Urinary Arsenic Species in Arsenic-Affected Area of West Bengal, India (Part II)

Hiroshi Tokunaga,* Tarit Roychowdhury, Tadashi Uchino, and Masanori Ando

National Institute of Health Sciences, 1–18–1, Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan

(Received June 23, 2003; Accepted August 28, 2003)

Arsenic-contamination of groundwater has long been reported in Mushidabad district of West Bengal, India. We visited 19 arsenic-affected families and 4 non-arsenic -affected families in that area during 20–22 February 2001 and collected 10 tubewell waters used for drinking and cooking and 89 urines from those families. The arsenic concentrations in waters ranged from 0.64 to 75.5 ppb. The average of arsenite, arsenate, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in urine were 23.1, 59.0, 24.6 and 127.4 ng/ml urine, respectively. The average of total arsenic was 234.1 ng/ml urine. On comparison of the ratio of (MMA + DMA) to total arsenic, the average proportion of (MMA + DMA) was 75.7%, but the proportions were from 4.2 to 57.2% for 11 persons. This result suggests that they might be damaged due to the arsenic-methylating capacity. When selecting the members of A to L families because of using the two same tubewell waters except 5 persons due to the luck of enough arsenic-methylating capacity, there were the good relationships ($p < 0.01$) between As(III) and MMA, As(III) and DMA, MMA and DMA, and DMA and total arsenic. The relationship between the arsenic concentration in tubewell waters and the averages of the total arsenic obtained from each families was not in agreement ($p > 0.05$). The result suggests the possibility of the contribution of other sources like foodstuffs regarding to the excretion of arsenic species from the urines.

Key words —— arsenic species, urine, tubewell water, West Bengal, India

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 sufferings of people are in Bangladesh and West Bengal, India.[1–3] Although the entire population are not drinking arsenic-contaminated water, but no doubt they are at risk. Groundwater arsenic contamination and sufferings of people have been reported all over the world. The arsenic contamination incident in well water of Taiwan (1961–1985) was well known.[4] Black-foot disease was noted, as were other arsenical manifestations such as hyperkeratosis, spotted melanosis and diffuse melanosis. Recently, the arsenic-contaminated groundwater in Vietnam has been reported.[5,6]

In 1987 Chakraborti A. K. and Saha K. C.[7] reported arsenical skin manifestation in 5 districts of West Bengal. During 1989 to nowadays, the group of Chakraborti D continues to report the arsenic calamity in West Bengal, India.[8–11] The district of Mushidabad is located to the border of Bangladesh and is one of the nine arsenic-affected districts, West Bengal. In order to estimate people's total exposure to arsenic, we visited the Jalangi block in Murshibad district during 4–7 December 2000 and collected 51 urines and hairs and the foodstuffs such as rice, potato and onion obtained from 12 arsenic-affected families and 6 tubewell waters used by those families. Therefore, we have already reported the arsenite, arsenate, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) in each urine and the inorganic arsenic in tubewell waters obtained from the Jalangi block in Murshibad district.[12] Also we estimated the arsenic in food composites and tubewell water from the villagers in Jalangi and Domkol block and reported that the dietary intakes of arsenic from the villagers in Jalangi and Domkol block were 801 and 658 μg for adult males, 718 and 588 μg for adult female and 432 and 351 μg for children, approximately 10 years of age, respectively.[13] Water contributes 76.8 and 71.4%, 74.1 and 68% and 76.9 and 71.2% of arsenic, with respect to the

*To whom correspondence should be addressed: National Institute of Health Sciences, 1–18–1, Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan. Tel.: +81-3-3700-1141 (ex. 255); Fax: +81-3-3707-6950; E-mail: tokunaga@nihs.go.jp

total intakes of arsenic from all sources by adult male, adult female and children in Jalangi and Domkol block.

In the present study, we visited a Domkal block in Murshidad during 22 to 24 February 2001 with Dr. Chakraborti group of Jadavpur University and collected 10 tubewell water and 89 urines from 19 arsenic-affected families and from 4 non-arsenic -affected families. We determined the inorganic arsenic in tubewell waters and the arsenic species in urines by using high-performance liquid chromatography and coupled plasma mass spectrometry (HPLC-ICP -MS) and reported the contribution of the inorganic arsenic to the arsenic species in urines and the evaluation of the arsenic-methylating capacity of each person.

MATERIALS AND METHODS

Reagents and Samples —— Sodium arsenite [As(III)], sodium arsenate [As(V)] 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 for arsenic, were obtained by weighing accurate amounts of As(III), As(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, by the appropriate dilution.

Spot urine samples were collected from 89 persons of both 19 arsenic-affected families marked at A to U families and 4 non-arsenic -affected families marked at V to Y families and were kept in polyethylene centrifuge tubes. The samples were not subjected to any chemical treatment. After collection, the samples were stored in a cooler icebox.

The tubewell water samples were collected from the hand tubewells, used by family members for drinking and cooking and other household purposes. The water samples were stored in polyethylene centrifuge tubes; one drop of concentrated nitric acid was added as a preservative, and they were then stored in a cooler icebox.

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

Table 1. ICP/MS Conditions

RF power (W)	1500
RF refraction (W)	1
Plasma gas flow (l/min)	15
Carier gas flow (l/min)	0.8
Monitoring mass	35 (Cl), 75 (As)
Integration interval (sec)	0.3
Scan number	1

Instrumentation —— A Model Agilent 7500 ICP mass spectrometer (Agilent, DE, U.S.A.) 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 ——

Arsenic Species in Urines: HPLC was performed under the following conditions: mobile phase 10 mmol/l phosphate buffer (pH 6.0), flow rate 1 ml/min, 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 defrosting, 20 μl of urine was 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.

