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

Antonio J Signes, Kasturi Mitra, Francisco M. Burló, Angel A Carbonell-Barrachina

► To cite this version:

Antonio J Signes, Kasturi Mitra, Francisco M. Burló, Angel A Carbonell-Barrachina. Contribution of water and cooked rice to an estimation of the dietary intake of inorganic arsenic in a rural village of West Bengal, India. Food Additives and Contaminants, 2007, 25 (01), pp.41-50. 10.1080/02652030701385233 . hal-00577460

HAL Id: hal-00577460

<https://hal.science/hal-00577460>

Submitted on 17 Mar 2011

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

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

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2006-364.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	04-Apr-2007
Complete List of Authors:	Signes, Antonio; Universidad Miguel Hernández, Agro-Food Technology Mitra, Kasturi; Ramakrishna Vivekanda Mission, Institute of Advanced Studies Burló, Francisco; Universidad Miguel Hernández, Agro-Food Technology Carbonell-Barrachina, Angel; Universidad Miguel Hernández, Agro-Food Technology
Methods/Techniques:	FAAS, Market basket survey, Metals analysis - AAS, Risk assessment
Additives/Contaminants:	Environmental contaminants, Environmental contaminants - Soils, Heavy metals - arsenic
Food Types:	Cooked foods, Rice

SCHOLARONE™
Manuscripts

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

Arsenic contamination of rice plants by arsenic polluted irrigation groundwater could result in high arsenic concentrations in cooked rice. The main objective of this study was to estimate the total and inorganic arsenic intakes in a rural population of West Bengal 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 $\mu\text{g L}^{-1}$). The results showed that the arsenic concentration in cooked rice was always higher than that in raw rice and ranged from 227 to 1642 $\mu\text{g kg}^{-1}$. 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 $\mu\text{g day}^{-1}$ for initial arsenic concentrations in the cooking water of 50, 250, and 500 $\mu\text{g As L}^{-1}$, respectively, compared to the tolerable daily intake which is 150 $\mu\text{g day}^{-1}$.

Key Words: arsenic speciation; cooked food; cooking water; organic arsenic; total arsenic.

Introduction

The world's two biggest cases of groundwater arsenic 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 arsenic concentrations which exceed by up to two orders of magnitude the threshold value of $10 \mu\text{g L}^{-1}$ recommended by the WHO (Norra et al. 2005). Arsenic 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 this study was located. Groundwater is the main source for drinking, cooking and other household purposes in these arsenic-affected districts. Even the agricultural system is mostly groundwater-dependent. In this way, a large amount of arsenic deposits on the irrigated lands.

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

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

Correct estimation of arsenic intake should consider not only the arsenic content of the raw product but also the content 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 arsenic concentration after cooking of seafood (Devesa et al. 2001) and vegetables (She and Kheng 1992; Díaz et al. 1989). In arsenic endemic areas, the high arsenic content in water used for cooking purposes is a further source of contamination, mainly as inorganic arsenic. This is indicated by

the high total arsenic contents found in the studies on total arsenic 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 arsenic. The actual amount of arsenic in cooked rice could be either increased, by chelation of arsenic in water by binding on rice grains, or decreased if water-soluble arsenic is released from rice into the water to be discarded (Bae et al. 2002).

In the literature there are data for total arsenic 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 inorganic arsenic (Ackerman et al. 2005; Laparra et al. 2005). However, knowledge of inorganic arsenic contents is essential for an evaluation of health risks. Inorganic arsenic has been classified by the International Agency for Research on Cancer (IARC) as a carcinogen to humans (Díaz et al. 2004; Tsuda et al. 1992). Nevertheless, there are no prior references to evaluate the influence of cooking on individual arsenic species in rice from arsenic affected areas such as West Bengal. Up to our knowledge there are only two studies dealing with speciation of arsenic in cooked rice, the first one carried out by Laparra et al. (2005) using Spanish rice and the second one carried out by Ackerman et al. (2005) using North-American rice.

