Food Additives and Contaminants, January 2008; 25(1): 41–50

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Contribution of water and cooked rice to an estimation of the dietary intake of inorganic arsenic in a rural village of West Bengal, India

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(*Received 5 December 2006; revised 4 April 2007; accepted 5 April 2007*)

Abstract
Arsenic contamination of rice plants by arsenic-polluted irrigation groundwater could result in high arsenic concentrations in cooked rice. The main objective of the study was to estimate the total and inorganic arsenic intakes in a rural population of West Bengal, India, through both drinking water and cooked rice. Simulated cooking of rice with different levels of arsenic species in the cooking water was carried out. The presence of arsenic in the cooking water was provided by four arsenic species (arsenite, arsenate, methylarsonate or dimethylarsinate) and at three total arsenic concentrations (50, 250 or 500 $\mu g\,l^{-1}$). The results show that the arsenic concentration in cooked rice is always higher than that in raw rice and range from 227 to 1642 $\mu g\,kg^{-1}$. The cooking process did not change the arsenic speciation in rice. Cooked rice contributed a mean of 41% to the daily intake of inorganic arsenic. The daily inorganic arsenic intakes for water plus rice were 229, 1024 and 2000 $\mu g\,day^{-1}$ for initial arsenic concentrations in the cooking water of 50, 250 and 500 μg arsenic l^{-1}, respectively, compared with the tolerable daily intake which is 150 $\mu g\,day^{-1}$.

Keywords: *Arsenic speciation, cooked food, cooking water, organic arsenic, total arsenic.*

Introduction

The world's two biggest cases of groundwater arsenic (As) contamination and the worst sufferings of people have been in Asia; in order of magnitude, these are Bangladesh and West Bengal, India (Rahman et al. 2003). Several million people in West Bengal consume water with As concentrations that exceed by up two orders of magnitude the threshold value of 10 $\mu g\ l^{-1}$ recommended by the WHO (Norra et al. 2005). As contamination of groundwater and illnesses of people have been reported in nine districts out of a total of 18 districts in West Bengal (Roychowdhury et al. 2003), including North-24-Parganas where the present study was located. Groundwater is the main source for drinking, cooking and other household purposes in these As-affected districts. Even the agricultural system is mostly groundwater dependent. In this way, a large amount of As deposits on the irrigated land.

Recently it has been considered that foods are responsible for an important part of As intake, and studies on total As (*t*-As) in food obtained from As-endemic areas have increased in recent years (Roychowdhury et al. 2003; Díaz et al. 2004). A failure to consider the contribution of food intake of As could introduce a substantial bias into the estimation of risks for the population of As-endemic areas (Díaz et al. 2004).

In most As-contaminated areas of West Bengal, the residents depend heavily on rice for their caloric intake (about 70% of the total), suggesting that if their rice is polluted with As it will become an important dietary source of this metalloid (Watanabe et al. 2001). As rice is cultivated in As-contaminated soils under anaerobic conditions (at which As is highly available for plant uptake), the As concentration in rice is high compared with other crops and regions (Carbonell-Barrachina et al. 1998; Abedin et al. 2002; Meharg 2004).

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ISSN 0265–203X print/ISSN 1464–5122 online
DOI: 10.1080/02652030701385233

Correct estimation of As intake should consider the As content not only of the raw product, but also of the contaminant in the product in the form in which it is consumed by the population (raw or cooked). Studies have shown changes in As concentration after cooking of seafood (Devesa et al. 2001) and vegetables (Díaz et al. 1989; She and Kheng 1992). In As-endemic areas the high As content in water used for cooking purposes is a further source of contamination, mainly as inorganic As (*i*-As). This is indicated by the high *t*-As contents found in the studies on *t*-As contents in cooked foods from contaminated areas (Concha et al. 1998; Bae et al. 2002; Roychowdhury et al. 2003; Sengupta et al. 2006). In many areas of West Bengal rice is washed and/or cooked with a substantial amount of water, which is sometimes contaminated with As. The actual amount of As in cooked rice could be either increased by chelation of As in water by binding on rice grains or decreased if water-soluble As is released from rice into the water to be discarded (Bae et al. 2002).

The literature has data for *t*-As contents in raw and cooked rice (Roychowdhury et al. 2003; Islam et al. 2004; Norra et al. 2005), but few values have been reported for *i*-As (Ackerman et al. 2005; Laparra et al. 2005). However, knowledge of *i*-As contents is essential for an evaluation of health risks. Inorganic As has been classified by the International Agency for Research on Cancer (IARC) as a carcinogen to humans (Tsuda et al. 1992; Díaz et al. 2004). Nevertheless, there are no prior references to evaluate the influence of cooking on individual As species in rice from As-affected areas such As West Bengal. To the present authors' knowledge only two studies deal with speciation of As in cooked rice, the first carried out by Laparra et al. (2005) using Spanish rice, and the second carried out by Ackerman et al. (2005) using North American rice.

The aim of the present study was to determine *t*- and *i*-As contents in cooked rice using As-polluted cooking water. Two factors were assayed in the cooking water: As speciation (arsenite, arsenate, methylarsonate or dimethylarsinate) and *t*-As concentration (50, 250 or 500 $\mu g l^{-1}$). Besides, the contribution of cooked rice and drinking water to the daily intake of *t*- and *i*-As of the inhabitants of an As-affected rural village of West Bengal was evaluated.

Materials and methods

Instrumentation

For As speciation analysis, a high-performance liquid chromatography (HPLC) system consisting of a Varian 9012 ternary pump (Varian, San Fernando, CA, USA), a Rheodyne 7125 injector and a 50 µl loop for sample introduction was used. Separations of As species were performed on a Hamilton PRP X-100 anion-exchange column (10 µm, 250 mm × 4.1 mm i.d.; Hamilton, Reno, NV, USA). A guard column packed with the same material (12–20 µm; 25 mm × 2.3 mm i.d.) preceded the analytical column. Hydride generation of volatile arsines before the detection was performed adding on-line solutions of HCl and $NaBH_4$ by means of a Gilson Minipuls 3 peristaltic pump. The quantification of As was performed on a hydride generation system (PSA 10.044, PS Analytical, Kent, UK) using an atomic fluorescence spectrometer system (AFS) (PSA 10.044 Excalibur, PS Analytical) equipped with a boosted-discharge hollow cathode lamp (Photron Pty. Ltd, Vic., Australia). The analogue signal output was connected to a computer equipped with chromatographic software (PS Analytical).

Determination of *t*-As was performed with a Unicam Model Solaar 969 atomic absorption spectrometer equipped with a continuous hydride generator Unicam Solaar VP90 (AAS-HG).

Other equipment used included a hot air oven (Selecta, Barcelona, Spain) with a maximum temperature of 250°C, a grinder (Moulinex, Valencia, Spain), a mechanical shaker Vibromatic (J. P. Selecta S.A., Barcelona, Spain), a centrifuge (Heraeus BioFuge, Heraeus Instruments, Hanau, Germany), a sand bath (Falc, Treviglio, Italy), model BS 70 with a maxim temperature of 200°C, a muffle furnace (Hobersal, Barcelona, Spain), and a lyophilizer (B. Biotech International, Christ Alpha 2–4, Osterode, Germany).

Reagents

Deionized water (18 MΩ cm) was used for the preparation of the reagents and standards. All glassware was treated with 10% v/v HNO_3 for 24 h and then rinsed three times with deionized water before use.

All chemicals were of, at least, pro analysis quality. Commercial standards of $NaAsO_2$ (sodium meta-arsenite) and $Na_2HAsO_4 \cdot 7H_2O$ (sodium hydrogen arsenate) were obtained from Panreac (Barcelona, Spain), while $CH_4AsNaO_3 \cdot 1.5\ H_2O$ (monosodium methylarsonate sesquihydrate, MA) and $(CH_3)_2AsO(ONa) \cdot 3H_2O$ (monosodium dimethylarsinate trihydrate, DMA) were from Supelco (Bellefonte, PA, USA) and Fluka (Buchs, Germany), respectively. Finally, anhydrous trifluoroacetic acid (TFA) was from Sigma (St Louis, MO, USA).

Table I. Total arsenic concentration in raw rice samples procured. All samples were from an arsenic-affected area at North-24-Parganas district, West Bengal.

Sample cultivar (number of samples)	Type of rice	Mean *t*-As ($\mu g\,kg^{-1}$)	Mean *i*-As ($\mu g\,kg^{-1}$)
Khitish, Initial Evolution Trial IET-4094 (3)	*Boro*[a]	272 ± 20	247 ± 7
Jaladhi-2, BAKU (3)	*Boro*[a]	410 ± 11	407 ± 8
Aditya Initial Evolution Trial IET-7613 (3)	*Aus*[b]	178 ± 14	165 ± 7
Biraj, CNM-539 (3)	*Aus*[b]	125 ± 12	116 ± 13
Khitish, Initial Evolution Trial IET-4094 (3)	*Aman*[c]	166 ± 16	140 ± 9
Ratna, IET-1411 (3)	*Aman*[c]	133 ± 14	120 ± 11
Khitish, Initial Evolution Trial IET-7328 (3)	*Aman*[c]	177 ± 16	163 ± 5

[a]*Boro*, irrigation done by groundwater during summer time (November to June).
[b]*Aus*,prekharif (April–September).
[c]*Aman*, irrigation done by rainwater (kharif: June–December).

Study area

The study was conducted in a village of North-24-Parganas district, approximately 25 km from Calcutta, India. The total area of the village is 5.0 km^2, with 22 270 people living in residential area of the village, with the remaining area being cultivated land. The average annual income of the villagers is US$350 per year. The main source of drinking water for the village is 100, mainly shallow, wells and tube wells used for drinking purposes. The village was chosen as the model village in the study because it was known that 70% of its tube wells had As concentrations above 0.05 $mg\,l^{-1}$ and it is highly affected by As contamination in the groundwater.

Food questionnaire

A 24-h dietary recall questionnaire was administered to homes in the studied village. It asked for information about the type and quantity of water and foods ingested the previous day, and how the foods were prepared for their consumption, raw or cooked. The design of the questionnaire was carried out by Miguel Hernandez University (Spain) and approved by the Ramakrishna Vivekananda Mission (West Bengal), a registered society with expertise in working with villagers in the studied area (Calcutta); it was administered by professionals from this society.

The number of interviewees was set at 115 (60 male and 55 female), with ages from above 12 to below 60 years. The selected interviewees were mainly farmers and housewives who normally eat at home, buy or obtain their food from local markets and/or farms, cook themselves, and take drinking water from surrounding tube wells. People working at nearby cities were not included in the survey; they are not representative of the endemic As area because they eat frequently at the city.

Rice samples

Commercial rice samples were collected in farms surrounding the previously cited rural village of the North-24-Parganas district. Rice samples from different periods of the year and varieties were analysed for *t*- and *i*-As concentrations (Table I). As expected *boro* rices contained higher *t*-As and *i*-As concentrations than *aus* and *aman* samples; these higher contents are related to the use of higher volume of As-polluted groundwater for irrigation of the *boro* rice plants (grown during summer time) compared with *aus* and *aman* plants. After a carefully study of information summarized in Table I, it was decided to select one of the *boro* varieties, the khitish rice (IET-4094), for the experiments because *boro* rices represent the highest potential health risks for humans.

Cooking conditions

Worldwide there are three common methods of cooking rice (Sengupta et al. 2006):

- The traditional method still used by more than 90% of the villagers in the Bengal delta: raw rice is washed until the washings become clear (five to six times), the washings are discarded and then the rice is boiled in excess water (five to six times the weight of raw rice) until cooked, finally discarding the remaining water (discard water) by tilting the pan against the lid before serving the rice.
- The rice is washed as above and boiled with a volume of water 1.5 times to twice the weight of rice until no water is left to discard.
- Unwashed rice is boiled with a volume of water 1.5 times to twice the weight of rice; the wash and discard steps are both omitted. This is the contemporary method.

The first experiment was conducted to investigate effect of (1) As species and (2) As concentration on the As content in the cooked rice. The rice was

cooked using deionized water or deionized water spiked with different levels (50, 250 or 500 $\mu g l^{-1}$) of only one of the following four As species: arsenite, arsenate, methylarsonate (MA) or dimethylarsinate (DMA).

The food survey carried out in this study showed that none of the three methods described above was the most popular in the studied rural village; the most popular rice cooking method was between the first and third methods (it is a hybrid in the evolution of the first method towards the third method). The unwashed rice is boiled with a volume of water 1.5 to four times the weight of rice; the wash step is omitted. Thus, the rice (250 g) was added to boiling water (750 ml) and kept under this heat condition until cooked; the remaining water was discarded. No additional ingredients were employed.

The second experiment was carried out to investigate the effect of As speciation in the cooking water on the As speciation in the cooked rice. Rice was cooked in the same way as described for the first experiment; cooking water was spiked with four As species (arsenite, arsenate, MA or DMA) but only one As concentration was studied 250 $\mu g\ As l^{-1}$.

Once cooked, all rice samples were frozen at $-20^{\circ}C$ and then freeze-dried. The lyophilized samples were ground in a domestic apparatus, and the resulting powder was vacuum-packed and kept in the freezer at $-20^{\circ}C$ until analysis. Total As concentrations were measured in dry raw rice, wet cooked rice, cooking water, and discarded starched water.

Quantification of t-As

A 0.250-g portion of lyophilized rice sample was weighed and digested using the ashing method previously described by Muñoz et al. (2000). Calibration standards were prepared using the same HCl concentration of the samples and certified materials. The instrumental conditions used for As determination by HG-AAS were as follows. Reducing agent: 1.4% (m/v) $NaBH_4$ in 0.4% NaOH, 5 $ml\,min^{-1}$; HCl solution: 10% (v/v), 10 $ml\,min^{-1}$; carrier gas: argon, 250 $ml\,min^{-1}$ flow rate; and for atomic absorption spectrometry, wavelength: 193.7 nm; spectral bandpass: 0.5 nm; hollow cathode lamp current setting 8 mA; air/acetylene flame with a fuel flow rate of 0.8 $l\,min^{-1}$.

