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Variation in Arsenic Speciation and Concentration in Paddy Rice Related to Dietary Exposure

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Ingestion of drinking water is not the only elevated source of arsenic to the diet in the Bengal Delta. Even at background levels, the arsenic in rice contributes considerably to arsenic ingestion in subsistence rice diets. We set out to survey As speciation in different rice varieties from different parts of the globe to understand the contribution of rice to arsenic exposure. Pot experiments were utilized to ascertain whether growing rice on As contaminated soil affected speciation and whether genetic variation accounted for uptake and speciation. USA long grain rice had the highest mean arsenic level in the grain at 0.26 μg As g^{-1} ($n = 7$), and the highest grain arsenic value of the survey at 0.40 μg As g^{-1}. The mean arsenic level of Bangladeshi rice was 0.13 μg As g^{-1} ($n = 15$). The main As species detected in the rice extract were As^{III}, DMA^{V}, and As^{V}. In European, Bangladeshi, and Indian rice 64 $\pm$ 1% ($n = 7$), 80 $\pm$ 3% ($n = 11$), and 81 $\pm$ 4% ($n = 15$), respectively, of the recovered arsenic was found to be inorganic. In contrast, DMA^{V} was the predominant species in rice from the USA, with only 42 $\pm$ 5% ($n = 12$) of the arsenic being inorganic. Pot experiments show that the proportions of DMA^{V} in the grain are significantly dependent on rice cultivar ($p = 0.026$) and that plant nutrient status is effected by arsenic exposure.

Introduction

Considerable concern exists globally about arsenic (As) in potable/drinking waters extracted from contaminated aquifers (*1*). The situation is at its worst in the Bengal Delta, with over 80 million people living in zones with arsenic above 50 μg L^{-1} in their groundwater (*1*). It is becoming apparent that ingestion of drinking water is not the only elevated source of arsenic to the diet in the Bengal Delta. Irrigation of paddy rice fields with arsenic-contaminated groundwater has led to arsenic build-up in paddy soil, with subsequent elevation in rice grain arsenic (*2*). Meharg and Rahman (*2*) calculated that, even at background levels (0.1−0.2 μg As g^{-1}), the arsenic in rice contributes considerably to arsenic ingestion in affected areas of Bangladesh. Also illustrating this point is the World Health Organization's (WHO) provisional maximum tolerable daily intake (MTDI) of arsenic of 2 μg kg^{-1} bodyweight per day (*3*). If rice grain with an arsenic level of 0.1 μg g^{-1}, which is typically found in the literature (Table 1), is consumed at a rate representative of a rice-based subsistence diet, 0.42 kg dry wt/day (*18*), then ingestion of 0.7 μg kg^{-1}, which is 35% of the MTDI, occurs. Levels far in excess of 0.1 μg As g^{-1} in rice grain have been repeatedly reported for Bangladesh and West Bengal (Table 1). The cooking of raw rice with contaminated water could further increase grain arsenic concentration by 10−35% (*19*).

Total dietary studies in Europe and the United States (*15, 20, 21*) show that rice is the primary source of arsenic in a nonseafood diet. The average 25−30-year-old male from the United States consumes approximately 140 g per day of grain product, which includes rice. This accounts for approximately 5% of mean food intake (*21*). However, for many ethnic groups in the United States, daily rice consumption would typically be much higher. In populations reliant on subsistence rice diets, such as Bangladesh, rice provides 73% of calorific intake (*22*).

The forms of arsenic present in rice for direct food use need to be better characterized, as inorganic species are believed to be more toxic than methylated species (*23*). The studies to date are relatively limited. Arsenite (As^{III}), arsenate (As^{V}), and dimethylarsinic acid (DMA^{V}) are the predominant species present (*14, 15, 24, 25*).

A wide survey of the variation in As speciation and concentration of different rice varieties from different parts of the world was conducted; the results were then related to dietary As exposure. Pot experiments were conducted to ascertain whether soil arsenic concentration affected rice As speciation and whether genetic variation accounts for uptake and speciation; effects on yields and nutrient status were also explored.

Methodology

Rice Survey Sample Selection. The sampling strategy was designed to assess variation in dietary arsenic intakes attributed to ingesting rice. By comparing and contrasting the As content of Bangladeshi rice against rice sourced from globally important rice producing regions, it was hoped that any existing arsenic elevation trends would become apparent. Rice was chosen to reflect differences in grain type (e.g., short, long, medium, and aromatic) and also in processing method (e.g., polishing, parboiling, and flaking).

By obtaining examples from large supermarkets in the city of Aberdeen (Scotland), it was possible to obtain a wide range of commercially available rice types which had been sourced from various locations worldwide and were marketed for direct human food use. Market surveys are widely used to monitor the safety of global food supplies (*4, 12, 13, 15, 21*).

Bangladeshi rice was obtained from a wholesale market, which supplies rice to shops and other smaller markets, in Dhaka (Bangladesh). The majority of the samples were Aman (wet season) rice. Two samples of Boro (dry season) rice, BRRI dhan 28 and 29, were also included as these are available for much of the year. Samples were representative of what residents would consume daily.

Sample Preparation. Duplicate subsamples were taken from each sample and powdered using a model MM2 ball mill (Retsch, Germany). NIST SRM 1568a rice flour used as certified SRM required no special preparation. To assess the water content of the rice, 0.5-g subsamples ($n = 20$) chosen to reflect origin and varietal variation were dried in an oven for 24 h at 100 °C. The average water content of the rice was 10 $\pm$ 0.1%, which agreed well with previous studies (*5*).

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TABLE 1. Summary of Arsenic Levels in Rice (reported as μg/g) in the Literature[a]

district	total As concentration, μg/g min	max	mean	*n*	arsenic contamination	survey range	reference	estimated contribution to MTDI (%)
Bangladesh								
Rajshahi	0.03	0.34	0.10	24	±	district	(4)	33
	0.03	0.28	0.11	17	±	market	(4)	37
	0.07	0.17	0.12		±	aman	(5)	39
	0.03	0.30	0.13	15	±	market basket	current study	43
	0.04	0.27	0.14	10	±	field	(6)	45
	0.11	0.33	0.18		±	boro	(5)	61
Pabna	<0.01	0.34	0.20	6	+	boro, district	(7)	67
	0.11	0.36	0.24	4	+	cooked food	(8)	79
Jessore	0.11	0.94	0.28	21	+	village	(4)	93
Nawabganj	<0.01	0.99	0.33	8	+	boro, district	(7)	110
Sonargaon	<0.05	1.23	0.46	12	+	field, district	(9)	153
Srinagar	<0.05	1.52	0.48	9	+	field, district	(9)	160
	0.09	1.84	0.50	13	±	wide	(2)	165
	0.05	1.66	0.53	6	±	field	(8)	177
Dinajpur			0.54	9	+	field, district	(9)	180
Gopalganj	<0.10	0.18	0.57	6	+	boro, district	(7)	190
Rajbari	0.05	2.05	0.76	8	+	boro, district	(7)	255
Faridpur	0.21	1.50	0.95	7	+	boro, district	(7)	317
China								
	0.31	0.70	0.49	11	+	contam. paddy soils	(10)	
			0.93		+	contam. paddy soils	(11)	
Taiwan								
	<0.10	0.14	0.05	137	±	barn	(12)	11
	<0.10	0.63	0.10	280	±	market basket	(12)	22
	0.06	0.17	0.12	6	-	wide	(13)	27
	0.19	0.22	0.20	3	-	farm	(13)	44
United States								
	0.11	0.34	0.24	7	-		(14)	36
	0.11	0.40	0.26	7	-	market basket	current study	39
	0.20	0.46	0.30	4	-	market basket	(15)	45
Vietnam								
	0.03	0.47	0.21	31	+	wide	(16)	
West Bengal								
	0.04	0.18	0.11	2	+	village	(17)	
	0.11	0.44	0.21	6	+	village	(17)	
	0.18	0.43	0.33	3	+	village	(17)	

