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EFFECT OF COOKING METHOD AND RICE TYPE ON ARSENIC CONCENTRATION IN COOKED RICE AND ESTIMATION OF ARSENIC DIETARY INTAKE IN A RURAL VILLAGE OF WEST BENGAL, INDIA

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3 **EFFECT OF COOKING METHOD AND RICE TYPE ON ARSENIC**
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5 **CONCENTRATION IN COOKED RICE AND ESTIMATION OF ARSENIC**
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7 **DIETARY INTAKE IN A RURAL VILLAGE OF WEST BENGAL, INDIA**
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28 **Running Title:** Dietary intake of arsenic in West Bengal, India
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ABSTRACT

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Arsenic contamination of rice plants can result in high total arsenic concentrations (t-As) in cooked rice, especially if arsenic-contaminated water is used for cooking. In this study we have examined two variables: a) the cooking method (water volume and inclusion of a washing step); b) rice type (atab and boiled). Cooking water and raw atab and boiled rice contained $40 \mu\text{g As l}^{-1}$, 185 and $315 \mu\text{g As kg}^{-1}$, respectively. In general, all cooking methods increased t-As from the levels in raw rice; however, raw boiled rice decreased its t-As by 12.7% when cooked by the traditional method but increased by 15.9% or 23.5%, when cooked by the intermediate or contemporary methods, respectively. Based on the best possible scenario (traditional cooking method leading to lowest level of contamination, and the atab rice type with lowest As content) t-As daily intake was estimated to be 328 μg , which was twice the tolerable daily intake of 150 μg .

Keywords: atab rice, