



**HAL**  
open science

## 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

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

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

4  
5  
6    Arsenic contamination of rice plants by arsenic polluted irrigation groundwater  
7    could result in high arsenic concentrations in cooked rice. The main objective of  
8    this study was to estimate the total and inorganic arsenic intakes in a rural  
9    population of West Bengal through both drinking water and cooked rice.  
10   Simulated cooking of rice with different levels of arsenic species in the cooking  
11   water was carried out. The presence of arsenic in the cooking water was provided  
12   by four arsenic species (arsenite, arsenate, methylarsonate, or dimethylarsinate)  
13   and at three total arsenic concentrations (50, 250, or 500  $\mu\text{g L}^{-1}$ ). The results  
14   showed that the arsenic concentration in cooked rice was always higher than that  
15   in raw rice and ranged from 227 to 1642  $\mu\text{g kg}^{-1}$ . The cooking process did not  
16   change the arsenic speciation in rice. Cooked rice contributed a mean of 41 % to  
17   the daily intake of inorganic arsenic. The daily inorganic arsenic intakes for water  
18   plus rice were 229, 1024, and 2000  $\mu\text{g day}^{-1}$  for initial arsenic concentrations in  
19   the cooking water of 50, 250, and 500  $\mu\text{g As L}^{-1}$ , respectively, compared to the  
20   tolerable daily intake which is 150  $\mu\text{g day}^{-1}$ .

21  
22  
23  
24  
25    **Key Words:** arsenic speciation; cooked food; cooking water; organic arsenic;  
26                    total arsenic.

## 1 Introduction

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

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

1 the high total arsenic contents found in the studies on total arsenic contents in  
2 cooked foods from contaminated areas (Concha et al. 1998; Bae et al., 2002;  
3 Roychowdhury et al. 2003; Sengupta et al. 2006). In many areas of West  
4 Bengal, rice is washed and/or cooked with a substantial amount of water, which  
5 is sometimes contaminated with arsenic. The actual amount of arsenic in cooked  
6 rice could be either increased, by chelation of arsenic in water by binding on rice  
7 grains, or decreased if water-soluble arsenic is released from rice into the water  
8 to be discarded (Bae et al. 2002).

9  
10 In the literature there are data for total arsenic contents in raw and cooked rice  
11 (Roychowdhury et al. 2003; Islam et al. 2004; Norra et al. 2005), but few values  
12 have been reported for inorganic arsenic (Ackerman et al. 2005; Laparra et al.  
13 2005). However, knowledge of inorganic arsenic contents is essential for an  
14 evaluation of health risks. Inorganic arsenic has been classified by the  
15 International Agency for Research on Cancer (IARC) as a carcinogen to humans  
16 (Díaz et al. 2004; Tsuda et al. 1992). Nevertheless, there are no prior references  
17 to evaluate the influence of cooking on individual arsenic species in rice from  
18 arsenic affected areas such as West Bengal. Up to our knowledge there are only  
19 two studies dealing with speciation of arsenic in cooked rice, the first one carried  
20 out by Laparra et al. (2005) using Spanish rice and the second one carried out  
21 by Ackerman et al. (2005) using North-American rice.

22  
23 The aim of the present study was to determine total and inorganic arsenic  
24 contents in cooked rice using arsenic polluted cooking water. Two factors were  
25 assayed in the cooking water: 1) arsenic speciation (arsenite, arsenate,  
26 methylarsonate, or dimethylarsinate) and 2) total arsenic concentration (50,  
27 250, or 500  $\mu\text{g L}^{-1}$ ). Besides, the contribution of cooked rice and drinking water  
28 to the daily intake of total and inorganic arsenic of the inhabitants of an arsenic-  
29 affected rural village of West Bengal was evaluated.

## 30 **Materials and Methods**

### 31 *Instrumentation*

32  
33 For arsenic speciation analysis, a high-performance liquid chromatography  
34 (HPLC) system consisting of a Varian 9012 ternary pump (Varian, San Fernando,  
35 CA, USA), a Rheodyne 7125 injector and a 50  $\mu\text{L}$  loop for sample introduction,  
36 was used. Separations of arsenic species were performed on a Hamilton PRP X-  
37

1 100 anion-exchange column (10  $\mu\text{m}$ , 250 mm  $\times$  4.1 mm i.d.; Hamilton, Reno,  
2 NV, USA). A guard column packed with the same material (12-20  $\mu\text{m}$ ; 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  $\text{NaBH}_4$  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  $\text{M}\Omega$  cm) was used for the preparation of the reagents and  
27 standards. All glassware was treated with 10% v/v  $\text{HNO}_3$  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  $\text{NaAsO}_2$  (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).