The aim of the present study was to determine total and inorganic arsenic contents in cooked rice using arsenic polluted cooking water. Two factors were assayed in the cooking water: 1) arsenic speciation (arsenite, arsenate, methylarsonate, or dimethylarsinate) and 2) total arsenic concentration (50, 250, or 500 $\mu\text{g L}^{-1}$). Besides, the contribution of cooked rice and drinking water to the daily intake of total and inorganic arsenic of the inhabitants of an arsenic-affected rural village of West Bengal was evaluated.

Materials and Methods

Instrumentation

For arsenic 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 arsenic species were performed on a Hamilton PRP X-

1 100 anion-exchange column (10 μm , 250 mm $\times$ 4.1 mm i.d.; Hamilton, Reno,
2 NV, USA). A guard column packed with the same material (12-20 μm ; 25 mm $\times$
3 2.3 mm i.d.) preceded the analytical column. Hydride generation of volatile
4 arsines prior to the detection was performed adding on-line solutions of HCl and
5 NaBH_4 by means of a Gilson Minipuls 3 peristaltic pump. The quantification of
6 arsenic was performed on a hydride generation system (PSA 10.044, PS
7 Analytical, Kent, U.K.) using an atomic fluorescence spectrometer system (AFS)
8 (PSA 10.044 Excalibur, PS Analytical) equipped with a boosted-discharge hollow
9 cathode lamp (Photron Pty. Ltd., Victoria, Australia). The analogical signal output
10 was connected to a computer equipped with chromatographic software (PS
11 Analytical).

12
13 Determination of total arsenic was performed with a Unicam Model Solaar 969
14 atomic absorption spectrometer equipped with a continuous hydride generator
15 Unicam Solaar VP90 (AAS-HG).

16
17 Other equipment used included a hot air oven (Selecta, Barcelona, Spain) with a
18 maximum temperature of 250 $^{\circ}\text{C}$, a grinder (Moulinex, Valencia, Spain), a
19 mechanical shaker Vibromatic (J.P. Selecta S.A., Barcelona, Spain), a centrifuge
20 (Heraeus BioFuge, Heraeus Instruments, Hanau, Germany), a sand bath (Falc,
21 Treviglio, Italy), model BS 70 with a maximum temperature of 200 $^{\circ}\text{C}$, a muffle
22 furnace (Hobersal, Barcelona, Spain) and a lyophilizer (B. Biotech International,
23 Christ Alpha 2-4, Osterode, Germany).

24 25 *Reagents*

26 Deionized water (18 $\text{M}\Omega\text{ cm}$) was used for the preparation of the reagents and
27 standards. All glassware was treated with 10% v/v HNO_3 for 24 h and then rinsed
28 three times with deionized water before use.

29
30 All chemicals were of, at least, pro analysis quality. Commercial standards of
31 NaAsO_2 (sodium meta-arsenite) and $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$ (sodium hydrogen arsenate)
32 were obtained from Panreac (Barcelona, Spain), while $\text{CH}_3\text{AsNaO}_3 \cdot 1.5\text{H}_2\text{O}$
33 (monosodium methylarsenate sesquihydrate, MA) and $(\text{CH}_3)_2\text{AsO}(\text{ONa}) \cdot 3\text{H}_2\text{O}$
34 (monosodium dimethylarsinate trihydrate, DMA) were from Supelco (Bellefonte,
35 PA, U.S.A.) and Fluka (Buchs, Germany), respectively. Finally, anhydrous
36 trifluoroacetic acid (TFA) was from Sigma (St. Louis, MO, USA).

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 km², with 22270 people living in residential area of the village with the remaining area being cultivated land. The average annual income of the villagers is 350 US\$ annum⁻¹. The main source of drinking water for the village is 100, mainly shallow, wells and tube wells, for drinking purposes. The village was chosen as the model village in our 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 in West Bengal. 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 the Miguel Hernandez University (Spain) and approved by the Ramakrishna Vivekananda Mission (West Bengal, India), a registered society with a wide expertise in working with villagers in the studied area (Calcutta), and it was administered by professionals from this society.