Inorganic Arsenic in Waters: The analytical column was removed from the HPLC system and HPLC pump was connected with the reodyne injector and the nebulizer of ICP mass spectrometer. After defrosting tubewell waters, these solutions were injected into the rheodyne injector and the peak area of arsenic was measured by ICP-MS for 1.5 min. The amounts of inorganic arsenic were calculated using working curves prepared by using 0, 5, 10 and 50 ppb of inorganic arsenic solutions.

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

Assay of Urinary Creatinine —— One milliliter of urine was diluted with MilliQ water to 10 ml, 0.5 ml of the diluted urine were put into the centrifuge tube and 3 ml of a solution (containing 0.8% sodium tangstate, 0.3% phosphoric acid and 0.2% sulfuric acid) for eliminating the 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 picric acid solution and 1 ml of 0.75 mol/l sodium hydroxide solution were added and mixed well. After standing for 20 min in a water bath at 25 to 30°C, the absorbance at 520 nm was determined. The creatinine standard solutions, which range from 0.025 to 0.10 mg/ml, were prepared and the working curve was made. The urinary creatinine was calculated using working curve.

RESULTS

During 22 to 24 February 2001, we visited Skrikrishnapur village, Mamudpur village and Bakshipur village in Domkal block of Murshidad district of West Bengal in India. The groundwaters of Skrikrishnapur and Mamudpur village were arsenic-contaminated. Each family who lived in the areas of the arsenic-contaminated underground water such as Skrikrishnapur and Mamudpur village had several patients having arsenical skin manifestations. The groundwaters of Bakshipur village had the low level of arsenic below 10 ppb. Each family member, their arsenical symptoms, gender and age from 89 persons studied, the amount of arsenic in their tubewell water and the depth of tubewell are listed in Table 2. The ages of investigated persons ranged from 3 to 60 years old. The numbers of male and of female were 49 and 40 persons, respectively.

The families from A to L in Skrikrishnapur village formed a big and relational family. They had two tubewell administrated in cooperation. The families from M to U were in Mamudpur village and used the different tubewell waters. The families from V to Y lived in the Bakshipur village where there was arsenic-free underground water whose arsenic amount was 2.58 ppb. We did not find any arsenical skin manifestations in four families who lived in Bakshipur village as control.

The Indian guideline of arsenic in drinking water is below 50 ppb. The amount of arsenic obtained from P family was 75.5 ppb and over 50 ppb of the Indian guideline of arsenic in drinking water.

On injecting 20 μl of As(III), As(V), MMA and DMA at 150 ppb, HPLC chromatogram is demonstrated in Fig. 1. The retention times (t_R) of As(III), DMA, MMA and As(V) were 111, 155, 214 and 416 seconds, respectively. HPLC chromatograms obtained from urine are shown in Fig. 2. The upper chromatogram gave the Chloride pattern in urine. Comparing the upper chromatogram with the lower one, the interference of argon chloride at the molecular weight of 75 was not observed on chromatogram. The peaks of As(III), DMA and MMA appeared on chromatogram.

The concentrations of creatinine (mg/dl), As(III) (ng/ml), As(V) (ng/ml), MMA (ng/ml), DMA (ng/ml), total arsenic (ng/ml) in urines, the ratio of (MMA + DMA) to total arsenic and the ratio of MMA to (MMA + DMA) obtained from 89 urines are shown in Table 3. We determined the urinary creatinine to correct the urinary arsenic species because of collecting the spot urines from the villagers.

DISCUSSION

The small numbers of the habitants in both Skrikrishnapur village and Mamudpur village of Domkal block had the arsenical skin manifestation such as Bowen's Disease, melanosis or keratosis as shown in Table 2. Their ages were over 15 years old. The big and relational families of A to M had two tubewell whose depth are 240 and 26 feet. The amounts of arsenic in waters from the 240-feet and 26-feet tubewell were 6.1 ppb and 25.8 ppb, respectively. The families from M to U who lived in Mamudpur village used 7 tubewells and their amounts of arsenic in waters ranged from 0.64 to 75.5 ppb. We observed one slightly arsenical patient who took the water at the arsenic concentration of 0.64 ppb. When the amounts of arsenic in drinking waters became higher, the number of people of having the arsenical symptom increased.

On the other hand, Dr. Chakraborti have already determined the amounts of arsenic in tubewell water and the arsenical skin manifestation of habitants in Bakshipur village of Domkal block and gotten the information about that the arsenic concentration in tubewell waters were low level. The families of V to Y who lived in Bakshipur village took the safe water at the arsenic concentration of 2.58 ppb and we could not find out the arsenical patients. So we selected those families as the control family.

Table 2–1. Sex, Age and Syptom of Subjects and Arsenic Concentration in Tubewell Waters from Arsenic-Contaminated Area