37

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

1 *Study Area*

2 The study was conducted in a village of North 24-Parganas district,  
3 approximately 25 km from Calcutta (India). The total area of the village is 5.0  
4 km<sup>2</sup>, with 22270 people living in residential area of the village with the  
5 remaining area being cultivated land. The average annual income of the villagers  
6 is 350 US\$ annum<sup>-1</sup>. The main source of drinking water for the village is 100,  
7 mainly shallow, wells and tube wells, for drinking purposes. The village was  
8 chosen as the model village in our study because it was known that 70 % of its  
9 tube wells had As concentrations above 0.05 mg L<sup>-1</sup> and it is highly affected by  
10 As contamination in the groundwater.

11  
12 *Food questionnaire*

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

21  
22 The number of interviewees was set at 115 (60 male and 55 female), with ages  
23 above 12 years old to below 60 years. The interviewees selected were mainly  
24 farmers and housewives who normally eat at home, buy or get their food from  
25 local markets and/or farms, cook themselves and take drinking water from  
26 surrounding tube wells. People working at nearby cities were not included in the  
27 survey; they are not representative of endemic arsenic area because they eat  
28 frequently at the city.

29

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

### 1 *Rice samples*

2 Commercial rice samples were collected in farms surrounding the previously  
3 cited rural village of the North 24-Parganas district. Rice samples from different  
4 periods of the year and varieties were analyzed for total and inorganic arsenic  
5 concentrations (**Table I**). As expected *boro* rices contained higher t-As and i-As  
6 concentrations than *aus* and *aman* samples; these higher contents are related to  
7 the use of higher volume of arsenic polluted groundwater for irrigation of the  
8 *boro* rice plants (grown during summer time) compared to *aus* and *aman* plants.  
9 After carefully study of information summarized in **Table I**, authors decided to  
10 select one of the *boro* varieties, the khitish rice (IET-4094), for their experiments  
11 because *boro* rices are those representing the highest potential health risks for  
12 humans.

### 14 *Cooking conditions*

15 Worldwide, there are three common methods of cooking rice (Sengupta et al.  
16 2006): a) The traditional method still used by more than 90% of the villagers in  
17 Bengal delta: raw rice is washed until the washings become clear (5–6 times),  
18 washings are discarded and then the rice is boiled in excess water (5–6 times  
19 the weight of raw rice) until cooked, finally discarding the remaining water  
20 (discard water) by tilting the pan against the lid before serving the rice; b) the  
21 rice is washed as in "a" and boiled with water of a volume 1.5–2 times the  
22 weight of rice until no water is left to discard; c) unwashed rice is boiled with  
23 water 1.5–2 times the weight of rice; the wash and discard steps are both  
24 omitted. This is the contemporary method.

25  
26 The **first experiment** was conducted to investigate effect of a) arsenic species  
27 and b) arsenic concentration on the arsenic content in the cooked rice. The rice  
28 was cooked using deionized water or deionized water spiked with different levels  
29 (50, 250 or 500  $\mu\text{g L}^{-1}$ ) of only one of the following four arsenic species: arsenite,  
30 arsenate, methylarsonate (MA), or dimethylarsinate (DMA).

31  
32 The food survey carried out in this study showed that none of the three methods  
33 described above was the most popular in the studied rural village; the most  
34 popular rice cooking method was in between methods "a" and "c" (it is a hybrid  
35 in the evolution of method "a" towards method "c"). The unwashed rice is boiled  
36 with a volume of water 1.5-4 times the weight of rice; the wash step is omitted.



1 Thus, the rice (250 g) was added to boiling water (750 mL) and kept under this  
2 heat conditions until cooked; the remaining water was discarded. No additional  
3 ingredients were employed.

4  
5 The **second experiment** was carried out to investigate the effect of arsenic  
6 speciation in the cooking water on the arsenic speciation in the cooked rice. Rice  
7 was cooked in the same way as described for the first experiment; cooking water  
8 was spiked with four arsenic species (arsenite, arsenate, MA, or DMA) but only  
9 one arsenic concentration was studied 250  $\mu\text{g As L}^{-1}$ .

10  
11 Once cooked, all rice samples were frozen at -20 °C and then freeze-dried. The  
12 lyophilized samples were ground in a domestic apparatus, and the resulting  
13 powder was vacuum-packed and kept in the freezer at -20 °C until analysis.  
14 Total arsenic concentrations were measured in dry raw rice, wet cooked rice,  
15 cooking water, and discarded starched water.

