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Urinary arsenic species in an arsenic-affected area of West Bengal, India (part III)

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Arsenic contamination of groundwater has long been reported in the Mushidabad district of West Bengal, India. We visited 13 arsenic-affected families in the Makrampur village of the Beldanga block in Mushidabad during 18–21 December 2001 and collected five shallow tubewell-water samples used general household purposes, four deep tubewell-water samples used for drinking and cooking purposes, and 44 urine samples from those families. The arsenic concentrations in the five shallow tubewell-water samples ranged from 18.0 to 408.4 ppb and those in the four deep tubewell-water samples were from 5.2 to 9.6 ppb. The average arsenite (arsenic(III)), dimethylarsinic acid (DMA), monomethylarsonic acid (MMA) and arsenate (arsenic(V)) in urine were 28.7 ng mg^{-1}, 168.6 ng mg^{-1}, 25.0 ng mg^{-1} and 4.6 ng mg^{-1} creatinine respectively. The average total arsenic was 227.0 ng mg^{-1} creatinine. On comparison of the ratio of (MMA + DMA) to total arsenic, the average proportion was 86.7 ± 9.2% (mean plus/minus to residual standard deviation, $n = 43$). The exception was data for one boy, whose proportion was 8.0%. One woman excreted the highest total arsenic, at 2890.0 ng mg^{-1} creatinine. When using 43 of the urine samples (the exception being the one sample obtained from the boy) there were significantly positive correlations ($p < 0.01$) between arsenic(III) and MMA, between arsenic(III) and DMA and between MMA and DMA. Copyright © 2005 John Wiley & Sons, Ltd.

KEYWORDS: arsenic species; urine; tubewell water; HPLC–ICP-MS; West Bengal; India; arsenic(III); arsenic(V); MMA; DMA

INTRODUCTION

Natural contamination of groundwater by arsenic has become a crucial water quality problem in many parts of the world. The world's two biggest cases of groundwater arsenic contamination and illnesses of people have been reported in Bangladesh and West Bengal, India.[1–3] The entirety of the subject areas is not yet affected, but they are running at risk. The arsenic contamination incident in well water in Taiwan (1961 to 1985) is well known.[4] Black-foot disease was noted, as were other arsenical manifestations, such as hyperkeratosis, spotted melanosis and diffuse keratosis. Recently, arsenic-contaminated groundwater in Vietnam has been reported.[5,6]

In 1987, Chakraborti and Saha[7] reported arsenical skin manifestation in five districts of West Bengal. From 1989 until now, the group of Chakraborti has continued to report the arsenic calamity in West Bengal, India.[8–11] Our present study is focused on the Murshidabad district. The district is located on the border of Bangladesh and is one of the nine arsenic-affected districts of West Bengal. In order to estimate people's total exposure to arsenic, we visited the Jalangi block in the Murshibad district during 4–7 December 2000 and collected 51 urine samples and hair samples and foodstuffs such as rice, potato and onion obtained from 12 arsenic-affected families and six tubewell-water samples used by those families. The arsenite (arsenic(III)), arsenate (arsenic(V)), monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in each urine sample and the inorganic arsenic in tubewell water samples obtained from the Jalangi block have been reported in our earlier publication.[12] Similarly, we visited the Damkal block in Murshidad during 22–24 February 2001 and collected 10 tubewell-water samples, 89 urine samples and foodstuffs such as rice, potato and onion from 19 arsenic-affected families and from four non-arsenic-affected families. The arsenic(III), arsenic(V), MMA and DMA concentrations in

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each urine samples and the inorganic arsenic in tubewell-water samples obtained from the Domkal block have been reported elsewhere.[13]

The dietary intakes of arsenic from the villagers in the Jalangi and Domkol blocks were respectively 801 μg day^{-1} and 658 μg day^{-1} for adult males, 718 μg day^{-1} and 588 μg day^{-1} for adult females, and 432 μg day^{-1} and 351 μg day^{-1} for children of approximately 10 years of age.[14] Water contributes 76.8% and 71.4% of arsenic in adult males, 74.1% and 68% of arsenic in adult females, and 76.9% and 71.2% of arsenic in children, with respect to the total intakes of arsenic from all sources in the Jalangi and Domkol blocks respectively.

