, occupation, education, years of residence, and drinking water sources. The analysis indicated a significant relationship ($p<0.05$) indicating that a combination of these factors could have a significant impact on the concentration of arsenic in hair samples in this study (see Appendix A24). The result also showed that the r square value of this relationship is 0.98 indicating there is 98%

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chance that the variation in hair arsenic concentration is casued due to combined of these factors.

Table 13: *Multivariate analysis*

Summary of Fit

R Square	0.989593
R Square Adj	0.927154
Root Mean Square Error	0.035004
Mean of Response	0.107621
Observations (or Sum Wgts)	29

Analysis of variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	24	0.466	0.019	15.848
Error	4	0.004	0.001	Prob > F
C. Total	28	0.470		0.0078*

Effect tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
As Water (mg/L)	1	1	0.062	50.7	0.0021*
Age	1	1	0.050	41.4	0.0030*
Gender	1	1	0.056	45.7	0.0025*
Education	3	3	0.056	15.4	0.0115*
Occupation	15	15	0.291	15.8	0.0081*
Years of residence	1	1	0.050	41.2	0.0030*
Water Source	2	2	0.101	41.6	0.0021*

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CHAPTER 6

DISCUSSIONS, CONCLUSIONS, AND RECOMMENDATIONS

Discussion

Data analyses derived from water arsenic analysis, hair arsenic analysis, and questionnaire survey analysis indicated that private well owners in the study area are exposed to low arsenic concentrations from drinking water sources. More than 50 percent of the water samples tested had arsenic concentration ranging from 0.001 mg/L to 0.027 mg/L. The highest prevalence of arsenic contamination was found in Wright, Worth, and Chickasaw counties. A study conducted by University of Iowa in 2009 indicated that 48% of total water tested in Iowa had arsenic concentrations above 0.001 mg/L. The study also found that arsenic contamination was prevalent in the north-central region of Iowa where 61% of the total samples tested had arsenic contamination (CHEEC, 2009). In addition, the arsenic concentration in the ground water of the upper Midwest is mainly due to the Late Wisconsinan glacial drift (Erickson & Barnes, 2005).

Correlational analysis of well depth data with water arsenic concentration did not show any relationship, indicating that arsenic is not dependent on depth of the well. However, most of the water samples collected were taken from well depths ranging from 100 to 200 feet. Since most of the private wells in this study had the same depth range, a relationship between well depth and arsenic concentration was not observed. A study done in Bangladesh, however, indicated a relationship between shallow tube wells with less than 50 m depth and arsenic concentrations of more than 0.05 mg/L (Kinniburgh &

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Smedley, 2001). Bangladesh is one of the most arsenic contaminated hot spot of the world. Arsenic is widely found in the groundwater of Bangladesh, therefore and it is difficult to compare the distribution of arsenic in different well depth with our study.

Hair sample analyses among participants exposed to arsenic of more than 0.001 mg/L showed that 14% of the sample exceeded the normal arsenic range. The sulfhydryl and keratin rich compounds present in the hair results limited mobility of arsenic and it is a good indicator for long term exposure. A study conducted by Hinwood et al. (2003) indicated that arsenic exposure via drinking water was positively correlated with hair arsenic concentration and the relation was statistically significant. Another study done by Uchino, Roychowdhury, Ando, and Tokunaga (2006) also showed that there was a positive correlation between water and hair arsenic concentration which was statistically significant.

The data analyses from the questionnaire survey showed that there were a slightly higher percentage of male participants (55%) than female participants (45%). The mean age of the female participants was 57 years and mean age of male participants was 61 years. Recruitment criteria focused on a population 18 years of age or older and living at the residence where the water sample was derived for more than one year. Most of the participants in the study were between age ranges of 52 to 66 years (76%). In regards to the total years of residence associated with the well site, the mean was 23 years. One participant was living at the current residence (well site) for 86 years with a range of 4 to 86 years. This indicates that most of the participants were exposed to arsenic via drinking water for many years.

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The majority of participants had a high school diploma (62%). Occupational status data indicated that seven participants were retired, with others having various occupations. Questions were asked that explored the basic knowledge level of participants in regards to understanding arsenic sources and health impacts. When asked if they had a basic "Knowledge about arsenic," 76% of the participants said "No" indicating that they did not know about arsenic from any source. Among the participants who said "Yes," most of them (58%) got that information about arsenic from various articles. This analysis shows that knowledge about arsenic is very limited in the potentially effected population and more educational outreach and awareness programs should be conducted to educate people about arsenic, especially the people drinking private well water who are responsible for their own water safety and quality.

Bivariate analysis between hair arsenic and years of residence showed a positive relationship but was not statistically significant. A similar study done by Hinwood et al. (2003) also showed non-significant results. In addition, age was not related with hair arsenic concentration indicating that living in arsenic affected areas alone does not lead to a directly proportional accumulation of arsenic in hair samples. Therefore, other factors that confound this relationship such as physiologic ability to move arsenic from the body proper to the hair should be examined to fully delineate these relationships.

Data analyses on hair arsenic concentrations with alcohol consumption and meat consumption information did not show any relationship. A study done by Saad and Hassanien (2001) found that there was no relationship between hair arsenic concentration and meat consumption except fish consumption which was statistically significant. The

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relationship between hair arsenic and smoking behavior could not be established because none of the participants were smokers. Saad and Hassanien (2001) showed non-significant relationship between hair arsenic and smoking habits such as active smokers and passive smokers. But at the same time hair arsenic was found more among the indoor passive smokers than outdoor passive smokers ($p<0.01$). In addition, the hair arsenic among molasses tobacco smokers was higher than that of cigarette smokers ($p<0.02$).

Data analyses on various health impacts due to arsenic in hair and water shows some relationship. The one-way ANOVA analysis with hair arsenic concentration and hair loss showed a significant relationship. This result indicates that people having high arsenic concentration in their hair sample have more chance of hair loss. So hair loss in people residing in arsenic affected areas could be an indicator for higher, long-term arsenic exposure. Various studies done on arsenic also show that hair loss is an indicator for arsenic poisoning (Hindmarsh, 2000; Tchounwou, Centeno, & Patlolla, 2004).

Hair is considered as an excretion site/biological sink for toxic chemicals due to the presence of sulfhydryl groups. Human hair has keratin rich tissues that contain cysteine which offer thiol groups for reaction with arsenic compounds. Since arsenic binds tightly to the sulfhydryl groups in particular (Yamato, 1988), its concentration in hair is much higher than in other tissues or biological fluids (Ya´n˜ez et al., 2005). According to Raab and Feldmann (2005) the arsenic speciation of hair samples contained dominantly inorganic arsenic (As (III)) with small amounts of sodium cacodylate (DMA (V)) and disodium monomethyl arsonate (MMA (V)).

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The chi-square test on hair arsenic concentration with kidney, liver, and lung conditions showed a positive relationship but was not statistically significant. In addition, there was no relationship observed between water arsenic with liver, lung, and kidney conditions. In contrast the study done by Chen, Chen, Wu, and Kuo (1992) indicated that there was a significant association between ingested arsenic and malignancy of liver, lung, bladder, and kidney among people residing in arsenic endemic areas. That study also showed that high exposure to arsenic in drinking water i.e > 0.10 mg/L was associated with mortality from liver, lung, and kidney cancer. The mortality rate was highest among people with an age of 30 years or more. Another study by Centeno et al. (2002) also indicated that ingestion of inorganic arsenic is associated with two types of liver cancer: hepatocellular carcinoma and angiosarcoma of the liver. In all of the above studies, the arsenic contamination was relatively high and the exposure period is long. Therefore, further studies should be conducted to correlate arsenic exposure and carcinogenic effects.

Self-reported dermatological concerns were found in 55% of the participants. The reported skin problems are dark spots, moles, warts, and melanoma. A one-way ANOVA analysis done on these skin problems and hair arsenic and water arsenic concentration showed no significant relationship. Previous studies done on chronic arsenic exposure show skin related disorders such as skin lesions, melanosis, keratosis, and skin cancer from chronic arsenic poisoning (Hall, 2002; Smith, Lingas, & Rahman, 2000). One of the highest correlations to arsenic exposure and epithelial tissue disease was found in Taiwan called “Black Foot Disease” wherein the population was drinking water with more than

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0.3 mg/L of arsenic. The prevalence of black foot disease was 8.9 per 1000 in this endemic area making resident higher chance of developing this necrotic change (Tseng, 1977).

Chronic arsenic exposure causes multisystem damage inside the body. Therefore, in an effort to identify any other health impacts to participants, additional general health questions were asked. However there were no relationships observed between hair arsenic and water arsenic with various other health issues such as pain in stomach, diabetes, heart problem, numbness on hand and feet, tiredness, depression, anxiety, and confusion of mind. Unlike this sample, a study done by Mazumdar (2008) showed that peripheral neuritis characterized by numbness, tingling, and weakness was present in 74 out of 156 people drinking arsenic contaminated water of 0.5-14.2 mg/L in West Bengal, India.

In this sample, results indicated that there was no significant relationship between diabetes and hair arsenic concentration. Since the mean concentration level of arsenic in hair and water is low, no correlation has been observed in this study. On the other hand, previous research done on diabetes and arsenic exposure showed a relationship between arsenic exposure at an average more than 0.01 mg/L and the presence of diabetes. A study done by Rahman, Tondel, Ahmad, and Axelson (1998) showed that the prevalence rate of diabetes mellitus (Type 1) to the subject exposed to arsenic of more than 0.01 mg/L concentration was 4.4 suggesting it is a risk factor for Type 1 or juvenile on-set diabetes. Another study done by Navas-Acien et al. (2008) indicated that the prevalence rate of type 2 diabetes was 7.7 among the people with chronic exposure to inorganic

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arsenic in drinking water. The study also found that participants with type 2 diabetes had a 26% higher level of total arsenic in their urine samples as compared to participants without type 2 diabetes. Many of the studies were focused on water arsenic concentration and prevalence of diabetes but very few focused on biological samples.

To examine the relative importance of a variety of factors in this sample population to arsenic concentration in hair a multivariate analysis was done with the covariates of water arsenic concentration, age, gender, education, occupation, years of residence, and water source. Findings indicate that this combination of factors is important and responsible for the accumulation of arsenic in hair. The most important factors were water arsenic ($p= 0.0021$), source of arsenic ($p= 0.0021$), gender ($p= 0.0025$), and years of residence ($p= 0.0030$) (see Table 13). A study done by Lindberg et al. (2007) also showed that age and gender were the major factors influencing arsenic methylation and that women had higher arsenic methylation efficiency than men during child bearing years indicating the influence of sex hormones.