#### 16 17 *Quantification of total arsenic*

18 A 0.250-g portion of lyophilized rice sample was weighed and digested using the  
19 ashing method previously described by Muñoz et al. (2000). Calibration  
20 standards were prepared using the same HCl concentration of the samples and  
21 certified materials. The instrumental conditions used for arsenic determination by  
22 HG-AAS were as follows: reducing agent: 1.4 % (m/v)  $\text{NaBH}_4$  in 0.4 % NaOH, 5  
23  $\text{mL min}^{-1}$ ; HCl solution: 10 % (v/v), 10  $\text{mL min}^{-1}$ ; carrier gas: argon, 250  $\text{mL}$   
24  $\text{min}^{-1}$  flow rate; and for atomic absorption spectrometry, wavelength: 193.7 nm;  
25 spectral bandpass: 0.5 nm; hollow cathode lamp current setting 8 mA;  
26 air/acetylene flame with a fuel flow rate of 0.8  $\text{L min}^{-1}$ .

27  
28 The certified reference materials (rice flour = NIST SRM 1568a, and bush,  
29 branches and leaves = GBW07603) used for testing this analytical method were  
30 provided by CYMIT Química, S.L. (Barcelona, Spain) and produced by the  
31 National Institute of Standards and Technology of U.S.A. and the Institute of  
32 Geophysical and Geochemical Exploration of China, respectively.

#### 33 34 *Quantification of arsenic species*

35 The method used for the extraction of the arsenic species was that described by  
36 Heitkemper et al. (2001). Dried and milled rice sample (0.5 g) was treated with  
37 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 045  $\mu\text{m}$  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  $\text{min}^{-1}$  flow rate. The elution  
9 order was arsenite, DMA, MA, and arsenate.

10  
11 50  $\mu\text{L}$  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

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

**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<sup>-1</sup> each of arsenite, arsenate, MAA and DMA. The IDL estimates were 1.7, 1.5, 1.1, and 1.4 µg L<sup>-1</sup>, 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<sup>-1</sup> 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<sup>-1</sup> 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<sup>-1</sup> and those reported in a previous study carried out by Heitkemper et al. (2001), 0.083 ± 0.06 mg kg<sup>-1</sup> 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<sup>-1</sup>, was compared with the certified value of the material, 0.290 ± 0.030 mg kg<sup>-1</sup>, and shows the goodness of the analytical method used.

#### *1<sup>st</sup> 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.

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

9  
10 As will be discussed later, results from our calculations suggest that cooked rice  
11 could be an important source of As if it is boiled using As-contaminated water.  
12 According to the experimental data, 1000 g of cooked rice will correspond to  
13 roughly 309 g of raw rice, and about 691 g of contaminated water. Therefore,  
14 this cooked rice will provide an additional and substantial burden of As to that  
15 coming from the drinking water.

16  
17 The amount of As in the cooked rice was 2.2-20.0 % higher than predicted (from  
18 raw rice and absorbed water) for initial As concentrations of 250 and 500  $\mu\text{g As L}^{-1}$ ,  
19 respectively. These results might suggest either that As in the water is  
20 chelated by rice grains, or that As becomes concentrated during the cooking  
21 process, because of evaporation.

22  
23 Díaz *et al.* (2004) stated that the total arsenic contents in raw food can alter in  
24 various ways during cooking treatments, with a consequent effect on the intake  
25 of this contaminant. Cooking treatments such as boiling and frying can alter total  
26 arsenic content by a) concentration of arsenic through loss of water, volatiles,  
27 and, to a lesser extent, certain macronutrients (carbohydrates, lipids, and  
28 proteins) and b) loss of As through solubilization.

29  
30 On the other hand, when the initial As concentration was 50  $\mu\text{g As L}^{-1}$ , the As  
31 content in the cooked rice was about 30 % lower than expected (**Table III**).  
32 These experimental observations indicate that the concentration of As through  
33 loss of water in the cooking process is not a good explanation. Besides, another  
34 experimental parameter sustaining this statement is that the ratio of As in initial  
35 water to that in the discarded water was close to 1 in samples cooked with water  
36 containing 250 and 500  $\mu\text{g As L}^{-1}$  (implying no As concentration due to water  
37 evaporation), while it was 0.4 for the initial concentration of 50  $\mu\text{g As L}^{-1}$ .