The number of interviewees was set at 115 (60 male and 55 female), with ages above 12 years old to below 60 years. The interviewees selected were mainly farmers and housewives who normally eat at home, buy or get 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 endemic arsenic 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 analyzed for total and inorganic arsenic 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 arsenic polluted groundwater for irrigation of the *boro* rice plants (grown during summer time) compared to *aus* and *aman* plants. After carefully study of information summarized in **Table I**, authors decided to select one of the *boro* varieties, the khitish rice (IET-4094), for their experiments because *boro* rices are those representing the highest potential health risks for humans.

Cooking conditions

Worldwide, there are three common methods of cooking rice (Sengupta et al. 2006): a) The traditional method still used by more than 90% of the villagers in Bengal delta: raw rice is washed until the washings become clear (5–6 times), washings are discarded and then the rice is boiled in excess water (5–6 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; b) the rice is washed as in "a" and boiled with water of a volume 1.5–2 times the weight of rice until no water is left to discard; c) unwashed rice is boiled with water 1.5–2 times 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 a) arsenic species and b) arsenic concentration on the arsenic content in the cooked rice. The rice was cooked using deionized water or deionized water spiked with different levels (50, 250 or 500 $\mu\text{g L}^{-1}$) of only one of the following four arsenic 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 in between methods "a" and "c" (it is a hybrid in the evolution of method "a" towards method "c"). The unwashed rice is boiled with a volume of water 1.5-4 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 conditions until cooked; the remaining water was discarded. No additional ingredients were employed.

The **second experiment** was carried out to investigate the effect of arsenic speciation in the cooking water on the arsenic speciation in the cooked rice. Rice was cooked in the same way as described for the first experiment; cooking water was spiked with four arsenic species (arsenite, arsenate, MA, or DMA) but only one arsenic concentration was studied 250 µg As L⁻¹.

Once cooked, all rice samples were frozen at -20 °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 °C until analysis. Total arsenic concentrations were measured in dry raw rice, wet cooked rice, cooking water, and discarded starched water.

Quantification of total arsenic

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 arsenic determination by HG-AAS were as follows: reducing agent: 1.4 % (m/v) NaBH₄ 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 National Institute of Standards and Technology of U.S.A. and the Institute of Geophysical and Geochemical Exploration of China, respectively.

Quantification of arsenic species

The method used for the extraction of the arsenic species was that described by Heitkemper et al. (2001). 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

1 capped HDPE centrifuge tube. The mixture was centrifuged and the supernatant
2 was collected and diluted to volume with deionized water. The TFA extracts were
3 filtered through a 0.45 μm nylon syringe filter prior to analysis by HPLC-HG-AFS.

4
5 The arsenic species (arsenite, arsenate, MA, and DMA) were determined in the
6 water extract using HPLC-HG-AAS. Separation of the arsenic compounds was
7 carried out in ca. 15 min in the anion-exchange column, using a 25 mM
8 phosphate buffer (pH 6.0) as mobile phase at 1.1 mL min⁻¹ flow rate. The elution
9 order was arsenite, DMA, MA, and arsenate.

10
11 50 μL of sample were injected in the HPLC system, following the instrumental
12 and analytical conditions described in **Table II**. Under these conditions, the
13 retention times were 3.1, 4.2, 5.1 and 8.6 min for arsenite, DMA, MA, and
14 arsenate, respectively. **Figure 1** shows the separation obtained in: a) a 50 $\mu\text{g L}^{-1}$
15 standard of arsenite, DMA, MA, and arsenate, b) the certified material NIST SRM
16 1568a, rice flour, c) raw rice, and d) rice cooked using arsenite-polluted water.

17
18 External calibration was accomplished using standard concentrations of 1, 10,
19 20, 30, 40, and 50 $\mu\text{g L}^{-1}$ of each of the four arsenic species studied (arsenite,
20 DMA, MA and arsenate).

21
22 Information on the certified material NIST SRM 1568a (rice flour) was used for
23 testing this analytical method.

24 *Statistical analyses*

25
26 All data were subjected to analysis of variance (ANOVA) and the Tukey least
27 significant difference multi-comparison test to determine significant differences
28 among samples (arsenic species and/or arsenic concentration). The statistical
29 analyses were done using SPSS 12.0 (SPSS Science, Chicago, USA).