	Sample	sex	age	symptom	As in water (ppb)	Depth of tubewell
1	A-1	M	55	+	6.1, 25.8	240 ft, 26 ft
2	A-2	F	46	+	6.1, 25.8	240 ft, 26 ft
3	A-3	M	20		6.1, 25.8	240 ft, 26 ft
4	A-4	F	28		6.1, 25.8	240 ft, 26 ft
5	A-5	F	9		6.1, 25.8	240 ft, 26 ft
6	A-6	F	22		6.1, 25.8	240 ft, 26 ft
7	B-1	M	50	+	6.1, 25.8	240 ft, 26 ft
8	B-2	F	35	+	6.1, 25.8	240 ft, 26 ft
9	B-3	M	28	+	6.1, 25.8	240 ft, 26 ft
10	B-4	M	19		6.1, 25.8	240 ft, 26 ft
11	B-5	M	16		6.1, 25.8	240 ft, 26 ft
12	B-6	M	14		6.1, 25.8	240 ft, 26 ft
13	B-7	M	9		6.1, 25.8	240 ft, 26 ft
14	B-8	F	11		6.1, 25.8	240 ft, 26 ft
15	C-1	M	40		6.1, 25.8	240 ft, 26 ft
16	C-2	M	13		6.1, 25.8	240 ft, 26 ft
17	C-3	F	11		6.1, 25.8	240 ft, 26 ft
18	C-4	F	3		6.1, 25.8	240 ft, 26 ft
19	D-1	F	42	+	6.1, 25.8	240 ft, 26 ft
20	D-2	M	9		6.1, 25.8	240 ft, 26 ft
21	E-1	M	39	+	6.1, 25.8	240 ft, 26 ft
22	E-2	M	5		6.1, 25.8	240 ft, 26 ft
23	E-3	M	5		6.1, 25.8	240 ft, 26 ft
24	E-4	F	8		6.1, 25.8	240 ft, 26 ft
25	E-5	F	35		6.1, 25.8	240 ft, 26 ft
26	E-6	F	55	+	6.1, 25.8	240 ft, 26 ft
27	E-7	F	16		6.1, 25.8	240 ft, 26 ft
28	E-8	M	14		6.1, 25.8	240 ft, 26 ft
29	F-1	F	25		6.1, 25.8	240 ft, 26 ft
30	F-2	F	11		6.1, 25.8	240 ft, 26 ft
31	F-3	M	9		6.1, 25.8	240 ft, 26 ft
32	F-4	M	7		6.1, 25.8	240 ft, 26 ft
33	G-1	F	21		6.1, 25.8	240 ft, 26 ft
34	G-2	F	3		6.1, 25.8	240 ft, 26 ft
35	H-1	F	35	+	6.1, 25.8	240 ft, 26 ft
36	H-2	F	16		6.1, 25.8	240 ft, 26 ft
37	I-1	M	43	+	6.1, 25.8	240 ft, 26 ft
38	I-2	F	35	+	6.1, 25.8	240 ft, 26 ft
39	I-3	M	18	+	6.1, 25.8	240 ft, 26 ft
40	I-4	M	15	+	6.1, 25.8	240 ft, 26 ft
41	J-1	M	30	+	6.1, 25.8	240 ft, 26 ft
42	J-2	F	27		6.1, 25.8	240 ft, 26 ft
43	J-3	F	8		6.1, 25.8	240 ft, 26 ft

The concentrations of creatinine, As(III), As(V), MMA and DMA obtained from 89 urines are shown in Table 3. The concentration of urinary creatinine ranged from 0.1 to 307.5 mg/dl. The urines from E-8 (boy, 14 years old) and J-4 (girl, 4 years old) had so the lower creatinines such as 0.1 and 0.8 mg/dl. Thomas *et al*. determined the arsenic in urines in a Millard Country, Utah, population of that is chronically exposed to arsenic from drinking water and already reported the correlation between arsenic in

Table 2–2. Sex, Age and Syptom of Subjects and Arsenic Concentration in Tubewell Waters from Arsenic-Contaminated Area

	Sample	sex	age	symptom	As in water (ppb)	Depth of tubewell
44	J-4	F	4		6.1, 25.8	240 ft, 26 ft
45	K-1	M	37	+	6.1, 25.8	240 ft, 26 ft
46	K-2	F	30		6.1, 25.8	240 ft, 26 ft
47	K-3	M	12		6.1, 25.8	240 ft, 26 ft
48	K-4	F	9		6.1, 25.8	240 ft, 26 ft
49	K-5	M	7		6.1, 25.8	240 ft, 26 ft
50	K-6	M	4		6.1, 25.8	240 ft, 26 ft
51	L-1	F	35		6.1, 25.8	240 ft, 26 ft
52	L-2	M	19	+	6.1, 25.8	240 ft, 26 ft
53	L-3	M	13		6.1, 25.8	240 ft, 26 ft
54	L-4	M	9		6.1, 25.8	240 ft, 26 ft
55	L-5	M	6.5		6.1, 25.8	240 ft, 26 ft
56	M-1	M	35	+	37.9	112 ft
57	M-2	F	25		37.9	112 ft
58	M-3	F	8		37.9	112 ft
59	M-4	F	5		37.9	112 ft
60	M-5	M	3		37.9	112 ft
61	M-6	F	60	+	37.9	86 ft
62	N-2	F	13		20.9	86 ft
63	O-1	M	38	+	48.1	66 ft
64	O-2	F	24		48.1	66 ft
65	O-3	F	7		48.1	66 ft
66	O-4	M	5		48.1	66 ft
67	P-1	F	36	+	75.5	66 ft
68	P-2	M	18		75.5	66 ft
69	P-3	M	16		75.5	66 ft
70	P-4	F	13		75.5	66 ft
71	Q-1	M	40	+	37.2	86 ft
72	Q-2	F	30	+	37.2	86 ft
73	Q-3	F	10		37.2	86 ft
74	Q-4	M	7		37.2	86 ft
75	S-1	M	45		37.2	86 ft
76	S-2	F	36		37.2	86 ft
77	S-3	M	16		37.2	86 ft
78	R-1	M	36	+	0.64	36 ft
79	R-2	F	29		0.64	36 ft
80	R-3	M	16		0.64	36 ft
81	R-4	M	12		0.64	36 ft
82	T-1	M	34		0.66	26 ft
83	U-1	M	30		0.66	26 ft
84	U-2	F	25		0.66	26 ft

drinking water and the mean total arsenic per creatinine.[14] If we corrected the arsenic species with the urinary creatinine, the corrected arsenic species would become the highest values. So we stopped to use the corrected arsenic species with urinary creatinine.