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

1  
2 Data on **Table IV** shows a significantly higher As concentration in the rice  
3 cooked using polluted cooking water containing arsenite. The mechanisms for  
4 capturing t-As and inorganic arsenic (i-As) might be related to the incorporation  
5 of water into food during cooking. This statement is supported by the fact that  
6 significantly more water was absorbed by the rice cooked with arsenite ( $639 \pm 17$   
7 mL) compared to the others ( $532 \pm 11$  mL). The high water retention capacity of  
8 the rice might be due to the high starch content in this cereal ( $\cong 90\%$ ), which  
9 incorporates a large quantity of water during its gelatinization in the boiling  
10 process (Tinarelli 1989).

11  
12 *2<sup>nd</sup> Experiment "arsenic speciation in cooked rice"*

13 Arsenic speciation of raw rice demonstrated that arsenic was mainly present as  
14 inorganic forms,  $206.8 \mu\text{g arsenite kg}^{-1}$  rice plus  $39.7 \mu\text{g arsenate kg}^{-1}$ , and  $25.5$   
15  $\mu\text{g DMA kg}^{-1}$  were also found. Considering that  $0.250$  kg of raw rice were used in  
16 our cooking simulations, the total amounts of  $51.7 \mu\text{g arsenite}$ ,  $9.9 \mu\text{g arsenate}$   
17 and  $6.4 \mu\text{g DMA}$  were incorporated into the cooking system from the raw rice.  
18 Besides, a total of  $187.5 \mu\text{g}$  of each species (arsenite, arsenate, MA, and DMA)  
19 were incorporated from the spiked cooking water in each of the different  
20 simulations. In summary, a total of  $255.5 \mu\text{g arsenic}$  was added to the cooking  
21 system from both rice and spiked cooking water.

22  
23 A control cooking experiment using arsenic-free water was carried out and  
24 showed that cooking caused an oxidation of arsenite to arsenate, and a complete  
25 transformation of the initial DMA into MA and inorganic arsenic. Arsenic was  
26 present in this cooked rice as  $110.4 \mu\text{g arsenate kg}^{-1}$ ,  $76.4 \mu\text{g arsenite kg}^{-1}$ , and  
27  $10.8 \mu\text{g MA kg}^{-1}$ .

28  
29 Data on **Table V** showed that when arsenic was present in the cooking water as  
30 inorganic forms (arsenite or arsenate), it was mainly present in the cooked rice  
31 as inorganic forms as well. However, about  $10\%$  of the final arsenic in the  
32 cooked rice was present under the form of non-added species; for example,  
33 when As was initially present in the cooking water as arsenite,  $23.8 \pm 0.4 \mu\text{g}$  of  
34 arsenite were transformed in arsenate. On the other hand, if As was initially  
35 present in the cooking water under organic forms (MA or DMA), no  
36 transformation between organic species occurs; however, a significant amount of  
37 arsenite was found.

1  
2  
3  
4  
5  
6 1  
7 2 To date, the only previous reference found dealing with the contents of i-As in  
8 3 cooked foods is the study carried out by Díaz *et al.* (2004) in Chile, with a  
9 4 maximum concentration of  $1.58 \mu\text{g g}^{-1}$  ww in maize boiled in water containing  
10 5  $0.572 \mu\text{g As mL}^{-1}$ . The lack of data concerning i-As content in cooked samples  
11 6 from arsenic endemic areas shows the novelty of the results reported in the  
12 7 present work.  
13 8

14 9 In general, from this section dealing with As speciation in the cooked rice it can  
15 10 be concluded stating that arsenic will be present in the cooked rice in the same  
16 11 form it was initially found in the cooking water and raw rice.  
17 12

### 13 *Estimation of arsenic intake and evaluation of risks*

14 14 From the data provided by the 24 h recall questionnaire administered to the  
15 15 inhabitants of the village in 24-North Parganas district [quantity of water (mL),  
16 16 rice ingested (g)] and the t-As and i-As concentrations spiked in the cooking  
17 17 water and found in raw and/or cooked rice analyzed (**Tables III and IV**), the  
18 18 intakes of t-As and i-As were calculated, and, expressed as  $\mu\text{g As day}^{-1}$ , are  
19 19 shown in **Table VI**. According to the food habits survey, the average daily water  
20 20 intake for adult (mean of males and females) was 2.5 L. This result is relatively  
21 21 low compared to results provided by Roychowdhury *et al.* (2003), who found that  
22 22 adult males, adult females and children (<10 years of age) consumed 4, 3, and 2  
23 23 L, respectively, at Murshidabad district, West Bengal. Besides, a mean rice intake  
24 24 of about 450 g for adults was used for our calculations, while Roychowdhury *et*  
25 25 *al.* (2003) found rice intakes of about 750 g.  
26 26