In the present study, we visited the Makrampur village in the Beldanga block in Murshidad during 18–21 December 2001 and collected five shallow tubewell-water samples, four deep tubewell-water samples and 44 urine samples from 13 arsenic-affected families. Most of the family members, especially the adults in our survey, have severe arsenical skin manifestations as they have consumed a high concentration of arsenic from their shallow tubewells for a long period. They have now stopped using the contaminated water from their shallow tubewells for drinking and cooking purposes for a short period and started to use the deep tubewell water (around 1000 ft), installed by the local government. We determined the inorganic arsenic, such as arsenic(III) and arsenic(V), in tubewell-water samples and the arsenic species in urine samples by using high-performance liquid chromatography and inductively coupled plasma mass spectrometry (HPLC–ICP-MS), and report the contribution of the inorganic arsenic with respect to the total arsenic in urine samples and the evaluation of the arsenic-methylating capacity of each person.

MATERIAL AND METHODS

Reagents and samples

Sodium arsenite and sodium arsenate were purchased from Wako Pure Chemical Industries (Osaka, Japan). MMA and DMA were obtained from Tri Chemical Lab. (Yamanashi, Japan). Other chemicals (analytical grade) were also from Wako Pure Chemical Industries (Osaka, Japan).

Stock standard solutions, each of 3750 ppm (50 mmol l^{-1}) for arsenic, were obtained by weighing accurate amounts of arsenic(III), arsenic(V), MMA and DMA and dissolving them in MilliQ water. These stock standard solutions were kept in the refrigerator at 4 °C until required.

Mixed standard solutions, one containing 30 ppb of each arsenic species and one containing 150 ppb of each arsenic species, were prepared daily from the stock standard solutions to the appropriate dilution.

Spot urine samples were collected from 44 villagers of 13 arsenic-affected families, identified as A to M, and kept in polyethylene centrifuge tubes. The samples were not subjected to any chemical treatment. After collection, the samples were stored in an icebox cooler.

The water samples were stored in polyethylene centrifuge tubes in an icebox cooler.

Both the urine samples and tubewell-water samples were transported from India to Japan by air and kept in a refrigerator at −30 °C in the laboratory before use.

Instrumentation

An Agilent 7500 ICP mass spectrometer (Agilent, DE, USA) was used for detecting the arsenic species. The operating conditions for ICP-MS are shown in Table 1.

The chromatograph had an STM-10A system controller with a Shimadzu 10AC HPLC pump, Shimadzu SIL-10A auto sampler and Shimadzu CTO-10AC column oven. The analytical column was a Gel PAK GL-IC-A15 (4.6 mm i.d. × 150 mm) packed with anion-exchange resin (Hitachi Kasei Co. Ltd. Tokyo, Japan).

HPLC–ICP-MS analysis

HPLC was performed under the following conditions for inorganic arsenic species, such as arsenic(III) and arsenic(V), in tubewell-water samples and arsenic species in urine samples: mobile phase 10 mmol l^{-1} phosphate buffer (pH 6.0), flow rate 1 ml min^{-1}, column temperature 35 °C and injection volume 20 μl. The outlet from the separation column was connected directly to the nebulizer of the ICP mass spectrometer using a polyethylene tube of 0.3 mm i.d.

After thawing 20 μl of water sample or urine sample, they were injected into the HPLC column and the peak areas of arsenic species were measured by ICP-MS for 8 min. The amounts of arsenic species were calculated using working curves prepared by using 0, 30 and 150 ppb solutions of arsenic species. The arsenic species in urinary samples were determined within at least 1 month because of degradation of arsenic species in urines.

After a day's work, the skimmer cone and the sampling cone of the ICP mass spectrometer were always cleaned with distilled water.

Detection limits of arsenic(III), DMA, MMA and arsenic(V) were all 0.2 ppb (20 μl injection) on an arsenic basis when the analyte concentrations corresponded to a signal-to-noise ratio of 3 was taken. The precision of analysis for these analytes ($n = 6$) was in the range of 2.6–3.5%

Table 1. ICP-MS conditions

RF power (W)	1500
RF refraction (W)	1
Plasma gas flow (l min^{-1})	15
Carrier gas flow (l min^{-1})	0.8
Monitoring mass	34 (Cl), 75 (As)
Integration interval (s)	0.3
Scan number	1

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at the concentration of 30 ppb. The average of duplicate measurements was used for all of the samples and standards.

Assay of urinary creatinine

Urine (1 ml) was diluted with MilliQ water to 10 ml; 0.5 ml of the diluted urine were put into a centrifuge tube and 3 ml of a solution (containing 0.8% sodium tungstate, 0.3% phosphoric acid and 0.2% sulfuric acid) for eliminating urinary protein was added. After standing for 10 min, the urinary solution was centrifuged for 10 min at 2500 rpm, 2 ml of supernatant was put into the test tube and 1 ml of 22 mmol l^{-1} picric acid solution and 1 ml of 0.75 mol l^{-1} sodium hydroxide solution were added and completely mixed. After standing for 20 min in a water bath at 25 to 30 °C, the absorbance at 520 nm was determined. Creatinine standard solutions ranging from 0.025 to 0.10 mg ml^{-1} were prepared and a working curve determined. The urinary creatinine was calculated using the working curve.