In this sample, the null hypothesis was rejected indicating that the participants consuming arsenic contaminated water have more chance to accumulate arsenic in their hair sample. In regards to potential health impacts, at a mean exposure level 0.006 mg/L in this sample study there were no correlations found. However hair loss was related to hair arsenic concentration at a mean exposure level 0.10 mg/kg but there was no relationship with the water arsenic concentration. This does illustrate that arsenic consumption and potential physiological impacts in addition to concentration in hair

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samples is occurring in Iowa. It should be examined with a larger sample size as this sample size limits the ability to make assumptions about the broader population exposure.

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Conclusions and Recommendations

The results illustrate that arsenic levels are present in a significant number of wells. Among private well owners who agreed to participate in the study, 58% showed arsenic positive water samples. Even though the study was on a small scale, a significant number of arsenic positive contaminations were found in the private wells. The results indicated that Chickasaw, Winnebago, and Wright counties had the highest number of arsenic positive private wells; in total, six water samples exceeded the maximum contaminant level of 0.01 mg/L. Based on the results of this water analysis, private well owners with water arsenic levels greater than 0.001 mg/l were selected for the hair analysis. Results of the hair analysis showed that four hair samples exceeded the normal arsenic range (0.08-0.25 mg/kg) but none of them had arsenic above the toxicity level (1 mg/kg). A correlation analysis of water arsenic and hair arsenic showed a positive relationship which was statistically significant ($p<0.05$). This indicates that individuals residing in rural areas and using private wells who are experiencing excessive hair loss should consider evaluating their drinking water for arsenic level, a parameter that is not routinely tested for as usually nitrates, bacteria, and sometimes pesticides are the more standard testing parameters.

The questionnaire based knowledge and health survey done with the participants showed that most of the people do not know about arsenic (76%) in drinking water. This indicates a pressing need to develop public health and education programs.

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The correlation analysis between years of residence and hair arsenic concentration indicated that people residing in arsenic affected areas for many years had a tendency for increased arsenic accumulation in hair but the result were not statistically significant ($p>0.05$).

Health questions focusing on kidney, lung, and liver conditions showed a trending relationship with hair arsenic concentration but the relationship was not statistically significant. In addition there was no statistically significant relationship observed between kidney, lung, and liver conditions and the water arsenic concentration. The arsenic contamination in drinking water and in the hair sample may be confounded by individual propensities to clear arsenic from the body through sulfhydryl group complexing, hair washing patterns, use of various cleaning agents. etc.; further studies in these areas could be valuable in helping clarify these relationships. Reported skin problems while observed among the participants were not directly impacted by the arsenic concentration in hair and water samples.

Additionally, other health conditions such as diabetes, stomach pain, heart problem, numbness in hands and feet, tiredness, depression, anxiety, and confusion of mind were not related to the hair arsenic concentration level or concentration of arsenic in drinking water. Finally a multivariate analysis done to find the combination of factors contributing to increasing hair arsenic concentration showed significant result when considered together with factors such as water arsenic concentration, age, gender, occupation, education, years of residence, and drinking water sources all responsible for hair arsenic accumulation.

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This study was conducted in north-central and north-eastern Iowa and involved only 50 participants. Even so, the fact that 58% of the wells tested positive for arsenic contamination indicates the need to widen geographically and numerically future studies. The self-report of general health conditions was a broad based means of examining health issues. Despite the fact that hair loss was based on two samples, the exposed individuals were exposed in a range more than 0.05 mg/L in India, Bangladesh, Taiwan and other high arsenic endemic areas (Hindmarsh, 2000). This study indicate hair loss due to chronic exposure problematic for hair loss as an early indicator of potentially significant bioaccumulation. Therefore, the fact that hair loss was detected and tied to arsenic water and hair arsenic concentrations indicates a need to expand this area of inquiry with a larger sample size and more subtle measures of biological impact (ie. additional physiological parameters such as blood glucose level, etc.). This would give a better picture of long-term subtle physiological impacts of low-dose exposures. Finally, the study clearly showed a need for additional public health education about the arsenic issue as respondents were lacking in a basic understanding of their responsibilities as private well owners for water testing and safety and an understanding of the health and well-being impacts of long term arsenic exposures.

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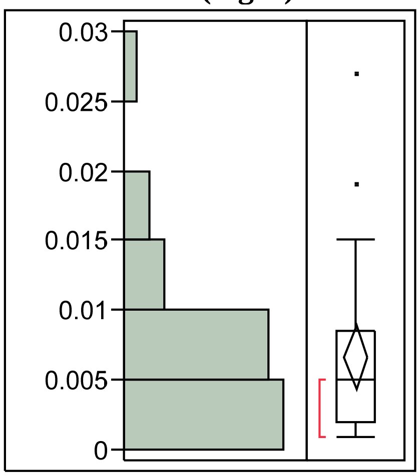
APPENDIX A

STATISTICAL ANALYSIS

A1: Distribution analysis of water arsenic concentration

Water arsenic (mg/L)



Quantiles

100.0%	maximum	0.027
99.5%		0.027
97.5%		0.027
90.0%		0.015
75.0%	quartile	0.0085
50.0%	median	0.005
25.0%	quartile	0.002
10.0%		0.001
2.5%		0.001
0.5%		0.001
0.0%	minimum	0.001

Summary Statistics

Mean	0.007
Std Dev	0.006
Std Err Mean	0.001
Upper 95% Mean	0.0092
Lower 95% Mean	0.004
N	29

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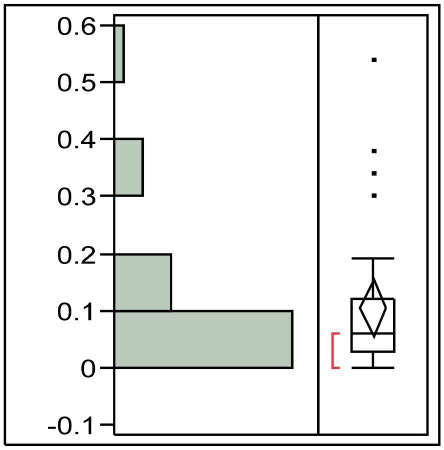
A2: Distribution analysis of hair arsenic concentration

Hair arsenic (mg/kg)



Quantiles

100.0%	maximum	0.54
99.5%		0.54
97.5%		0.54
90.0%		0.34
75.0%	quartile	0.12
50.0%	median	0.06
25.0%	quartile	0.03
10.0%		0
2.5%		0
0.5%		0
0.0%	minimum	0

Summary Statistics

Mean	0.108
Std Dev	0.130
Std Err Mean	0.024
Upper 95% Mean	0.157
Lower 95% Mean	0.059
N	29

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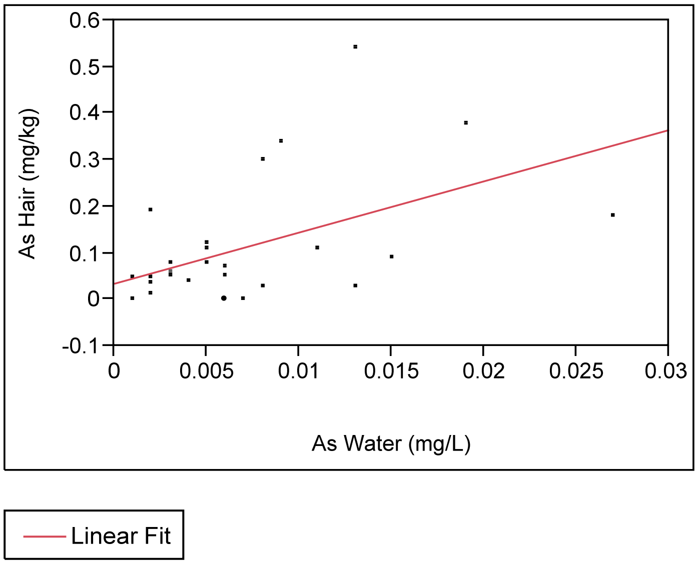
A3: Bivariate fit of hair arsenic by water arsenic



Linear Fit
As Hair (mg/kg) = 0.0339395 + 11.014194*As Water (mg/L)

Summary of Fit

R-Square	0.26
R-Square Adj	0.24
Root Mean Square Error	0.114
Mean of Response	0.107
Observations (or Sum Wgts)	29

Analysis of Variance

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	1	0.12230807	0.122308	9.4712
Error	27	0.34867076	0.012914	**Prob > F**
C. Total	28	0.47097883		0.0047*

Parameter Estimates

Term	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Intercept	0.0339395	0.031914	1.06	0.2970
As Water (mg/L)	11.014194	3.578912	3.08	0.0047*

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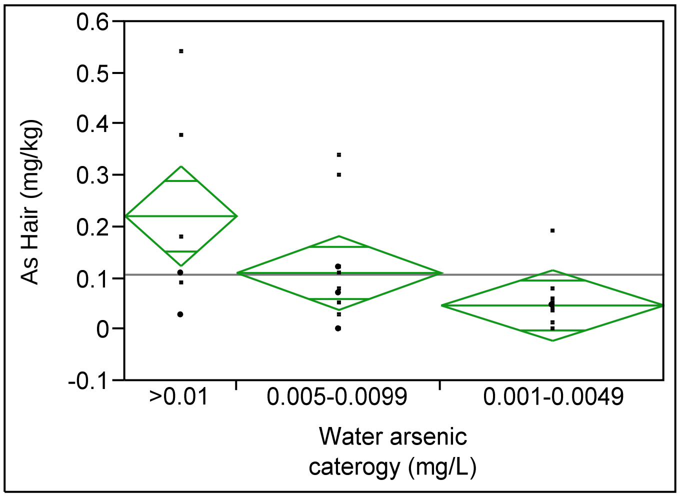
A4: One way analysis of hair arsenic by water arsenic category



Summary of Fit

R-square	0.257786
Adj Rsquare	0.200692
Root Mean Square Error	0.115952
Mean of Response	0.107621
Observations (or Sum Wgts)	29

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Water arsenic caterogy (mg/L)	2	0.12141167	0.060706	4.5152	0.0207*
Error	26	0.34956716	0.013445		
C. Total	28	0.47097883			

Means for Oneway ANOVA

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
>0.01	6	0.221667	0.04734	0.1244	0.31897
0.005-0.0099	11	0.110909	0.03496	0.0390	0.18277
0.001-0.0049	12	0.047583	0.03347	-0.0212	0.11639

Std Error uses a pooled estimate of error variance

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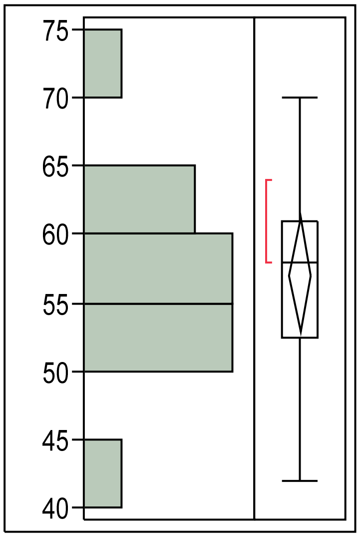
A5: Descriptive analysis of age and gender



