

**BIOMONITORING PILOT STUDY  
HAIR ARSENIC LEVELS IN CLIENTS ATTENDING THE SPECIAL  
SUPPLEMENTAL NUTRITION PROGRAM FOR WOMEN, INFANTS  
AND CHILDREN (WIC) PROGRAM**

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## Summary

Hair arsenic levels measured in Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) participants were within the range found in other studies with no known sources of environmental exposure. The total number of participants that agreed to hair arsenic testing was 386. The average arsenic hair level in children of 230 ppb was higher than the adult hair level of 89 ppb. Five children exceeded the 1000 ppb level of concern, but no common risk factors were identified. In children, age and ownership of pets were significantly associated with arsenic hair levels. In adults, ethnicity and ancestry were associated with hair levels, with Asians lower than Hawaiian Pacific Islanders and those reporting multiple races. The factors influencing hair levels in adults of different races are unknown.

## Background

In 2005, an investigation conducted by the Hawaii Department of Health (HDOH) found high levels of total arsenic in soil in a number of locations around the town of Kea`au (HDOH 2007). The arsenic is most likely from past use of sodium arsenite, an herbicide used in sugar production between approximately 1915-1950. Of particular concern was elevated levels of arsenic found in two large (acres) community gardens.

DOH and the Agency for Toxic Disease Registry (ATSDR) conducted an Exposure Investigation to assess exposure to inorganic arsenic in people using the community gardens (ATSDR 2008). Speciated arsenic was measured in urine of approximately 30 people of Filipino ancestry over a period of three months. The average urinary arsenic levels of the Kea`au participants were higher than the 20 µg/L urinary screening level chosen for the study. Difficulties were encountered during the study because some participants ate seafood, including seaweed, just prior to testing. Seafood contains an organic form of arsenic that is not toxic but can interfere with the interpretation of the results. Additionally their diet was high in rice, another source of arsenic. DOH concluded that the dietary contribution of arsenic in this population overwhelmed the potential contribution from the contaminated soil. The limited pilot study in Kea`au highlighted the difficulties in assessing whether individuals are at risk of excessive inorganic arsenic exposure from soil when the diet is high in naturally occurring arsenic.

Because of the widespread contamination of former agriculture lands in Hawaii with arsenic, additional testing of people for arsenic exposure is warranted. The goal is to gather more

information on background levels of arsenic in a population with no known exposure to arsenic other than dietary to compare against people living on arsenic contaminated soil. The goal of this study is to gather more information on background levels of arsenic in a population with no known exposure to arsenic other than dietary. Ultimately, information of this type will be used to compare against people living on arsenic contaminated soil.

## Hair Testing

In 2008, DOH committed to conducting a hair mercury biomonitoring project in clients of Special Supplemental Nutrition Program for Women, Infants, and Children (WIC). The hair samples collected for this project provided an opportunity to also measure arsenic levels.

Arsenic can be measured in urine, hair, or blood to evaluate exposure. Measurement of arsenic in blood is not a reliable indicator of chronic exposure to low levels of arsenic since it is cleared from the blood within a few hours and reflects only very recent exposure. Urine arsenic is the most reliable method for measuring arsenic exposure, particularly exposures occurring within a few days of the specimen collection. However, the arsenic must be speciated to distinguish between exposure to inorganic arsenic and its metabolites and the relatively non toxic form of organic arsenic commonly found in seafood. Even with speciation, DOH's pilot study in Kea'au found that seafood consumption appeared to increase one of the metabolites associated with inorganic arsenic, making interpretation difficult.

Hair provides a convenient specimen for biomonitoring because it is non-invasive and readily obtainable. Inorganic arsenic concentrates in hair due to its high affinity for keratin (Hindmarsh 2003). Based on animal studies, arsenobetaine does not accumulate in hair (Vahter et al. 1983, as cited by Hughes 2006), so consumption of seafood should not be a complicating factor in arsenic hair analysis.

Absorbed arsenic accumulates in hair with inorganic arsenic as the predominant form. Studies have shown a good correlation between hair arsenic levels and groundwater concentrations (Kurttio et al., 1998; Mandal et al. 2004; Gault et. al., 2008 Agusa et al., 2006). Background levels are less than 1000 ppb (Hindmarsh, 2002, as cited by Hughes 2006). However, hair arsenic levels are prone to external contamination (hair products, air, dust etc.), which cannot be distinguished from internally deposited arsenic in the hair shaft. Thus external contamination may overestimate the exposure.

We measured arsenic in the hair samples collected from WIC participants to collect preliminary data on "background" hair arsenic levels in unexposed individuals not known to be living on contaminated soil. We also evaluated other sources of exposure (determined by questionnaire) and demographic factors that make a contribution to hair arsenic. Because this study was conducted in participants attending WIC clinics, the results of this investigation are applicable only to the WIC population and may not be representative of the general population. Although the background reference range cannot be applied to the general population, this was a preliminary effort to characterize arsenic exposures in Hawaii. Ultimately, information of this type will be used to compare against people living on arsenic contaminated soil.