[a] Contribution of inorganic arsenic to Bangladeshi MTDI assumes that 80% of total arsenic is inorganic, that body weight is 60 kg, and that consumption rate is 0.5 kg per day. Contribution to U.S. MTDI assumes that 42% of total arsenic is inorganic, body weight is 70 kg, and consumption rate is 0.5 kg per day. Contribution to Taiwanese MTDI assumes that 62% of total arsenic is inorganic (Schoof et al. (*13*)), body weight is 70 kg, and consumption rate is 0.5 kg per day.

Chemicals. AnalaR Nitric acid (HNO_3) (70%) and trifluoroacetic acid (TFA) ($C_2HF_3O_2$) (99%) were obtained from Fisher Scientific and Lancaster, respectively. Monosodium arsenate (Na_2HasO_4) and sodium arsenite ($NaAsO_2$), reagent grade, were purchased from Merck.

Methylarsonic acid (MA^V), was purchased from Chem Service MC, West Chester, and dimethylarsinic acid (DMA^V) was purchased from Sigma Chemicals. Rhodium (Rh) was a plasma standard solution ($RhCl_3$) from AlfaAesar. Indium (In) was prepared in-house from indium chloride. The HPLC mobile phase was prepared using ammonium hydrophosphate and ammonium dihydrogen orthophosphate AnalaR from BHD chemicals Ltd. (Poole, England).

Extraction Procedures. For concentrations of total arsenic, milled subsamples (0.1−0.2 g) were weighed into quartz glass digestion tubes, steeped in 2.5 mL of nitric acid, and allowed to predigest/stand overnight at room temperature. The samples were digested at 120 °C, until clear, and then evaporated to dryness at 160 °C. The residue was resuspended in 1.2% nitric acid to a weight of 10 g. Rhodium (100 μL of 100 μg L^{-1}) was added as the internal standard.

To speciate arsenic in rice, TFA extractions were performed. TFA hydrolyzes starch in the grain during digestion, which can result in a more successful recovery than a methanol/water extraction (*26*). Additionally, TFA extraction is not plagued by argon chloride inference problems as found with hydrochloric acid digestions (*14*). Due to shifts in redox potential TFA can reduce arsenate to arsenite; therefore, this method is only suitable in determining the levels of total inorganic arsenic. Spiking experiments have shown that TFA has little effect in the conversion of MA^V or DMA^V to inorganic arsenic (*26*).

Milled subsamples (0.25 g) were weighed into quartz digestion tubes and 2 mL of 2 M TFA was added. The mixture was allowed to stand overnight. The tubes were then placed on a heating block at 100 °C for 6 h. The digest was evaporated to dryness at 160 °C and made up to a weight of 10 g with distilled water.

Total Arsenic Detection. An ICP-MS 7500 (Agilent Technologies) was used to determine total arsenic concentration. Arsenic sensitivity was monitored daily and optimized when required. The elements measured were As (m/z 75) and Rh (m/z 103). The following m/z 77, 78, 82 were measured to identify polyatomic $Ar^{40}Cl^{35}$ interferences on m/z 75. Corrections for interference from $Ar^{40}Cl^{35}$ were not found to be necessary for the samples analyzed. Concentration was determined using a five-point calibration of 0, 1, 5, 10, and 20 μg of As L^{-1}. Subsamples were randomized prior to analysis. Standards were run after every set of 10 subsamples.

Arsenic Speciation. Chromatographic columns were obtained from Hamilton and consisted of a precolumn (11.2 mm, 12−20 μm) and a PRP-X100 10-μm anion-exchange

column (150 × 4.1 mm). A HP1100 HPLC system (Agilent Technologies, Stockport, Cheshire, UK) with cooled auto-sampler and a Peltier controlled column compartment was used for all of the analyses. The auto-sampler was set to 4 °C and the column was set to 30 °C. Injection volume was set at 100 μL of sample and the HPLC mobile phase flow rate was maintained at 1 mL min^{-1}. The mobile phase employed for anion-exchange chromatography, adapted from Heitkemper et al. (*14*), consisted of 6.66 mM ammonium hydrophosphate ($NH_4H_2PO_4$) and 6.66 mM ammonium nitrate (NH_4NO_3), adjusted to pH 6.2 using ammonia. Samples were centrifuged at 150 × G and the supernatant was injected directly onto the HPLC column. Each analysis was performed within 3 days of sample extraction to minimize any changes in speciation during prolonged storage, although samples can be stored for over three months at 4 °C in the dark without significant transformation occurring (*24*). Retention time for the As species was determined using a species mix comprising standards of 50 μg L^{-1} As^{III}, As^{V}, DMA^{V}, and MA^{V}.

Postcolumn Detection of Arsenic. Postcolumn, element-specific detection of arsenic was achieved using an ICP-MS 7500 (Agilent Technologies). The outlet of the HPLC was connected to a T-piece via Teflon tubing. Internal standard was pumped using the ICP-MS's peristaltic pump and mixed with the column effluent in the T-piece, which connected directly to the inlet of the ICP-MS neubulizer. The elements measured were As (m/z 75) and In (m/z 115). Argon chloride interference effects on m/z 75 were monitored. Chromatographic peaks were integrated and their concentration was determined using a five-point calibration of DMA^{V} (0, 5, 10, 15, and 25 μg of As L^{-1}).

Pot Experiment. American rice (*Oryza sativa*) samples were obtained from the International Rice Research Institute (IRRI) and were chosen on the basis of their known variation in susceptibility to straighthead disease, a plant disorder linked to arsenic contamination (*27*). Cultivars Nortai, Star Bonnet, Star Bonnet Short Straw, Lebonnet, and Belle Patna were resistant to straighthead disease, while Dawn was susceptible.

Rice seeds ($n = 10$ for each cultivar) were germinated and then transferred to 1-L pots containing uniformly packed Tillycorthie clay-rich subsoil, with a total As concentration of 31.3 μg g^{-1}, as used and characterized in the experiments of Abedin et al (*26*). The pots were painted black and had no drainage holes. The soils were either untreated or had monosodium arsenate added to a concentration of 100 μg As g^{-1} soil dry weight. The plants were then grown in a tropical greenhouse (average temperature 25 °C) with sodium lighting providing a supplement of 150 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) until plants had set seed (4−6 months). Five rice plants replicated each treatment. Pots were watered daily with deionized water to maintain flooded conditions (i.e., saturation to permanent immersion of the soil up to 3−4 cm of solution). After seed set, seeds and shoots were harvested. Biomass was obtained along with the total number of grains and the number of filled grains for each plant. Seeds were then husked and seed dry weight was determined.