RESULTS

The groundwaters of Makrapur village were highly contaminated with arsenic. Several members from each family in our survey had arsenical skin manifestations. Gender, age and arsenical symptoms for each of 44 subjects are listed in Table 2, along with the depth and arsenic concentrations in the tubewell waters the subjects utilized. The age of the subjects ranged from 3 to 66 years old. The numbers of male and female subjects were 26 and 18 respectively. Of the 44 subjects, 24 had the arsenical manifestations such as hyperkeratosis, melanosis and Bowen's disease on their palms, chests and backs.

Most of the families in our surveyed area had their own shallow tubewell. Those who do not have their own tubewells used to collect the water from their neighbor's tubewell or from the panchayet (local government) tubewell, deep in nature, installed near their house. The water was supplied from the deep tubewell to the villagers through the pipeline beside the road, 5 h per day.

Families A to E formed a big and relational family. They had one shallow tubewell and one deep tubewell administrated in cooperation. Thirteen within 14 families used the water from the shallow and the deep tubewells.

The concentrations of arsenic in the waters obtained from three shallow tubewells were 61.0, 120.9 and 408.4 ppb, exceeding the Indian guideline of arsenic in drinking water (50 ppb). The concentrations of arsenic in deep tubewell-waters ranged from 5.2 to 9.6 ppb.

The depth of each tubewell used by the families and the inorganic arsenic concentration (arsenic(III), arsenic(V), total arsenic and arsenic(III)/total arsenic) in water are shown in Table 3. The families from A to E had two shallow tubewells of A–E (TW) and A–E (115 ft) on their site. Each water sample included arsenic(III) and arsenic(V).

Figure 1a shows the HPLC–ICP-MS chromatogram of arsenic species when 20 µl of a standard solution containing 150 ppb of arsenic(III), arsenic(V), MMA and DMA. The HPLC chromatogram is demonstrated in the retention times t_R of arsenic(III), DMA, MMA and arsenic(V) were 113 s, 153 s, 215 s and 421 s respectively. The t_R of arsenocholine and arsenobetaine was 82 s and 95 s respectively (data not shown). HPLC–ICP-MS chromatogram obtained from a urine sample is shown in Figure 1b. The peaks of arsenobetaine, arsenic(III), DMA and MMA appeared on the chromatogram.

The concentrations of creatinine (mg ml^{-1}), arsenic(III) (ng/mg creatinine), arsenic(V) (ng mg^{-1} creatinine), MMA (ng mg^{-1} creatinine), DMA (ng mg^{-1} creatinine), total arsenic (ng mg^{-1} creatinine) in urine samples, the ratio of (MMA + DMA) to total arsenic obtained from 44 urine samples are shown in Table 4.

DISCUSSION

The groundwater of the Makrampur village of the Beldanga block is highly contaminated with arsenic and the local government is supplying safe water with respect to arsenic to the villagers from a deep aquifer (around 1000 ft). Of 44 inhabitants from 13 arsenic-affected families, 24 had arsenical skin manifestations, such as Bowen's disease, melanosis or keratosis, as shown in Table 2. In our survey, we did not find any arsenic-affected people below 17 years old. Chakraborti and coworkers[15,16] reported that the age of the arsenic-affected people in West Bengal, India, and in Bangladesh has exceeded 11 years old. So our data coincide with their findings. Of 13 families, 12 used two sources of water from shallow and deep tubewells. The families started to use the deep tubewell water for cooking and drinking purposes after stopping using their old arsenic-contaminated shallow tubewells. Families A to E in Makrampur village formed a relational family. They had two shallow tubewells administrated in cooperation. As shown in Table 3, one tubewell marked as A–E (TW) has not been used because of its high arsenic concentration (577.9 ppb). Another tubewell-water has been used by the household. The high concentration of arsenic (577.9 ppb) suggests that family members would have got long-term damage on their bodies during the usage of this contaminated water for a long period of time, and this is why 10 out of 14 members have arsenic manifestations on their skin. The arsenic concentrations of the five shallow tubewell-water samples ranged from 18.0 to 408.4 ppb and their depth was from 45 to 152 ft. The arsenic concentrations of the four deep tubewell-water samples ranged from 5.2 to 9.6 ppb, below the WHO recommended standard of 10 ppb arsenic in drinking water. The percentage of arsenic(III) in the five shallow tubewell-water samples ranged from 75.6 to 98.6% of total arsenic concentrations. The percentage of arsenic(III) in the shallow tubewell-water samples was higher than that in the deep tubewell-water samples.