## Study Population

The target population for this study is women and children enrolled in the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC). The WIC program serves children from 0 to 5 years of age, pregnant and postpartum teens and adult women. Women do not have to be pregnant to participate. WIC currently serves approximately 26, 949 women, infants, and children in Hawaii (including over 2404 pregnant women). The initial goal was to enroll only WIC clients. However, there was interest from mothers, guardians, and grandmothers of WIC clients not enrolled in the program to be tested. We included these participants in the testing, but subsequently don't have some demographic information

## Recruitment into Study and Procedures

Staff from the Biomonitoring Project coordinated with the WIC Program to set up information tables at various WIC clinics throughout the islands to recruit volunteers to participate in the study. The primary goal of the project was to measure mercury in hair samples of WIC clients. Clients were told that they would be given their hair mercury results along with any nutritional counseling if the results were elevated. If the client agreed to participate in the mercury testing, DOH then offered hair arsenic testing. It was explained that these results would not be provided back to the participant because there was not a reliable mean of explaining the health significance of the results. Out of 643 total participants, 426 participants agreed to be tested for arsenic. There were several reasons given for declining arsenic testing including not knowing what arsenic was and not receiving the results of the test.

Each participant who agreed to be in the study was asked to fill out a questionnaire and an informed consent form (see attached). After informed consent had been completed, the biomonitoring staff collected hair and put it into a labeled Ziploc bag. Hair was transported to the Hawaii State Laboratory with chain of custody procedures.

### Privacy

Each participant was given a unique numerical identifier linked to her WIC identification. The DOH principal investigator (Barbara Brooks, Ph.D.), UH research assistant (Yesid Romero Ph.D., MD), WIC nutritionist (Sher Pollack, M.S., R.D.) and WIC epidemiologist (Dr. Don Hayes) had access to the arsenic results linked to personal contact information.

Reports produced from this information gave group information and did not identify specific individuals. Confidential information is kept in locked cabinets or on password protected computers.

### Informed Consent

Informed consent was obtained from each survey participant following guidelines approved by the University of Hawaii Committee on Human Studies and Hawaii Department of Health. Prior to testing, each participant and a parent or legal guardian of each minor participant was required to sign an informed consent. Pregnant and postpartum preteens ages 12 to 17 were also required to sign an assent form. A copy of these forms is attached.

### Survey Forms

In addition to completing consent/assent forms, each participant was asked a few questions to gather information on risk factors for exposure to arsenic through fish consumption and arsenic through food pathways, contact with contaminated soil, or use of arsenic containing products.

### WIC demographic information

The WIC program maintains a database of demographic and health information for WIC clients that is updated at every visit. This information was used to evaluate associations of hair arsenic with various demographic information.

### Sample Collection

Approximately 50-100 strands of hair (about 1/8 inch in diameter) were collected at the neck nape using stainless steel scissors. The hair strands were tied with dental floss at the scalp end of the hair and stored in a labeled Ziploc bag. After hair collection the hair samples were either stored in a file cabinet in a room that required a password to enter or immediately transported to the Hawaii State Laboratory with Chain of Custody form. Prior to arsenic analysis, the first 1 to 3 cm of the scalp end was cut and analyzed for mercury. The remaining hair sample was stored at room temperature in the Ziploc bag until arsenic analysis

## **Arsenic Analysis**

A digestion vessel was first tared on an analytical balance. A portion of hair was then cut from the provided hair sample using a pair of ceramic scissors and placed directly into the digestion vessel where the weight of the sample to be digested was recorded.

There was no standard length cut for each person because the amount of hair varied especially with young children. For very young children, it was difficult to get enough hair to get a sample weight of 50-100 mg so lower sample weights were sometimes used. The average hair sample weight for children was 38 mg with a range of 2 mg to 106 mg.

The hair was not washed prior to digestion. Midway through the project, the procedure was revised to allow the use of the same centrifuge tubes for digestion and ICP-MS analysis, to eliminate a transfer step, and eliminate excess digestate that needed to be stored

### Analyses prior to 12/21/2009

A hair sample (approximately 100 mg) was digested in 2:1 concentrated nitric acid – 30% hydrogen peroxide (2 mL) for 1 hour at 70°C in a plastic screw-capped centrifuge tube. Once cool, an aliquot (1 mL) of the clear digestate was removed, diluted with 2% nitric acid (9 mL) containing the internal standard and analyzed.

### Analyses including and after 12/21/2009

A hair sample (approximately 50 mg) was digested in 2:1 concentrated nitric acid – 30% hydrogen peroxide (1 mL) for 1 hour at 70°C in a plastic screw-capped centrifuge tube. Once cool the clear digestate was diluted with 2% nitric acid (9 mL) containing the internal standard and analyzed.



### Analysis:

The digested hair samples were analyzed by Inductively Coupled Plasma/Dynamic Reaction Cell/Mass spectrometry (ICP/DRC/MS) using the Validated CDC method for total arsenic in urine (CDC Method CTL-TMS-2.01, 2005) that was revalidated to use oxygen as the reaction gas.

### Sample Precision

The arsenic limit of quantitation (LOQ) is 25 ppb for hair. The method precision is 5.1% at 15 ppb and 1.6% at 150 ppb for matrix matched aqueous QCs using oxygen as the reaction gas.

### Quality Control and Quality Assurance:

Aqueous, matrix matched quality control samples (QCs) were analyzed at two levels, low (15 ppb) and high (150 ppb), prior to running a sample batch and immediately after a sample batch was complete. Two human hair reference samples were also analyzed prior to running a sample batch at two levels, low (~500 - 600 ppb) and high (~2000 - 2500 ppb).

The human hair reference material was acquired from CTQ (Centre de toxicology du Quebec) which administers the Quebec Multielement Quality Assessment Scheme (QMEQAS), the hair arsenic PT program that DOH was participating in at the time of the arsenic project. For this project DOH selected and purchased lots with appropriate mean arsenic concentrations to use for our low and high reference levels

During a sample batch check, standards were analyzed every 5 – 6 samples at various levels within the calibration range for calibration verification.

## **Statistical Analysis**

The Excel data analysis package and SAS Version 9.2 were used to evaluate the data. A generalized linear model was used to perform the multiple linear regression analysis. A test for interaction was done to look for confounding variables. Because most biomonitoring data do not follow a normal distribution, the arsenic values were log transformed before being used for data analysis. Values that were less than the limit of quantitation were divided by the square root of 2 prior to data analysis. Significant outliers that were more than the geometric mean plus 3 standard deviation of the log transformed data were removed (but discussed qualitatively) prior to data analysis. Independent sample t tests, linear regression or analysis of variance followed by Tukeys test were calculated for the log of hair arsenic concentration versus demographic and survey factors.

## **Results**

The total number of participants that agreed to hair arsenic testing was 426. There was insufficient hair samples for 35 of these participants and 5 other samples were not tested for unknown reasons. Hair arsenic results were obtained for 124 children and 262 adults. Demographic information (age, race, gender) was missing for several children and adults. Figure 1 presents a frequency histogram for all hair arsenic results. There was considerable variability in hair arsenic results in the children. No adults exceeded 1000 ppb, while 5 children had levels greater than 1000 ppb. The geometric mean, 95% confidence interval and range of values for

children and adults are shown in Table 1.

## Frequency Hair Arsenic Results

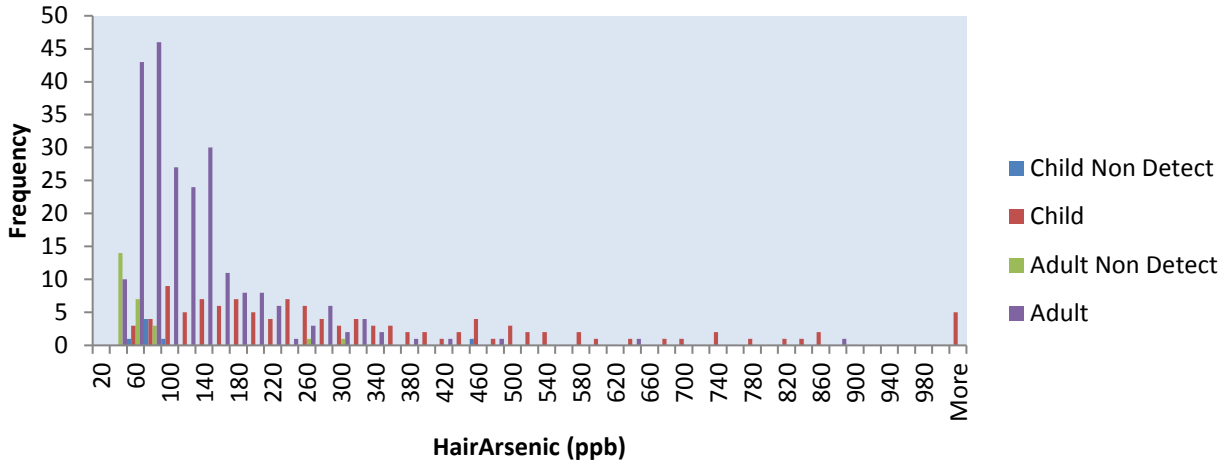


Figure 1-Frequency Hair Arsenic Results

Statistical outliers that were greater than the geometric mean plus 3 standard deviations of the log transformed data were removed prior to data analysis. They are discussed qualitatively in the sections below. Statistical outliers included one infant with a hair arsenic result of 3830 ppb and one adult with a result of 866.7 ppb.

Table 1-Descriptive Statistics for Hair Arsenic Levels in Children and Adults<sup>a</sup>

Age Group <sup>a</sup>	Number	Average Age (yrs)	Geo Mean(ppb)	(95% CI)	Range (ppb)	Number of Non Detects
Infants and Children	123	3.2 (0.6-12.5)	230.2*	198.8-266.5	Nd-1970	7
Adults	261	30.4 (17.8-56.6)	88.7	82.0-95.9	Nd-625.3	26

<sup>a</sup> Outliers removed. One child and one adult with hair levels that were more than the geometric mean and 3 times the standard deviation were removed from the dataset.

\* statistically significant ( $p < 0.001$ ).

The average age of the children participants was 3.2 years and the average age of the adults was 30.4 years. A preliminary test for the equality of variances indicates that the variances of the two groups were significantly different  $F=1.6$ .  $F_{crit}=1.3$ ,  $p < 0.001$ . Therefore, a two-sample t-test for hair arsenic levels was performed that does not assume equal variances. The means of the hair arsenic levels were significantly different ( $p < 0.001$ ) with a t statistic of 11.3.