All samples for chemical analysis were oven-dried overnight and then ball milled. Arsenic analysis on seeds and shoot was determined as above. Nitrogen and carbon content was determined using a NCS analyzer (Fison 1500, Elemental Microanalysis). Phosphorus was determined using flow injection colorimetric analysis (*28*).

Statistics. All statistics were performed using general linear modeling (GLM) and conducted using Minitab v.14 (State College, PA). Total arsenic levels in rice data were ranked before analysis to normalize distribution.

TABLE 2. Analysis of Variance of Market Rice Origin and Color on the Levels of Grain Arsenic

	total [As]			
	degrees freedom	adjusted means square	*F*	*P*
Bangladeshi, U.S., Indian, or European rice	3	1158.22	20.31	<0.001
brown or white rice	1	0.47	0.01	>0.05
error	36	57.04		

Results and Discussion

Arsenic Level of Rice Grain. Evidence from pot experiments (*26, 29−31*) and actual field samples clearly show that growing rice on arsenic-contaminated land (*2, 4, 7−9, 11, 16, 17*) results in elevated arsenic levels in rice grain. In the market rice survey we found highly significant differences ($P < 0.001$) (Tables 2−4, and Figure 1) in total arsenic concentration between Bangladeshi, U.S., Indian, and European rice. No differences were observed between white (polished) and brown rice (unpolished) ($P > 0.05$). U.S. long-grain rice had the highest mean grain arsenic level of 0.26 μg As g^{-1} ($n = 7$) and the highest grain arsenic value of the survey at 0.40 μg As g^{-1} (Figure 1). This agrees well with other U.S. rice surveys, which range from mean values of 0.24 to 0.30 μg g^{-1} ($n = 11$ samples) (Table 1).

The mean arsenic level from Bangladeshi rice was 0.13 μg As g^{-1} ($n = 15$), in a range from 0.03 to 0.30 μg As g^{-1} (Figure 1). This is similar to a previous wet season rice survey (*5*), with a mean arsenic grain level of 0.12 μg As g^{-1} (0.07 to 0.17 μg As g^{-1}). However, large variability exists in the mean arsenic values of other Bangladeshi rice surveys, 0.10−0.95 μg As g^{-1} ($n = 151$ samples) (Table 1) with the maximum level of arsenic in a rice grain sample being 2.05 μg As g^{-1} reported in Islam et al (*7*). This is mirrored by the large variability in soil arsenic level of individual paddy fields in Bangladesh (3.1−42.5 μg As g^{-1}) (*2*).

European rice had a mean arsenic level of 0.18 μg As g^{-1} ($n = 7$), in a range from 0.13 to 0.22 μg As g^{-1} (Figure 1). Basmati rice from India possessed the lowest mean arsenic level at 0.05 μg As g^{-1}, with little variation, only in the range of 0.03 to 0.08 μg As g^{-1} (Figure 1). Rice surveys in arsenic-affected areas in the Murshidabad district, West Bengal show greater elevation and more variation in arsenic levels ranging from 0.04 to 0.43 μg As g^{-1} (*17*). Indian, Bangladeshi, European, and U.S. rice from this survey would contribute 18, 46, 63, and 91%, respectively, to the MTDI, assuming a consumption rate of 0.42 kg dry wt/day and a body weight of 60 kg. The consumption of 0.42 kg dry wt of the Indian and Bangladeshi rice from this survey combined with 2 L (*32*) of water containing 50 μg of As per L, the safe limit in these countries, would equate to a dietary exposure for the people of India and Bangladesh of 121 and 154.6 μg As per day, respectively.

The ICP-MS detection limit for total As analysis for the market rice survey was 0.1 μg As L^{-1} and from the pot experiment 0.46 μg As L^{-1}. Assuming sample weights of 0.1 g are maintained, these analyses had detection limits of 0.01 and 0.05 μg As per g of rice, respectively. The combined recovery of As from the NIST CRM 1568a rice flour was 97 ± 4% ($n = 5$) and the spike recovery (1 mL of 100 μg As/L) was 111 ± 3% ($n = 5$).

Quantification of Arsenic Species in Market Rice. The main species detected were As^{III}, DMA^{V}, and As^{V} (Tables 3 and 4 and Figure 2); quantifiable amounts of MA^{V} were observed in one Indian sample and also in the CRM. In European, Bangladeshi, and Indian rice 64 ± 1 ($n = 7$), 80 ± 3 ($n = 11$), and 81 ± 4% ($n = 15$), respectively, of the