The certified reference materials (rice flour = NIST SRM 1568a; and bush, branches and leaves = GBW07603) used for testing this analytical method were provided by CYMIT Química, S.L. (Barcelona, Spain) and produced by the US National Institute of Standards and Technology and the Institute of Geophysical and Geochemical Exploration of China, respectively.

Quantification of as species

The method used for the extraction of the As species was that described by Heitkemper et al. (2001). A dried and milled rice sample (0.5 g) was treated with 3 ml of 2 M TFA. The mixture was allowed to stand for 6 h at 100°C in a 60-ml capped HDPE centrifuge tube. The mixture was centrifuged and the supernatant collected and diluted to volume with deionized water. The TFA extracts were filtered through a 0.45 μm nylon syringe filter before analysis by HPLC-HG-AFS.

The As species (arsenite, arsenate, MA and DMA) were determined in the water extract using HPLC-HG-AAS. Separation of the As compounds was carried out in about 15 min in the anion-exchange column using 25 mM phosphate buffer (pH 6.0) as the mobile phase at a 1.1 $ml\,min^{-1}$ flow rate. The elution order was arsenite, DMA, MA and arsenate.

A total of 50 μl of sample were injected in the HPLC system following the instrumental and analytical conditions described in Table II. Under these conditions the retention times were 3.1, 4.2, 5.1 and 8.6 min for arsenite, DMA, MA and arsenate, respectively. Figure 1 shows the separation obtained in: (1) a 50 $\mu g l^{-1}$ standard of arsenite, DMA, MA and arsenate; (2) the certified material NIST SRM 1568a, rice flour; (3) raw rice; and (4) rice cooked using arsenite-polluted water.

External calibration was accomplished using standard concentrations of 1, 10, 20, 30, 40 and 50 $\mu g l^{-1}$ of each of the four As species studied (arsenite, DMA, MA and arsenate).

Information on the certified material NIST SRM 1568a (rice flour) was used to test this analytical method.

Statistical analyses

All data were subjected to analysis of variance (ANOVA) and the Tukey least-significant difference multi-comparison test to determine significant differences among samples (As species and/or As concentration). Statistical analyses were performed using SPSS 12.0 (SPSS Science, Chicago, IL, USA).

Results and discussion

Analytical quality assurance

Total As. The analytical characteristics for the *t*-As methodology were as follows: detection limit, 7 $\mu g\,kg^{-1}$; precision, 2%; accuracy for rice flour (NIST SRM 1568a), found value = $0.29 \pm 0.04\ mg\,kg^{-1}$ (certified value = $0.29 \pm 0.03\ mg\,kg^{-1}$); accuracy for bush, branches and leaves (GBW07603), found value = $1.18 \pm 0.03\ mg\,kg^{-1}$ (certified value = $1.25 \pm 0.10\ mg\,kg^{-1}$).

Table II. Instrumental and analytical conditions for HPLC-HG-AFS.

	HPLC
Column	Hamilton PRP-X100
Guard column	Hamilton PRP-X100
Mobile phase	10 mM K_2HPO_4/KH_2PO_4 adjusted to pH 6.0 (isocratic)
Injection volume	50 µl
Flow rate	0.8 ml min^{-1}
	HG-AFS
Reducing agent	1.4% (w/v) $NaBH_4$ in 0.4% (w/v) NaOH
Flow rate of reducing agent	1.0 ml min^{-1}
HCl	1.5 M
HCl flow rate	1.5 ml min^{-1}
Carrier gas	Argon
Carrier gas flow rate	200 ml min^{-1}
Hydrogen flow rate	60 ml min^{-1}
Resonance wavelength	193.7 nm

As speciation. An estimate of the instrumental detection limit (IDL) for each of the four As species was calculated based on three times the standard deviation of peak area measurements for replicate 50 µl injections of an As standard containing 2.0 µg l^{-1} each of arsenite, arsenate, MAA and DMA. The IDL estimates were 1.7, 1.5, 1.1 and 1.4 µg l^{-1}, respectively. Finally, estimates of the method detection limits (MDLs) were calculated using the IDLs multiplied by a dilution factor of 10 (0.5 g of rice diluted to a final volume of 5 ml). The MDLs were 17, 15, 11 and 14 µg As kg^{-1} fresh matter for arsenite, DMA, MA and arsenate, respectively. The average fortification recoveries (for a 0.75 µg As spike on 10 ml of a 20 µg l^{-1} standard) through the method were 94, 89, 90 and 92% for arsenite, DMA, MA and arsenate, respectively. No significant reduction/oxidation reactions were observed in these fortification studies.