Total arsenic in urine ranged from 5.8 to 1707.7 ng/ml urine and the average was 234.1 ng/ml urine. Total arsenic in urines from R and U families, whose tubewell water arsenic concentration were 0.64 and 0.66 ppb, respectively, ranged from 43.7 to 216.1 ng/ml urine and their average was 116.1 ng/ml urine. On the other hand, the total arsenic in urines from the control families, whose

Table 2–3. Sex, Age and Syptom of Subjects and Arsenic Concentration in Tubewell Waters from Arsenic-Free Area

	Sample	sex	age	symptom	As in water (ppb)	Depth of tubewell
85	85. V-1	M	75		2.58	52 ft
86	86. W-1	M	35		2.58	52 ft
87	87. W-2	F	30		2.58	52 ft
88	88. W-3	M	8		2.58	52 ft
89	89. W-4	M	10		2.58	52 ft
90	90. X-1	M	27		2.58	52 ft
91	91. X-2	F	23		2.58	52 ft
92	92. X-3	F	3		2.58	52 ft
93	93. Y-1	F	35		2.58	52 ft
94	94. Y-2	F	13		2.58	52 ft
95	95. Y-3	F	11		2.58	52 ft
96	96. Y-4	F	9.5		2.58	52 ft

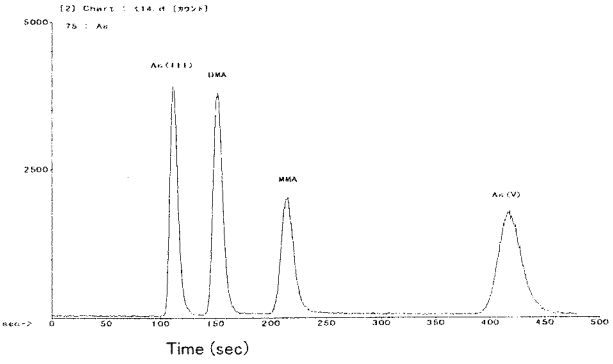

Fig. 1. HPLC Chromatogram of As(III), DMA, MMA and As(V) at 150 ppb

As(III): 111 sec, DMA: 152 sec, MMA: 214 sec, As(V): 416 sec. HPLC conditions; Detector: Agilent 7500 model ICP/MS (*m*/*z* 35 and 75), Column: Gel PAK GL-IC-A15 (4.6 mm i.d. × 150 mm), Mobil phase: 10 mM phosphate buffer (pH 6.0), Column temperature: 35°C, Flow rate: 1 ml/min.

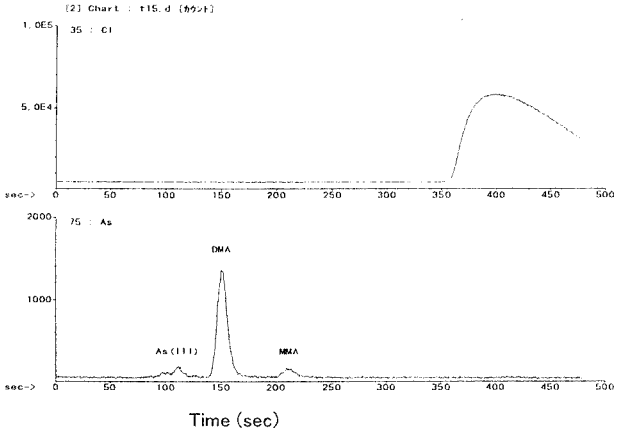

Fig. 2. HPLC Chromatogram of As Species in Urine

As(III): 111 sec, DMA: 152 sec, MMA: 214 sec.

tubewell water arsenic concentration were 2.58 ppb, were from 18.7 to 58.6 ng/ml urine and their average was 36.7 ng/ml urine. The families from R to U took the tubewell waters at the low level of arsenic on comparison with the control families. But their total arsenics in urines were higher than those from the control families. This result suggests that the source of arsenic might have other pathways like food composites in addition to the drinking water. We have already reported the arsenic in food composites obtained both from Mamudpur village of Domkal block and from Bakshipur village of Domkal block.[13] The food composites included the vegetables, cereals and bakery goods, spices and miscellaneous. The averages of arsenic in all categories collected from Mamudpur village and Bakshipur village were 236.89 and 146.14 μg/kg, respectively. These results suggest that the intake of arsenic both from the drinking water and from the food composites should be considered on total exposure assessment.

Aposhian *et al*. have already reported that the metabolic pathway of inorganic arsenic in rabbit liver passed through As(V) → As(III) → MMA → DMA at the presence of glutathione, S-adenosyl-L-methionine, As(III) methyltransferase and MMA(III) methyltransferase.[15] In order to estimate the function of arsenic-methylating enzyme at each person, the ratios of (MMA + DMA) to total arsenic were used. Del Rago *et al*.[16] investigated the arsenic species in control urines (arsenic concentration of drinking water was 25 ppb) from Santa Ana, Cohuila, Mexico and exposed urines (arsenic concentration of drinking water was the average 415 ppb) from