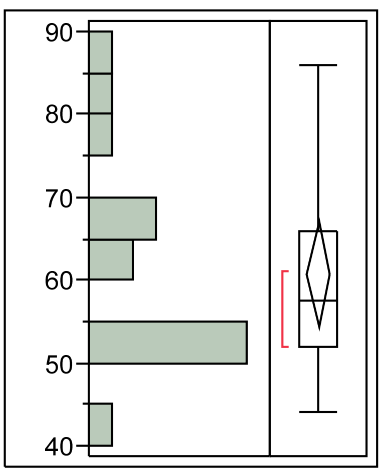
Age and Female population

Quantiles

100.0%	maximum	70
99.5%		70
97.5%		70
90.0%		67.6
75.0%	quartile	61
50.0%	median	58
25.0%	quartile	52.5
10.0%		46
2.5%		42
0.5%		42
0.0%	minimum	42

Summary Statistics

Mean	57.076923
Standard Deviation	6.8855106
Standard Error Mean	1.909697
Upper 95% Mean	61.237795
Lower 95% Mean	52.916051
N	13



Age and Male population

Quantiles

100.0%	maximum	86
99.5%		86
97.5%		86
90.0%		81.8
75.0%	quartile	66
50.0%	median	57.5
25.0%	quartile	52
10.0%		49.6
2.5%		44
0.5%		44
0.0%	minimum	44

Summary Statistics

Mean	60.75
Standard Deviation	11.919172
Standard Error Mean	2.9797931
Upper 95% Mean	67.101279
Lower 95% Mean	54.398721
N	16

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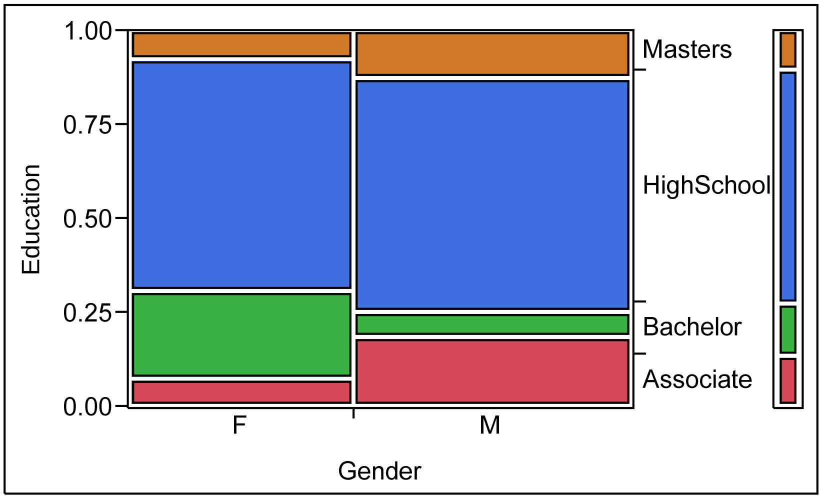
A6: Contingency analysis of education by gender

Mosaic Plot



Contingency Table: Gender by Education

Count Total % Col % Row %	Associate	Bachelor	High School	Masters	
F	1 3.45 25.00 7.69	3 10.34 75.00 23.08	8 27.59 44.44 61.54	1 3.45 33.33 7.69	13 44.83
M	3 10.34 75.00 18.75	1 3.45 25.00 6.25	10 34.48 55.56 62.50	2 6.90 66.67 12.50	16 55.17
	4 13.79	4 13.79	18 62.07	3 10.34	29

Tests

N	DF	-Log Like	R-Square (U)
29	3	1.1722858	0.0375

Test	Chi-Square	Prob>Chi-Sq
Likelihood Ratio	2.345	0.5040
Pearson	2.269	0.5184

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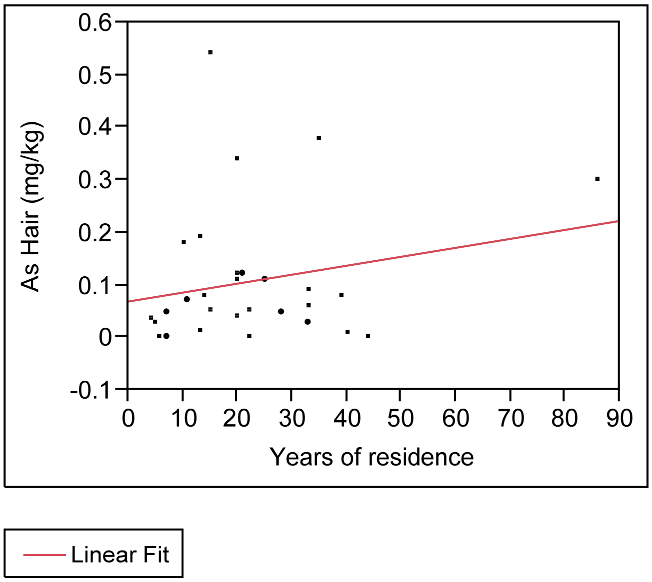
A7: Bivariate fit of hair arsenic concentration by years of residence



Linear Fit
As Hair (mg/kg) = 0.0687801 + 0.0017053*Years of residence

Summary of Fit

RSquare	0.047507
RSquare Adj	0.01223
Root Mean Square Error	0.128899
Mean of Response	0.107621
Observations (or Sum Wgts)	29

Analysis of Variance

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**
Model	1	0.02237497	0.022375	1.3467
Error	27	0.44860385	0.016615	**Prob > F**
C. Total	28	0.47097883		0.2560

Parameter Estimates

Term	**Estimate**	**Std Error**	**t Ratio**	**Prob>|t|**
Intercept	0.0687801	0.041148	1.67	0.1062
Years of residence	0.0017053	0.00147	1.16	0.2560

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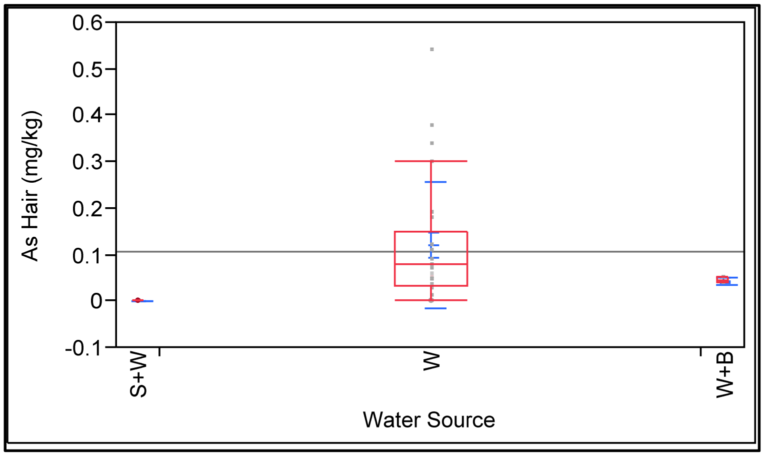
A8: One-way analysis hair arsenic (mg/kg) by drinking water source



Summary of Fit

R-square	0.075681
Adj R-square	0.00458
Root Mean Square Error	0.129397
Mean of Response	0.107621
Observations (or Sum Wgts)	29

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Water Source	2	0.03564427	0.017822	1.0644	0.3595
Error	26	0.43533456	0.016744		
C. Total	28	0.47097883			

Means for One-way ANOVA

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
S+W	2	0.000000	0.09150	-0.1881	0.18808
W	25	0.121240	0.02588	0.0680	0.17444
W+B	2	0.045000	0.09150	-0.1431	0.23308

Note:

S+W= Water Supply and Well water

W= Well water

W+B: Well water and bottled water

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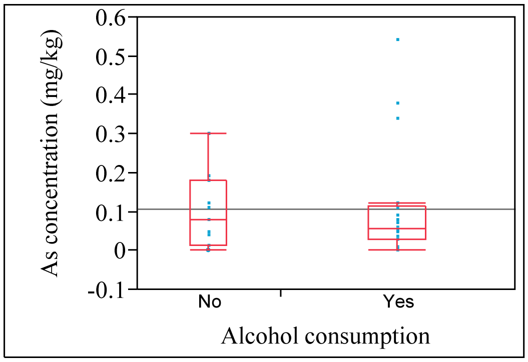
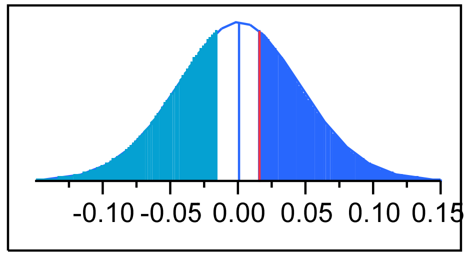
A9: One-way analysis of hair arsenic (mg/kg) by alcohol consumption and meat consumption

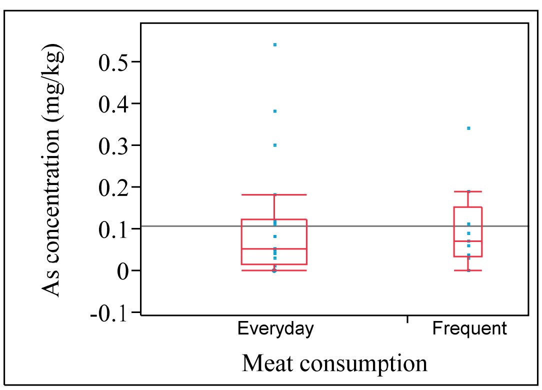


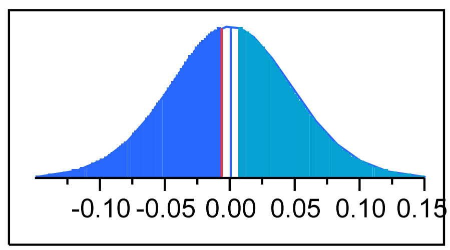


t- Test (Yes-No)

Assuming unequal variances

Difference	0.01521	t Ratio	0.335455
Std Err Dif	0.04533	DF	26.92501
Upper CL Dif	0.10823	Prob > |t|	0.7399
Lower CL Dif	-0.07782	Prob > t	0.3699
Confidence	0.95	Prob < t	0.6301





t- Test (Frequent-Everyday)

Assuming unequal variances

Difference	-0.00702	t Ratio	-0.14893
Std Err Dif	0.04715	DF	20.77016
Upper CL Dif	0.09110	Prob > |t|	0.8830
Lower CL Dif	-0.10514	Prob > t	0.5585
Confidence	0.95	Prob < t	0.4415