30 **Results and discussion**

31 *Analytical Quality Assurance*

32
33 **Total arsenic.** The analytical characteristics for the total arsenic methodology
34 were as follows: detection limit, 7 $\mu\text{g kg}^{-1}$; precision 2 %; accuracy for rice flour
35 (NIST SRM 1568a), found value = $0.29 \pm 0.04 \text{ mg kg}^{-1}$ (certified value = $0.29 \pm$
36

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

0.03 mg kg⁻¹); accuracy for bush, branches and leaves (GBW07603), found value = 1.18 ± 0.03 mg kg⁻¹ (certified value = 1.25 ± 0.10 mg kg⁻¹).

Arsenic speciation. An estimate of the instrumental detection limit (IDL) for each of the four arsenic species was calculated based on 3 times the standard deviation of peak area measurements for replicate 50 µL injections of an arsenic 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. No significant reduction/oxidation reactions were observed in these fortification studies.

There are no food certified reference materials available for arsenic species and/or inorganic arsenic. 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 arsenic species.

Finally, the total arsenic concentration (sum of the four studied arsenic 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.

1st Experiment "total arsenic 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-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 later, results from our 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 $\mu\text{g As L}^{-1}$, 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 total arsenic 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 total arsenic content by a) concentration of arsenic through loss of water, volatiles, and, to a lesser extent, certain macronutrients (carbohydrates, lipids, and proteins) and b) loss of As through solubilization.

On the other hand, when the initial As concentration was 50 $\mu\text{g As L}^{-1}$, 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 $\mu\text{g As L}^{-1}$ (implying no As concentration due to water evaporation), while it was 0.4 for the initial concentration of 50 $\mu\text{g As L}^{-1}$.

Data on **Table IV** shows a significantly higher As concentration in the rice cooked using polluted cooking water containing arsenite. The mechanisms for capturing t-As and inorganic arsenic (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 to the others (532 ± 11 mL). The high water retention capacity of the rice might be due to the high starch content in this cereal ($\cong 90\%$), which incorporates a large quantity of water during its gelatinization in the boiling process (Tinarelli 1989).

2nd Experiment "arsenic speciation in cooked rice"

Arsenic speciation of raw rice demonstrated that arsenic was mainly present as inorganic forms, $206.8 \mu\text{g arsenite kg}^{-1}$ rice plus $39.7 \mu\text{g arsenate kg}^{-1}$, and $25.5 \mu\text{g DMA kg}^{-1}$ were also found. Considering that 0.250 kg of raw rice were used in our cooking simulations, the total amounts of $51.7 \mu\text{g arsenite}$, $9.9 \mu\text{g arsenate}$ and $6.4 \mu\text{g DMA}$ were incorporated into the cooking system from the raw rice. Besides, a total of $187.5 \mu\text{g}$ 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 $255.5 \mu\text{g arsenic}$ was added to the cooking system from both rice and spiked cooking water.

A control cooking experiment using arsenic-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 inorganic arsenic. Arsenic was present in this cooked rice as $110.4 \mu\text{g arsenate kg}^{-1}$, $76.4 \mu\text{g arsenite kg}^{-1}$, and $10.8 \mu\text{g MA kg}^{-1}$.

Data on **Table V** showed that when arsenic 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 arsenic in the cooked rice was present under the form of non-added species; for example, when As was initially present in the cooking water as arsenite, $23.8 \pm 0.4 \mu\text{g}$ 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 $1.58 \mu\text{g g}^{-1}$ ww in maize boiled in water containing $0.572 \mu\text{g As mL}^{-1}$. The lack of data concerning i-As content in cooked samples from arsenic endemic areas shows 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 arsenic will be present in the cooked rice in the same form it was initially found in the cooking water and raw rice.

Estimation of arsenic intake and evaluation of risks

From the data provided by the 24 h recall questionnaire administered to the inhabitants of the village in 24-North Parganas district [quantity of water (mL), rice ingested (g)] and the t-As and i-As concentrations spiked in the cooking water and found in raw and/or cooked rice analyzed (**Tables III and IV**), the intakes of t-As and i-As were calculated, and, expressed as $\mu\text{g As day}^{-1}$, 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 to 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 our calculations, while Roychowdhury *et al.* (2003) found rice intakes of about 750 g.

