Malaysian Journal of Science (**23**): 227-234 (2004)

Arsenic concentration in hair as an indicator of exposure

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ABSTRACT Elevated concentrations of the extremely toxic arsenic in ground water occur in many parts of the world with largest population being exposed in the entire Bengal delta. Exposure to arsenic can occur through food, water, and air. One of the biological indicators of exposure to arsenic is its accumulation in hair. The arsenic concentrations in hair in this study indicate that the exposure decreased in the following order: West Bengal (India) > Thailand > Malaysia (Kuala Lumpur) > Denmark > Malaysia (Rural); however only in India, Thailand and Kuala Lumpur there is an indication of chronic arsenic exposure. Our findings also indicate difficulties in distinguishing between external exposure and exposure due to intake of water and food. Hair is a promising indicator of arsenic exposure, but there is a need for further research to exactly define how arsenic accumulates in hair and whether the ingested form (organic and inorganic) of arsenic has an appreciable effect on the accumulation rate in hair.

(arsenic, hair, water, food)

INTRODUCTION

Since the discovery of mass scale arsenic (*As*) poisoning in the Bengal Delta Plain (Bangladesh and West Bengal India) a decade ago with an estimated population of 40 million people at risk of drinking *As* contaminated water above 10 µg/l, *As* in drinking water has caught the attention of both researchers and policy makers [1]. WHO changed the Maximum Contaminant Level (MCL) of *As* in drinking water from 50 µg/l to a provisional guideline value of 10 µg/l in 1993 [2]. Only recently many industrial countries have adopted the new lowered guideline value. *As* is a documented human carcinogen and there are reported incidences of *As* poisoning through elevated concentrations in air (mostly due to burning of coal containing elevated concentrations of *As*) [3] and drinking water (mostly groundwater with elevated concentrations of *As*) [3, 4, 5, 6], whereas limited data is available on *As* toxicity through food.

According to [2] the estimated mean daily intake of *As* from food is approximately 40 µg, of which about 10 µg is inorganic *As*. Drinking water generally has less than 10 µg in areas without natural sources of *As*, common values are 5 µg/l or less. The estimated intake from air is generally less than 1 µg/day. The toxicity of *As* depends on the species with inorganic *As* regarded much more toxic than organic *As* compounds.

Exposure to *As* through drinking water can only be perceived by measuring the *As* content in water since *As* is colourless and odourless. The same holds good in case of exposure through food and air. Samples of urine, blood, hair and nails can also be used to assess the human exposure to *As*, since exposure to *As* results in elevated concentrations of *As* in these biological samples. Acute and sub-acute *As* exposures can easily be assessed by measuring *As* in the urine. An *As* concentration in excess of 50 µg/l (or 100 µg in 24-hour urine) in the absence of any sea food or fish ingestion indicates the symptoms of acute or sub-acute toxicity [7]. However, hair and finger/toe nails gives more qualified results for chronic exposure to *As* and according to [2] a hair *As* concentration below 1 µg/g indicates a normal value for individuals exposed to acceptable dietary levels. According to the literature survey made by [8], the natural *As* concentration in hair, finger/toe nails, blood and urine are 0.02-3.7 µg/g, 0.2-3 µg/g, 1.7-10 µg/l, 2-8 µg/l respectively, whereas according to the literature review by [9] the reported world average *As* concentration in human hair is in the range of 0.01-0.4 µg/g.