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Table 2. Sex, age and syptom of inhabitants and arsenic in tubewell waters

	Sample	Sex	Age	Symptom	As in water (ppb)	Tubewell Depth
1	A-1	M	66	+	61.0, 9.6	115 ft, deep tubewell
2	A-2	F	57	+	61.0, 9.6	115 ft, deep tubewell
3	A-3	M	17	+	61.0, 9.6	115 ft, deep tubewell
4	A-4	M	21	+	61.0, 9.6	115 ft, deep tubewell
5	B-1	M	36	+	61.0, 9.6	115 ft, deep tubewell
6	B-2	F	32	+	61.0, 9.6	115 ft, deep tubewell
7	C-1	M	31	+	61.0, 9.6	115 ft, deep tubewell
8	C-2	F	26	+	61.0, 9.6	115 ft, deep tubewell
9	C-3	M	4		61.0, 9.6	115 ft, deep tubewell
10	D-1	M	26	+	61.0, 9.6	115 ft, deep tubewell
11	D-2	F	20		61.0, 9.6	115 ft, deep tubewell
12	D-3	M	5		61.0, 9.6	115 ft, deep tubewell
13	D-4	M	2		61.0, 9.6	115 ft, deep tubewell
14	E-1	M	24	+	61.0, 9.6	115 ft, deep tubewell
15	F-1	M	50	+	9.6	Deep tubewell
16	F-2	F	46	+	9.6	Deep tubewell
17	F-3	F	17		9.6	Deep tubewell
18	F-4	F	11		9.6	Deep tubewell
19	F-5	F	24	+	9.6	Deep tubewell
20	F-6	M	25		9.6	Deep tubewell
21	F-7	F	22		9.6	Deep tubewell
22	G-1	M	61	+	120.9, 5.2	72 ft, deep tubewell
23	G-2	F	55	+	120.9, 5.2	72 ft, deep tubewell
24	G-3	M	19		120.9, 5.2	72 ft, deep tubewell
25	G-4	M	15		120.9, 5.2	72 ft, deep tubewell
26	G-5	F	30	+	120.9, 5.2	72 ft, deep tubewell
27	G-6	F	8		120.9, 5.2	72 ft, deep tubewell
28	H-1	F	45	+	120.9, 5.2	72 ft, deep tubewell
29	I-1	M	55	+	18, 6.6	152 ft, deep tubewell
30	I-2	F	47	+	18, 6.6	152 ft, deep tubewell
31	I-3	M	26		18, 6.6	152 ft, deep tubewell
32	I-4	M	22		18, 6.6	152 ft, deep tubewell
33	I-5	M	16		18, 6.6	152 ft, deep tubewell
34	J-1	M	35		18, 6.6	152 ft, deep tubewell
35	J-4	M	4		18, 6.6	152 ft, deep tubewell
36	K-1	F	40	+	408.4, 6.2	45 ft, deep tubewell
37	K-2	M	18	+	408.4, 6.2	45 ft, deep tubewell
38	L-1	M	45	+	18, 6.6	152 ft, deep tubewell
39	L-2	F	41		18, 6.6	152 ft, deep tubewell
40	L-3	M	18		18, 6.6	152 ft, deep tubewell
41	M-1	M	55	+	18, 6.6	152 ft, deep tubewell
42	M-2	F	47	+	18, 6.6	152 ft, deep tubewell
43	M-3	M	25		18, 6.6	152 ft, deep tubewell
44	M-4	F	15		18, 6.6	152 ft, deep tubewell

As shown in Table 4, the concentrations of urinary creatinine in 44 samples ranged from 0.07 to 1.456 mg ml^{-1}. Thomas and coworkers[17] reported on arsenic in urines in the Millard County, Utah, population chronically exposed to arsenic from drinking water; a correlation was found between arsenic in drinking water and the mean total arsenic per creatinine. Thus, we corrected the urinary arsenic species by using the urinary creatinine.