TABLE 3. Arsenic Speciation of Supermarket Rice by Origin

rice type	grain	total arsenic μg As g^{-1}	DMA^V μg As g^{-1}	MA^V μg As g^{-1}	$As^{III} + As^V$ μg As g^{-1}	species sum μg As g^{-1}	extraction efficiency (%)	organic arsenic (%)	inorganic arsenic (%)	contribution to MTDI (%)[a]
					United States					
white	long	0.17 ± 0.01	0.05	<LOD	0.05	0.10	59 ± 3	31 ± 1	27 ± 2	18
		0.21	0.17	<LOD	0.02	0.19	92	82	10	8[b]
		0.23 ± 0.02	0.09	<LOD	0.07	0.16	68 ± 5	37 ± 7	31 ± 2	25
		0.28 ± 0.01	0.10	<LOD	0.11	0.21	73 ± 7	34 ± 3	39 ± 4	39
		0.28	0.17	<LOD	0.09	0.26	93	60	33	33[b]
		0.30	0.17	<LOD	0.09	0.26	86	56	30	33[b]
		0.34	0.24	<LOD	0.10	0.33	97	69	28	34[b]
		0.40 ± 0.01	0.26	<LOD	0.08	0.34	85 ± 0	65 ± 1	20 ± 1	29
brown	long	0.11 ± 0.00	0.04	<LOD	0.06	0.10	91 ± 5	32 ± 2	59 ± 2	21
		0.16	0.06	<LOD	0.10	0.16	99	38	61.38	35
		0.29 ± 0.04	0.11	<LOD	0.12	0.23	81 ± 0	40 ± 2	42 ± 0	43
		0.34 ± 0.02	0.15	<LOD	0.14	0.29	86 ± 16	45 ± 11	41 ± 5	50
					India					
white	basmati	0.03 ± 0.00	<LOD	<LOD	0.02	0.02	69 ± 5	6 ± 1	63 ± 6	7
		0.04 ± 0.00	0.01	<LOD	0.02	0.02	57 ± 5	14 ± 0	43 ± 4	7
		0.04 ± 0.01	0.01	<LOD	0.03	0.03	86 ± 8	23 ± 4	63 ± 4	11
		0.04 ± 0.00	<LOD	<LOD	0.03	0.03	74 ± 9	9 ± 9	64 ± 1	11
		0.04 ± 0.00	0.01	<LOD	0.02	0.03	73 ± 1	25 ± 3	48 ± 4	7
		0.05 ± 0.00	0.01	<LOD	0.03	0.04	88 ± 5	23 ± 4	65 ± 1	11
		0.05 ± 0.01	0.01	0.01	0.02	0.04	61 ± 11	25 ± 10	36 ± 2	7
		0.06 ± 0.00	<LOD	<LOD	0.04	0.04	71 ± 1	4 ± 4	67 ± 5	14
		0.06 ± 0.00	0.01	<LOD	0.03	0.04	65 ± 10	19 ± 4	45 ± 5	11
brown	basmati	0.07 ± 0.01	<LOD	<LOD	0.04	0.04	67 ± 8	6 ± 3	61 ± 4	14
red	long	0.08 ± 0.00	0.01	<LOD	0.05	0.06	72 ± 8	6 ± 3	65 ± 6	18
					Italy					
risotto	medium	0.22 ± 0.01	0.08	<LOD	0.14	0.22	103 ± 2	38 ± 1	65 ± 1	50
		0.22 ± 0.01	0.09	<LOD	0.12	0.21	96 ± 1	41 ± 1	55 ± 0	43
brown	medium	0.19 ± 0.03	0.05	<LOD	0.10	0.15	77 ± 2	24 ± 4	53 ± 2	36
					Spain					
paella		0.17 ± 0.01	0.05	<LOD	0.08	0.13	78 ± 3	30 ± 5	48 ± 2	29
					Taiwan					
white		0.19	0.03	0.015	0.12	0.16	84	23	61	42[c]
		0.20	0.03	0.02	0.11	0.16	81	23	58	41[c]
		0.76	0.05	0.06	0.51	0.61	81	14	67	181[c]
					Thailand					
jasmine	long	0.11 ± 0.01	0.03	<LOD	0.08	0.11	98 ± 7	24 ± 6	74 ± 1	29
					Europe					
pudding		0.13 ± 0.00	0.04	<LOD	0.08	0.12	93 ± 1	30 ± 3	62 ± 2	29
flaked		0.14 ± 0.01	0.04	<LOD	0.06	0.10	72 ± 2	27 ± 1	44 ± 1	21
ground		0.20 ± 0.01	0.06	<LOD	0.1	0.16	79 ± 4	28 ± 2	51 ± 3	36
					Canada					
wild rice	long	0.02 ± 0.01	<LOD	<LOD	0.01	0.01	83 ± 5	12 ± 12	71 ± 17	4
		0.11	0.01	<LOD	0.08	0.09	84	8	76	30[b]

[a] Contribution of inorganic arsenic to MTDI assumes a body weight of 70 kg and a consumption rate of 0.5 kg per day. [b] Ref 14. [c] Ref 13.

recovered arsenic was inorganic (Figure 2). Schoof et al. (*13*) report similar values of 61, 58, and 67% for Taiwanese rice. In contrast DMA^V was the predominant species in rice from the United States, with only 42 ± 5% (n = 12) inorganic arsenic (Figure 2). These data concur with Schoof et al. (*15*) who report that DMA^V contributes 54% (n = 4) to the arsenic in the grain of U.S. rice. This study found highly significant differences (P < 0.001) (Table 5) in the percentage of recovered inorganic arsenic between Bangladeshi, U.S., Indian, and European market rice. No significant differences were observed between white (polished) and brown (unpolished) rice (P > 0.05).

Thailand is the largest exporter of rice in the world, supplying the global market with more than 6 000 000 t of milled rice each year (*33*). We only had one sample of Thai rice, which had a total arsenic concentration of 0.11 μg g^{-1}; however, we found that 91% of the arsenic detected was inorganic.

The HPLC−ICP-MS analysis detection limit for speciation ranged between 0.04 and 0.30 μg As L^{-1}, determined as three times the standard deviation on the measured 5 μg of As L^{-1} standard solution. This is comparable with other arsenic speciation studies (*24, 34*). Therefore, assuming sample weights of 0.25 g are maintained, our analysis has detection limits between 0.002 and 0.012 μg As g^{-1} rice. NIST CRM 1568a rice flour was used to validate the method. Although not certified for arsenic species, its speciation had been characterized prior to this study (Table 6). The mean total recovery ((sum of species recovered from HPLC separation obtained from the TFA extraction/total [As] from nitric acid extraction) × 100) for the analysis was 80 ± 12% (n = 16), which is consistent with other studies (Table 6). Procedural blanks revealed no As contamination.

Pot Experiment Results

Yields. Arsenic application had no statistical effect (at P > 0.05) on straw biomass compared to the control, where there were varietal differences on straw biomass (P = 0.005) (Figure 3, Table 7). Application of arsenic did, however, statistically reduce husked grain yield (P = 0.004), which also showed

TABLE 4. Arsenic Speciation and Dietary Exposure of Bangladeshi Market Rice

rice variety	rice distribution	origin (district)	grain	total arsenic μg As g^{-1}	DMA$^{V)}$ μg As g^{-1}	As^{III} + As^{V} μg As g^{-1}	species sum μg As g^{-1}	extraction efficiency (%)	organic arsenic (%)	inorganic arsenic (%)	contribution to MTDI (%)[a]
Chinigura	speciality	Chapai	medium	0.03 ± 0.00	0.01	0.01	0.02	97 ± 23	49 ± 19	48 ± 4	4
Bashphoo	local	Bhairab	medium	0.04 ± 0.00	<LOD	0.03	0.03	70 ± 14	1 ± 1	69 ± 13	13
Kataribogh	speciality	Dinajpur	long	0.06 ± 0.00	0.01	0.04	0.05	77 ± 4	17 ± 4	60 ± 0	17
Parija	local	Dinajpur	long	0.07 ± 0.00	0.01	0.03	0.04	53 ± 3	11 ± 3	42 ± 1	13
Bashphool	local	Dhaka	medium	0.09 ± 0.01	<LOD	0.05	0.05	59 ± 3	4 ± 4	55 ± 1	21
Nazirshai	common	Sherpur	long	0.09 ± 0.00	0.02	0.06	0.07	80 ± 8	17 ± 8	63 ± 3	25
Parija	local	Natore	long	0.10 ± 0.00	0.01	0.07	0.08	84 ± 8	15 ± 9	69 ± 0	29
Parija	local	Chapai	long	0.10 ± 0.00	0.03	0.04	0.07	70 ± 3	25 ± 1	44 ± 2	17
Nazirshail	common	Dhaka	long	0.14 ± 0.01	0.01	0.09	0.10	70 ± 7	3 ± 3	64 ± 7	38
BRRI dhan28	common	Natore	long	0.15 ± 0.00	0.01	0.10	0.10	69 ± 5	6 ± 1	63 ± 5	42
Zami	speciality	Sylhet	medium	0.17 ± 0.01	0.02	0.09	0.11	60 ± 7	10 ± 8	53 ± 1	38
Parija	local	Bogra	long	0.20 ± 0.02	0.04	0.11	0.15	75 ± 2	21 ± 2	54 ± 1	46
Parija	local	Rajshahi	long	0.21 ± 0.02	0.05	0.12	0.17	83 ± 1	24 ± 0	59 ± 1	50
Miniket	common	Kushtia	long	0.22 ± 0.01	0.04	0.19	0.23	103 ± 4	16 ± 0	86 ± 4	79
BRRI dhan29	common	Tangail	long	0.30 ± 0.01	0.03	0.21	0.24	82 ± 2	11 ± 2	71 ± 0	88

[a] Contribution of inorganic arsenic to MTDI assumes a body weight of 60 kg and a consumption rate of 0.5 kg per day.