The relationship between hair *As* and drinking water *As* concentrations differs between studies. Table 1 shows hair concentrations in populations from different parts of the world and the corresponding *As* concentrations in water. The literature review by [10], cites a study which reports that an *As* concentration of 400 μg/l in drinking water gives rise to hair *As* of approximately 3 μg/g. The same literature review cites other studies where a concentration of 100 μg/l *As* in drinking water corresponds to 0.26 μg/g *As* in hair in California and Nevada; 0.09 μg/g in Taiwan; 0.94 μg/g in Bangladesh, and 1 μg/g in Finland. The results obtained by [10] for 100 μg/l *As* in drinking water correspond to approximately 1 μg/g *As* in hair, while 400 μg/l corresponds with approximately 3 mg/g hair, thus agreeing with the results of one of the studies in their literature review. The results obtained by [11] indicates that an increase in the *As* concentration in drinking water of 10 μg/l and an increase in the daily dose of 10-20 μg/d from drinking water correspond to a 0.1 μg/g increase in the hair *As*. The above literature review indicates that except for Taiwan and US (California and Nevada) drinking 100 μg/l *As* contaminated water results in approximately 1 μg/g *As* concentration in hair. According to [12] the average *As* concentration in men was higher than in women. Furthermore the average *As* concentration in men/boys in the age group below 20 years was higher compared to other age groups. This may be due to the greater physical activity leading to a higher consumption of water.

Toxicology studies have shown that inorganic *As* is absorbed far more rapidly when inhaled than when entering via the gastrointestinal tract or following deposition on intact skin [13]. The authors found no significant difference between the hair *As* concentration of workers (mean 0.042 μg/g) exposed to an air *As* concentration of 15 μg/m^3 compared to administrative controls (mean 0.033 μg/g) with air *As* concentrations of 2 μg/m^3. The *As* concentration in heavy smokers with one-two pack cigarettes per day was 0.076-0.106 μg/g. According to [14], the *As* concentration in hair of a population exposed to *As* in a province of inner Mongolia due to burning of *As* rich coal with an *As* content of 2170 μg/g was 3.08 μg/g. In the controlled population with coal *As* content of 2.5 μg/g the *As* content in hair was 0.97 μg/g. Estimated mean daily intake of *As* per person was 31 μg in the polluted city and only 1.34 μg in the control area. There was no significant difference between concentration of *As* in the hair of men and women.

The presence of other elements, such as selenium in the diet can have an effect on *As* concentration in hair. According to [15], with a supplementation of Se and discontinuation of *As* contaminated drinking water the *As* concentration in hair decreased from 2.57 +/-0.16 to 0.68+/-0.06 μg/g, whereas in the control population with a placebo tablet and with discontinuation of *As* contaminated drinking water the *As* concentration decreased only to 1.25+/-0.16 μg/g indicating the positive effect of Se on hair *As* concentration.

How *As* enters the hair from the blood is still unknown, and hair samples from different persons adsorb *As* differently [16]. According to the literature review by [17] one study reports that the *As* in the hair of guinea pigs injected with sodium arsenite reached well beyond where it could reach by growth alone. The same was found in another study which detected high levels of *As* 4 cm from the scalp hair even though the hair growth was only 3.5 mm since the onset of ingestion. The study also investigated whether washing could move the hair *As* by capillary action and found that although there was some spreading by washing most of the applied *As* remained at the point of deposition on the hair even after washing with various solutions. The explanation from both the studies was that injected *As* is secreted in the sweat and/or sebaceous secretions and becomes deposited on the surface of the hair where it remains firmly attached for the life of the hair. Hair can concentrate *As* from the solution in which it is suspended. A human hair placed in a solution of sodium arsenite for 10 days (10 μg/mL) increased its *As* content from one to 50 μg/g. 60% of this *As* remained after 15 days soaking in distilled water. Even though washing techniques eventually removed *As* from the hair, the study could not distinguish between external and internal contamination. Hair levels of 12 μg/g have been reported in corpses buried in *As* contaminated soils. Neither can location of *As* in a cross-section of hair determine whether *As* is ingested or derived from external contamination.

The literature review by [17] also cites a study with 31 persons in Alaska who drank bottled water and washed their hair with *As* contaminated well-water (≥ 100 μg/l) who had mean *As* level of

5.7 µg/g in hair, while another person who drank and washed in water with *As* content less than 100 µg/l had a mean level of 0.46 µg/g. A study from Japan showed similar results of elevated *As* concentration in hair from washing with contaminated water. The results obtained by [17] show that two of the worst cases had hair *As* concentrations of 47 and 4.2 µg/g even though both of them lived in the same house indicating the extreme variability of hair *As* level at similar level of exposure.