As shown in Figure 1b, an arsenic-containing compound was detected at $t_R = 95$ s, which coincides with t_R of arsenobetaine. Detection of arsenobetaine in some urine samples may be attributed to the fact that people in this survey

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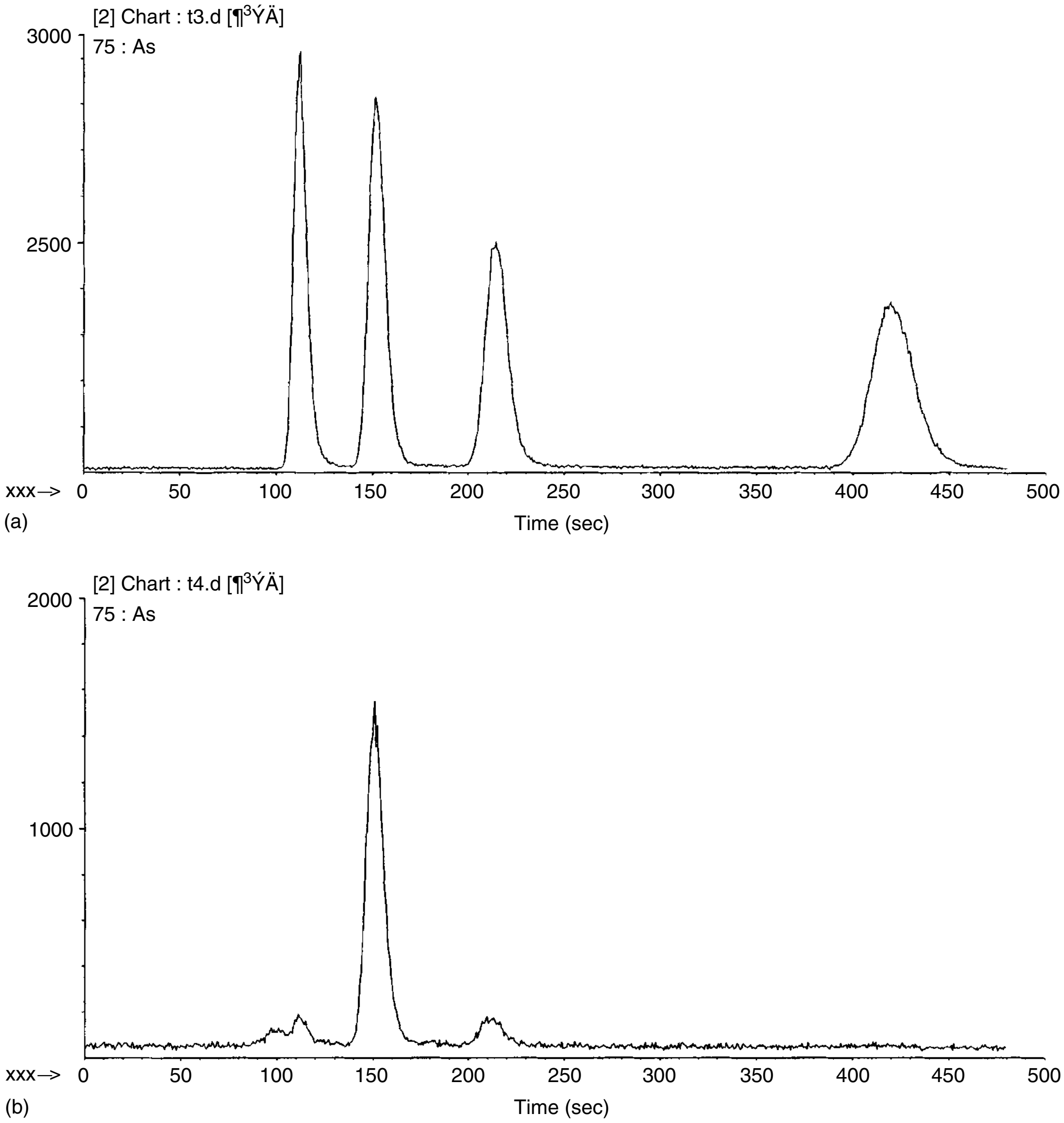

Figure 1. (a) HPLC chromatogram of arsenic(III), DMA, MMA and arsenic(V) at 150 ppb. arsenic(III): 113 s; DMA: 153 s; MMA: 215 s; arsenic(V): 421 s. (b) HPLC chromatogram of arsenic species in urine. HPLC conditions: detection, Agilent 7500 model ICP-MS (*m/z*: 75); column, Gel PAK-GL-IC-A15 (4.6 mm i.d. × 150 mm); mobile phase, 10 mM phosphate buffer (pH 6.0); column temperature, 35 °C; flow rate, 1 ml min^{-1}.