There are no food certified reference materials available for As species and/or *i*-As. The quality criterion adopted for testing the current analytical method, therefore, was the overlapping between the ranges of *i*-As found in a certified rice flour sample (NIST SRM 1568a): 0.082 ± 0.09 mg kg^{-1} and those reported in a previous study carried out by Heitkemper et al. (2001), 0.083 ± 0.06 mg kg^{-1} using the same method of extraction for the As species.

Finally, the *t*-As concentration (sum of the four studied As species) of the NIST SRM 1568a, 0.268 ± 0.028 mg kg^{-1}, was compared with the certified value of the material, 0.290 ± 0.030 mg kg^{-1}, and shows the goodness of the analytical method used.

First experiment 't-As in cooked rice'

The ratio of added water to raw rice was 3:1 (750 ml: 250 g), which is typical of the cooking habits in West Bengal and Bangladesh (Misbahuddin 2003). According to Bae et al. (2002) the water:rice ratio ranges from 3.2:1 to 4.0:1 in Bangladesh, which was significantly higher than the ratio used, for example, in Japan, 1.3:1.

Tables III and IV show the effect of *t*-As concentration and As speciation initially present in the cooking water on the *t*-As concentration in the cooked rice. In general, no effect of *t*-As concentration (Table III) or As speciation (Table IV) was found on the volumes of absorbed water in the cooked rice (absorbed water was measured by drying cooked rice and raw rice at 70°C until constant weights and subtracting these two water contents); although the rice cooked with arsenite retained significantly more water (639 ± 17 ml) than the others (mean of 533 ± 17 ml).

As will be discussed below, results from the calculations suggest that cooked rice could be an important source of As if it is boiled using As-contaminated water. According to the experimental data, 1000 g of cooked rice will correspond to roughly 309 g of raw rice, and about 691 g of contaminated water. Therefore, this cooked rice will provide an additional and substantial burden of As to that coming from the drinking water.

The amount of As in the cooked rice was 2.2–20.0% higher than predicted (from raw rice and absorbed water) for initial As concentrations of 250 and 500 µg As l^{-1}, respectively. These results might suggest either that As in the water is chelated by rice grains, or that As becomes concentrated during the cooking process, because of evaporation.

Díaz et al. (2004) stated that the *t*-As contents in raw food can alter in various ways during cooking treatments, with a consequent effect on the intake of this contaminant. Cooking treatments such as boiling and frying can alter *t*-As content by (1) concentration of As through loss of water, volatiles and, to a lesser extent, certain macronutrients