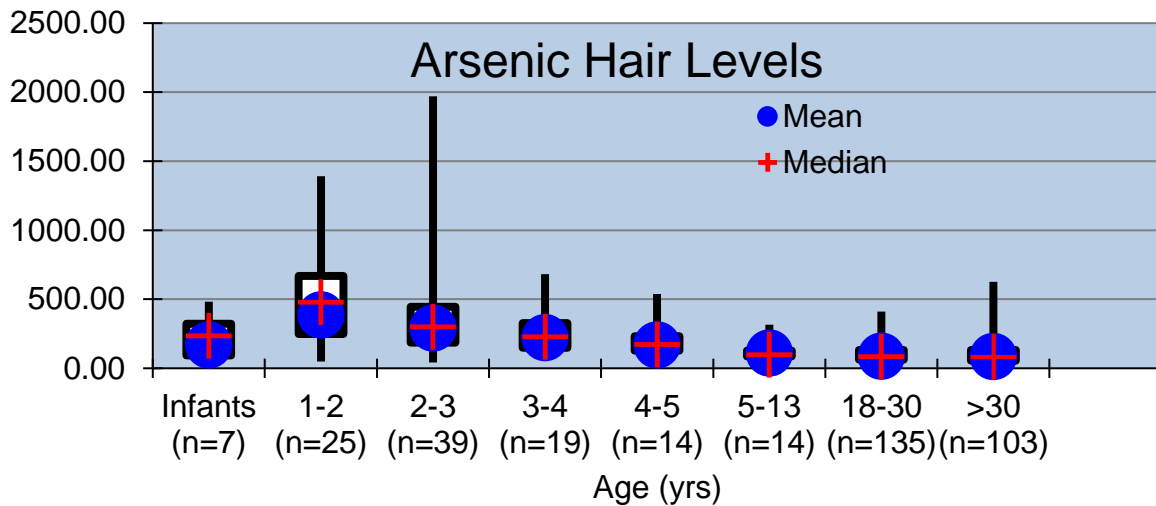
Table 2 shows the average hair arsenic levels in children and adults by age, race, ancestry and gender. Analysis of variance followed by Tukey's test showed a significant association ( $p < 0.05$ ) of hair arsenic levels with age in children, and race in adults. The statistical significant

associations are discussed in more detail in the following sections.

Age

Figure 2 shows a box plot of the geometric mean of the age categories. Boxes represent 25<sup>th</sup> and 75<sup>th</sup> percentile. The ends of the whiskers show the minimum and maximum. The highest average arsenic levels were found in toddlers aged 1-2 years old. In children older than 1, the arsenic levels decreased with age. Looking at children only, Anova and Tukeys test showed that children aged 1-2 years have significantly higher ( $p < 0.001$ ) hair arsenic levels than children older than 4 years. Children aged 2-3 years have significantly ( $p < 0.001$ ) higher arsenic levels than the children older than 5. Children aged 1-5 years differed from the adults ( $p < 0.001$ ).

Linear regression of the log of the children’s hair arsenic concentration versus age showed a negative and significant correlation ( $p < 0.001$ ,  $r^2 = 0.17$ ). Adults showed no correlation between age and log arsenic hair levels. A potential confounder with age and hair arsenic levels in children is the weight of hair used for analysis. With the very small children, the lab was unable to collect 50 mg of hair. Linear regression analysis between age and hair sample weight showed a significant association in children ( $r^2 = 0.10$ ,  $p < 0.001$ ), but not in adults. According to the lab, the small sample weight would not bias the results high, but adds uncertainty because there was no hair left for reanalysis. Additionally, pilot studies by the lab showed that arsenic analysis was accurate over a wide range of hair weights that spanned those of the children for this study.



**Figure 2-Arsenic Hair Levels in Children and Adults**

Race

Arsenic hair levels categorized by self- identified race and ancestry are shown in Table 2. For confidentiality, results for race and ancestry with less than 5 participants were not reported. The racial makeup of the adults is shown in Figure 3. Adult participants reporting single-race Hawaiian or Pacific Islander had the highest average hair arsenic level of 115 ppb followed by participants reporting Multiple races at 95 ppb. They were both significantly higher than single

race Asians whose average hair arsenic level was 71 ppb. No other differences were significant. In children hair levels did not differ significantly, although single race Asian hair levels were lower than the other racial categories. When looking at self reported ancestry, hair levels were significantly higher ( $p < 0.05$ ) in adult Hawaiians than Chinese and Filipino participants. No differences were seen in children.

### Self -Identified Race (Percentage) of Adult Participants

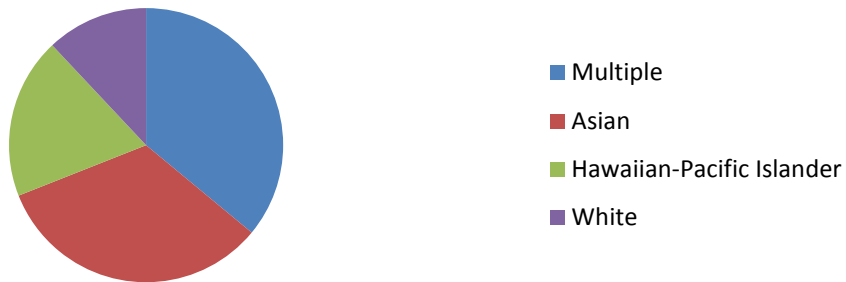


Figure 3-Self identified racial makeup of adult participants

#### Gender

All the adults were females. In children, boys had higher hair arsenic levels but the difference was not significant ( $P=0.07$ )

#### Location

Table 3 shows the hair arsenic levels by place of residency at the time of hair sampling. The majority of the participants were from Oahu. Due to budget constraints, limited sampling was conducted in neighbor islands. Some of the children and adults from the island of Hawaii were living in areas known to have high arsenic soil levels but there were no significant differences in hair arsenic levels with zip code or island. However there were very few samples from the island of Hawaii.