Table 3. Concentration of inorganic arsenic in tubewell water

	Tubewell	As(III) (ppb)	As(V) (ppb)	Total As (ppb)	As(III)/total As (%)
1	A (TW)	577.9	13.0	590.9	97.8
2	A (115 ft)	57.5	3.5	61.0	94.3
3	A (deep)	7.8	1.8	9.6	81.3
4	G (72 ft)	110.2	10.7	120.9	91.1
5	G (deep)	1.7	3.5	5.2	32.7
6	I (152 ft)	13.6	4.4	18.0	75.6
7	I (deep)	1.1	5.5	6.6	16.7
8	K (45 ft)	402.8	5.6	408.4	98.6
9	K (deep)	2.0	4.2	6.2	32.3

area consume freshwater shrimps. In our study we considered the total arsenic concentration in urine as the sum of arsenic(III), DMA, MMA and arsenic(V) concentrations, neglecting the presence of the small amount of arsenobetaine which clearly did not come from the arsenic-contaminated water. The concentrations of arsenic(III), DMA, MMA and arsenic(V) in the 44 urine samples ranged from <0.2 to 237.4 ng mg^{-1}, <0.2 to 2166.4 ng mg^{-1}, <0.2 to 430.6 ng mg^{-1} and <0.2 to 89.7 ng mg^{-1} creatinine respectively. The respective averages are 28.7 ng mg^{-1}, 168.6 ng mg^{-1}, 25.0 ng mg^{-1} and 4.6 ng mg^{-1} creatinine. The woman identified as K-1, who is 40 years old, excreted the highest amount of total arsenic, at 2890.0 ng mg^{-1} creatinine, in her urine. The arsenic concentration in the shallow tubewell water used by the K family

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Table 4. Urinary creatinine and arsenic species obtained from families A to M

			Arsenic (ng mg^{-1} creatinine)					
	Sample	Creatinine (mg ml^{-1})	As(III)	DMA	MMA	As(V)	Total As	(MMA + DMA)/Total As (%)
2	A-1	0.236	11.9	251.8	32.0	<0.2	295.8	96.0
3	A-2	0.443	<0.2	31.1	5.4	<0.2	36.5	100.0
4	A-3	0.956	2.7	26.6	5.9	<0.2	35.2	92.3
5	A-4	1.456	12.4	65.8	8.0	<0.2	86.2	85.6
6	B-1	1.413	2.1	31.2	13.7	<0.2	47.1	95.4
7	B-2	0.279	1.7	29.7	17.7	<0.2	49.0	96.6
8	C-1	0.421	7.3	72.6	14.5	<0.2	94.4	92.3
9	C-2	0.625	21.5	203.7	14.7	<0.2	239.9	91.0
10	C-3	0.120	1.2	19.8	<0.2	<0.2	21.0	94.4
11	D-1	0.873	15.4	100.6	2.0	<0.2	118.1	86.9
12	D-2	0.540	26.6	115.0	<0.2	0.4	142.0	81.0
13	D-3	0.894	35.1	289.0	<0.2	19.3	343.4	84.2
14	D-4	0.277	11.7	92.0	<0.2	<0.2	103.7	88.7
15	E-1	0.958	15.6	249.5	14.5	<0.2	279.6	94.4
16	F-1	0.475	44.8	157.4	12.9	<0.2	215.1	79.2
17	F-2	0.471	32.3	159.9	15.4	<0.2	207.7	84.4
18	F-3	0.855	112.5	325.8	15.8	1.3	455.5	75.0
19	F-4	0.696	41.3	150.7	18.8	<0.2	210.7	80.4
20	F-5	0.294	16.9	140.9	11.1	3.4	172.4	88.2
21	F-6	1.077	49.7	438.0	48.6	1.1	537.4	90.5
22	F-7	0.577	29.3	105.8	9.1	<0.2	145.1	79.2
23	G-1	0.817	20.3	102.5	20.5	<0.2	143.2	85.9
24	G-2	0.070	3.1	18.6	<0.2	<0.2	21.8	85.6
25	G-3	0.277	9.5	49.3	14.8	<0.2	73.7	87.1
26	G-4	0.279	8.2	47.9	12.4	<0.2	68.5	88.0
27	G-5	0.086	6.3	14.2	<0.2	<0.2	20.5	69.2
28	G-6	0.403	14.4	34.3	6.8	<0.2	55.5	74.1
29	H-1	0.804	32.3	322.6	40.0	5.3	400.3	90.6
30	I-1	0.173	0.6	18.6	1.6	<0.2	20.8	97.3
31	I-2	0.598	4.1	28.5	8.8	<0.2	41.4	90.0
32	I-3	1.290	59.4	170.6	34.9	<0.2	264.9	77.6
33	I-4	0.201	5.1	27.7	1.3	<0.2	34.1	85.1
34	I-5	0.995	7.6	23.5	3.6	<0.2	34.7	78.2
35	J-1	0.362	45.0	64.2	25.5	11.9	146.6	61.2
36	J-4	0.308	21.4	71.3	<0.2	<0.2	92.7	76.9
37	K-1	0.803	237.4	2166.4	430.6	55.5	2890.0	89.9
38	K-2	0.154	79.2	220.1	73.0	5.6	377.8	77.6
39	L-1	0.470	36.0	171.6	41.9	<0.2	249.4	85.6
40	L-2	0.484	29.2	113.2	22.7	<0.2	165.0	82.3
41	L-3	1.160	11.8	0.0	8.9	89.7	110.4	8.0
42	M-1	0.319	10.1	82.9	13.7	<0.2	106.8	90.6
43	M-2	0.213	7.6	62.4	9.3	<0.2	79.3	90.5
44	M-3	1.319	81.6	342.6	52.7	3.2	480.1	82.3
45	M-4	0.425	40.4	210.5	17.7	4.6	273.2	83.5
	Average	0.590	28.7	168.6	25.0	4.6	227.0	83.9
	Max.	1.456	237.4	2166.4	430.6	89.7	2890.0	100.0
	Min.	0.070	<0.2	<0.2	<0.2	<0.2	20.5	8.0