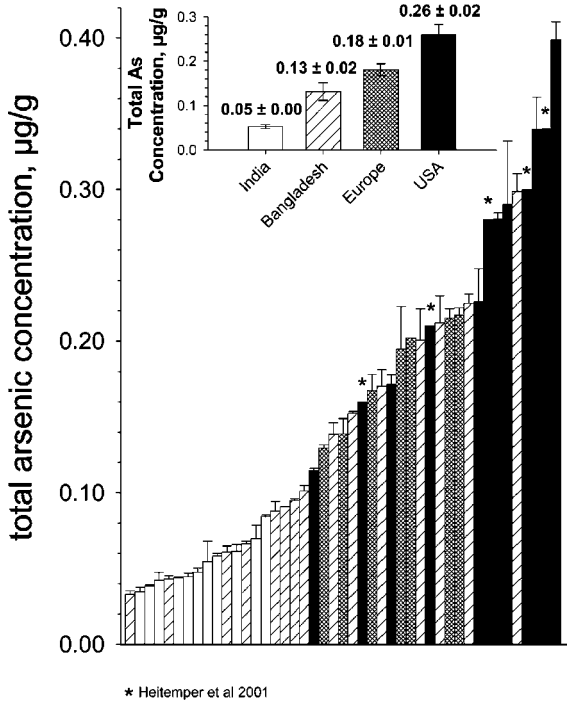

FIGURE 1. Total arsenic in market rice. The legend shows the mean (±SE) of total arsenic levels in rice for India (white), Bangladesh (striped), Europe (cross-hatched), and the United States (black).

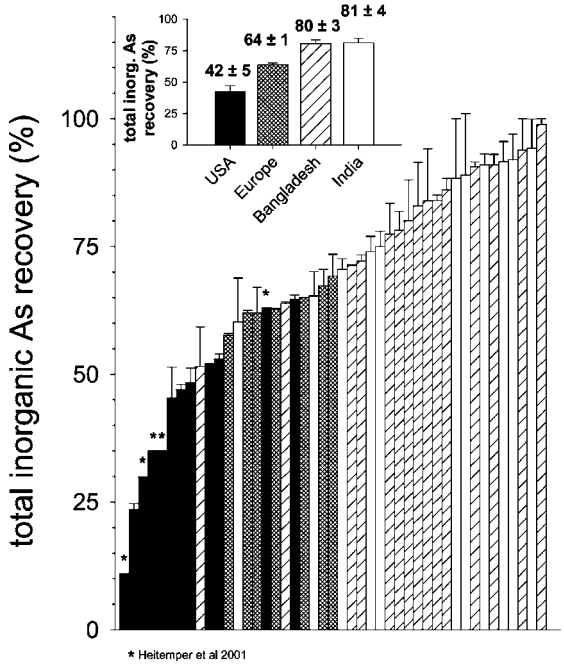

FIGURE 2. Percentage inorganic As in market rice, from species sum. The legend shows the mean (±SE) of the percentage inorganic arsenic levels in rice from the United States (black), Europe (cross-hatched), Bangladesh (striped), and India (white).

strong varietal influence ($P < 0.001$). There were no significant varietal × arsenic interactions for straw or grain weight. The percentage of filled grains (Table 7) was significantly different for the variety term. The range of percentage unfilled grains was considerable, with Belle Patna having ~10% unfilled grains and Star Bonnet Short Straw, at the other extreme, having ~70% unfilled.

Arsenic Translocation to Shoot and Grain. In all arsenic-treated plants, shoot arsenic increased compared to the control, but the magnitude of this increase varied considerably among varieties (Figure 4). Shoot arsenic only increased ~50% for Star bonnet, but increased 400% for Labelle. All statistical terms were statistically significant, including variety × arsenic interaction (Table 8). All statistical terms were also significant for grain arsenic, but unlike for shoots, for some varieties grain arsenic remained nearly constant (e.g., Belle Patna, Lebonnet, Dawn), while others had increased grain arsenic with arsenic treatment (Labelle, Star Bonnet, Nortai, Star Bonnet Short Straw). Arsenic levels in grain varied considerably in non-arsenic-amended plants, with Star Bonnet Short Straw having the lowest grain arsenic at 0.14 μg g^{-1} dry wt, and Lebonnet having the highest at 0.32 μg g^{-1}

TABLE 5. Analysis of Variance of Market Rice Origin and Color Effects on Arsenic Speciation

	% inorganic As[a]			
	degrees freedom	adjusted means square	*F*	*P*
Bangladeshi, U.S., Indian, or European rice	3	1182.5	7	<0.001
brown or white rice[b]	1	2	0.01	0.914
error	36	168.9		

[a] % inorganic As = ([inorganic As recorded by HPLC−ICP-MS]/[total As]) × 100. [b] White = polished rice. Brown = unpolished rice.

TABLE 6. Comparison of the Speciation Analysis of Rice Flour Reference Material NIST CRM 1568a as Reported in the Literature, Using Different Extraction Techniques

total arsenic μg As g^{-1}	n	DMA^V μg As g^{-1}	MA^V μg As g^{-1}	total inorganic As μg As g^{-1}	species sum μg As g^{-1}	extraction efficiency	DMA arsenic (%)	inorganic arsenic (%)	extraction	ref
0.29	3	0.17 ± 0.009	0.008	0.09 ± 0.002	0.27	94	63	34	2M TFA	14
0.31		0.165 ± 0.008	0.014	0.11 ± 0.003	0.29	94	57	38	methanol:water	25
0.29	16	0.16 ± 0.024	0.002	0.08 ± 0.014	0.24 ± 0.04	80 ± 12	54	26	2M TFA	this study
0.29	13	0.16 ± 0.004	0.013	0.106	0.28	96	57	38	α-amylase + methanol/water	24