Table 3–1. Arsenic Species in Urines Obtained from Skrikrishnapur Village

	Sample	As(III) ng/ml	As(V) ng/ml	MMA ng/ml	DMA ng/ml	Total As ng/ml	(MMA + DMA) /Total As (%)	MMA/ (MMA + DMA)(%)
1	A-1	10.0	0.0	17.4	119.1	146.5	93.2	12.7
2	A-2	6.5	0.0	5.1	77.3	88.9	92.7	6.2
3	A-3	10.0	0.0	9.7	104.7	124.4	92.0	8.5
4	A-4	4.0	12.0	6.7	71.2	93.9	83.0	8.6
5	A-5	19.1	0.0	19.9	132.6	171.6	88.9	13.0
6	A-6	6.5	0.0	5.3	53.5	65.3	90.0	9.0
7	B-1	9.5	0.0	7.6	37.0	54.1	82.4	17.0
8	B-2	13.1	7.4	12.2	65.5	98.2	79.1	15.7
9	B-3	21.9	2.2	26.6	30.9	81.6	70.5	46.3
10	B-4	15.2	0.0	20.7	82.9	118.8	87.2	20.0
11	B-5	81.1	0.0	58.9	167.1	307.1	73.6	26.1
12	B-6	50.2	0.0	28.2	106.9	185.3	72.9	20.9
13	B-7	17.9	0.0	17.2	117.2	152.3	88.2	12.8
14	B-8	33.8	0.0	24.1	132.2	190.1	82.2	15.4
15	C-1	0.0	6.4	10.1	36.1	52.6	87.8	21.9
16	C-2	14.8	0.0	11.4	123.0	149.2	90.1	8.5
17	C-3	2.6	0.0	3.0	29.7	35.3	92.6	9.2
18	C-4	12.9	11.8	9.3	116.3	150.3	83.6	7.4
19	D-1	6.7	8.1	7.9	74.0	96.7	84.7	9.6
20	D-2	5.7	0.0	6.8	66.9	79.4	92.8	9.2
21	E-1	9.7	108.5	10.1	88.3	216.6	45.4	10.3
22	E-2	5.4	18.7	7.1	47.1	78.3	69.2	13.1
23	E-3	5.9	38.1	8.5	4.9	57.4	23.3	63.4
24	E-4	9.0	19.8	16.0	228.7	273.5	89.5	6.5
25	E-6	1.1	1.8	0.0	2.9	5.8	50.0	0.0
26	E-7	166.1	13.6	132.7	1005.3	1317.7	86.4	11.7
27	E-8	70.9	13.9	131.0	884.7	1100.5	92.3	12.9
28	F-1	2.8	0.0	2.0	17.8	22.6	87.6	10.1
29	F-2	9.1	0.0	4.1	48.1	61.3	85.2	7.9
30	F-3	10.7	0.0	4.2	49.6	64.5	83.4	7.8
31	F-4	6.2	0.0	20.5	69.9	96.6	93.6	22.7
32	G-1	11.3	0.0	6.2	39.3	56.8	80.1	13.6
33	G-2	13.8	0.0	6.7	57.6	78.1	82.3	10.4
34	H-1	4.3	17.6	7.1	55.8	84.8	74.2	11.3
35	H-2	12.8	0.0	13.0	80.1	105.9	87.9	14.0
36	I-1	12.2	0.0	9.8	64.0	86.0	85.8	13.3
37	I-2	7.4	31.2	6.3	2.6	47.5	18.7	70.8
38	I-3	11.6	0.0	7.2	70.3	89.1	87.0	9.3
39	I-4	15.5	0.0	9.4	81.0	105.9	85.4	10.4
40	J-1	17.7	0.0	14.0	96.6	128.3	86.2	12.7
41	J-3	10.9	0.0	5.9	61.8	78.6	86.1	8.7
42	J-4	2.1	7.0	0.0	1.4	10.5	13.3	0.0
43	K-1	2.8	0.0	2.1	21.2	26.1	89.3	9.0
44	K-2	28.4	0.0	18.5	213.7	260.6	89.1	8.0
45	K-3	6.4	0.0	4.4	45.3	56.1	88.6	8.9
46	K-4	13.5	0.0	9.6	173.9	197.0	93.1	5.2
47	K-5	8.3	0.0	3.6	55.6	67.5	87.7	6.1
48	K-6	4.2	0.0	3.3	23.4	30.9	86.4	12.4
49	L-2	17.7	102.9	16.9	0.0	137.5	12.3	100.0
50	L-3	18.5	0.0	12.1	58.2	88.8	79.2	17.2

Table 3–1. Continued

	Sample	As(III) ng/ml	As(V) ng/ml	MMA ng/ml	DMA ng/ml	Total As ng/ml	(MMA + DMA) /Total As (%)	MMA/ (MMA + DMA)(%)
51	L-4	2.5	0.0	3.0	17.2	22.7	89.0	14.9
52	L-5	3.7	8.2	1.7	13.3	26.9	55.8	11.3
	average	16.6	8.3	15.5	104.3	144.7	78.3	16.0
	Max	166.1	108.5	132.7	1005.3	1317.7	93.6	100.0
	Min	0.0	0.0	0.0	0.0	5.8	12.3	0.0