**Table 2-Hair Arsenic Levels by age, race, ethnicity and gender**

Characteristic	Children				Adults			
	N	Geo Mean (ppb)	Min	Max	N	Geo Mean (ppb)	Min	Max
<b>Age</b>								
Infants	7	169.1	ND	483.3				
c1 (1-2 yrs) <sup>ac</sup>	25	386.9	ND	1390.0				
c2 (2-3 yrs) <sup>bc</sup>	39	292.8	ND	1970.0				
c3 (3-4 yrs) <sup>c</sup>	19	222.0	62.2	682.6				
c4 (4-5 yrs) <sup>c</sup>	14	168.9	ND	539.0				
c5 (5-13 yrs)	14	112.7	42.4	314.5				
A1 (18-30)					118	88.3	ND	410.0
A2 (31-46)					119	87.5	ND	625.3
<b>Race</b>								
American Indian or Alaska Native	<5				<5			
Asian	27	184.5	ND	831.0	78	70.6	ND	625.3
Black or African American	0							
Hawaiian or Pacific Islander <sup>d</sup>	14	209.6	84.2	735.1	45	114.9	ND	410.0
Caucasian or White	11	263.9	80.8	1390.0	28	82.7	ND	274.0
Multiple <sup>d</sup>	65	273.8	ND	1970.0	84	95.1	ND	472.4
<b>Ancestry</b>								
Chinese	<5				9	53.5	ND	134.0
Filipino	22	218.5	ND	853.0	64	73.7	ND	625.3
Hawaiian or Part-Hawaiian <sup>e</sup>	9	238.4	84.2	620.2	33	124.2	ND	472.4
Japanese					8	72.5	ND	182.0
Korean	<5				<5			
Laotian					<5			
Marshallese					<5			
Micronesian	<5				18	122.9	46.1	410.0
Other Pacific Islander	<5				<5			
Samoan	5	216.1	100.0	539.0	<5			
Thai	<5				<5			
Vietnamese					<5			
Multiple	56	271.5	ND	1970.0	61	90.0	ND	324.0
<b>Gender</b>								
Male	48	292.1	ND	1970.0				
Female	66	223.1	ND	1390.0				

<sup>a</sup> significantly (p<0.05) different than children older than 4 years

<sup>b</sup> significantly (p<0.001) different than children older than 5 years

<sup>c</sup> significantly (p<0.05) different than adults

<sup>d</sup> Adults Hawaiian and Multiple significantly (p<0.001) different than Asians

<sup>e</sup> Adult Hawaiian significantly different (p<0.05) than Chinese and Filipino ancestry

ND=Non detect

**Table 3-Hair Arsenic Levels by Place of Residence**

Island	Children				Adults			
	N	Geomean	Min	Max	N	Geomean	Min	Max
<b>Oahu</b>	112	222.8	42.4	1970.0	230	90.7	ND	625.3
<b>Maui</b>	5	266.9	123.9	502.0	7	79.9	34.0	242.0
<b>Hawaii</b>	6	372.9	170.0	850.7	20	69.5	ND	222.8
<b>Kauai</b>					4	100.5	45.6	172.0

Survey Factors

We investigated several risk factors and their association with hair arsenic levels. The variables and percentage of children and adults that answered yes to each of the arsenic survey questions are shown in Table 4. All Yes answers were postulated to influence hair arsenic levels in the positive direction. Most of the participants reported eating fish and rice, known sources of dietary arsenic. For variables with several frequency categories (rice, shellfish, contact with soil, fish consumption) linear regression was performed. For other variables that are only yes or no, a two-sample t-test was performed that does not assume equal variances. The only factor that showed a significant association with children’s log hair arsenic concentration was the ownership of pets that spend time outdoors (p=0.007, with a t statistic of 2.76). To look for possible confounder variables, we checked for interaction of age, gender, race, ancestry and exposure to soil in children, and there was no interaction. No differences were seen in adults.

**Table 4-Number and percentage of participants answering yes to survey questions.**

Survey Question	Children	% (Yes)	Adults	% (Yes)
Treated hair	NA		117	44.8
Pets that go outside <sup>a</sup>	40	32.5	72	27.7
Eats rice	119	96.7	257	98.8
Exposure to smoke	39	32.0	104	40.0
Eats Shellfish	76	61.8	223	85.8
Contact with soil	78	63.4	61	23.5
Eats homegrown vegetables	35	28.5	68	26.2
Uses pesticides	NA		27	10.4
Works with arsenic treated wood	NA		7	2.7
Ate fish in the past month	114	92.7	257	98.5

<sup>a</sup> Children with pets significantly different than children without pets  
Average hair levels of children with pets is 290 ppb and without pets 206 ppb.

### Adult Outlier and Children with Hair Levels above 1000 ppb

Five children had hair levels that exceeded 1000 ppb. The highest adult had a hair level of 867 ppb. The survey questions responses are summarized below. Only 1 child reported a smoker in the house and ownership of pets that may spend some time outdoors. There is no obvious factor that is associated with the high levels. Because the children were so young, only a very small (in milligrams) hair sample was analyzed and no hair was available for a retest. WIC staff contacted the family of child 1 to offer retesting, but they declined. It is possible that the elevated levels may reflect external contamination, because the hair was not washed prior to analysis.

**Table 5-Adult Outlier and Children above 1000 ppb**

Category	Hair Level (ppb)	Age	Contact with soil	Smokers in house	Pets that spend time outdoors	Rice
Adult	867	36	yes	no	no	yes
Child	3830	<1	yes	yes	no	no
Child	1390	1	2x week	no	yes	4x week
Child	1420	3	no	no	no	2x week
Child	1970	2	2x week	no	no	daily
Child	1186	2	no	no	no	daily

### **Discussion**

Our study provides information on factors influencing hair arsenic levels in a population not known to be living on contaminated soil. Hair arsenic levels differed by age, gender, and ethnicity and the ownership of pets.