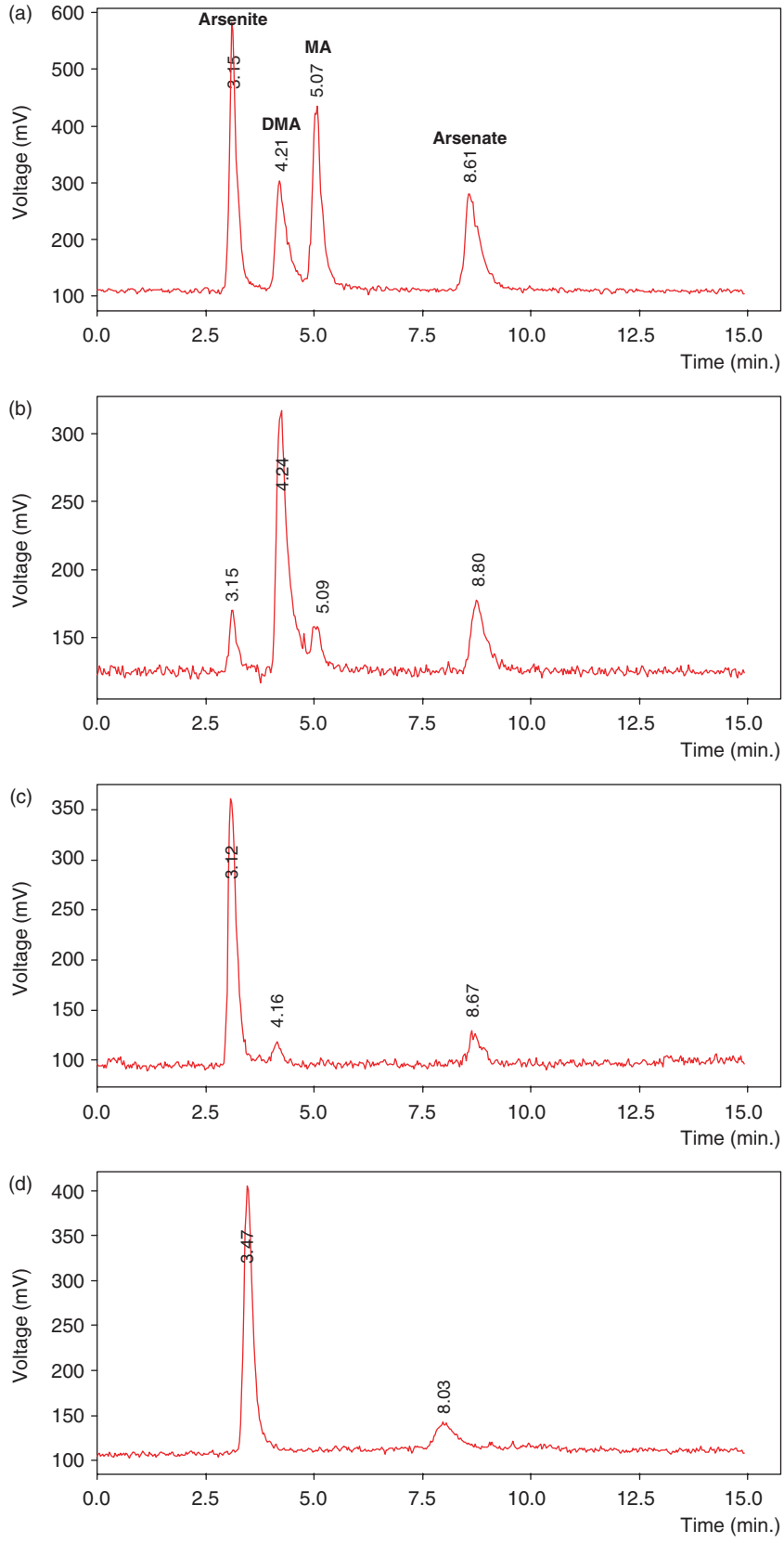

Figure 1. Example of HPLC-HG-AAS chromatograms of arsenic species: (a) standard of 50 $\mu g l^{-1}$ of arsenite, DMA, MA and arsenate; (b) NIST SRM 1568a, rice flour; (c) raw rice; and (d) cooked rice using cooking water polluted with arsenite.

Table III. Effect of the arsenic concentration (50, 250 or 500 $\mu g\,l^{-1}$) present in cooking water on the variables under study in the cooking of rice.

Variable	As in cooking water ($\mu g\,l^{-1}$)	Mean ± ES*
Absorbed water in cooked rice (ml)	50	548 ± 15^{a}**
	250	583 ± 16^{a}
	500	548 ± 15^{a}
Discarded water (ml)	50	202 ± 15^{a}
	250	167 ± 16^{a}
	500	202 ± 15^{a}
As in cooked rice ($\mu g\,kg^{-1}$)	50	227 ± 22^{a}
	250	874 ± 24^{b}
	500	1642 ± 22^{c}
As in discarded water ($\mu g\,l^{-1}$)	50	128 ± 14^{a}
	250	234 ± 14^{b}
	500	492 ± 14^{c}
As retention in cooked rice (%)	50	$(-)30.1 \pm 2.5^{a}$
	250	80.2 ± 2.6^{b}
	500	91.4 ± 2.5^{b}

* Mean ± ES, mean value of three replicates ± standard error.
** Values with the same letters were not significantly different at $p < 0.05$ for the variable studied (Tukey multiple range test).

Table IV. Effect of the arsenic species (arsenite, arsenate, MA or DMA) present in cooking water on the variables under study in the cooking of rice.

Variable	As species	Mean ± ES*
Absorbed water in cooked rice (ml)	Arsenite**	639 ± 17^{a}***
	Arsenate	496 ± 17^{b}
	MA	562 ± 18^{b}
	DMA	540 ± 17^{b}
Discarded water (ml)	Arsenite	111 ± 17^{a}
	Arsenate	254 ± 17^{b}
	MA	188 ± 18^{b}
	DMA	210 ± 17^{b}
As in cooked rice ($\mu g\,kg^{-1}$)	Arsenite	1001 ± 25^{a}
	Arsenate	855 ± 25^{b}
	MA	$915 \pm 27^{a,b}$
	DMA	886 ± 25^{b}
As in discarded water ($\mu g\,l^{-1}$)	Arsenite	230 ± 16^{a}
	Arsenate	298 ± 16^{b}
	MA	308 ± 17^{b}
	DMA	302 ± 16^{b}
As retention in cooked rice (%)	Arsenite	56.4 ± 2.9^{a}
	Arsenate	41.8 ± 2.9^{b}
	MA	50.0 ± 3.1^{ab}
	DMA	40.4 ± 2.9^{b}

* Mean ± ES, mean value of three replicates ± standard error.
** Values with the same letters were not significantly different at $p < 0.05$ for the variable studied (Tukey multiple range test).
*** MA, methylarsonate; DMA, dimethylarsinate.

(carbohydrates, lipids and proteins); and (2) loss of As through solubilization.