Table 3–2. Arsenic Species in Urines Obtained from Mamudpur Village

	Sample	As(III) ng/ml	As(V) ng/ml	MMA ng/ml	DMA ng/ml	Total As ng/ml	(MMA + DMA) /Total As (%)	MMA/ (MMA + DMA)(%)
60	M-1	82.7	0.0	107.2	317.9	507.8	83.7	25.2
61	M-2	21.4	1020.0	50.6	12.5	1104.5	5.7	80.2
62	M-3	0.0	158.9	24.8	313.8	497.5	68.1	7.3
63	M-4	11.0	1624.5	27.3	44.9	1707.7	4.2	37.8
64	M-6	57.0	245.7	62.5	342.0	707.2	57.2	15.5
65	N-2	7.3	120.6	52.4	265.2	445.5	71.3	16.5
66	O-1	130.5	10.2	122.3	154.4	417.4	66.3	44.2
67	O-2	18.9	68.8	91.3	360.0	539.0	83.7	20.2
68	O-3	46.0	4.1	60.6	373.4	484.1	89.7	14.0
69	O-4	97.6	782.4	32.1	38.7	950.8	7.4	45.3
70	P-1	48.2	0.0	57.0	169.4	274.6	82.4	25.2
71	P-2	85.2	5.1	86.6	317.1	494.0	81.7	21.5
72	P-3	55.2	15.8	62.2	280.7	413.9	82.8	18.1
73	P-4	14.9	140.4	32.5	21.6	209.4	25.8	60.1
74	Q-1	18.6	0.0	15.8	90.7	125.1	85.1	14.8
75	Q-2	10.4	0.0	16.1	88.0	114.5	90.9	15.5
76	Q-3	32.4	0.0	15.5	88.1	136.0	76.2	15.0
77	Q-4	15.5	0.0	9.1	78.4	103.0	85.0	10.4
78	R-1	7.0	0.0	8.0	82.8	97.8	92.8	8.8
79	R-2	6.7	0.0	6.4	93.7	106.8	93.7	6.4
80	R-3	5.4	6.6	4.4	27.3	43.7	72.5	13.9
81	R-4	31.8	0.0	11.5	172.8	216.1	85.3	6.2
82	S-1	44.7	13.0	35.7	312.1	405.5	85.8	10.3
83	S-2	53.0	48.8	69.9	445.4	617.1	83.5	13.6
84	S-3	74.6	13.5	86.0	181.0	355.1	75.2	32.2
85	T-1	1.7	0.0	4.6	25.2	31.5	94.6	15.4
86	U-1	5.2	10.9	7.2	25.9	49.2	67.3	21.8
87	U-3	4.7	0.0	3.7	41.7	50.1	90.6	8.1
	average	24.5	58.3	25.5	136.1	243.3	75.2	18.9
	Max	166.1	1624.5	132.7	1005.3	1707.7	94.6	100.0
	Min	0.0	0.0	0.0	0.0	5.8	4.2	0.0

Nazareno, Durango, Mexico and reported that the ratios of (MMA + DMA) to total arsenic were 86.8% and 66.2%, respectively. In our previous study,[12] we also reported that the normal ratios of (MMA + DMA) to total arsenic ranged from 72.0 to 96.5%. As shown in Tables 3-1 and 3-2, these ratios from urines of E-1, E-3, E-6, I-2, J-4, L-2, L-5, M-2, M-4, M-6, O-4 and P-4 were 45.4, 23.3, 50.0, 18.7, 13.3, 12.3, 55.8, 5.7, 4.2, 57.2, 7.4 and 25.8 %, respectively. As shown in Table 3-3, the ratios of (MMA + DMA) to total arsenic ranged from 84.0 to 96.3%. So the result suggests that those habitants

Table 3–3. Arsenic Species in Urines Obtained from Bakshipur Village

	Sample	As(III) ng/ml	As(V) ng/ml	MMA ng/ml	DMA ng/ml	Total As ng/ml	(MMA + DMA) /Total As (%)	MMA/ (MMA + DMA)(%)
89	V-1	3.6	0.0	6.4	22.1	32.1	88.8	22.5
90	V-2	1.2	0.0	4.7	26.1	32	96.3	15.3
91	W-1	6.3	0.0	7.2	27.1	40.6	84.5	21.0
92	W-2	2	0.0	3	13.7	18.7	89.3	18.0
93	W-3	2.4	0.0	4.9	18.3	25.6	90.6	21.1
94	X-1	6.3	0.0	11	22	39.3	84.0	33.3
95	Y-1	5	0.0	7.8	45.8	58.6	91.5	14.6
96	Y-2	3.7	0.0	5.3	26.1	35.1	89.5	16.9
97	Y-4	7.2	0.0	7.4	33.7	48.3	85.1	18.0
	average	4.2	0.0	6.4	26.1	36.7	88.8	20.1
	Max	7.2	0.0	11.0	45.8	58.6	96.3	33.3
	Min	1.2	0.0	3.0	13.7	18.7	84.0	14.6

might be damaged on both As(III) methyltransferase and MMA(III) methyltransferase. Especially, the urines of M-2, M-4 and O-4 contained the total arsenic of 1104.5, 1707.7 and 950.8 ng/ml urine and the ratios of As(V) to total arsenic were 92.3, 95.1 and 82.2%, respectively. These habitants had so the lower arsenic-methylating capacity that most part of inorganic arsenic might be excreted without metabolizing the inorganic arsenic to the organic arsenic. But we could not find out the arsenic manifestation such as Bowen's disease, hyperkeratosis and melanosis from three habitants. Also we estimated the ratio of MMA to (MMA + DMA) in urine. The ratios obtained from urines of A-1 to U-3 ranged from 0 to 100% and those average was 18.2%. The ratios obtained from urines of V-1 to Y-4 who lived in the control area ranged from 14.6 to 33.3% and those average was 20.1%. The ratios of MMA to (MMA + DMA) from E-3, I-2, L-2, M-1 and P-4 were 63.4, 70.8,100.0, 80.2 and 60.4%, respectively. The result suggests that these five habitants might have the low activity of MMA(III) methyltransferase.