27 27 Our results proved that the general assumption that As is present in the drinking  
28 28 water and foods mainly under inorganic forms, arsenite and/or arsenate, is  
29 29 correct; the As intakes estimated in this study show that the daily i-As intake  
30 30 represents between 82 and 99 % of the daily T-As intake. This is due to the  
31 31 types of foods analyzed, water and rice, as indicated earlier, i-As are the major  
32 32 species.  
33 33

34 34 Díaz *et al.* (2004) studied the contribution of water, bread, and vegetables to the  
35 35 dietary intake of arsenic in a rural village of Northern Chile. They studied these  
36 36 items in two different periods, in which the water used by the population for  
37 37 drinking and cooking purposes contained  $0.572$  (first period) or  $0.041 \mu\text{g mL}^{-1}$

1 (second period). The foods studied contribute to 5 % (first period) or 30 %  
2 (second period) of the total As intake. Consequently, Díaz et al. (2004)  
3 concluded that the significance of the intake of As from food increases as the  
4 concentration of As in water decreases. This result should be taken into account  
5 in non-arsenic endemic areas. The main difference between the study by Díaz et  
6 al. (2004) and the present study carried out in West Bengal is the amount of rice  
7 being consumed by the Indian population, 450 g day<sup>-1</sup>. With these initial  
8 considerations, only rice consumption will contribute 45.0 %, 38.6 %, and 37.2  
9 % to the final total As intake for cooking water containing 50, 250, and 500 µg L<sup>-1</sup>  
10 <sup>1</sup>, respectively (**Table VI**).

11  
12 Assuming a body weight of 70 kg for adult people in West Bengal, the reference  
13 intakes stated by the FAO/WHO are equivalent to 150 µg i-As day<sup>-1</sup> for adult  
14 people (Díaz et al. 2004; WHO 1989).

15  
16 In India, the studies carried out by Roychowdhury *et al.* (2002-2003) in West  
17 Bengal also provide data for t-As intake from water and from raw and cooked  
18 food. Assuming that at least 50 % of the t-As in food sample is i-As, the  
19 maximum intake obtained from water and foods was 708 µg day<sup>-1</sup> in adult males,  
20 which is 4.7 times greater than the TDI. In this case, the daily intake contributed  
21 by foodstuffs (rice, vegetables, and spices) was 189 µg, 27 % of the daily t-As  
22 intake.

23  
24 In the present study, the daily arsenic intakes obtained from water plus rice were  
25 227, 1018, and 1989 µg As day<sup>-1</sup> for initial As concentrations in the cooking  
26 water of 50, 250, and 500 µg As L<sup>-1</sup>, respectively, which are 1.5, 6.8, and 13.3  
27 times greater than the TDI. Similar figures are obtained when inorganic species  
28 are considered (**Table VI**); 229, 1024, and 2000 µg As day<sup>-1</sup> for initial As  
29 concentrations in the cooking water of 50, 250, and 500 µg As L<sup>-1</sup>, respectively,  
30 which are 1.5, 6.8, and 13.3 times greater than the TDI.

## 31 32 **Conclusions**

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

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

### 7 8 **Acknowledgements**

9 This paper has been produced with the financial assistance of the European Union  
10 (Development of a low cost technology for in-situ treatment of groundwater for  
11 potable and irrigation purposes, TIPOT, ASI/B7-301/2598/24-2004/79013), MEC  
12 (CTM2005-2425-E), and Caja de Ahorros del Mediterráneo (CAM-2006). The  
13 contents of this document are the sole responsibility of UMH and can under no  
14 circumstances be regarded as reflecting the position of the European Union  
15 and/or CAM.

### 16 17 **References**

- 18  
19 Abedin MJ, Feldman J, Meharg AA. 2002. Uptake kinteics of arsenic species in rice  
20 plants. *Plant Physiology* 128:1120-1128.
- 21 Ackerman AH, Creed PA, Parks AN, Fricke MW, Schwegel CA, Creed JT,  
22 Heitkemper DT, Vela NP. 2005. Comparison of a chemical and enzymatic  
23 extraction of arsenic from rice and an assessment of the arsenic absorption  
24 from contaminated water by cooked rice. *Environmental Science and  
25 Technology* 39:5241-5246.
- 26 Bae M, Watanabe C, Inaoka T, Sekiyama M, Sudo N, Bokul MH, Ohtsuka R. 2002.  
27 Arsenic in cooked rice in Bangladesh. *Lancet* 360:1839-1840.
- 28 Carbonell-Barrachina AA, Aarabi MA, DeLaune RD, Gambrell RP, Patrick WH Jr.  
29 1998. The influence of arsenic chemical form and concentration on *Spartina*  
30 *patens* and *Spartina alterniflora* growth and tissue arsenic concentration.  
31 *Plant and Soil* 198:33-43.
- 32 Concha G, Nermell B, Vahter M. 1998. Metabolism of inorganic arsenic in children  
33 with chronic high arsenic exposure in northern Argentina. *Environmental  
34 Health Perspectives* 106:355-359.