On the other hand, when the initial As concentration was 50 $\mu g\ As\,l^{-1}$, the As content in the cooked rice was about 30% lower than expected (Table III). These experimental observations indicate that the concentration of As through loss of water in the cooking process is not a good explanation. Besides, another experimental parameter sustaining this statement is that the ratio of As in initial water to that in the discarded water was close to 1 in samples cooked with water containing 250 and 500 $\mu g\ As\,l^{-1}$ (implying no As concentration due to water evaporation), while it was 0.4 for the initial concentration of 50 $\mu g\ As\,l^{-1}$.

Data on Table IV show a significantly higher As concentration in the rice cooked using polluted cooking water containing arsenite. The mechanisms for capturing *t*-As and *i*-As might be related to the incorporation of water into food during cooking. This statement is supported by the fact that significantly more water was absorbed by the rice cooked with arsenite (639 ± 17 ml) compared with the others (532 ± 11 ml). The high water retention capacity of the rice might be due to the high starch content in this cereal (≅90%), which incorporates a large quantity of water during its gelatinization in the boiling process (Tinarelli 1989).

Second experiment 'As speciation in cooked rice'

As speciation of raw rice demonstrated that As was mainly present as inorganic forms, 206.8 μg arsenite kg^{-1} rice plus 39.7 μg arsenate kg^{-1}, and 25.5 μg DMA kg^{-1} were also found. Considering that 0.250 kg of raw rice were used in the cooking simulations, the total amounts of 51.7 μg arsenite, 9.9 μg arsenate and 6.4 μg DMA were incorporated into the cooking system from the raw rice. Besides, a total of 187.5 μg of each species (arsenite, arsenate, MA and DMA) were incorporated from the spiked cooking water in each of the different simulations. In summary, a total of 255.5 μg As was added to the cooking system from both rice and spiked cooking water.

A control cooking experiment using As-free water was carried out and showed that cooking caused an oxidation of arsenite to arsenate, and a complete transformation of the initial DMA into MA and *i*-As. As was present in this cooked rice as 110.4 μg arsenate kg^{-1}, 76.4 μg arsenite kg^{-1}, and 10.8 μg MA kg^{-1}.

Data on Table V showed that when As was present in the cooking water as inorganic forms (arsenite or arsenate), it was mainly present in the cooked rice as inorganic forms as well. However, about 10% of the final As in the cooked rice was

Table V. Content of arsenic species (arsenite, arsenate, MA and DMA) in cooked rice. In the present cooking process water containing only one arsenic species, at 250 $\mu g l^{-1}$, and a cooking ratio of water:rice of 3:1 (750 ml water: 250 g rice) were used.

Initial As species	As species	Added As (%)	Found As (%)
Arsenite	Arsenite	93.6 (rice + water)[a]	89.9
	Arsenate	3.9 (rice)	10.1
	MA	0	0
	DMA	2.5 (rice)	0
Arsenate	Arsenite	20.2 (rice)	9.9
	Arsenate	77.3 (rice + water)	90.1
	MA	0	0
	DMA	2.5 (rice)	0
MA	Arsenite	20.2 (rice)	6.9
	Arsenate	3.9 (rice)	12.4
	MA	73.4 (water)	80.7
	DMA	2.5 (rice)	0
DMA	Arsenite	20.2 (rice)	7.6
	Arsenate	3.9 (rice)	10.7
	MA	0	0
	DMA	75.9 (rice + water)	81.7

[a]Total of 255.5 μg of As was added to each system (187.5 μg from cooking water and 68.0 μg from raw rice). From this total amount 236.5, 231.9, 242.6 and 234.7 μg were found after cooking in the arsenite, arsenate, MA and DMA systems, respectively.

Table VI. Daily dietary arsenic intake from water and cooked rice. It is assumed that each person drinks daily about 2.5 L of water and eats daily about 0.450 kg of cooked rice.

As in drinking/cooking water ($\mu g l^{-1}$)	AsIDW (μg As day^{-1})	AsIDW (% TAI)	As in cooked rice ($\mu g kg^{-1}$)	AsICR (μg As day^{-1})	AsICR (% TAI)	DAsI (μg As day^{1})
Total arsenic						
50	125	55.0	227	102	45.0	227
250	625	61.4	874	393	38.6	1018
500	1250	62.8	1642	739	37.2	1989
Inorganic arsenic						
50	125	54.6	231	104	45.4	229
250	625	61.0	887	399	39.0	1024
500	1250	62.5	1667	750	37.5	2000

AsIDW, arsenic intake from drinking water; AsICR, arsenic intake from cooked rice; DAsI, total arsenic daily intake.

present under the form of non-added species. For example, when As was initially present in the cooking water as arsenite, 23.8 ± 0.4 μg of arsenite were transformed in arsenate. On the other hand, if As was initially present in the cooking water under organic forms (MA or DMA), no transformation between organic species occurs; however, a significant amount of arsenite was found.