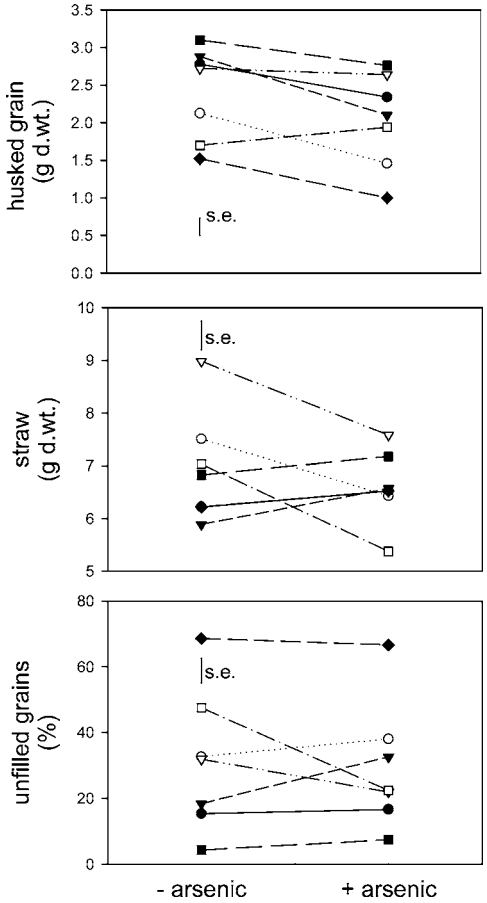

FIGURE 3. Yields of rice grown in a control soil and one amended with arsenic. Results are shown for seven different rice cultivars grown in a no-arsenic-added control soil (−arsenic) and a soil amended with arsenic (+arsenic). Symbols denote different rice cultivars: black diamond = Starbonnet SS; black square = Nortai; black triangle = Labelle; black circle = Belle Patna; white square = Starbonnet; white triangle = Lebonnet; white circle = Dawn.

dry wt. There is also a clear pattern that the three varieties with highest grain arsenic in the no-added-arsenic control did not increase grain arsenic on arsenic amendment, while the four varieties with lower grain arsenic in the unamended treatments all enhanced grain arsenic. The explanation for this may be found in Figure 5 where shoot arsenic is plotted against grain arsenic. Arsenic concentrations in grain rise sharply with increasing shoot arsenic up to a threshold shoot concentration of 2 mg kg^{-1} arsenic dry wt., beyond which grain arsenic plateaus at higher shoot arsenic concentrations.

Plant Nutrient Status. Shoot carbon (C) generally tends to increase on arsenic exposure (Figure 4), with both arsenic and variety terms being highly significant (Table 7). For some varieties (Belle Patna, Dawn, Star Bonnet), shoot C stays constant or decreases slightly. The increase in C is clearer for

TABLE 7. Analysis of Variance of the Yields of Rice Grown in a Control Soil and One Amended with Arsenic

	degrees freedom	adjusted means square	*F*	*P*
husked grain weight				
variety	6	3.58	13.41	<0.001
arsenic	1	2.3446	8.78	0.004
variety × arsenic	6	0.3041	1.14	0.352
error	55	0.2669		
percent grain filled				
variety	6	3791.4	18.06	<0.001
arsenic	1	60	0.29	0.595
variety × arsenic	6	395.9	1.89	0.1
error	55	209.9		
straw weight				
variety	6	5.489	3.58	0.005
arsenic	1	2.555	1.67	0.202
variety × arsenic	6	2.281	1.49	0.2
error	55	1.534		

the grain, with all varieties increasing on arsenic exposure, with highly significant arsenic and variety terms in the statistical model (Table 7). Grain and shoot C increase by a maximum of ~10%, whereas shoot and grain nitrogen (N) increases are much more pronounced (Table 7); Lebonnet's shoot N content increase was the most dramatic, rising by 78%. For grain this increase was highly significant for the variety and arsenic terms of the GLM model, while all three terms of the model were highly significant for shoots. With the larger increase in N than C, C:N ratio fell considerably in the arsenic-treated plants, with shoots showing a highly significant variety × arsenic interaction.

Grain phosphorus (P), in contrast to N and C, fell consistently on arsenic exposure, with both variety and arsenic terms in the GLM model being significant. The situation for shoot P was more complicated with the P content of some varieties rising and others falling. Only the variety term of the model was significant (Table 7).

Quantifying Arsenic Species from the Pot Experiment. The main species detected were As^{III} and DMA^V. The proportion of DMA^V in the grain is significantly dependent on cultivar (P = 0.026) (Tables 9 and 10). Despite a significant increase in grain arsenic between the control and spike treatments, the proportion of DMA^V is not significantly altered (P > 0.05) (Table 10) and no significant interaction between cultivar and arsenic treatment was observed (P > 0.05) (Table 10). Control treatments of Lebonnet, Dawn, Nortai, and Star Bonnet had proportions of DMA^V of 7 ± 1, 26 ± 2, 23 ± 20, and 33 ± 18%, while for Star Bonnet Short Straw and Belle Patna proportions of DMA^V were 54 ± 2 and 56 ± 2% (Table 9). In the spiked treatment only Lebonnet showed a rise in the proportion of DMA^V, from 7 ± 1 to 22 ± 1%.

Cultivar had a significant effect (P = 0.008) (Table 9) on the percentage of arsenic recorded by HPLC−ICP-MS compared to total arsenic. Arsenic treatment had a highly