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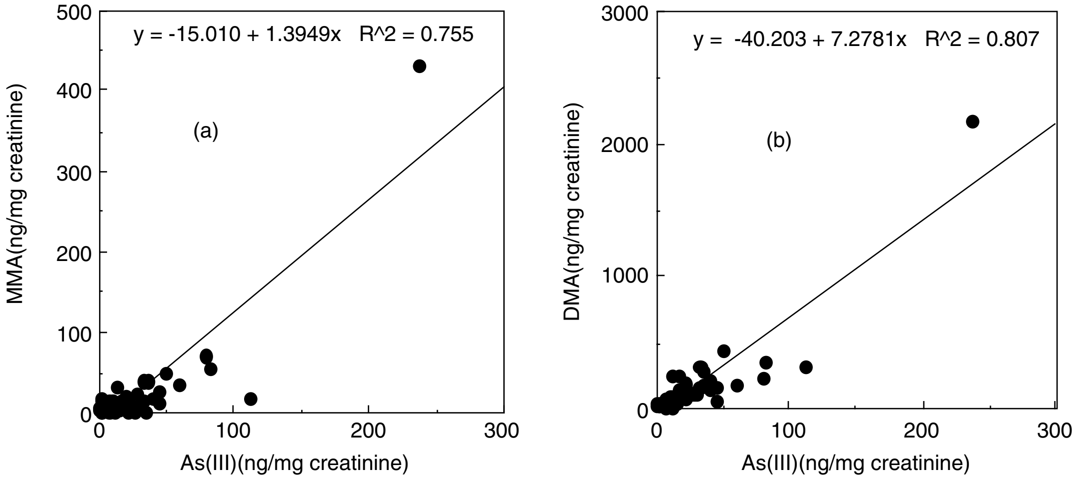
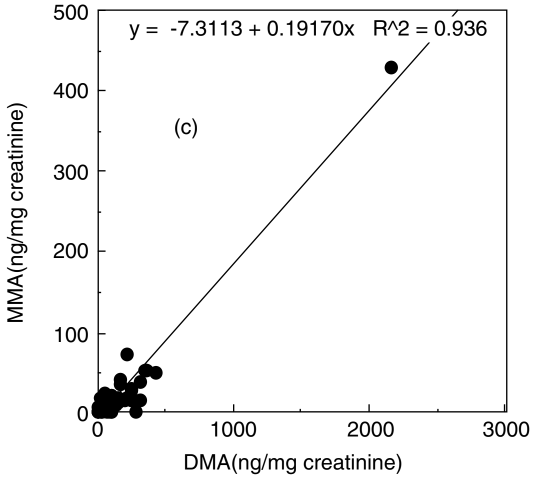
was 408.4 ppb. On comparing the urinary total arsenic of K-2 (377.8 ng mg^{-1} creatinine) with that of K-1 (2890.0 ng mg^{-1} creatinine), we can conclude that K-1 must recently have taken the arsenic-contaminated water from the shallow tubewell-water instead of the deep tubewell-water.