To date the only previous reference found dealing with the contents of *i*-As in cooked foods is the study carried out by Díaz et al. (2004) in Chile, with a maximum concentration of 1.58 $\mu g g^{-1}$ w/w in maize boiled in water containing 0.572 μg As ml^{-1}. The lack of data concerning *i*-As content in cooked samples from As endemic areas show the novelty of the results reported in the present work.

In general, from this section dealing with As speciation in the cooked rice it can be concluded stating that As will be present in the cooked rice in the same form it was initially found in the cooking water and raw rice.

Estimation of As intake and evaluation of risks

From the data provided by the 24-recall questionnaire administered to the inhabitants of the village in North-24-Parganas district — quantity of water (ml) and rice ingested (g) — and the *t*-As and *i*-As concentrations spiked in the cooking water and found in raw and/or cooked rice analysed (Tables III and IV), the intakes of *t*-As and *i*-As were calculated, and, expressed as μg As day^{-1}, are shown in Table VI. According to the food habits survey, the

average daily water intake for adult (mean of males and females) was 2.5 L. This result is relatively low compared with results provided by Roychowdhury et al. (2003), who found that adult males, adult females and children (<10 years of age) consumed 4, 3 and 2 L, respectively, at Murshidabad district, West Bengal. Besides, a mean rice intake of about 450 g for adults was used for the calculations, while Roychowdhury et al. (2003) found rice intakes of about 750 g.

The results proved that the general assumption that As is present in the drinking water and foods mainly under inorganic forms, arsenite and/or arsenate is correct; the As intakes estimated in this study show that the daily *i*-As intake represents between 82 and 99% of the daily *t*-As intake. This is due to the types of foods analysed, water and rice, which and as indicated earlier, *i*-As are the major species.

Díaz et al. (2004) studied the contribution of water, bread and vegetables to the dietary intake of As in a rural village of Northern Chile. They studied these items in two different periods, in which the water used by the population for drinking and cooking purposes contained 0.572 (first period) or 0.041 $\mu g\,ml^{-1}$ (second period). The foods studied contribute to 5% (first period) or 30% (second period) of the *t*-As intake. Consequently, Díaz et al. concluded that the significance of the intake of As from food increases as the concentration of As in water decreases. This result should be taken into account in non-As endemic areas. The main difference between the study of Díaz et al. and the present study carried out in West Bengal is the amount of rice being consumed by the Indian population: 450 $g\,day^{-1}$. With these initial considerations, only rice consumption will contribute 45.0, 38.6 and 37.2% to the final *t*-As intake for cooking water containing 50, 250, and 500 $\mu g\ l^{-1}$, respectively (Table VI).

Assuming a body weight of 70 kg for adults in West Bengal, the reference intakes stated by the FAO/WHO are equivalent to 150 μg *i*-As day^{-1} for adults (WHO 1989, Díaz et al. 2004).

In India the studies carried out by Roychowdhury et al. (2002, 2003) in West Bengal also provide data for *t*-As intake from water and from raw and cooked food. Assuming that at least 50% of the *t*-As in food sample is *i*-As, the maximum intake obtained from water and foods was 708 $\mu g\,day^{-1}$ in adult males, which is 4.7 times greater than the TDI. In this case, the daily intake contributed by foodstuffs (rice, vegetables and spices) was 189 μg, 27% of the daily *t*-As intake.

In the present study the daily As intakes obtained from water plus rice were 227, 1018, and 1989 μg As day^{-1} for initial As concentrations in the cooking water of 50, 250 and 500 μg As l^{-1}, respectively, which are 1.5, 6.8 and 13.3 times greater than the TDI. Similar figures are obtained when inorganic species are considered (Table VI); 229, 1024 and 2000 μg As day^{-1} for initial As concentrations in the cooking water of 50, 250 and 500 μg As l^{-1}, respectively, which are 1.5, 6.8 and 13.3 times greater than the TDI.

Conclusions

The results suggest that rice cooked with As-contaminated water might be an important source of As, especially *i*-As, and that the conditions of the cooking process (the ratio of rice:water and the volume of the discarded water) could affect the amount of this element in the final cooked rice. A dose–response association between As exposure and any health effects might underestimate the health risk of As if the intake of this element from sources other than drinking water is not included. Finally, if cooking water containing low levels of As can be provided to villagers, even if their vegetables are still contaminated with As, cooked items will have an As concentration lower than expected due to migration of some of the As to the cooking water.

Acknowledgements

The paper was produced with the financial assistance of the European Union (Development of a Low Cost Technology for *In-situ* Treatment of Groundwater for Potable and Irrigation Purposes, TIPOT, ASI/B7-301/2598/24-2004/79013), MEC (CTM2005-2425-E), and Caja de Ahorros del Mediterráneo (CAM-2006). The contents of this paper are the sole responsibility of UMH and can under no circumstances be regarded as reflecting the position of the European Union and/or CAM.

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