Numerous studies have shown that background hair arsenic levels are typically less than 1000 ppb in uncontaminated areas (summarized by Wu and Chen, 2010). In our study, five young children exceeded 1000 ppb, while all adults were less than the benchmark. The average hair levels measured in our study are within the range found in other studies without direct arsenic exposure (Table 6).

**Table 6-Comparison of Hair Arsenic Concentrations in Different Countries**

Country	N samples	Hair Arsenic Concentration (ppb)	Range	Direct As Exposure	Reference
Egypt	100	353 (children) 233 (adults)		no	Saad (2001)
Korea	655	110 (children)	10-910	no	Park (2007)
Italy	263	90	20-930	no	Senofonte et al 2000
Uruguay	180	130 (children) 40 (adults)		No	Kordas et al (2)010)
Japan	100	75	8-329	no	Yamato, (1998
Japan	250	230	<720	no	Sera et al (2002).
Sweden	114	90	34-319	no	Rodushkin I, Axelsson (2000)
Cambodia	19	280	100-570	no	Gault et al. (2008)

Young children in our study had significantly higher levels of hair arsenic concentrations compared to adults. This has been reported in other studies (Saad et al., 2001; Hindwood et al., 2003; Kordas et al., 2010; and Wu and Chen, 2010). Whether the differences are due to metabolic factors in young children, higher exposures or some other factor is not currently known. Studies have shown that arsenic levels in hair reflect blood levels. Blood and hair arsenic levels in young children may be higher due to less efficient elimination of arsenic into the urine (Wu and Chen, 2010), although others have found that children may be more efficient than adults (Sun et al 2007). In addition to metabolic differences, toddlers display hand to mouth activities increasing the chance of potential ingestion of contaminants in soil and dust. On a body weight basis, they are also exposed to higher levels of dietary arsenic. However our study did not find an association between hair arsenic levels and exposure to soil or dietary exposure to arsenic from rice, shellfish and fish.

As discussed above, no associations were found between arsenic hair levels and the frequency of fish, rice, and shellfish consumption. In contrast, the Kea'au Exposure Investigation (ATSDR 2008) found urinary arsenic levels to be elevated in a primarily Filipino population consuming rice, fish and seaweed. These results can be explained by the fact that hair and urine measure different forms of arsenic. Inorganic trivalent arsenic is the main form found in hair, while urine mainly contains the methylated metabolites monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA) (Yanez et al., 2005; Nicolis et al., 2009). In the Keaau study, more than 80% of the inorganic arsenic in urine was the metabolite, DMA. DMA can be a direct metabolite of arsenous acid and arsenosugars found in seafood and seaweed, and is also found in fish and rice (Yanez et al., 2005; Meharg et al., 2008).

Our study showed that male children had higher levels of hair arsenic than females, although only borderline significant ( $p < 0.07$ ). Others have also shown that males have higher arsenic hair levels than females (Hindwood et al., 2003; Kordas, et al., 2010; Wu and Chen, 2010; Maden et al., 2011). Numerous studies have shown that men are more susceptible to the arsenic-related skin effects than females (discussed in Lindberg et al., 2008). In humans, efficient methylation from inorganic arsenic to DMA is associated with a high rate of arsenic excretion and lower tissue concentrations (Gardner et al., 2010). Lindberg demonstrated that males were less efficient in methylating arsenic, which can lead to a higher retention of arsenic in the body. Our results indicate that even young male children may be less efficient in metabolizing arsenic compared to female children.

Children with pets showed statistically higher arsenic hair levels than children without pets. One reason could be that pets bring outside soil indoors. However, our study did not find a significant association with contact with soil, and there is no indication that soil was contaminated with arsenic. Hindwood (2003) did find an association with arsenic hair levels and soil contamination, but the arsenic hair levels were at least an order of magnitude higher than those measured in our study. Additional work is needed to confirm these results.



Finally, the only significant association in adults with hair arsenic was ethnicity and ancestry. Hawaiian- Pacific Islander and those reporting Multiple races had higher arsenic levels than Asians. Participants reporting Hawaiian ancestry were higher than Filipinos and Chinese. Whether it is biological or environmental is not known. No other factor showed an association with arsenic hair levels in adults including dietary sources of arsenic.

The relationship between arsenic metabolism and ethnicity has not been extensively studied. Genetic factors in the metabolism of arsenic can explain some of the differences in people's ability to methylate and eliminate arsenic (Schlebusch et al., 2013). Brima et al., (2006) found total concentrations of urinary and fingernail arsenic samples of a Somali Black-African population were significantly different from the Asian (India, Pakistan, and Bangladesh) and White groups. The urinary arsenic was lower, while the fingernail arsenic was higher. There was no difference in arsenic hair samples in the 3 groups. The authors suggested that the differences may be due to diet, because the Somali Black-African group had a diet that was heavily meat based which differed from the other groups. Additional work is needed to evaluate biological and environmental factors influencing hair levels in the Asian and Hawaiian-Pacific Islander and those reporting multiple races.

## **Conclusion**

Hair arsenic levels measured in WIC participants were within the range found in other studies with no known source of environmental exposure. There was considerable variability in the hair arsenic results especially in children. Five children exceeded the 1000 ppb level of concern, but no common risk factors were identified. In children, age and ownership of pets were significantly associated with arsenic hair levels. Children's arsenic hair levels were higher than adults. In adults, ethnicity and ancestry were associated with arsenic hair levels, with Asians lower than Hawaiian Pacific Islanders and those reporting multiple races. The factors influencing arsenic hair levels in people of different races are unknown.