Our 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 analyzed, water and rice, 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 arsenic 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 $0.041 \mu\text{g mL}^{-1}$

(second period). The foods studied contribute to 5 % (first period) or 30 % (second period) of the total As intake. Consequently, Díaz et al. (2004) 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-arsenic endemic areas. The main difference between the study by Díaz et al. (2004) 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 total As intake for cooking water containing 50, 250, and 500 µg L⁻¹, respectively (**Table VI**).

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

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 µg 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 arsenic 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

Our results suggest that rice cooked with arsenic-contaminated water might be an important source of arsenic, especially inorganic arsenic, and that the conditions of the cooking process (ratio rice:water and volume of water discarded) could affect the amount of this element in final cooked rice. A dose-response association between arsenic exposure and any health effects might

underestimate the health risk of arsenic if the intake of this element from sources other than drinking water is not included. Finally, if cooking water containing low levels of arsenic can be provided to villagers, even if their vegetables are still contaminated with arsenic, cooked items will have an arsenic concentration lower than expected due to migration of some the arsenic to the cooking water.

Acknowledgements

This paper has been 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 document are the sole responsibility of UMH and can under no circumstances be regarded as reflecting the position of the European Union and/or CAM.

References

- Abedin MJ, Feldman J, Meharg AA. 2002. Uptake kintetics of arsenic species in rice plants. *Plant Physiology* 128:1120-1128.
- Ackerman AH, Creed PA, Parks AN, Fricke MW, Schwegel CA, Creed JT, Heitkemper DT, Vela NP. 2005. Comparison of a chemical and enzymatic extraction of arsenic from rice and an assessment of the arsenic absorption from contaminated water by cooked rice. *Environmental Science and Technology* 39:5241-5246.
- Bae M, Watanabe C, Inaoka T, Sekiyama M, Sudo N, Bokul MH, Ohtsuka R. 2002. Arsenic in cooked rice in Bangladesh. *Lancet* 360:1839-1840.
- Carbonell-Barrachina AA, Aarabi MA, DeLaune RD, Gambrell RP, Patrick WH Jr. 1998. The influence of arsenic chemical form and concentration on *Spartina patens* and *Spartina alterniflora* growth and tissue arsenic concentration. *Plant and Soil* 198:33-43.
- Concha G, Nermell B, Vahter M. 1998. Metabolism of inorganic arsenic in children with chronic high arsenic exposure in northern Argentina. *Environmental Health Perspectives* 106:355-359.