Hair washing procedure for analysis may have an effect on the measured *As* concentration in hair. By washing some of the external contamination is removed and a study reported a difference of 3 mg/g between washed and unwashed hair (unwashed 6 µg/g and washed 3.1 µg/g) [17].

From the above literature review it is evident that *As* in hair varies for each individual and depends on the type of exposure, but it is clear that intake of *As* will result in elevated *As* concentrations in hair.

The aim of the present study is to assess the human exposure to *As* in Denmark, Malaysia, West Bengal (India) and Thailand and to investigate whether there is any indication of chronic toxicity of *As* in the studied areas based on the *As* concentrations in the hair.

METHODS

Collection, Preparation and Analysis of Hair samples

Hair samples from Thailand, Denmark, and West Bengal (India): samples from these areas were collected from individuals with no attempt to control the location from where hair was extracted. In all cases a sample of the person's hair was cut and stored in either individual plastic bottles or polyethylene bags and sealed to avoid further contamination. An universally accepted standard procedure is lacking for preparation of hair samples for *As* analysis. Here all samples were washed in 0.1% Triton-x and rinsed with running distilled water for eventual removal of surface contamination. The samples were dried at 100 °C. The washed and dried hair was cut into 2-5 mm lengths for digestion. The acid digestion was carried out using 2 ml of HNO_3 and 0.2 ml of $HClO_4$ added to 0.2 g of hair. The samples were gently heated until a clear solution appeared. The samples were then analysed with a continuous Hydride Generation method on an Atomic Absorption spectrometer (Perkin Elmer 5000 AAS). The detection limit of the method was 1 µg/L.

Hair samples from Malaysia: hair samples were collected from donors by single cutting from the occipital region with a pair of clean stainless steel scissors in accordance with the IAEA protocols. During collection of the hair samples, each individual was asked to complete a questionnaire detailing name, sex, age, occupation and dietary habits. The hair samples were cut to lengths of about 2-5 mm. The hair samples were then washed according to the procedure recommended by the IAEA: wash hair in acetone, three times in water and once more in acetone. The samples were then dried overnight in an oven at 60°C.

The internal standards were prepared by pipetting about 1µl of known concentration solution of methylmercury onto ashless filter paper and packed in polyethylene envelopes after careful drying.

Samples, standard and blank were irradiated in the TRIGA reactor of the Malaysian Institute for Nuclear Technology and Research for 6 hours in a neutron flux of $2.3 \cdot 10^{12}$ $n \cdot cm^{-2}.s^{-1}$. The irradiated samples were then cooled for 2 days prior to counting. After cooling, the samples, standards and blanks were counted for 1000s using HPGe detector. For calculation of *As* concentrations, the 559.1 kev peak of ^{76}As ($t_{1/2}$= 26.44 h) was used. Gamma ray energy calibration was made with standard sources prior to every set of experiments. The nuclides were quantified by comparing net photopeak area with those of the internal standards.

Collection, Preparation and Analysis of water samples

Water samples were collected in 20 ml plastic bottles and later on preserved using HCl. The samples were analysed with a continuous Hydride Generation method on an Atomic Absorption spectrometer (Perkin Elmer 5000 AAS). The detection limit of the method was 1 µg/l.

Table 1. Reported arsenic (*As*) concentrations in hair and drinking water around the world.