## References

Agusa T, Kunito T, Fujihara J, Kubota R, Minh TB, Kim Trang PT, Iwata H, Subramanian A, Viet PH, Tanabe S. (2006) Contamination by arsenic and other trace elements in tube-well water and its risk assessment to humans in Hanoi, Vietnam. *Environ Pollut* 139(1): 95–106

Agency for Toxic Substances and Disease Registry. (2008). Health Consultation. Exposure Investigation Report.. Kea`au 8.5 and 9.5 Mile Camps. Kea`au Hawaii County, Hawaii. [http://www.atsdr.cdc.gov/HAC/pha//Keaau8.5and9.5MileCamps/KEAAU\\_8.5\\_AND\\_9.5\\_MILE\\_CAMPS\(EI-HC\)8-15-2008.pdf](http://www.atsdr.cdc.gov/HAC/pha//Keaau8.5and9.5MileCamps/KEAAU_8.5_AND_9.5_MILE_CAMPS(EI-HC)8-15-2008.pdf)

Brima EI, Haris PI, Jenkins RO, Polya DA., Gault AG, Harrington CF. (2006). Understanding arsenic metabolism through a comparative study of arsenic levels in the urine, hair and fingernails of healthy volunteers from three unexposed ethnic groups in the United Kingdom. *Toxicol Appl Pharmacol* 216(1): 122–130

Gardner RM, Nermell B, Kippler M, Grandér M, Li L, Ekström EC, Rahman A, Lönnerdal B, Hoque AM, Vahter M. (2010). Arsenic methylation efficiency increases during the first trimester of pregnancy independent of folate status. *Reprod Toxicol* 31: 210–218

Gault AG, Rowland HAL, Charnock JM, Wogelius RA, Gomez-Morilla I, Vong S, Leng M, Samreth S, Sampson ML, Polya DA. (2008). Arsenic in hair and nails of individuals exposed to arsenic-rich groundwaters in Kandal province, Cambodia *Sci Total Environ.* 393(1): 168-176

Hawaii Department of Health 2007. Soil Arsenic Assessment Study - Kea`au, Hawai`i: State of Hawai`i Department of Health, Hazard Evaluation and Emergency Response Office, December 2007. Available at URL: <http://hawaii.gov/health/environmental/hazard/docs/keaausoilarsenicassessmentstudydec2007.pdf>

Hindmarsh, JT. (2002). Caveats in hair analysis in chronic arsenic poisoning. *Clin Biochem.* 35(1): 1-11. (as cited by Hughes 2006)

Hinwood AL, Sim MR, Jolley D, de Klerk N, Bastone EB, Gerostamoulos J, Drummer OH. (2003) Hair and toenail arsenic concentrations of residents living in areas with high environmental arsenic concentrations. *Environ Health Perspect* 111(2): 187-193.

Hughes M.F. (2006). Biomarkers of exposure: a case study with inorganic arsenic. *Environ. Health Perspect.* 114(11): 1790–1796.

Kordas K, Queirolo EI, Ettinger AS, Wright R, Stoltzfus RJ. (2010) Prevalence and predictors of exposure to multiple metals in preschool children from Montevideo, Uruguay. *Sci Total Environ* 408(20): 4488-4494

Kurttio P, Komulainen H, Hakala E, Kahelin H, Pekkanen J.(1998) Urinary excretion of arsenic species after exposure to arsenic present in drinking water. *Arch Environ Contam Toxicol* 34(3): 297–305

Lindberg AL, Rahman M, Persson LA, Vahter M. (2008) The risk of arsenic induced skin lesions in Bangladeshi men and women is affected by arsenic metabolism and the age at first exposure. *Toxicol Appl Pharmacol* 230(1): 9-16

Maden N, Singh A, Smith LS, Maharjan M, Shrestha S. (2011) Factors associated with arsenicosis and arsenic exposure status in Nepal: implications from community based study. *J Community Health* 36(1): 76-82

Mandal BK, Ogra Y, Anzai K, Kazuo T, Suzuki KT. (2004) Speciation of arsenic in biological samples. *Toxicol Appl Pharmacol* 198(3): 307– 318

Meharg AA, Lombi E, Williams PN, Scheckel KG, Feldmann J, Raab A, Zhu Y, Islam R. (2008) Speciation and localization of arsenic in white and brown rice grains. *Environ Sci Technol* 42(4): 1051-1057

Nicolis I, Curis E, Deschamps P, Benazeth, S. (2009) Arsenite medicinal use, metabolism, pharmacokinetics and monitoring in human hair. *Biochimie* 91(10): 1260-1267

Park HS, Shin KO, Kim JS. (2007). Assessment of reference values for hair minerals of Korean preschool children. *Biol Trace Elem Res.* 116(2): 119-130

Rodushkin I, Axelsson MD. (2000) Application of double focusing sector field ICP-MS for multielemental characterization of human hair and nails. Part II. A study of the inhabitants of northern Sweden. *Sci Total Environ* 262(1-2): 21–36

Saad A, Hassanien MA. (2001) Assessment of arsenic level in the hair of the nonoccupational Egyptian population: pilot study. *Environ Int* 27(6): 471–478

Schlebusch CM, Lewis Jr, CM, Vahter M, Engström K, Tito RY, et al. (2013) Possible positive selection for an arsenic-protective haplotype in humans. *Environ Health Perspect* 121(1): 53–58