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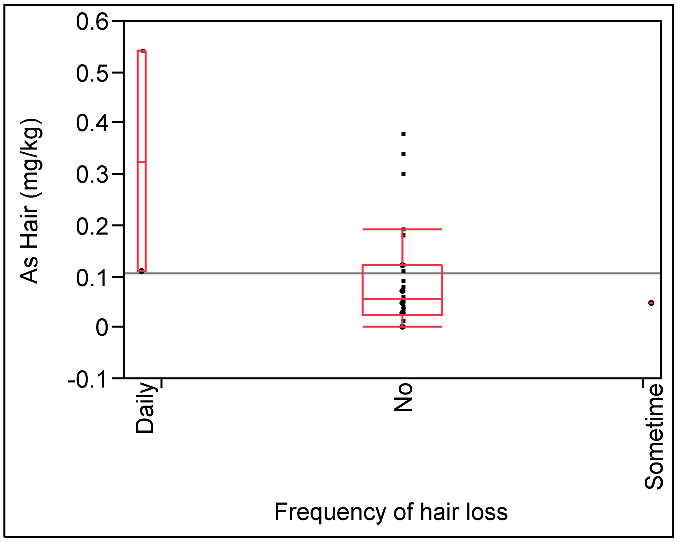
A10: One-way analysis of hair Arsenic (mg/kg) by hair loss



Summary of Fit

Rsquare	0.219512
Adj Rsquare	0.159474
Root Mean Square Error	0.118904
Mean of Response	0.107621
Observations (or Sum Wgts)	29

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Frequency of hair loss	2	0.10338544	0.051693	3.6562	0.0399*
Error	26	0.36759338	0.014138		
C. Total	28	0.47097883			

Means for One-way ANOVA

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Daily	2	0.325000	0.08408	0.1522	0.49782
No	26	0.093154	0.02332	0.0452	0.14109
Sometime	1	0.049000	0.11890	-0.1954	0.29341

Std Error uses a pooled estimate of error variance

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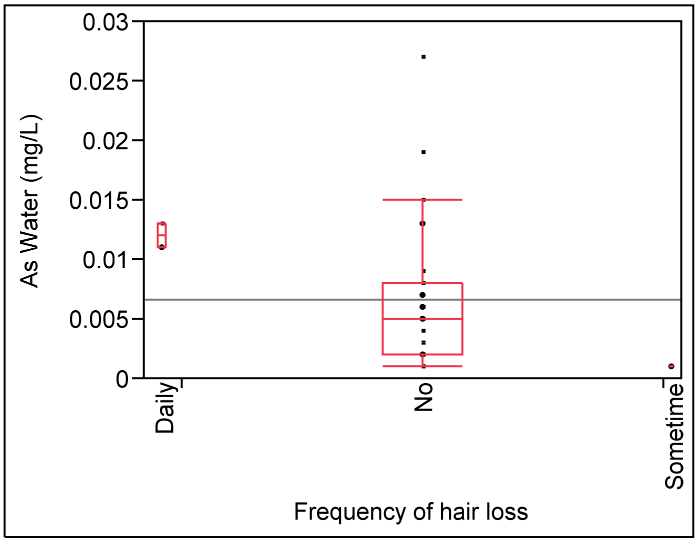
A11: One-way analysis of water Arsenic (mg/L) by hair loss



Summary of Fit

Rsquare	0.088977
Adj Rsquare	0.018898
Root Mean Square Error	0.005944
Mean of Response	0.00669
Observations (or Sum Wgts)	29

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Frequency of hair loss	2	0.00008971	0.000045	1.2697	0.2978
Error	26	0.00091850	0.000035		
C. Total	28	0.00100821			

Means for One-way ANOVA

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Daily	2	0.012000	0.00420	0.0034	0.02064
No	26	0.006500	0.00117	0.0041	0.00890
Sometime	1	0.001000	0.00594	-0.0112	0.01322

Std Error uses a pooled estimate of error variance

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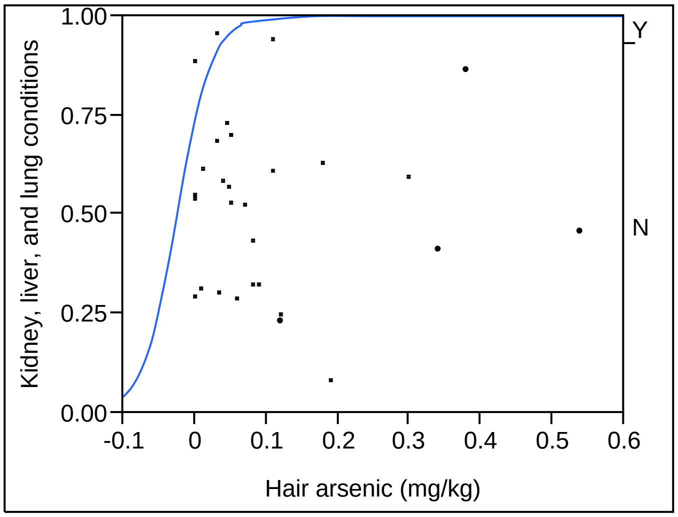
A12: Logistic fit of kidney, liver, and lung conditions by hair arsenic (mg/kg)



Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	1.7144957	1	3.428991	0.0641
Full	5.5631936			
Reduced	7.2776893			

R quare (U)	0.2356
AICc	15.5879
BIC	17.861
Observations (or Sum Wgts)	29

Measure	Training	Definition
Entropy RSquare	0.2356	1-Loglike(model)/Loglike(0)
Generalized RSquare	0.2826	(1-(L(0)/L(model))^(2/n))/(1-L(0)^(2/n))
Mean -Log p	0.1918	∑ -Log(ρ[j])/n
RMSE	0.2401	√ ∑(y[j]-ρ[j])²/n
Mean Abs Dev	0.1136	∑ |y[j]-ρ[j]|/n
Misclassification Rate	0.0690	∑ (ρ[j]≠ρMax)/n
N	29	n

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq
Intercept	1.0406822	0.9839889	1.12	0.2902
Hair arsenic(mg/kg)	41.936799	34.471391	1.48	0.2238

For log odds of N/Y

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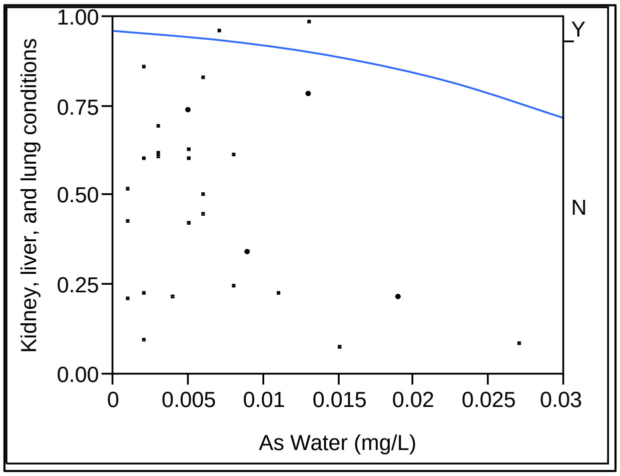
A13: Logistic fit of kidney, liver, and lung conditions by water arsenic (mg/L)



Whole Model Test

Model	-Log Likelihood	DF	ChiSquare	Prob>ChiSq
Difference	0.2730286	1	0.546057	0.4599
Full	7.0046607			
Reduced	7.2776893			

R Square (U)	0.0375
AICc	18.4709
BIC	20.7439
Observations (or Sum Wgts)	29

Measure	Training	Definition
Entropy RSquare	0.0375	1-Loglike(model)/Loglike(0)
Generalized RSquare	0.0473	(1-(L(0)/L(model))^(2/n))/(1-L(0)^(2/n))
Mean -Log p	0.2415	∑ -Log(ρ[j])/n
RMSE	0.2533	√ ∑(y[j]-ρ[j])²/n
Mean Abs Dev	0.1268	∑ |y[j]-ρ[j]|/n
Misclassification Rate	0.0690	∑ (ρ[j]≠ρMax)/n
N	29	n

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq
Intercept	3.2124615	1.1778424	7.44	0.0064*
As Water (mg/L)	-76.093666	96.552456	0.62	0.4306

For log odds of N/Y

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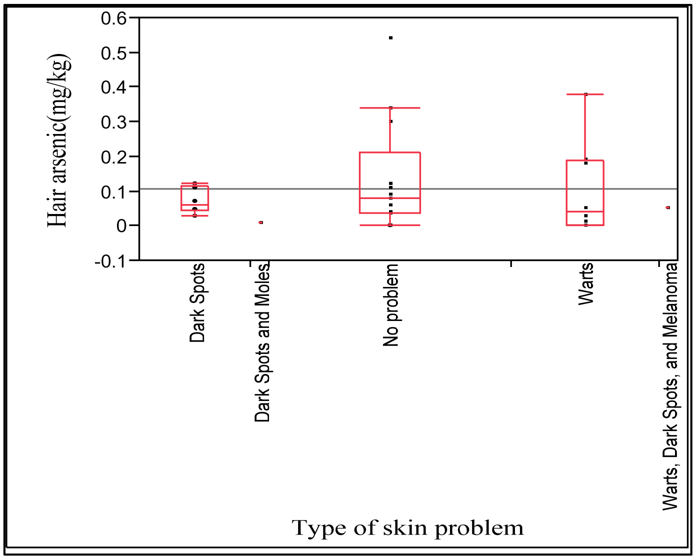
A14: One-way analysis of hair arsenic by type of skin problem



Summary of Fit

Rsquare	0.070233
Adj Rsquare	-0.08473
Root Mean Square Error	0.135077
Mean of Response	0.107621
Observations (or Sum Wgts)	29

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type of skin problem	4	0.03307820	0.008270	0.4532	0.7691
Error	24	0.43790063	0.018246		
C. Total	28	0.47097883			

Means for One-way ANOVA

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Dark Spots	6	0.070833	0.05515	-0.0430	0.18465
Dark Spots and Moles	1	0.010000	0.13508	-0.2688	0.28879
No problem	13	0.138077	0.03746	0.0608	0.21540
Warts	8	0.105125	0.04776	0.0066	0.20369
Warts, Dark Spots, and Melanoma	1	0.050000	0.13508	-0.2288	0.32879