Place	*As* concentration in hair (μg/g)				*As* concentration in water (μg/L)	Reference
	Type	Median	Mean	Range		
Bangladesh	All	2.49	4.05			[18]
Bangladesh	All			1.1-19.84	10 - 900	Cited by [19]
Bangladesh	All	6.4	8.57	0.26-79.49		[7]
Chile; Antofagasta	All			4-83.4		Cited by [4]
Egypt	M		0.303			[20]
	F		0.292			
	C		0.353			
	A		0.233			
	NS		0.294			
	PS (I)		0.376			
	PS (O)		0.141			
	AS		0.36			
	CG		0.209			
	M T		0.459			
Finland		0.96		0.06-12.5	17 - 510	[11]
Finland		0.05		B.D.-0.18	4 - 44	
Iceland		0.04		≤0.0037-0.2		[21]
India, (WB)				1.81-31.05		[4]
India, (WB)		1.32	1.48	0.18-20.34		[18]
Inner Mongolia	M			≤ 1-9.5	≤100 - 800	[10]
Iran			0.073	≤0.0155-0.427		[21]
Iran, Najaf Ajad village	All	0.2±0.07			50	[12]
	M	0.281				
	F	0.119				
	All	4.9±0.5			550	
	M	5.53				
	F	4.19				
	All	5.6			740	
	M	7.18				
	F	2.89				
UK, Cornwall	All		2.51	0.89-14.56		[9]
	M		2.54	0.92-12.69		
	F		2.49	0.89-14.56		
UK, Glasgow			0.65	≤0.2-8.17		[21]
UK Oxfordshire & Wiltshire	All		0.7	≤ 0.70-9.03		[9]
	M		0.37	≤ 0.7-1.56		
	F		1.07	≤ 1.72-9.03		
US Lassen county, CA		0.01-2				Cited by [4]
US, Millard county UT		0.1-4.7				Cited by [4]
US AL Fairbanks		1				Cited by [4]

(F: female, M: male, C: child, A: adults, NS: non-smoker, PS: passive smoker, As: active smoker, CG: cigarette, MT: molasses tobacco)

RESULTS & DISCUSSION

Relationship between hair As concentration and corresponding As concentration in drinking water in West Bengal:

Figure 1 shows the measured *As* concentrations in hair and the corresponding *As* concentrations in water from West Bengal, India. The figure also shows the expected *As* concentrations in hair with corresponding *As* concentrations in drinking water based on the literature values of *As* concentration in water and the resulting *As* concentration in hair (straight-line with square points). Only few results were in accordance with the literature values, whereas more than half of the samples lie far above the expected values.

There could be many reasons for this. Probably one the most important is the amount of water consumed, which could be higher in the present

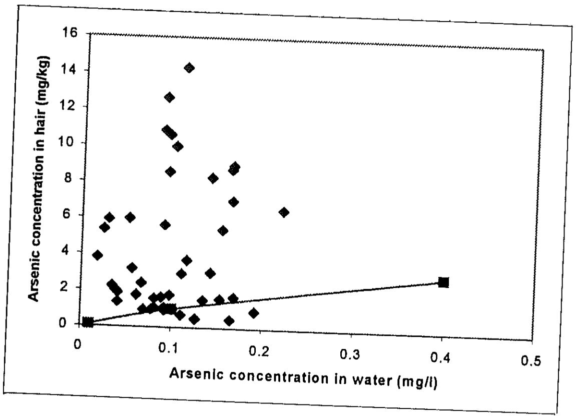

Figure 1. *As* concentration in hair and the corresponding *As* concentrations in water from West Bengal, India. The line with square points shows the expected *As* concentrations in hair corresponding to As concentrations in water (derived from [10], and [11]).