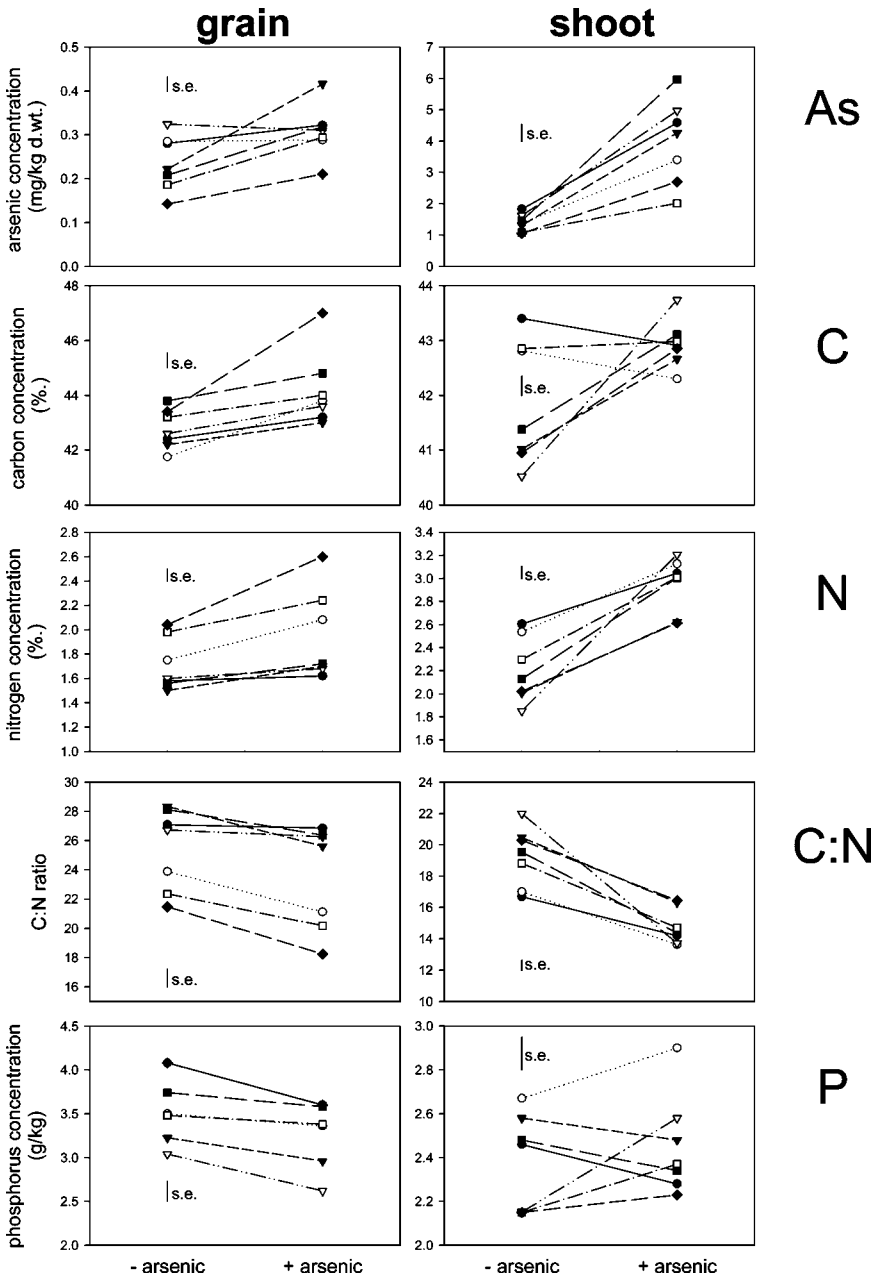

FIGURE 4. Dynamics of As, C, N, and P in rice grown in a control soil and one amended with arsenic. Results are shown for seven different rice cultivars grown in a no-arsenic-added control soil (−arsenic) and one amended with arsenic (+arsenic). Symbols denote different rice cultivars: black diamond = Starbonnet SS; black square = Nortai; black triangle = Labelle; black circle = Belle Patna; white square = Starbonnet; white triangle = Lebonnet; white circle = Dawn.

significant effect ($P = 0.001$) (Table 9) on the percentage of arsenic recovered. Interactions between cultivar and arsenic treatment were highly significant ($P < 0.000$). Although it is unknown whether the losses result from extraction or separation, plants grown on the arsenic-spiked soil generally exhibited decreased recoveries. This effect was most prevalent in Dawn, Nortai, and Star Bonnet, which showed recovery deceases from ~100% to ~50% with arsenic-grown plants. However, Belle Patna showed increased recovery by 27% from control to spiked treatments. The CRM mean recovery for the analysis was 81% ($n = 6$).

Arsenic Intake for Rice. Table 1 compiles all the available studies (that the authors were aware of) which report arsenic levels in rice from West Bengal and Bangladesh, with additional information on arsenic rice levels from other regions of the world. In total, Table 1 provides unequivocal evidence that arsenic is elevated in regions of West Bengal and Bangladesh where paddy fields are irrigated with arsenic-contaminated water.

What are the health implications of elevated arsenic in rice? The only national or international standard set for food is the Australian limit of 1 mg kg^{-1} d wt (*35*). This level is somewhat spurious in the context of a subsistence rice diet. Australians have a high standard of living and health. The level of 1 mg kg^{-1} was set with regard to high seafood intake of Australians, since many seafood items are arsenic-elevated. These seafood items typically contain the relatively nontoxic forms such as arseno-sugars or arsenobetaine (*23, 34*). Rice grain arsenic speciation is dominated by the more toxic forms of inorganic arsenic and DMA^V. Furthermore, this Australian standard was not set with regard to the provisional Maximum Tolerable Daily Intake (MTDI) for inorganic arsenic of 2 μg kg^{-1} body mass given by the WHO in 1993 (*3*). The WHO has since failed to ratify or address the MTDI (*36*).

TABLE 8. Analysis of Variance of the Dynamics of As, C, N, and P in Rice Grown in a No-Arsenic-Added Control Soil and One Amended with Arsenic, with Different Rice Cultivars

	degrees freedom	adjusted means square		*F*		*P*	
		grain	shoot	grain	shoot	grain	shoot
			arsenic				
variety	6	0.03	6.25	5.17	4.41	<0.001	<0.001
arsenic	1	0.09	117.77	18.24	83.08	<0.001	<0.001
variety × arsenic	6	0.01	3.50	2.50	2.47	0.033	0.035
error	55	0.01	1.42				
			carbon				
variety	6	9.12	2.54	6.56	4.18	<0.001	0.002
arsenic	1	35.44	20.68	25.53	34.03	<0.001	<0.001
variety × arsenic	6	2.76	4.96	1.98	8.16	0.084	<0.001
error	55	1.39	0.61				
			nitrogen				
variety	6	0.84	0.45	17.91	8.31	<0.001	<0.001
arsenic	1	0.93	9.60	19.92	176.98	<0.001	<0.001
variety × arsenic	6	0.08	0.23	1.63	4.22	0.156	0.001
error	55	0.05	0.05				
			carbon:nitrogen				
variety	6	98.90	16.45	13.65	8.46	<0.001	<0.001
arsenic	1	62.17	352.86	8.58	181.52	<0.001	<0.001
variety × arsenic	6	3.35	8.63	0.46	4.44	0.833	0.001
error	55	7.25	1.94				
			phosphorus				
variety	6	1.49	379805.00	5.79	3.44	<0.001	0.006
arsenic	1	1.49	99217.00	4.45	0.90	0.039	0.348
variety × arsenic	6	0.05	128407.00	0.20	1.16	0.976	0.340
error	55	0.26	110550.00				

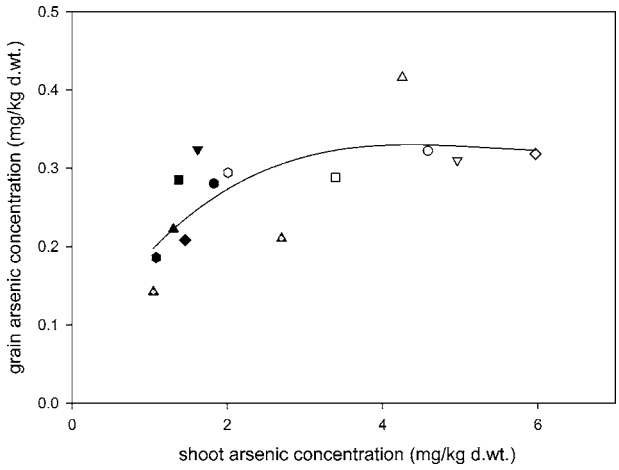

FIGURE 5. Comparing shoot As concentration against grain arsenic concentration.