Std Error uses a pooled estimate of error variance

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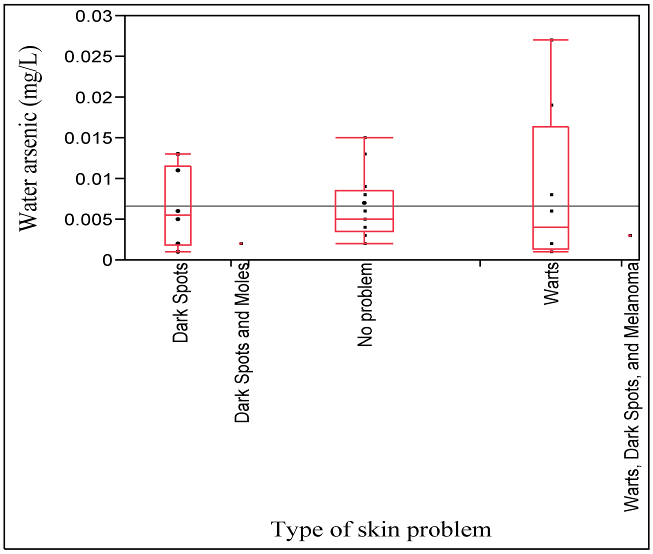
A15: One-way analysis of water arsenic by type of skin problem



Summary of Fit

Rsquare	0.055686
Adj Rsquare	-0.1017
Root Mean Square Error	0.006298
Mean of Response	0.00669
Observations (or Sum Wgts)	29

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Type of skin problem	4	0.00005614	0.000014	0.3538	0.8388
Error	24	0.00095206	0.000040		
C. Total	28	0.00100821			

Means for One-way ANOVA

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
Dark Spots	6	0.006333	0.00257	0.0010	0.01164
Dark Spots and Moles	1	0.002000	0.00630	-0.0110	0.01500
No problem	13	0.006538	0.00175	0.0029	0.01014
Warts	8	0.008250	0.00223	0.0037	0.01285
Warts, Dark Spots, and Melanoma	1	0.003000	0.00630	-0.0100	0.01600

Std Error uses a pooled estimate of error variance

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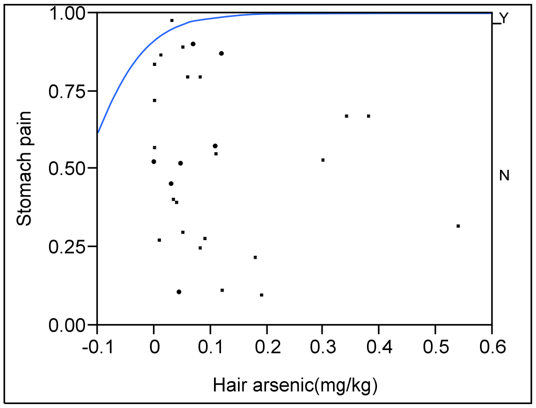
A16: Logistic fit of pain in stomach By hair arsenic



Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	0.4077388	1	0.815478	0.3665
Full	3.9421140			
Reduced	4.3498528			

RSquare (U)	0.0937
AICc	12.3458
BIC	14.6188
Observations (or Sum Wgts)	29

Measure	Training	Definition
Entropy RSquare	0.0937	1-Loglike(model)/Loglike(0)
Generalized RSquare	0.1070	(1-(L(0)/L(model))^(2/n))/(1-L(0)^(2/n))
Mean -Log p	0.1359	∑ -Log(ρ[j])/n
RMSE	0.1815	√ ∑(y[j]-ρ[j])²/n
Mean Abs Dev	0.0654	∑ |y[j]-ρ[j]|/n
Misclassification Rate	0.0345	∑ (ρ[j]≠ρMax)/n
N	29	n

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq
Intercept	2.34950304	1.3813284	2.89	0.0890
Hair arsenic(mg/kg)	18.7213099	29.775395	0.40	0.5295

For log odds of N/Y

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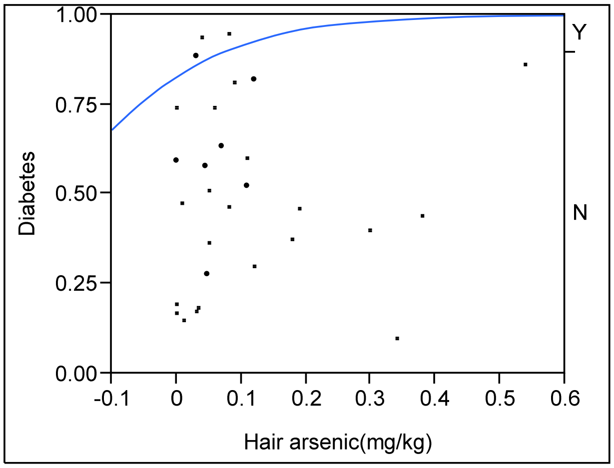
A17: Logistic fit of diabetes by hair arsenic



Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	0.5177875	1	1.035575	0.3089
Full	9.1274447			
Reduced	9.6452322			

R Square (U)	0.0537
AICc	22.7164
BIC	24.9895
Observations (or Sum Wgts)	29

Measure	Training	Definition
Entropy R Square	0.0537	1-Loglike(model)/Loglike(0)
Generalized R Square	0.0722	(1-(L(0)/L(model))^(2/n))/(1-L(0)^(2/n))
Mean -Log p	0.3147	∑ -Log(ρ[j])/n
RMSE	0.3021	√ ∑(y[j]-ρ[j])²/n
Mean Abs Dev	0.1813	∑ |y[j]-ρ[j]|/n
Misclassification Rate	0.1034	∑ (ρ[j]≠ρMax)/n
N	29	n

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq
Intercept	1.56353637	0.8318399	3.53	0.0602
Hair arsenic(mg/kg)	8.15098992	10.638003	0.59	0.4435

For log odds of N/Y

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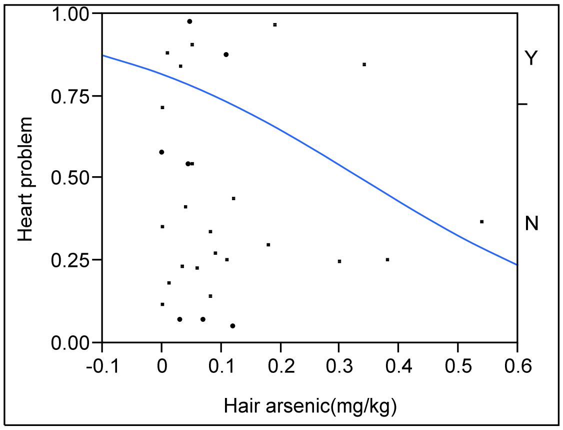
A18: Logistic fit of heart problem by hair arsenic



Whole Model Test

Model	-Log Likelihood	DF	Chi Square	Prob>Chi Sq
Difference	1.023496	1	2.046992	0.1525
Full	16.057580			
Reduced	17.081076			

R Square (U)	0.0599
AICc	36.5767
BIC	38.8498
Observations (or Sum Wgts)	29

Measure	Training	Definition
Entropy R Square	0.0599	1-Loglike(model)/Loglike(0)
Generalized R Square	0.0985	(1-(L(0)/L(model))^(2/n))/(1-L(0)^(2/n))
Mean -Log p	0.5537	∑ -Log(ρ[j])/n
RMSE	0.4290	√ ∑(y[j]-ρ[j])²/n
Mean Abs Dev	0.3684	∑ |y[j]-ρ[j]|/n
Misclassification Rate	0.2414	∑ (ρ[j]≠ρMax)/n
N	29	n

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq
Intercept	1.49463524	0.5887574	6.44	0.0111*
Hair arsenic(mg/kg)	-4.4474043	3.2048741	1.93	0.1652

For log odds of N/Y

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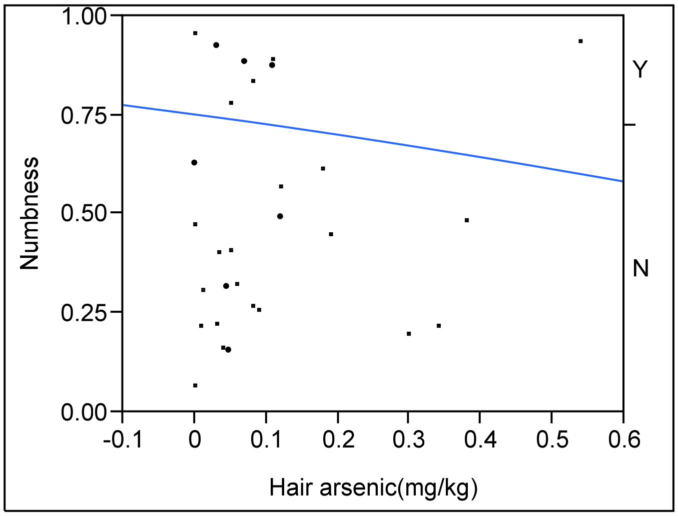
A19: Logistic fit of numbness in hand or feet by hair arsenic



Whole Model Test

Model	-Log Likelihood	DF	Chi Square	Prob>ChiSq
Difference	0.084948	1	0.169895	0.6802
Full	16.996128			
Reduced	17.081076			

RSquare (U)	0.0050
AICc	38.4538
BIC	40.7268
Observations (or Sum Wgts)	29

Measure	Training	Definition
Entropy R Square	0.0050	1-Loglike(model)/Loglike(0)
Generalized R Square	0.0084	(1-(L(0)/L(model))^(2/n))/(1-L(0)^(2/n))
Mean -Log p	0.5861	∑ -Log(ρ[j])/n
RMSE	0.4454	√ ∑(y[j]-ρ[j])²/n
Mean Abs Dev	0.3969	∑ |y[j]-ρ[j]|/n
Misclassification Rate	0.2759	∑ (ρ[j]≠ρMax)/n
N	29	n

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq
Intercept	1.11015173	0.5505795	4.07	0.0438*
Hair arsenic(mg/kg)	-1.2946926	3.0982308	0.17	0.6760

For log odds of N/Y

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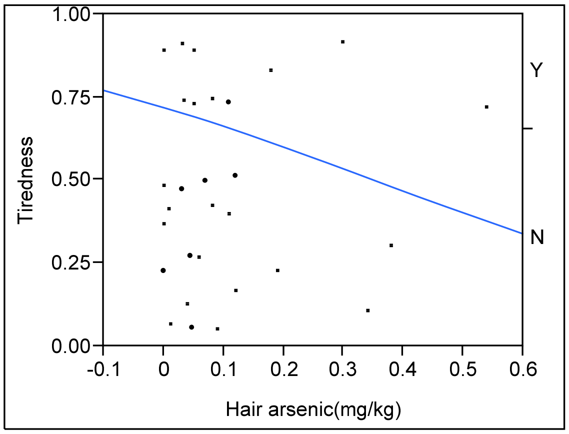
A20: Logistic fit of tiredness by hair arsenic



Whole Model Test

Model	-Log Likelihood	DF	ChiSquare	Prob>ChiSq
Difference	0.403728	1	0.807457	0.3689
Full	18.277659			
Reduced	18.681388			