- 1  
2  
3  
4 1 Devesa V, Macho ML, Jalón M, Urieta I, Muñoz O, Súañer MA, López F, Vélez D,  
5  
6 2 Montoro, R. 2001. Arsenic in cooked fish products: Study on the effect of  
7  
8 3 cooking on total and inorganic contents. *Journal of Agricultural and Food*  
9  
10 4 *Chemistry* 49:4132-4140.  
11  
12 5 Díaz OP, Leyton I, Muñoz O, Núñez N, Devesa V, Súañer MA, Vélez D, Montoro, R.  
13  
14 6 2004. Contribution of water, bread, and vegetables (raw and cooked) to  
15  
16 7 dietary intake of inorganic arsenic in a rural village of Northern Chile.  
17  
18 8 *Journal of Agricultural and Food Chemistry* 52:1773-1779.  
19  
20 9 Díaz O, Recabarren E, Piñeiro M. 1989. Contents in raw potato, cooked potato  
21  
22 10 and industrialized products as mashed potatoes. *Reviews in Clinical*  
23  
24 11 *Nutrition* 17:116-121.  
25  
26 12 Heitkemper DT, Vela NP, Stewart KR, Westphal CS. 2001. Determination of total  
27  
28 13 and speciated arsenic in rice by ion chromatography and inductively  
29  
30 14 coupled plasma mass spectrometry. *Journal of Analytical Atomic*  
31  
32 15 *Spectrometry* 16:299-306.  
33  
34 16 Islam MR, Islam S, Jahiruddin M, Islam MA. 2004. Effects of irrigation water  
35  
36 17 arsenic in the rice-rice cropping system. *Journal of Biological Sciences*.  
37  
38 18 4(4):542-546.  
39  
40 19 Laparra JM, Vélez D, Barberá R, Farré R, Montoro R. 2005. Bioavailability of  
41  
42 20 inorganic arsenic in cooked rice: Practical aspects for human health risk  
43  
44 21 assessments. *Journal of Agricultural and Food Chemistry* 53:8829-8833.  
45  
46 22 Meharg AA. 2004. Arsenic in rice. Understanding a new disaster for South-East  
47  
48 23 Asia. *Trends in Plant Sciences* 9:415-417.  
49  
50 24 Misbahuddin M. 2003. Consumption of arsenic through cooked rice. *Lancet*  
51  
52 25 361:435-436.  
53  
54 26 Muñoz O, Devesa V, Súañer MA, Vélez D, Montoro R, Urieta I, Macho ML, Jalón M.  
55  
56 27 2000. Total and inorganic arsenic in fresh and processed fish products.  
57  
58 28 *Journal of Agricultural and Food Chemistry* 48:4369-4376.  
59  
60 29 Norra S, Berner ZA, Agarwala P, Wagner F, Chandrasekharam D, Stüben D.  
30  
31 30 2005. Impact of irrigation with As rich groundwater on soil and crops: A  
32  
33 31 geochemical case study in West Bengal Delta Plain, India. *Applied*  
34  
35 32 *Geochemistry*. 20:1890-1906.  
36  
37 33 Rahman MM, Mandal BK, Chowdhury TR, Sengupta MK, Chowdhury UK, Lodh D,  
38  
39 34 Chanda CR, Basu GK, Mukherjee, Saha KC, Chakraborti D. 2003. Arsenic  
40  
41 35 groundwater contamination and sufferings of people in North 24-Parganas,  
42  
43 36 one of the nine arsenic affected districts of West Bengal, India. *Journal of*  
44  
45 37 *Environmental Science and Health A* 38:25-59.