We have already reported in our earlier publications[12,13] that the average of urinary arsenic(III), DMA, MMA and arsenic(V) in the Jalangi block were 92.0 ng mg^{-1}, 391.4 ng mg^{-1}, 62.1 ng mg^{-1} and 45.4 ng mg^{-1} creatinine respectively. And those in the Domkal block were 24.5 ng ml^{-1}, 136.1 ng ml^{-1}, 25.5 ng ml^{-1} and 58.3 ng ml^{-1} urine respectively. The arsenic concentrations in the six shallow tubewell-water samples in the Jalangi block and in the eight tubewell-water samples in the Domkal block ranged from 7.3 to 170.0 ppb and from 0.64 to 75.5 ppb respectively. When compared with the Jalangi and Domkal populations, the population studied in Makrampur village, Beldanga block, consumed groundwaters with a lower concentration of arsenic through their deep tubewell waters. This must be reflected in the lower concentrations of arsenic species than those from the subjects of our previous studies. Vahter[18] reviewed the urinary arsenic species obtained from areas with arsenic-affected underground water, such as Taiwan, California, Santa Ana (Mexico), Toconao and San Antonio, and reported that the urinary inorganic arsenic to total arsenic ranged from 10 to 30%, MMA ranged from 10 to 20%, and DMA ranged from 60 to 70%. Also, Hsueh *et al.*[19] estimated the urinary arsenic species from previous cumulative exposure to arsenic through consuming artesian well water among healthy residents in an arseniasis hyperendemic area in Taiwan. They reported that, in the case of cumulative exposure to arsenic exceeding 10 mg l^{-1} year^{-1}, the average percentage (plus/minus the standard error) of (arsenic(III) + arsenic(V)), of MMA and of DMA to total arsenic in urine amples



Relationship beteen As(III) and MMA in urines

Relationship between As(III) and DMA in urines



Relationship between DMA and MMA in urines

Figure 2. Relationship between urinary As species.

was 10.27% (±0.60%, $n = 148$), 23.21% (±1.12%, $n = 148$), and 66.50% (±1.39%, $n = 148$) respectively. In our current data, shown in Table 4, the average (plus/minus the residual standard deviation) of the ratio of (MMA + DMA) to total arsenic was 86.7% (±9.2%), with the exception of the data for the L-3 urine sample. Our data are similar to the ratio of (MMA + DMA) to total arsenic obtained by Hsueh *et al.*[19] and Vahter.[18] The exception (L-3 in Table 4) was an 18-year-old boy who had a markedly low ratio of (MMA + DMA) to total arsenic (8.0%). The amount of total arsenic in his urine sample was 110.4 ng mg^{-1} creatinine and most of the arsenic species was arsenic(V), at 89.7 ng mg^{-1} creatinine. It may be speculated that the boy has a low methylating capacity of inorganic arsenic in the liver. We could not observe any arsenic symptoms from this boy, as shown in Table 2.

We estimated the relationships between each arsenic species, except for the data obtained from the boy identified as L-3. As shown in Figure 2, there were significantly positive correlations between the concentrations of arsenic(III) and MMA, arsenic(III) and DMA, and MMA and DMA ($p = 0.01$). We have already reported similar observations in our earlier publications.[12,13] The presence of these positive correlations does not contradict the postulation that the metabolic pathway of inorganic arsenic follows as arsenic(V) → arsenic(III) → MMA → DMA in the case of humans.

CONCLUSIONS

Based on the results from our field surveys of tubewell-water samples and human urine samples obtained from the Makrampur village, in the Beldanga block of the Murshidad district, we obtained the following findings.

(1) The arsenic concentrations in five shallow tubewell-water samples ranged from 18.0 to 408.4 ppb. The arsenic concentrations in four deep tubewell-water samples ranged from 5.2 to 9.6 ppb.
(2) We found 22 arsenic-affected villagers out of 44 villagers, belonging to 13 families.
(3) The concentrations of arsenic(III), DMA, MMA and arsenic(V) in urine samples obtained from the 44 villagers ranged from <0.2 to 237.4 ng mg^{-1}, <0.2 to 2166.4 ng mg^{-1}, <0.2 to 403.6 ng mg^{-1} and < 0.2 to 89.7 ng mg^{-1} creatinine respectively, and the averages were 28.7 ng mg^{-1}, 168.6 ng mg^{-1}, 25.0 ng mg^{-1} and 4.6 ng mg^{-1} creatinine respectively. The average of total arsenic was 227.0 ng mg^{-1} creatinine.
(4) One boy had a low arsenic-methylating capacity and directly excreted 81.2% of arsenic(V) against the total arsenic in his urine. One woman excreted the highest amount of total arsenic at 2890.0 ng mg^{-1} creatinine in her urine.
(5) When estimating the arsenic species in 43 urine samples obtained from families A to M, the correlations between arsenic(III) and MMA, between arsenic(III) and DMA or between MMA and DMA in urine samples were significant ($p = 0.01$).

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