RSquare (U)	0.0216
AICc	41.0169
BIC	43.2899
Observations (or Sum Wgts)	29

Measure	Training	Definition
Entropy R Square	0.0216	1-Loglike(model)/Loglike(0)
Generalized R Square	0.0379	(1-(L(0)/L(model))^(2/n))/(1-L(0)^(2/n))
Mean -Log p	0.6303	∑ -Log(ρ[j])/n
RMSE	0.4684	√ ∑(y[j]-ρ[j])²/n
Mean Abs Dev	0.4387	∑ |y[j]-ρ[j]|/n
Misclassification Rate	0.3448	∑ (ρ[j]≠ρMax)/n
N	29	n

Parameter Estimates

Term	Estimate	Std Error	Chi Square	Prob> Chi Sq
Intercept	0.94403907	0.526801	3.21	0.0731
Hair arsenic(mg/kg)	-2.6894576	3.0205709	0.79	0.3733

For log odds of N/Y

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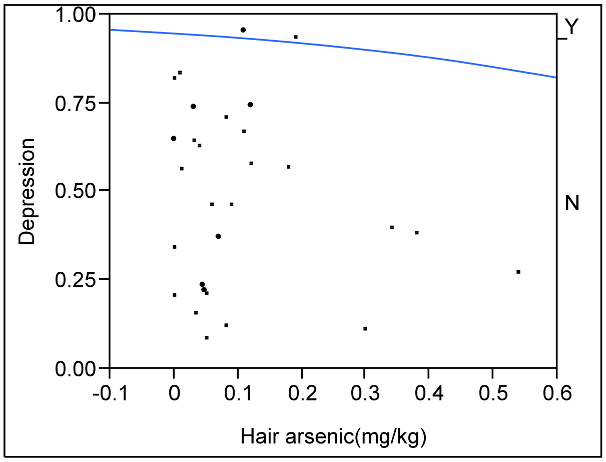
A21: Logistic fit of depression by hair arsenic



Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	0.1018647	1	0.203729	0.6517
Full	7.1758246			
Reduced	7.2776893			

RSquare (U)	0.0140
AICc	18.8132
BIC	21.0862
Observations (or Sum Wgts)	29

Measure	Training	Definition
Entropy RSquare	0.0140	1-Loglike(model)/Loglike(0)
Generalized RSquare	0.0177	(1-(L(0)/L(model))^(2/n))/(1-L(0)^(2/n))
Mean -Log p	0.2474	∑ -Log(ρ[j])/n
RMSE	0.2534	√ ∑(y[j]-ρ[j])²/n
Mean Abs Dev	0.1279	∑ |y[j]-ρ[j]|/n
Misclassification Rate	0.0690	∑ (ρ[j]≠ρMax)/n
N	29	n

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq
Intercept	2.88535315	1.0041095	8.26	0.0041*
Hair arsenic(mg/kg)	-2.255226	4.7173057	0.23	0.6326

For log odds of N/Y

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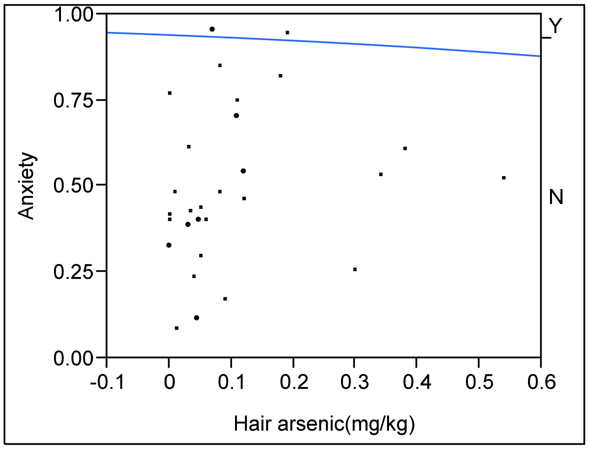
A22: Logistic fit of anxiety by hair arsenic



Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	0.0302888	1	0.060578	0.8056
Full	7.2474005			
Reduced	7.2776893			

RSquare (U)	0.0042
AICc	18.9563
BIC	21.2294
Observations (or Sum Wgts)	29

Measure	Training	Definition
Entropy RSquare	0.0042	1-Loglike(model)/Loglike(0)
Generalized RSquare	0.0053	(1-(L(0)/L(model))^(2/n))/(1-L(0)^(2/n))
Mean -Log p	0.2499	∑ -Log(ρ[j])/n
RMSE	0.2534	√ ∑(y[j]-ρ[j])²/n
Mean Abs Dev	0.1282	∑ |y[j]-ρ[j]|/n
Misclassification Rate	0.0690	∑ (ρ[j]≠ρMax)/n
N	29	n

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq
Intercept	2.75527167	0.9797408	7.91	0.0049*
Hair arsenic(mg/kg)	-1.2998976	5.0815308	0.07	0.7981

For log odds of N/Y

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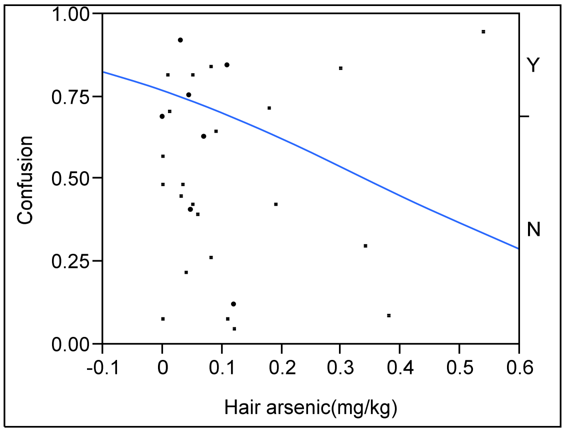
A23: Logistic fit of confusion by hair arsenic



Whole Model Test

Model	-LogLikelihood	DF	ChiSquare	Prob>ChiSq
Difference	0.665906	1	1.331813	0.2485
Full	17.296006			
Reduced	17.961912			

RSquare (U)	0.0371
AICc	39.0536
BIC	41.3266
Observations (or Sum Wgts)	29

Measure	Training	Definition
Entropy RSquare	0.0371	1-Loglike(model)/Loglike(0)
Generalized RSquare	0.0632	(1-(L(0)/L(model))^(2/n))/(1-L(0)^(2/n))
Mean -Log p	0.5964	∑ -Log(ρ[j])/n
RMSE	0.4514	√ ∑(y[j]-ρ[j])²/n
Mean Abs Dev	0.4074	∑ |y[j]-ρ[j]|/n
Misclassification Rate	0.3103	∑ (ρ[j]≠ρMax)/n
N	29	n

Parameter Estimates

Term	Estimate	Std Error	ChiSquare	Prob>ChiSq
Intercept	1.20278902	0.5523088	4.74	0.0294*
Hair arsenic(mg/kg)	-3.5095667	3.0951007	1.29	0.2568

For log odds of N/Y

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A24: Multivariate analysis on response hair arsenic

Summary of Fit

R Square	0.989593
R Square Adj	0.927154
Root Mean Square Error	0.035004
Mean of Response	0.107621
Observations (or Sum Wgts)	29

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	24	0.46607757	0.019420	15.8489
Error	4	0.00490126	0.001225	**Prob > F**
C. Total	28	0.47097883		0.0078*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.5266912	0.098319	5.36	0.0059*
As Water (mg/L)	18.074677	2.536906	7.12	0.0021*
Age	-0.011613	0.001804	-6.44	0.0030*
Gender[F]	-0.101597	0.015014	-6.77	0.0025*
Education[Associate]	0.0390828	0.025399	1.54	0.1987
Education[Bachelor]	-0.057371	0.024657	-2.33	0.0805
Education[High School]	-0.122137	0.020103	-6.08	0.0037*
Occupation[Artist]	0.2222322	0.045825	4.85	0.0083*
Occupation[Cabinet Maker]	0.0893738	0.038412	2.33	0.0805
Occupation[Copier Repair Tech]	-0.039087	0.03769	-1.04	0.3583
Occupation[Factory Worker]	-0.441623	0.067464	-6.55	0.0028*
Occupation[Farmer]	-0.243043	0.036203	-6.71	0.0026*
Occupation[House wife]	0.0099405	0.029288	0.34	0.7514
Occupation[Inspector]	-0.185571	0.061753	-3.01	0.0397*
Occupation[Production]	0.2519578	0.034034	7.40	0.0018*
Occupation[Retired]	0.1403444	0.027083	5.18	0.0066*
Occupation[Route Driver]	-0.058328	0.038191	-1.53	0.2014
Occupation[Secretary]	0.1690033	0.044994	3.76	0.0198*
Occupation[Self-Employed]	-0.115692	0.041453	-2.79	0.0493*
Occupation[Social Worker]	0.1777324	0.038121	4.66	0.0096*
Occupation[Store Manager]	0.0642683	0.042676	1.51	0.2065
Occupation[Teacher]	-0.111519	0.031855	-3.50	0.0249*
Years of residence	0.0047249	0.000736	6.42	0.0030*

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Term	Estimate	Std Error	t Ratio	Prob>|t|
Water Source [S+W]	0.3437325	0.053283	6.45	0.0030*
Water Source [W]	0.0911659	0.020628	4.42	0.0115*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
As Water (mg/L)	1	1	0.06219849	50.7612	0.0021*
Age	1	1	0.05079625	41.4557	0.0030*
Gender	1	1	0.05610547	45.7886	0.0025*
Education	3	3	0.05690428	15.4802	0.0115*
Occupation	15	15	0.29186645	15.8798	0.0081*
Years of residence	1	1	0.05054726	41.2525	0.0030*
Water Source	2	2	0.10199274	41.6190	0.0021*

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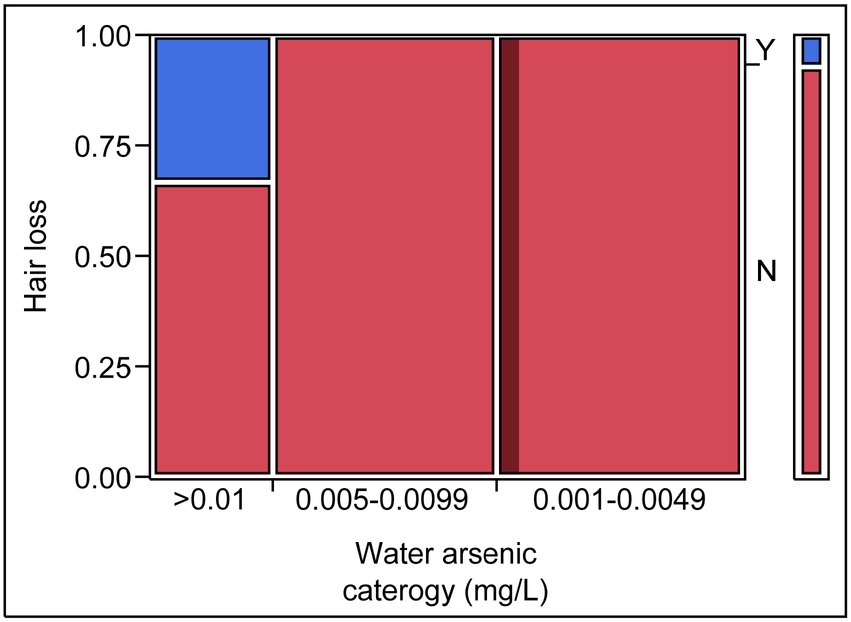
A25: Contingency analysis of hair loss by water arsenic category



Contingency Table

Water arsenic caterogy (mg/L) By Hair loss

Count Total % Col % Row %	N	Y	
>0.01	4 13.79 14.81 66.67	2 6.90 100.00 33.33	6 20.69
0.005-0.0099	11 37.93 40.74 100.00	0 0.00 0.00 0.00	11 37.93
0.001-0.0049	12 41.38 44.44 100.00	0 0.00 0.00 0.00	12 41.38
	27 93.10	2 6.90	29

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Tests

N	DF	-LogLike	RSquare (U)
29	2	3.4586043	0.4752

Test	ChiSquare	Prob>ChiSq
Likelihood Ratio	6.917	0.0315*
Pearson	8.235	0.0163*

Warning: 20% of cells have expected count less than 5, ChiSquare suspect.