- 1  
2  
3  
4  
5 1 Roychowdhury T, Tokunaga H, Ando M. 2003. Survey of arsenic and other heavy  
6 2 metals in food composites and drinking water and estimation of dietary  
7 3 intake by the villagers from an arsenic-affected area of West Bengal, India.  
8 4 Science of the Total Environment 308:15-35.  
9  
10 5 Roychowdhury T, Uchino T, Tokunaga H, Ando M. 2002. Survey of arsenic in food  
11 6 composites from an arsenic-affected area of West Bengal, India. Food  
12 7 Chemical Toxicology 40:1611-1621.  
13  
14 8 Sengupta MK, Hossain MA, Mukherjee A, Ahamed S, Das B, Nayak B, Chakraborti  
15 9 D. 2006. Arsenic burden of cooked rice: Traditional and modern methods.  
16 10 Food and Chemical Toxicology. 44:1823-2829.  
17  
18 11 She LK, Kheng LC. 1992. Arsenic contents in some Malaysian vegetables.  
19 12 Pertanika 15:171-173.  
20  
21 13 Tinarelli A. 1989. El Arroz. Madrid (Spain): Ediciones Mundi-Prensa.  
22  
23 14 Tsuda T, Babazono A, Ogawa T, Hamad H, Mino Y, Aoyama H, Kuramatani T,  
24 15 Hotta N, Harada M, Inomata S. 1992. Inorganic arsenic: A dangerous  
25 16 enigma for mankind. Applied Organometallic Chemistry 6:309-322.  
26  
27 17 Watanabe C, Inaoka T, Kadono T, Nagano M, Nakamura S, Ushijima K,  
28 18 Murayama N, Miyazaki K, Ohtsuka R. 2001. Males in rural Bangladeshi  
29 19 communities are more susceptible to chronic arsenic poisoning than  
30 20 females: analyses based on urinary arsenic. Environmental Health  
31 21 Perspectives 109:1265-70.  
32  
33 22 WHO. 1989. Evaluation of Certain Food Additives and Contaminants; 33rd Report  
34 23 of the Joint FAO/WHO Expert Committee on Food Additives; Technical  
35 24 Report Series 776; Geneva (Switzerland): WHO.  
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

1 **Table I.** Total arsenic concentration in raw rice samples procured. All samples  
 2 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 <sup>†</sup>	272 $\pm$ 20	247 $\pm$ 7
Jaladhi-2, BAKU (3)	Boro <sup>†</sup>	410 $\pm$ 11	407 $\pm$ 8
Aditya Initial Evolution Trial IET-7613 (3)	Aus <sup>‡</sup>	178 $\pm$ 14	165 $\pm$ 7
Biraj, CNM-539 (3)	Aus <sup>‡</sup>	125 $\pm$ 12	116 $\pm$ 13
Khitish, Initial Evolution Trial IET-4094 (3)	Aman <sup>γ</sup>	166 $\pm$ 16	140 $\pm$ 9
Ratna, IET-1411 (3)	Aman <sup>γ</sup>	133 $\pm$ 14	120 $\pm$ 11
Khitish, Initial Evolution Trial IET-7328 (3)	Aman <sup>γ</sup>	177 $\pm$ 16	163 $\pm$ 5

4 <sup>†</sup> Boro: irrigation done by groundwater during summer time (November to June).

5 <sup>‡</sup> Aus: prekharif (April to September). <sup>γ</sup> Aman: irrigation done by rainwater

6 (kharif: June to December).

1  
2  
3  
4  
5 1 **Table II.** Instrumental and analytical conditions for HPLC-HG-AFS.  
6  
7 2  
8

<b>HPLC</b>	
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 $\mu\text{L}$
Flow rate	0.8 $\text{mL min}^{-1}$
<b>HG-AFS</b>	
Reducing agent	1.4% (w/v) $\text{NaBH}_4$ in 0.4% (w/v) $\text{NaOH}$
Flow rate of reducing agent	1.0 $\text{mL min}^{-1}$
HCl	1.5 M
HCl flow rate	1.5 $\text{mL min}^{-1}$
Carrier gas	argon
Carrier gas flow rate	200 $\text{mL min}^{-1}$
Hydrogen flow rate	60 $\text{mL min}^{-1}$
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 <sup>†</sup>
<b>Absorbed Water in Cooked Rice (mL)</b>		
	50	548 $\pm$ 15 <sup>a†</sup>
	250	583 $\pm$ 16 <sup>a</sup>
	500	548 $\pm$ 15 <sup>a</sup>
<b>Discarded Water (mL)</b>		
	50	202 $\pm$ 15 <sup>a</sup>
	250	167 $\pm$ 16 <sup>a</sup>
	500	202 $\pm$ 15 <sup>a</sup>
<b>As in Cooked Rice (<math>\mu\text{g kg}^{-1}</math>)</b>		
	50	227 $\pm$ 22 <sup>a</sup>
	250	874 $\pm$ 24 <sup>b</sup>
	500	1642 $\pm$ 22 <sup>c</sup>
<b>As in Discarded Water (<math>\mu\text{g L}^{-1}</math>)</b>		
	50	128 $\pm$ 14 <sup>a</sup>
	250	234 $\pm$ 14 <sup>b</sup>
	500	492 $\pm$ 14 <sup>c</sup>
<b>As Retention in Cooked Rice (%)</b>		
	50	(-)30,1 $\pm$ 2,5 <sup>a†</sup>
	250	80,2 $\pm$ 2,6 <sup>c</sup>
	500	91,4 $\pm$ 2,5 <sup>c</sup>