- 1 Devesa V, Macho ML, Jalón M, Urieta I, Muñoz O, Súñer MA, López F, Vélez D,
2 Montoro, R. 2001. Arsenic in cooked fish products: Study on the effect of
3 cooking on total and inorganic contents. *Journal of Agricultural and Food*
4 *Chemistry* 49:4132-4140.
- 5 Díaz OP, Leyton I, Muñoz O, Núñez N, Devesa V, Súñer MA, Vélez D, Montoro, R.
6 2004. Contribution of water, bread, and vegetables (raw and cooked) to
7 dietary intake of inorganic arsenic in a rural village of Northern Chile.
8 *Journal of Agricultural and Food Chemistry* 52:1773-1779.
- 9 Díaz O, Recabarren E, Piñeiro M. 1989. Contents in raw potato, cooked potato
10 and industrialized products as mashed potatoes. *Reviews in Clinical*
11 *Nutrition* 17:116-121.
- 12 Heitkemper DT, Vela NP, Stewart KR, Westphal CS. 2001. Determination of total
13 and speciated arsenic in rice by ion chromatography and inductively
14 coupled plasma mass spectrometry. *Journal of Analytical Atomic*
15 *Spectrometry* 16:299-306.
- 16 Islam MR, Islam S, Jahiruddin M, Islam MA. 2004. Effects of irrigation water
17 arsenic in the rice-rice cropping system. *Journal of Biological Sciences*.
18 4(4):542-546.
- 19 Laparra JM, Vélez D, Barberá R, Farré R, Montoro R. 2005. Bioavailability of
20 inorganic arsenic in cooked rice: Practical aspects for human health risk
21 assessments. *Journal of Agricultural and Food Chemistry* 53:8829-8833.
- 22 Meharg AA. 2004. Arsenic in rice. Understanding a new disaster for South-East
23 Asia. *Trends in Plant Sciences* 9:415-417.
- 24 Misbahuddin M. 2003. Consumption of arsenic through cooked rice. *Lancet*
25 361:435-436.
- 26 Muñoz O, Devesa V, Súñer MA, Vélez D, Montoro R, Urieta I, Macho ML, Jalón M.
27 2000. Total and inorganic arsenic in fresh and processed fish products.
28 *Journal of Agricultural and Food Chemistry* 48:4369-4376.
- 29 Norra S, Berner ZA, Agarwala P, Wagner F, Chandrasekharam D, Stüben D.
30 2005. Impact of irrigation with As rich groundwater on soil and crops: A
31 geochemical case study in West Bengal Delta Plain, India. *Applied*
32 *Geochemistry*. 20:1890-1906.
- 33 Rahman MM, Mandal BK, Chowdhury TR, Sengupta MK, Chowdhury UK, Lodh D,
34 Chanda CR, Basu GK, Mukherjee, Saha KC, Chakraborti D. 2003. Arsenic
35 groundwater contamination and sufferings of people in North 24-Parganas,
36 one of the nine arsenic affected districts of West Bengal, India. *Journal of*
37 *Environmental Science and Health A* 38:25-59.

- 1 Roychowdhury T, Tokunaga H, Ando M. 2003. Survey of arsenic and other heavy
2 metals in food composites and drinking water and estimation of dietary
3 intake by the villagers from an arsenic-affected area of West Bengal, India.
4 Science of the Total Environment 308:15-35.
- 5 Roychowdhury T, Uchino T, Tokunaga H, Ando M. 2002. Survey of arsenic in food
6 composites from an arsenic-affected area of West Bengal, India. Food
7 Chemical Toxicology 40:1611-1621.
- 8 Sengupta MK, Hossain MA, Mukherjee A, Ahamed S, Das B, Nayak B, Chakraborti
9 D. 2006. Arsenic burden of cooked rice: Traditional and modern methods.
10 Food and Chemical Toxicology. 44:1823-2829.
- 11 She LK, Kheng LC. 1992. Arsenic contents in some Malaysian vegetables.
12 Pertanika 15:171-173.
- 13 Tinarelli A. 1989. El Arroz. Madrid (Spain): Ediciones Mundi-Prensa.
- 14 Tsuda T, Babazono A, Ogawa T, Hamad H, Mino Y, Aoyama H, Kuramatani T,
15 Hotta N, Harada M, Inomata S. 1992. Inorganic arsenic: A dangerous
16 enigma for mankind. Applied Organometallic Chemistry 6:309-322.
- 17 Watanabe C, Inaoka T, Kadono T, Nagano M, Nakamura S, Ushijima K,
18 Murayama N, Miyazaki K, Ohtsuka R. 2001. Males in rural Bangladeshi
19 communities are more susceptible to chronic arsenic poisoning than
20 females: analyses based on urinary arsenic. Environmental Health
21 Perspectives 109:1265-70.
- 22 WHO. 1989. Evaluation of Certain Food Additives and Contaminants; 33rd Report
23 of the Joint FAO/WHO Expert Committee on Food Additives; Technical
24 Report Series 776; Geneva (Switzerland): WHO.

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

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

[†] Boro: irrigation done by groundwater during summer time (November to June).

[‡] Aus: prekharif (April to September). ^γ Aman: irrigation done by rainwater (kharif: June to December).