Warning: Average cell count less than 5, LR ChiSquare suspect.

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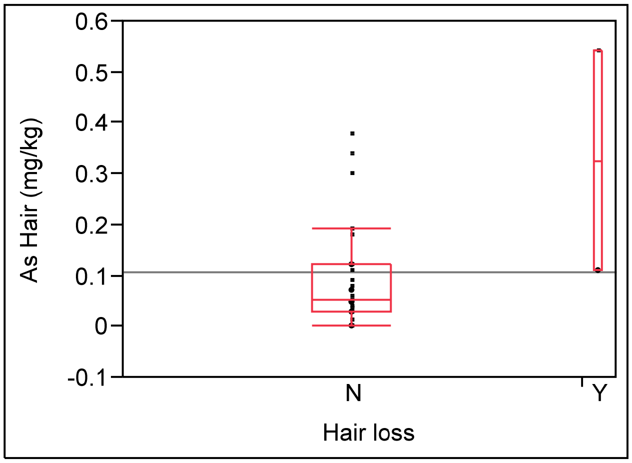
A26: One-way analysis of hair arsenic (mg/kg) by hair loss

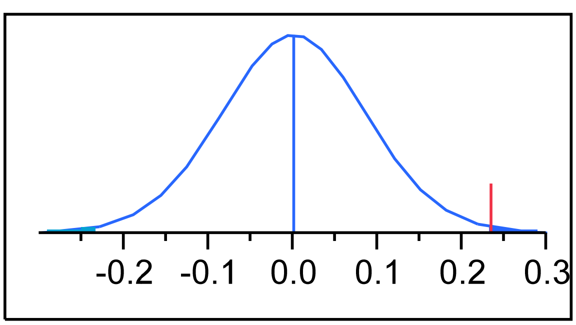


Summary of Fit

Rsquare	0.215526
Adj Rsquare	0.186471
Root Mean Square Error	0.116979
Mean of Response	0.107621
Observations (or Sum Wgts)	29

t-Test (Y-N)
Assuming equal variances

Difference	0.233481	t Ratio	2.723593
Std Err Dif	0.085726	DF	27
Upper CL Dif	0.409376	Prob > |t|	0.0112*
Lower CL Dif	0.057587	Prob > t	0.0056*
Confidence	0.95	Prob < t	0.9944



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Analysis of Variance

Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**	**Prob > F**
Hair loss	1	0.10150809	0.101508	7.4180	0.0112*
Error	27	0.36947074	0.013684		
C. Total	28	0.47097883			

Means for One-way ANOVA

Level	**Number**	**Mean**	**Std Error**	**Lower 95%**	**Upper 95%**
N	27	0.091519	0.02251	0.04533	0.13771
Y	2	0.325000	0.08272	0.15528	0.49472

Std Error uses a pooled estimate of error variance

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APPENDIX B

INVITATION LETTER

Participant ID:

Long-term, low-dose exposure of people residing in arsenic affected areas of Iowa: A cross-sectional analytical study

Principal Investigator: Junu Shrestha, Graduate Student, Office of Environmental Programs, University of Northern Iowa

Faculty Supervisor: Dr. Catherine Zeman, Associate Professor and Director Health Division, School of HPELS and Recycling and Reuse Technology Transfer Center, University of Northern Iowa

Dear Sir/ Madam,

I am writing to invite you to participate in a research project. The purpose of this research is to examine the potential effects of low dose arsenic exposure to human health. Arsenic is a common element that occurs naturally in the environment and its presence can be detected in plants, foods (such as fish), soil, air and water. In the United States, arsenic is generally found in low concentrations and a small amount is thought to pose no threat to human health. According to the Iowa Geological Survey, 2009, it has been estimated that 450,000 Iowans currently use private wells as their drinking water source. According to the Iowa Statewide Rural Well Water Survey, arsenic at low levels may be present in as

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many as 48% of the samples. Therefore, it is important to check the arsenic concentration level in your drinking water. You have been invited to participate in our research, and in return you will receive a free water arsenic testing report and may be chosen for a free hair analysis.

This research will be completed in three steps. First you will be asked for your water sample to test the arsenic concentration in your private well. If we find a measurable arsenic level, then we will again contact you to complete an arsenic study survey that includes general information, exposure evaluation information, and health profile information. We will also ask you to provide us few strands of your hair to check for any indication that arsenic levels in your well water are resulting in a concentration in your hair.

Finally, we will provide you with the results of a free arsenic test report of your private well water and hair sample analysis, if one was provided.

Your participation is totally voluntarily, but if you agree to participate then you could know more about the water quality in your private well. You will also help me (a graduate student at University of Northern Iowa) with my research.

If you agree to participate in the research, please sign participant informed consent forms. Take one copy for your record. In addition, please fill out participant basic information form. After filling out the form, put that form and another copy of participant informed consent form into the self- addressed, stamped envelope that we provided.

Please reply us within 10 days after you receive this mail.

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You will be hearing back from the research team in about three weeks once you have agreed to participate in this study. If you do not hear anything after three weeks then please feel free to contact me at junu@uni.edu, or contact number 309-750-8302.

If you need any further information, then please contact me at junu@uni.edu, cell 309-750-8302 or my faculty supervisor, Dr. Catherine Zeman at School of HELPS, University of Northern Iowa, cell 319-273-7090. You can also contact the office of IRB, University of Northern Iowa at 319-273-6148 for answers to questions about the rights of research participants and the participant review process.

Your participation will be greatly appreciated.

Sincerely,

Junu Shrestha,

Graduate Student, University of Northern Iowa

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APPENDIX C

INFORMED CONSENT FORM

UNIVERSITY OF NORTHERN IOWA
HUMAN PARTICIPANTS REVIEW

PARTICIPANT INFORMED CONSENT

Participant ID:

Project Title: Long-term, low-dose exposure of people residing in Arsenic Affected Areas of Iowa: A Cross-sectional analytical study

Name of Investigator(s): Junu Shrestha, Graduate Student, University of Northern Iowa

Dear Respondent,

You are invited to participate in a research project conducted through the University of Northern Iowa. The University requires that you give your signed agreement to participate in this project. The following information is provided to help you make an informed decision about whether or not to participate.

Arsenic is a naturally occurring common element found in the environment. Its presence can be detected in plants, foods (such as fish), soil, air and water. In the United States, arsenic is generally found in low concentrations; however, moderate to high concentrations do occur in some areas throughout the nation. Low level concentrations

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are commonly are found throughout the West and in parts of the Midwest and Northeast. Arsenic at low levels may be present in 48% of the samples in Iowa. This study is examining long-term, low-dose exposure of arsenic for people residing in arsenic affected areas. Since your private well might contain arsenic, your participation will provide important information in this study.

As a participant of this study, you are asked to complete arsenic study survey that focuses on:

1. General information

2. Exposure evaluation

3. Health profile

In addition to the arsenic study survey, you will be invited to participate in a hair sample analysis for long-term arsenic exposure. The hair samples taken will be used for laboratory analysis and do not pose any threat to the participants.

The answers you provide on the survey will be kept confidential. Your survey will be destroyed once your responses have been tallied. There are no foreseeable risks to you as a participant in this project; nor are there any direct benefits. But you will know about arsenic concentration status in your drinking water and hair if you participate in this research and your participation is extremely valued.

Arsenic analysis report of water and hair sample will be provided to you at the middle and completion of the research respectively.

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If you have questions about the study you may contact or desire information in the future regarding your participation or the study generally, you can contact me at 309- 750-8302 or my faculty advisor, Dr. Catherine Zeman at the School of Health, Physical Education & Leisure Services, University of Northern Iowa 319-273-7090. You can also contact the office of the IRB, University of Northern Iowa, at 319-273-6148, for answers to questions about rights of research participants and the participant review process.

I am fully aware of the nature and extent of my participation in this project as stated above and the possible risks arising from it. I hereby agree to participate in this project. I acknowledge that I have received a copy of this consent statement. I am 18 years of age or older.

______________________________ __________________

(Signature of participant) (Date)

____

(Printed name of participant)

___________________________ __________________

(Signature of investigator) (Date)

____________________________ __________________

(Signature of instructor/advisor) (Date)

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APPENDIX D

PARTICIPANT BASIC INFORMATION FORM

Participant ID

1. How long have you been drinking the water from your private well	< 1 year	about 1 year	> 1 year
2. Which option do you prefer for the arsenic study survey?	Mailing	Please provide your address below	
	Online	Please provide your email address below	
	Telephone	Please provide your daytime phone number	

3. Are you willing to provide a few strands of your hair? Yes No

Your information will be confidential and will not be disclosed in any circumstances

If Yes, then Please fill in the blanks below

How long is your hair?	< 2 inch	2-6 inch	> 6 inch

4. Do you want your hair analysis report? Yes No

Thank you for your willingness to participate in this research.

Your co-operation is greatly appreciated.

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APPENDIX E

ARSENIC STUDY SURVEY

Date

GENERAL PROFILE

1	Age	**2**	Gender	Male	Female
3	Highest education	**4**	Occupation		
5	Number of family members				

EXPOSURE EVALUATION

6 How long have you been living in this place?

7 Major source of drinking water?
- Water supply
- Individual Wells
- Bottled
- Others (Please specify)

8 How much water do you drink each day — Liters

9 How many times in a week do you bathe/shower?

10 Do you know about arsenic found in drinking water?
- Yes
- No

If yes how? Media Internet Newspaper Articles

Others , Please specify (
)

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11	Do you smoke?	Yes No	If Yes, how often ( )
12	Do you drink alcohol?	Yes No	If Yes, how often ( )
13	Do others in your family smoke?	Yes No	If Yes, how often ( )
14	Do others in your family drink alcohol?	Yes No	If Yes, how often ( )
15	How frequently do you eat meat and fish?	Everyday Frequent Once a week Your comments	

HEALTH PROFILE

16	Do you take any health supplements?	Yes	No
	If Yes, What type of health supplements?	Multivitamins Calcium	
	If others, please describe ( )		
17	Have you ever experienced any kind of pain in your stomach after drinking water?		Yes No
	If yes, how often		
18	Have you ever gone to see doctor for the case of diabetes?		Yes No

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19 Do you or any of your family members have any type of following skin problems or lesions?