We estimated the presence of arsenic species in urines among the habitants having the normal metabolism for arsenic. At first we selected the A to L families who lived in Skrikrishnapur village because these families used the same two tubewell water below the Indian guideline of arsenic in drinking water at 50 ppb and were relational each other. But we eliminated the data of arsenic species obtained from E-1, E-3, E-6, I-2, J-4, L-2 and L-5 caused by the possibility of damage to the arsenic-methylating enzymes. We could use 45 data out of 52 data for analysis. The ratios of (MMA + DMA) to total arsenic of 45 data ranged from 69.2 and 93.6% and their average was 85.6%. Also the ratios of MMA to (MMA + DMA) were from 5.2 to 46.3% and their average was 12.8%. The averages of As(III), As(V), MMA and DMA were 2.9, 16.9, 118.0 and 156.0 ng/ml urine, respectively. We estimated the relationship between the arsenic species each other in 45 urines obtained from the normal arsenic metabolism in A to L families because of using the same two tubewell waters. Statistically, there was not a good correlation between As(V) and the other three species. But, as shown in Figs. 3a to 3c, the relationships between As(III) and MMA, As(III) and DMA, MMA and DMA, and DMA and total As were in good agreement ($p < 0.01$). Next we used the data of arsenic

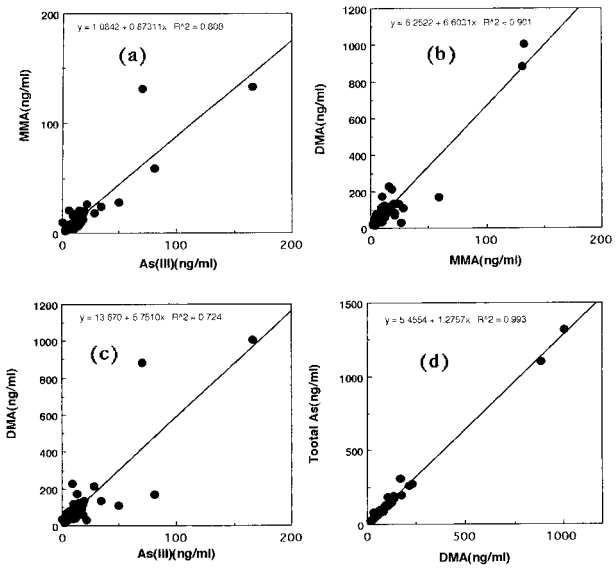

Fig. 3. Relationship between Arsenic Species Each Other in Urines

species obtained from the habitants of M-1 to U-3 who lived in Mamudpur village and took the drinking waters from the different tubewells. In that case we eliminated 5 data from M-2, M-4, M-6, O-4 and P-4 caused by the ratio of (MMA + DMA) to total As having 5.7, 4.2, 57.2, 7.4 and 25.8%, respectively. When estimating the correlation between arsenic species each other using 26 out of 31 data, there were the statistic correlations between As(III) and MMA, As(III) and DMA, MMA and DMA, and DMA and total As ($p < 0.05$). In our previous study,[12] we reported the same correspondence regarding to the relationships between each arsenic species without As(V) using 37 habitants who had the normal arsenic metabolism. These evidence might reflect the arsenic metabolism such as the metabolic pathway of As(V) → As(III) → MMA → DMA.

We estimated the relationship between total arsenic in urines and age and could not find out a good correlation statistically between total arsenics and ages.

In order to estimate the difference of the arsenic metabolism between the adult male and adult female, we picked up the couples from 10 families of A, B, I, K, O, Q, R, S, V and W and showed the arsenic species of each person. Total arsenics from male urine ranged from 26.1 to 507.8 ng/ml urine and those average was 176.3 ng/ml urine. Also total arsenics from female urines were from 18.7 to 1104.5 ng/ml urine and those average was 275.3 ng/ml urine. The averages of total arsenics from female urines were 1.5 times higher than that from male urines. There were the good correlations between MMA from male and female and total arsenic from male and female ($p < 0.01$).

In order to estimate the relationship between the arsenic in drinking waters and the total arsenic in urines, we counted the average of total arsenics obtained from each family member and showed the result in the Fig. 4. In the case of the families of A to L, we used the average of arsenic concentrations in two tubewell waters. There was no correlation statistically between the arsenic in drinking waters and the total arsenic in urines ($p < 0.05$). The result suggests the possibility of the contribution of other sources like foodstuffs except the drinking water regarding to the excretion of arsenic species from the urines.

In conclusion, as results of our field surveys of tubewell waters and human urines obtained from the arsenic-affected district, Mushidabad in West Bengal, India, we got the following findings.

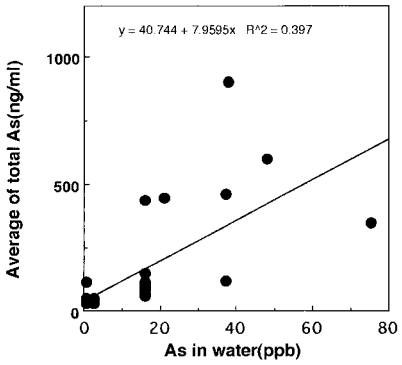

Fig. 4. Relationship between As in Tubewell Waters and Average of Total as in Urines from Families

The arsenic concentrations in tubewell waters ranged from 0.66 to 75.5 ppb.

The concentrations of As(III), As(V), MMA and DMA in urines obtained from 81 persons, who lived in the arsenic-affected area, ranged from 0 to 166.1, from 0 to 1624.5, from 0 to 132.7 and from 0 to 127.4 ng/ml urine, respectively and those average were 23.1, 59.0, 24.6 and 127.4 ng/ml urine, respectively. The average of total arsenic was 234.1 ng/ml urine.

On comparison with the ratio of (MMA + DMA) to total arsenic, the average was 77.6%, but the ratios obtained from 11 persons were from 4.2 to 57.2%. The result suggests that those habitants might be damaged on both As(III) methyltransferase and MMA(III) methyltransferase.

On comparison with the ratio of MMA to (MMA + DMA), the ratios of MMA to (MMA + DMA) from E-3, I-2, L-2, M-1 and P-4 were 63.4, 70.8,100.0, 80.2 and 60.4%, respectively. The result suggests that these five habitants might have the low activity of MMA(III) methyltransferase.