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

HPLC	
Column	Hamilton PRP-X100
Guard column	Hamilton PRP-X100
Mobile phase	10 mM $\text{k}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ 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

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

Variable	As in Cooking Water ($\mu\text{g L}^{-1}$)	Mean $\pm$ ES [†]
Absorbed Water in Cooked Rice (mL)		
	50	548 $\pm$ 15 ^{a†}
	250	583 $\pm$ 16 ^a
	500	548 $\pm$ 15 ^a
Discarded Water (mL)		
	50	202 $\pm$ 15 ^a
	250	167 $\pm$ 16 ^a
	500	202 $\pm$ 15 ^a
As in Cooked Rice ($\mu\text{g kg}^{-1}$)		
	50	227 $\pm$ 22 ^a
	250	874 $\pm$ 24 ^b
	500	1642 $\pm$ 22 ^c
As in Discarded Water ($\mu\text{g L}^{-1}$)		
	50	128 $\pm$ 14 ^a
	250	234 $\pm$ 14 ^b
	500	492 $\pm$ 14 ^c
As Retention in Cooked Rice (%)		
	50	(-)30,1 $\pm$ 2,5 ^{a†}
	250	80,2 $\pm$ 2,6 ^c
	500	91,4 $\pm$ 2,5 ^c

[†]Mean $\pm$ ES: Mean value of three replicates $\pm$ 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 the cooking water on the variables under study in the cooking of rice.

Variable	As Species	Mean $\pm$ ES [†]
Absorbed Water in Cooked Rice (mL)	Arsenite [‡]	639 $\pm$ 17 ^{a+}
	Arsenate	496 $\pm$ 17 ^b
	MA	562 $\pm$ 18 ^b
	DMA	540 $\pm$ 17 ^b
Discarded Water (mL)	Arsenite	111 $\pm$ 17 ^a
	Arsenate	254 $\pm$ 17 ^b
	MA	188 $\pm$ 18 ^b
	DMA	210 $\pm$ 17 ^b
As in Cooked Rice ($\mu\text{g kg}^{-1}$)	Arsenite	1001 $\pm$ 25 ^a
	Arsenate	855 $\pm$ 25 ^b
	MA	915 $\pm$ 27 ^{ab}
	DMA	886 $\pm$ 25 ^b
As in Discarded Water ($\mu\text{g L}^{-1}$)	Arsenite	230 $\pm$ 16 ^a
	Arsenate	298 $\pm$ 16 ^b
	MA	308 $\pm$ 17 ^b
	DMA	302 $\pm$ 16 ^b
As Retention in Cooked Rice (%)	Arsenite	56,4 $\pm$ 2,9 ^{a+}
	Arsenate	41,8 $\pm$ 2,9 ^b
	MA	50,0 $\pm$ 3,1 ^{ab}
	DMA	40,4 $\pm$ 2,9 ^b

[†]Mean $\pm$ ES: Mean value of three replicates $\pm$ standard error.

[‡]MA=methylarsonate; DMA=dimethylarsinate. ⁺Values with the same letters were not significantly different at $p < 0.05$ for the variable studied (Tukey multiple range test).

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 µg 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) [†]	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 were 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 people drinks daily about 2.5 L of water and eats daily about 0.450 kg of cooked rice.

TOTAL ARSENIC						
As in drinking/cooking water ($\mu\text{g L}^{-1}$)	AsIDW ($\mu\text{g As day}^{-1}$)	AsIDW (% TAI)	As in cooked rice ($\mu\text{g kg}^{-1}$)	AsICR ($\mu\text{g As day}^{-1}$)	AsICR (% TAI)	DAsI ($\mu\text{g As day}^{-1}$)
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						
As in drinking/cooking water ($\mu\text{g L}^{-1}$)	AsIDW ($\mu\text{g As day}^{-1}$)	AsIDW (% TAI)	As in cooked rice ($\mu\text{g kg}^{-1}$)	AsICR ($\mu\text{g As day}^{-1}$)	AsICR (% TAI)	DAsI ($\mu\text{g As day}^{-1}$)
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.

FIGURE CAPTIONS

Figure 1. Example of HPLC-HG-AAS chromatograms of arsenic species. (a) Standard of 50 µg L⁻¹ of arsenite, DMA, MA, and arsenate. (b) NIST SRM 1568a, rice flour. (c) Raw rice. (d) Cooked rice using cooking water polluted with arsenite.

1