Corns
Warts
Dark spots

If others, please describe ( )

20 Does your heart feel like it is racing at times? Yes No

If Yes, how often ( )

21 Do you have frequent noticeable loss of hair? Yes No

If Yes, how often daily weekly monthly

22 Do you feel any numbness in your hand or feet? Yes No

If yes, how often daily weekly sometimes

23 Do you feel tired or exhausted when you do daily household activities? Yes No

If yes, how often ( )

24 Have you ever been diagnosed with kidney, liver, and lung conditions as separate problems? Yes No

If yes, please explain ( )

25 Do you suffer from depression? Yes No

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26	Do you suffer from anxiety?	Yes	No
27	Have you ever experienced a state of confusion or forgetfulness during your daily activities?		Yes No

THANK YOU FOR YOUR PARTICIPANTION IN THE RESEARCH

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APPENDIX F

HAIR SAMPLE COLLECTION MANUAL

Please follow the following few steps for collection of hair samples:

1. First of all **RELAX**! You have to cut only a few strands of your hair.
2. Use a pair of scissors with blunted safety tips to cut the hairs.
3. Please make sure that you cut enough strands of hair all together to make a pea size ball when rolled-up.
4. As indicated on the photos, identify the appropriate spots for hair collection at the **nape of your neck** first (as shown in PHOTO 1). Cut a **few strands** of hair as close to the **scalp** as possible (as shown in PHOTO 2).
5. Now identify hair on the **side** of your head (PHOTO 3). Cut a few strands of hair as close to your scalp as possible (PHOTO 4).
6. Please repeat step 4 on the other side of your head.
7. Again identify and cut a few strands of hair from the very top portion of your head and cut as close as possible to the scalp (PHOTO 5).
8. Please make sure that you have cut enough strands of hair to make a pea size ball when rolled-up together.
9. Place the hair into the zip-lock bag and seal the bag properly.
10. Put the bag into the postage paid, pre-addressed envelope and place in the mail.
11. Thank You for your assistance with this research.
12. For more information call Junu Shrestha (Phone No. 309-750-8302) and Dr. Catherine Zeman (319 273-7090)

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APPENDIX G

LETTER FOR PARTICIPATION IN QUESTIONNAIRE SURVEY AND HAIR SAMPLE COLLECTION

Dear Participant,

Thank You for your interest on the research and agreeing to participate on our first step of water analysis. You participation is greatly valued and gives us important information to fulfill our research goal. I would like to assure that the information you provide will be used only for this research.

As the second step of this research, we have approached you for the arsenic study survey and hair sample collection. You will be asked to answer the questions about your general information, exposure evaluation and health profile. Upon your agreement you are requested to provide us few strands or your hair. Please follow the hair sampling collection manual for efficiency.

If you have any question on hair sampling collection manual, then please contact me at junu@uni.edu or call me at 309-750-8302.

Thank you once again for helping in this research. Your cooperation is greatly appreciated.

Sincerely,

Junu Shrestha

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APPENDIX H

ARSENIC ANALYSIS REPORT FOR WATER SAMPLE

Dear Participant,

I would like to thank you for participating in this research. The information you provided was very helpful. Since many private wells in areas of Iowa have low levels of arsenic concentration, this study is vital to monitor any health impacts from using this water. As you requested an arsenic testing report of your water and hair sample, we are presenting this report for your reference.

Water Arsenic concentration: <0.01 mg/L

(0.01 mg/L arsenic = 1 drop of arsenic in 16 gallons of water).

Your water does not contain measurable arsenic level and is safe for drinking and other household purposes.

I would like to thank you once again for your cooperation with and commitment to this study.

Sincerely,

Junu Shrestha
Graduate Student, Office of Environmental Programs
Contact No. 309-750-8302 Email: junu@uni.edu and

Dr. Catherine Zeman
Associate Professor and Director Health Division, (Faculty Advisor)
School of HPELS and Recycling and Reuse Technology Transfer Center
203 Wellness/Recreation Center
University of Northern Iowa, Cedar Falls, IA 50614-0241

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ARSENIC ANALYSIS REPORT FOR WATER SAMPLE

Dear Participant,

I would like to thank you for participating in this research. The information you provided was very helpful. Since many private wells in areas of Iowa have low levels of arsenic concentration, this study is vital to monitor any health impacts from using this water. As you requested an arsenic testing report of your water sample, we are presenting this report for your reference.

Water Arsenic concentration: >0.01 mg/L

(0.01 mg/L arsenic = 1 drop of arsenic in 16 gallons of water).

Since your water sample has been detected with measurable arsenic level, I would encourage you to participate in the second step of the research. Please read the attached letter for further information.

I would like to thank you once again for your cooperation with and commitment to this study.

Sincerely,

Junu Shrestha
Graduate Student, Office of Environmental Programs
Contact No. 309-750-8302 Email: junu@uni.edu and

Dr. Catherine Zeman
Associate Professor and Director Health Division, (Faculty Advisor)
School of HPELS and Recycling and Reuse Technology Transfer Center
203 Wellness/Recreation Center
University of Northern Iowa, Cedar Falls, IA 50614-0241

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ARSENIC ANALYSIS REPORT OF WATER SAMPLE (>0.01 mg/L)

Dear Participant,

I would like to thank you for participating in this research. The information you provided was very helpful. Since many private wells in areas of Iowa have low levels of arsenic concentration, this study is vital to monitor any health impacts from using this water.

The arsenic test for your well indicates that your drinking water contains moderately high arsenic contamination. The result shows that your drinking water contains >0.01 mg/L arsenic concentration. This level exceeds the EPA standard of 0.01 mg/L. Drinking water with arsenic contamination could impact your health in the future if you continue to drink the water. Therefore I suggest you to contact the County Health Department in your area.

Dr. Catherine Zeman is also available to discuss this with you should you wish. Her contact number is 319-273-7090

I would like to thank you once again for your cooperation with and commitment to this study.

Sincerely,

____

Junu Shrestha
Graduate Student, Office of Environmental Programs
Contact No. 309-750-8302 Email: junu@uni.edu and

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____

Dr. Catherine Zeman
Associate Professor and Director Health Division, (Faculty Advisor)
School of HPELS and Recycling and Reuse Technology Transfer Center
203 Wellness/Recreation Center
University of Northern Iowa, Cedar Falls, IA 50614-0241

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Arsenic Analysis Report for Hair Sample

Dear Participant,

I would like to thank you for participating in this research. The information you provided was very helpful in completing this research. Since many private wells in areas of Iowa have low levels of arsenic concentration, this study was vital to monitor any health impacts from using this water. As you requested an arsenic testing report of your hair sample, we are presenting this report for your reference.

Hair Arsenic concentration: >0.25 mg/kg

(1mg/kg= 1 milligram of arsenic in 1 kilogram of the hair sample)

The normal range of arsenic in hair sample is 0.08-0.250 mg/kg. The report indicates that your hair contains a comparatively high arsenic level but does not exceed the toxicity level of 1mg/kg. Since your water arsenic concentration is more than the maximum contaminant level of 0.01 mg/L, a possible water purification method such as reverse osmosis method is suggested.

We also suggest you contact your County Health Department for a list of companies that can aid you in treating your drinking water.

I would like to thank you once again for your cooperation with and commitment to this study.

Sincerely,

Junu Shrestha
Graduate Student, Office of Environmental Programs
Contact No. 309-750-8302 Email: junu@uni.edu and

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Dr. Catherine Zeman
Associate Professor and Director Health Division, (Faculty Advisor)
School of HPELS and Recycling and Reuse Technology Transfer Center
203 Wellness/Recreation Center
University of Northern Iowa, Cedar Falls, IA 50614-0241

Please feel free to contact us if you need more information on arsenic in drinking water and hair.

If you want to know more about the research

Please contact:

Dr. Catherine Zeman
Associate Professor and Director Health Division, (Faculty Advisor)
School of HPELS and Recycling and Reuse Technology Transfer Center
203 Wellness/Recreation Center
University of Northern Iowa, Cedar Falls, IA 50614-0241

If you want to know more about arsenic contamination status of Iowa
Please contact:
Dr. Michael D. Wichman
Associate Director, Environmental Health Programs
State Hygienic Laboratory, University of Iowa
UI Research Park - Coralville
Iowa City, IA 52242-5002

You can also contact your medical doctor or the County Health Department, if you have further medical questions.

Thank You for your support to complete this research.

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ARSENIC ANALYSIS REPORT FOR HAIR SAMPLE

Dear Participant,

I would like to thank you for participating in this research. The information you provided was very helpful in completing this research. Since many private wells in areas of Iowa have low levels of arsenic concentration, this study was vital to monitor any health impacts from using this water. As you requested an arsenic testing report of your hair sample, we are presenting this report for your reference.

Hair Arsenic concentration: <0.25 mg/kg

(1mg/kg= 1 milligram of arsenic in 1 kilogram of the hair sample)

The normal range of arsenic in hair sample is 0.08-0.250 mg/kg. The report indicates that your hair contains normal arsenic level and safe from arsenic related health problems.

I would like to thank you once again for your cooperation and commitment to this study.

Sincerely,

Junu Shrestha
Graduate Student, Office of Environmental Programs
Contact No. 309-750-8302 Email: junu@uni.edu and

Dr. Catherine Zeman
Associate Professor and Director Health Division, (Faculty Advisor)
School of HPELS and Recycling and Reuse Technology Transfer Center
203 Wellness/Recreation Center
University of Northern Iowa, Cedar Falls, IA 50614-0241

Please feel free to contact us if you need more information on arsenic in drinking water and hair.

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If you want to know more about the research

Please contact:

Dr. Catherine Zeman

Associate Professor and Director Health Division, (Faculty Advisor)

School of HPELS and Recycling and Reuse Technology Transfer Center

203 Wellness/Recreation Center

University of Northern Iowa, Cedar Falls, IA 50614-0241

If you want to know more about arsenic contamination status of Iowa

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State Hygienic Laboratory, University of Iowa

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Iowa City, IA 52242-5002

You can also contact your medical doctor or the County Health Department, if you have further medical questions.

Thank You for your support to complete this research.

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