When estimating the arsenic species in urines obtained from A to L families, the relationship between As(III) and MMA, As(III) and DMA or MMA and DMA in urines were in good agreement ($p < 0.01$).

The MMA and total arsenic in urines between male and female of 10 couples showed a good correlation. The averages of total arsenics from female urines were 1.5 times higher than that from male urines.

The relationship between the average of total arsenic in urines obtained from each family member and the arsenic concentrations in tubewell waters was not in agreement.

Acknowledgements We would like to express our sincere gratitude to Dr. Chakraborti and his coworkers of School of Environmental Studies, Jadavpur University, Kolkata, West Bengal, India. We acknowledge the help of the Ministry of Environment for the financial support of Global Environment Research Found.

REFERENCES

1) Chowdhury, U. K., Biswas, B. K., Roy Chowdury, T., Samanta, G., Mandal, B. K., Basu, G. K., Chanda, C. R., Lodh, D., Saha, K. C., Mukherjee, S. C., Roy, S., Kabir, S., Quamarzzaman, Q. and Chakraborti, D. (2000) Groundwater arsenic contamination in Bangladesh and West Bengal, India. *Environ. Health Perspect.*, **108**, 393–397.
2) Smith, A. H., Lingas, E. O. and Rahman, M. (2000) Contamination of drinking water of arsenic in Bangladesh. A public health emergency. *Bull. World Health Organ.*, **78**, 1093–1103.
3) Mandal, K. M., Ogra, Y. and Suzuki, K. T. (2001) Identification of dimethylarsinous and monomethylarsonous acid in human urine of the arsenic-affected areas in West Bengal, India. *Chem. Res. Toxicol.*, **14**, 371–378
4) Tseng, W. P., Chu, H. M., Fong, J. M., Lin, C. S. and Yen, S. (1968) Prevalence of skin cancer in an endemic area of chronic arsenicism in Taiwan. *J. Natl. Cancer Inst.*, **40**, 453–463.
5) Berg, M., Tran, H. C., Nguyen, T. C., Pham, H. V., Schertenkeib, R. and Giger, W. (2001) Arsenic contamination of groundwater and drinking water in Vietnam: A human health threat. *Environ. Sci. Technol.*, **35**, 2621–2626.
6) Christen, K. (2001) The arsenic threat worsens. *Environ. Sci. Technol.*, **35**, 286A–291A .
7) Chakraborti, A. K. and Saha, K. C. (1987) Arsenical dermatitis from tubewell water in West Bengal. *Ind. J. Med. Res.*, **85**, 326–334.
8) Chowdhury, T., Basu, G. K., Mandal, B. K., Biswas, B. K., Chowdhury, U. K., Chanda, C. R., Lodh, D., Roy, S. L., Saha, K. C., Roy, S., Kabir, S. and Quamruzzaman, Chakraborti D. (1999) Arsenic poisoning in the Ganges delta. *Nature* (London), **401**, 545–546.
9) Chowdhury, U. K., Biswas, B. K., Chowdhury, T. R., Samanta, G., Mandal, B. K., Basu, G. C., Chanda, C. R., Lodh, D., Saha, K. C., Mukherjee, S. K., Roy, S., Kabir, S., Quamruzzaman, Q. and Chakraborti, D. (2000) Groundwater arsenic contamination in Bangladesh and West Bengal, India. *Environ Health Perspect.*, **108**, 393–397.
10) Rahman, M. M., Chowdhury, U. K., Mukherjee, S. C., Mondal, B. K., Paul, K., Lodh, D., Biswas, B. K., Chanda, C. R., Basu, G. K., Saha, K. C., Roy, S., Das, R., Palit, S. K., Quamruzzaman, Q. and Chakraborti, D. (2001) Chronic arsenic toxicity in Bangladesh and West Bengal, India — a review and commentary. *J. Toxicol. Clin. Toxicol.*, **39**, 683–700.
11) Rahman, M. M., Mukherjee, D., Sengupta, M. K., Chowdhury, U. K., Lodh, D. C., Roy, S., Selim, M., Quamruzzaman, Q., Milton, A. H., Shahidullah, S. M., Rahman, M. T. and Chakraborti, D. (2002) Effectiveness and reliability of arsenic field testing kits: are the million dollar screening projects effective or not? *Environ. Sci. Technol.*, **36**, 5385–5394.
12) Tokunaga, H., Roychowdhury, T., Chandraskaran, N., Uchino, T. and Ando, M. (2002) Urinary arsenic species in an arsenic-affected area of West Bengal, India. *Appl. Organometal. Chem.*, **16**, 406–414.
13) Roychowdhury, T., Uchino, T., Tokunaga, H. and Ando, M. (2002) Survey of arsenic in food composites from an arsenic-affected area of West Bengal, India. *Food Chem. Toxicol.*, **40**, 1611–1621.
14) Calderson, R. L., Hudgens, E., Le, X. C., Schreinemachers, D. and Thomas, D. J. (1999) Excretion of arsenic in urine as a function of exposure to arsenic in drinking water. *Environ. Health Perspect.*, **107**, 663–667.
15) Zakharyan, R., Wu, Y., Bogdan, G. M. and Aposhiam H. V. (1995) Assay, partial purification, and properities of arsenite methyltransferrase and monomethylarsonic acid methyltransferase of rabbit liver. *Chem. Res. Toxicol.*, **8**, 1029–1038.
16) Del Razo, L. M., Garcia-Vargas, G. G., Vargas, H., Albores, A., Gonsebatt, M. E., Montero, R., Ostrosky-Wegman, P., Kelsh, M. and Cebrian, M. E. (1997) altered profile of urinary arsenic metabolites in adults with chronic arsenicism. A pilot study. *Arch. Toxicol.*, **71**, 211–217.