Senofonte O, Violante N, Caroli S. (2000) Assessment of reference values for elements in human hair of urban schoolboys. *J Trace Elem Med Biol* 14(1): 6-13

Sera K, Futatsugawa S, Murao S. (2002) Quantitative analysis of untreated hair samples for monitoring human exposure to heavy metals. *Nuclear Instruments and Methods in Physics Research Section B-Beam Interactions with Materials and Atoms* 189: 174-179

Sun G, Xu Y, Li X, Jin Y, Li B, Sun X. (2007) Urinary arsenic metabolites in children and adults exposed to arsenic in drinking water in Inner Mongolia, China. *Environ Health Perspect.* 115(4): 648–652

Vahter M., Marafante E., Dencker L. (1983) Metabolism of arsenobetaine in mice, rats and rabbits. *Sci Total Environ* 30: 197–211. (as cited by Hughes, 2006)

Wu B, and Chen T. (2010) Changes in hair arsenic concentration in a population exposed to heavy pollution: follow-up investigation in Chenzhou City, Hunan Province, southern China. *J Environ Sci* 22(2): 283-289

Yáñez J, Fierro V, Mansilla H, Figueroa L, Cornejo L, Barnes RM. (2005) Arsenic speciation in human hair: a new perspective for epidemiological assessment in chronic arsenicism . *J Environ Monit* 7(12): 1335 –1341

Yamato N. (1988) Concentrations and chemical-species of arsenic in human-urine and hair. *Bull Environ Contam Toxicol* 40(5): 633-640

**Attachments**



## Arsenic Exposure Survey

We would like to know a little bit about you, your contact with soil, and your dietary habits. Please take a few minutes to answer these questions as completely as possible.

1. Do you color, straighten or otherwise treat your hair?  Yes  No
2. Do you smoke?  Yes  No
3. Do you have pets that spend time outdoors?  Yes  No
4. Do you eat rice?  Yes  No
5. If "yes," how often?
  - Several times a day
  - Daily or almost daily
  - Twice a week
  - Once a week
  - More than once a month
  - Other: \_\_\_\_\_

### In the last 3 months:

6. Did you eat shellfish (for example: shrimp, scallops, lobster, mussels)?  
 Yes  No
7. If "yes," how often?
  - Several times a day
  - Daily or almost daily
  - Twice a week
  - Once a week
  - More than once a month
  - Other: \_\_\_\_\_
8. Did you have any contact with the soil in your yard (gardening, yard work, etc.)?  
 Yes  No
9. If "yes," how often?
  - Daily or almost daily
  - Twice a week
  - Once a week
  - More than once a month
  - Other: \_\_\_\_\_

-please turn survey over-

10. Have you used any pesticides or garden sprays?  Yes  No

11. Did you eat any vegetables grown in your garden?  Yes  No

12. If "yes," how often?

- Daily or almost daily
- Twice a week
- Once a week
- More than once a month
- Other: \_\_\_\_\_

13. Have you worked with chemically-treated wood (for example: chromium copper arsenate)?

- Yes  No

14. What is your current occupation? \_\_\_\_\_

**Thank you for your help!**



## Arsenic Exposure Survey

We would like to know a little bit about your child, their contact with soil, and their dietary habits. Please take a few minutes to answer these questions about the child who is taking part in this project.

1. Do you have pets that spend time outdoors?  Yes  No
2. Does your child eat rice?  Yes  No
3. If "yes," how often?
  - Several times a day
  - Daily or almost daily
  - Twice a week
  - Once a week
  - More than once a month
  - Other: \_\_\_\_\_

### **In the last three months:**

4. Did your child eat shellfish (for example: shrimp, scallops, lobster, mussels)?  
 Yes  No
5. If "yes," how often?
  - Daily or almost daily
  - Twice a week
  - Once a week
  - More than once a month
  - Other: \_\_\_\_\_
6. Did your child have any contact with the soil in your yard?  Yes  No
7. If "yes," how often?
  - Daily or almost daily
  - Twice a week
  - Once a week
  - More than once a month
  - Other: \_\_\_\_\_
8. Did your child eat any vegetables grown in your garden?  Yes  No
9. If "yes," how often?
  - Daily or almost daily
  - Twice a week
  - Once a week
  - More than once a month
  - Other: \_\_\_\_\_

**Thank you for your help!**



## Consent Statement

For Staff Use Only

Date: \_\_\_\_\_ Time: \_\_\_\_\_

ID# \_\_\_\_\_ Clinic: \_\_\_\_\_

I have read this form or it has been read to me. I have had a chance to ask questions about this project and my questions have been answered. I agree to be part of this project. **I have marked the parts below that I will do.**

1a. Yes  No

**Give some hair to test for mercury.**

1b. Yes  No

Answer a few questions about my or my child's fish-eating habits.

2a. Yes  No

**Have the hair also tested for arsenic.**

2b. Yes  No

Answer a few questions about my or my child's hobbies and habits that are related to arsenic exposure.

I \_\_\_\_\_ (print name), agree to hair testing and answering a few questions for:

(\_\_\_) Myself

(\_\_\_) My child

I need a \_\_\_\_\_ language interpreter to translate my test results for me.

I want my mercury results sent to my health care provider: \_\_\_\_\_

Physician's Name

\_\_\_\_\_  
Address

\_\_\_\_\_  
Signature

\_\_\_\_\_  
Address

\_\_\_\_\_  
Phone