case due to hot climate compared to the literature values. In the literature water consumption was around 2 L/d, whereas in case of West Bengal the realistic estimate for daily water consumption would be around 3.5-4 L/d due to the climatic conditions and nature of work, since most of the people work in the fields. A second reason could be that the persons in the present case also used the *As* and iron contaminated water for washing hair and hence may have higher *As* concentrations in hair due to precipitation or adsorption of *As* compounds compared to the literature value. The literature review shows that As can be adsorbed into the hair when exposed to *As* contaminated water externally and that it is difficult to distinguish between external exposure compared to internal exposure. A third reason could be that person's smoking habits have an effect on the *As* concentration in hair, especially if tobacco is grown locally on contaminated soils and hence have a high *As* concentration. A fourth reason could be a high dietary intake from food items originating from the same contaminated soils, since *As* would accumulate in the food items from the soil. The *As* intake through food is widely varied depending on the type of food consumption. Based on the market basket survey of total *As* the US Food and Drug Administration has estimated that adults ingest on average about 53 µg As/d from the diet [2]. A Japanese study showed a daily intake of *As* at 182 µg, which is very high compared to the other mentioned studies, and is largely attributed to the consumption of seafood by Japanese. According to a study conducted in West Bengal, India [22] the average intake of *As* through food is 172 µg/d, which is similar to the *As* consumption in the Japanese study. It is not attributed to seafood consumption as in case of Japan but is mostly due to elevated concentrations of *As* in the food grains, vegetables etc. From the literature review it is not clear if there is any difference between accumulation of *As* in hair depending on whether it is organic or inorganic *As*. It may be expected that the accumulation of ingested inorganic *As* in hair could be higher compared to organic *As*, because inorganic *As* is more toxic compared to easily excreted and virtually non-toxic organic *As*. Based on this assumption the accumulation of *As* in hair from food grains and vegetables would be high compared to seafood consumption, since the inorganic *As* in food grains and vegetables is high compared to seafood. The intake of food *As* would also contribute to the hair *As* concentration, and hence higher *As* concentrations in hair from individuals eating contaminated food could be expected compared to the dose response curve for ingested *As* through drinking water alone.

A statistical analysis to assess whether there is any difference between hair *As* concentration between men and women revealed no such difference. It was also found that people from the same family have quite different hair *As* concentrations indicating that persons being from the same family may not consume the same amount and quality of water depending on their occupation. For example, children, youngsters and men are away from home most of the time and hence may consume water from other sources than the elderly people and women who usually remain at home. This could also be one of the reasons why the measured *As* concentrations do not coincide with the literature values for dose-response values.

These results show that the *As* concentration in hair depends on many factors, but it is demonstrated that elevated *As* concentrations both in food and drinking water will be reflected in hair and hence hair concentrations can be used as an indicator of exposure to As.

Arsenic concentrations in hair due to exposure through food and drinking water:
The obtained hair *As* concentrations from Denmark, Malaysia, Thailand and West Bengal (India) are shown in Table 2. The hair *As* concentrations from these different areas decreased in the following order: West Bengal (India) > Thailand > Malaysia (Kuala Lumpur) > Denmark > Malaysia (Rural). From the available information on various routes of exposure to *As*, people from Denmark and Malaysia are mainly exposed through food, whereas people from Thailand and India are exposed through both food and drinking water with drinking water being the major route of exposure. If the food intake of *As* is significant in Thailand and West Bengal it may explain why the hair concentrations being higher than what could be expected according to Figure 1.

The *As* concentration in drinking water in India was in the range of BD-0.29 mg/l, whereas the As concentration in drinking water from Thailand is unknown. The hair samples from Thailand were collected from the Ronphiboon Area where there are reported cases of arsenicosis and where according to [23], the average *As* concentration in the groundwater (both shallow and deep) is approximately 350 µg/L. The As concentration in drinking water from Malaysia and Denmark is below 10 µg/L. Based on this information the highest As concentrations in hair are expected from Thailand and West Bengal and according to the obtained results highest hair As concentrations were found in West Bengal, India followed by Thailand. As discussed above (Figure 1) even though higher As concentrations in drinking water results in higher As concentrations in hair there are many other factors that affect the concentration of As in hair and hence higher As concentrations may be found in hair with lower As concentrations in drinking water.

The average and median hair *As* concentration found in Thailand and West Bengal were above 1 µg/g and according to WHO's definition of chronic toxicity the studied population is exposed to chronic *As* poisoning.