<sup>†</sup>Mean  $\pm$  ES: Mean value of three replicates  $\pm$  standard error. <sup>‡</sup> 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 <sup>†</sup>
<b>Absorbed Water in Cooked Rice (mL)</b>	Arsenite <sup>‡</sup>	639 $\pm$ 17 <sup>a‡</sup>
	Arsenate	496 $\pm$ 17 <sup>b</sup>
	MA	562 $\pm$ 18 <sup>b</sup>
	DMA	540 $\pm$ 17 <sup>b</sup>
<b>Discarded Water (mL)</b>	Arsenite	111 $\pm$ 17 <sup>a</sup>
	Arsenate	254 $\pm$ 17 <sup>b</sup>
	MA	188 $\pm$ 18 <sup>b</sup>
	DMA	210 $\pm$ 17 <sup>b</sup>
<b>As in Cooked Rice (<math>\mu\text{g kg}^{-1}</math>)</b>	Arsenite	1001 $\pm$ 25 <sup>a</sup>
	Arsenate	855 $\pm$ 25 <sup>b</sup>
	MA	915 $\pm$ 27 <sup>ab</sup>
	DMA	886 $\pm$ 25 <sup>b</sup>
<b>As in Discarded Water (<math>\mu\text{g L}^{-1}</math>)</b>	Arsenite	230 $\pm$ 16 <sup>a</sup>
	Arsenate	298 $\pm$ 16 <sup>b</sup>
	MA	308 $\pm$ 17 <sup>b</sup>
	DMA	302 $\pm$ 16 <sup>b</sup>
<b>As Retention in Cooked Rice (%)</b>	Arsenite	56,4 $\pm$ 2,9 <sup>a‡</sup>
	Arsenate	41,8 $\pm$ 2,9 <sup>b</sup>
	MA	50,0 $\pm$ 3,1 <sup>ab</sup>
	DMA	40,4 $\pm$ 2,9 <sup>b</sup>

<sup>†</sup>Mean  $\pm$  ES: Mean value of three replicates  $\pm$  standard error.

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

1 **Table V.** Content of arsenic species (arsenite, arsenate, MA, and DMA) in cooked  
 2 rice. In the present cooking process, water containing only one arsenic species,  
 3 at 250  $\mu\text{g L}^{-1}$ , and a cooking ratio of water:rice of 3:1 (750 mL water:250 g rice)  
 4 were used.  
 5

Initial As Species	As Species	Added As (%)	Found As (%)
<b>Arsenite</b>	Arsenite	93.6 (rice+water) <sup>†</sup>	89.9
	Arsenate	3.9 (rice)	10.1
	MA	0	0
	DMA	2.5 (rice)	0
<b>Arsenate</b>	Arsenite	20.2 (rice)	9.9
	Arsenate	77.3 (rice+water)	90.1
	MA	0	0
	DMA	2.5 (rice)	0
<b>MA</b>	Arsenite	20.2 (rice)	6.9
	Arsenate	3.9 (rice)	12.4
	MA	73.4 (water)	80.7
	DMA	2.5 (rice)	0
<b>DMA</b>	Arsenite	20.2 (rice)	7.6
	Arsenate	3.9 (rice)	10.7
	MA	0	0
	DMA	75.9 (rice+water)	81.7

6 <sup>†</sup> A total of 255.5  $\mu\text{g}$  of As were added to each system (187.5  $\mu\text{g}$  from cooking  
 7 water and 68.0  $\mu\text{g}$  from raw rice). From this total amount 236.5, 231.9, 242.6,  
 8 and 234.7  $\mu\text{g}$  were found after cooking in the arsenite, arsenate, MA, and DMA  
 9 systems, respectively.

1 **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  
 2 and eats daily about 0.450 kg of cooked rice.

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

3 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  $\mu\text{g L}^{-1}$  of arsenite, DMA, MA, and arsenate. (b) NIST SRM 1568a, rice flour. (c) Raw rice. (d) Cooked rice using cooking water polluted with arsenite.

For Peer Review Only

1