The proportions of inorganic and organic arsenic in rice grain varies from sample to sample. Until data on the speciation of arsenic in rice are robust enough to effectively predict the risk associated with rice consumption, to ensure complete safety, total arsenic must be assumed to represent the value of inorganic arsenic in rice. Therefore, the daily maximum tolerable intake of arsenic by rice consumption must not exceed 2 μg kg^{-1} body mass. By modeling the rice consumption typical of a rice-based diet at various grain arsenic levels (Tables 1, 3, and 4) the importance of rice to the MTDI becomes apparent. A Bangladeshi weighing 60 kg and drinking 3 L of water containing 50 μg L^{-1} arsenic, the safe limit in Bangladesh, ingests 2.5 μg per kg body mass per day, before eating. Rice in the contaminated areas has an

TABLE 9. Cultivar Effects on Arsenic Speciation in Rice Grown in a Control and an Arsenic-Amended Soil

	As −		As +	
rice cultivar	recovery (%)	DMA (%)	recovery (%)	DMA (%)
Belle Patna	45 ± 6	56 ± 2	72 ± 6	56 ± 4
Dawn	104 ± 13	26 ± 2	52 ± 5	29 ± 4
Lebonnet	63 ± 4	7 ± 1	53 ± 5	22 ± 1
Nortai	95 ± 1	23 ± 20	48 ± 2	36 ± 10
Starbonnet	94	33 ± 18	69 ± 7	31 ± 10
Starbonnet SS	73 ± 5	54 ± 2	79 ± 4	56 ± 4

TABLE 10. Analysis of Variance of Cultivar Effects on Arsenic Speciation in Rice Grown in a Control and an Arsenic-Amended Soil

	degrees freedom	adjusted means square	*F*	*P*
		% DMA[a]		
cultivar	5	863.2	3.99	0.026
As treatment	1	143.1	0.66	>0.05
cultivar × As treatment	5	200.6	0.93	>0.05
error	11	216.2		
		% Recovery[b]		
cultivar	5	378.82	5.61	0.008
As treatment	1	1569.38	23.23	0.001
cultivar × As treatment	5	957.46	14.18	0.000
error	11	67.55		

[a] % DMA = ([DMA recorded by HPLC−ICP-MS]/[total As]) × 100. [b] % Recovery = ([As recorded by HPLC−ICP-MS]/[total As]) × 100.

average of ~200 μg kg^{-1} arsenic (Table 1). If a 60-kg Bangladeshi consumes 0.5 kg of rice per day (*18*), rice will contribute 1.7 μg per kg body mass per day. When added to the consumption in water, total consumption is twice the WHO's maximum tolerable arsenic intake. Localized elevation of arsenic in rice grain only further exacerbates the problem.

Modeling of the recovered inorganic arsenic from the Bangladeshi samples, assuming a consumption rate of 0.5 kg and an average weight of 60 kg (Table 4) shows great variation in the contribution to MTDI (4−88%). The two samples with the highest inorganic arsenic level, 79 and 88% of the MTDI, were varieties of Minikat (grown in districts close to the Indian border) and BRRI dhan29 (a high-yielding variety). Both are commonly available and would be consumed in large quantities (Table 4).

If the model is expanded to cover the rest of the market rice, and the body weight term is increased to 70 kg to reflect a more typical European and American weight, then the importance of speciation becomes apparent. Even though the American rice in this study had the highest total grain levels, when they are modeled the contribution of the recovered inorganic As to the MTDI ranged from only 8 to 50% (Table 3). European rice ranged from 21 to 50% and Indian from 7 to 18% (Table 3). Therefore the health effects resulting from dietary exposure of As from rice will be greater in Bangladesh than in the United States, Europe, and India (excluding West Bengal).

American Rice. The amount of arsenic that has entered the environment as a result of arsenic pesticide and herbicide use is largely unknown. Arsenic pesticide application in the United States was most prevalent in the early- to mid-20th century. However, records on usage volume, pesticide type, and distribution are incomplete (*37*). Estimates suggest that in the 1990s monosodium methylarsonate (MSMA) and disodium methylarsonate (DSMA) herbicide application

alone resulted in an input exceeding 1000 metric t of arsenic into the environment (*38*). Usage of arsenic herbicides is prevalent in Mississippi and Arkansas, traditional cotton (*Gossypium* spp.) growing areas where arsenic compounds were widely used. Some of these areas have subsequently switched to rice production. Although bioavailability and toxicity of organoarsenicals is low, there is evidence of transformations in surface waters, adjacent soils, and groundwaters of herbicide-applied areas (*38*). Methylated arsenicals can be readily demethylated in soil (*39*).

The arsenic−rice situation in the United States is quite unusual in that rice varieties were specifically selected to grow on arsenical-pesticide-treated soils, where rice yields were known to be affected by arsenic, which caused the physiological disorder "straighthead" (*40*). Symptoms range in severity from an increase in blank florets to complete grain failure (*41*). A rice panicle with unfilled grains is clearly visible as it remains upright, hence the name. Wauchope et al. (*30*) found that under U.S. growth conditions in a field trial, rice sprayed with arsenical pesticides could accumulate up to 2.5 μg As g^{-1} in the grain. To our knowledge no surveys have been conducted to determine the levels of arsenic in rice grown on soils previously treated with arsenical pesticides or herbicides entering the U.S. market.

Our research shows that American rice cultivars grown in arsenic-amended soils pass elevated arsenic levels on to their shoots, which results in increases in the grain. The straighthead-susceptible variety, Dawn (*27*), apart from having the highest grain arsenic level in the control treatment, did not vary from the other straighthead-resistant cultivars (*27*). Nutrient dynamics within the grain are complex: under arsenic stress, not only do carbon and nitrogen increase, and phosphorus decreases as was observed in our study, but enzyme activity is additionally altered (*42*). Different cultivars react differently to arsenic stress (*27*). The market survey and the pot experiment showed that American long-grain rice varieties have high levels of DMA^V in the grain compared to other rice varieties, reducing their contribution to the inorganic MTDI. Whether the biomethylation process occurs in the rhizosphere or within the plant is unclear. However, for the proportion of DMA^V found in the grain to be directly linked to the proportion of bioavailable soil arsenic the ratio of bioavailable soil DMA^V would have to be very high to compensate for the low rice root uptake kinetics of DMA^V compared to inorganic arsenic (*43*).

Rice cultivar has an effect on the proportion of DMA^V in the grain and U.S. rice has higher proportions of DMA^V in the grain than Indian, Bangladeshi, and European rice. An emphasis must be given to estimating the total dietary exposure of As to the peoples of Southeast Asia and reducing As intakes to those exceeding the daily permissible thresholds. More work is needed in characterizing the speciation of arsenic in rice and other commonly eaten foods. Producing rice grains that possess a high organic/inorganic arsenic ratio could help to reduce inorganic arsenic exposure. Increases in shoot arsenic level appear to result in sharp increases in grain arsenic level. However, the pot experiment showed that a threshold is reached at a shoot arsenic concentration of approximately 2 mg As kg^{-1} d. wt., after which a plateau effect is observed (Figure 5) resulting in small increases in grain As level compared to shoot As level increases. Further understanding of this mechanism of shoot-to-grain arsenic translocation could be an important tool in reducing grain arsenic levels.

The conclusion of the rice survey was that there is clear variation in As speciation and concentration in rice grown in different countries. When this variation is related to dietary exposure it is evident that countries whose rice is elevated in inorganic As and who are reliant on rice as a dietary staple are most at risk. The conclusion from the pot experiment is that As speciation is not significantly affected by soil As concentration, but that genetic variation does account for differences in uptake and speciation.

Acknowledgments

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