In Denmark and Malaysia there very few reported incidences of elevated concentrations of *As* in drinking water, or polluted air due to *As* and hence the major route of exposure would be through food. The lowest hair As concentrations were found in Denmark and from the rural areas of Malaysia and most of the samples are within the range of the normal *As* concentration range of 0.01-0.4 µg/g, although none of the measured values were below 0.2 µg/g. This indicates that all the investigated people are exposed to *As*, but none of them were above one µg/g, being the indicator of chronic *As* exposure. The highest value found in Denmark was 0.86 µg/g and according to the interview there was reported use of a shampoo for the treatment of dandruff and a high consumption of seafood. *As* is a known compound used for the treatment of dandruff. The second highest was 0.64 µg/g from a person who smokes 20-25 cigarettes per day. There were three other persons with *As* concentration in the range of 0.4-0.6, but the available information does not indicate any particular reason for the elevated As concentrations. In the rest the *As* concentration was below 0.4 µg/g.

The *As* concentrations from Kuala Lumpur city (Malaysia) were higher than from Denmark and rural areas of Malaysia, with the highest concentration being 1.73 µg/g. If 1 µg/g is taken as the indicator for chronic *As* poisoning the highest value from Kuala Lumpur city indicates that some of the persons may be

Table 2. *As* concentrations in hair of subjects from different countries.

Country	Median (µg/g)	Average (µg/g)	Range (µg/g)	No. of samples
Denmark	0.3	0.4	0.2 -0.9	14
Thailand	1.1	1.8	0.4 -5.8	14
Malaysia (Kuala Lumpur),	0.7	0.8	0.2 -1.8	25
Malaysia (Rural)	0.3	0.3	0.1-0.6	66
West Bengal, India	2.4	4.2	0.5 -14.4	43

suffering from chronic *As* toxicity. One of the reasons for the higher concentrations in Kuala Lumpur city compared to the rural areas could be differences in food consumption. As fish is one of the main sources of protein for the general population in Malaysia, and as the city dwellers have a higher income compared to the villagers, there is a possibility of higher fish consumption in the city area compared to the rural population. As explained earlier seafood contains higher *As* concentration, but only 5% of *As* in the fish is found as inorganic *As*. So far it is not certain whether there is any difference between the accumulation of *As* in hair depending on whether it is organic *As* or inorganic *As*. The results from this study indicate strongly that exposure to inorganic *As* will result in higher hair *As* concentration than the same intake of organic *As*.

The above results indicate that higher *As* concentrations in hair are a result of exposures to *As*, but it is difficult to comment whether the elevated *As* concentrations in hair are due to direct intake by food and water, or from external contamination as in case of the highest *As* concentration on a person in Denmark.

CONCLUSIONS

1. The *As* concentrations in hair indicate that people in Denmark, Thailand, Malaysia and West Bengal, India are exposed to *As* and the exposure decreases in the following order: West Bengal (India) > Thailand > Malaysia (Kuala Lumpur) > Denmark > Malaysia (Rural).
2. The results from Denmark and rural areas in Malaysia indicates that chronic exposure to *As* rarely occurs due to dietary exposure to *As* since the measured concentrations were well below the value suggested by WHO as an indicator of chronic exposure.
3. Based on the present knowledge it is difficult to conclude whether the higher *As* concentrations in Kuala Lumpur compared to rural Malaysia are due to higher consumption of fish in the city population compared to the villagers.
4. The results also indicate that exposure to *As* can be investigated by measuring hair *As* concentrations although the results do not indicate whether the exposure is due to direct intake or external contamination.
5. High individual hair concentrations found may be attributed to a combination of high intake with water and food, smoking and even external contamination from washing in *As* rich water.
6. Although chronic exposure to *As* relatively easy can be determined by measuring *As* concentration in hair more research is needed to exactly define how *As* accumulates in hair and whether the ingested form of *As* has an effect on the accumulation.

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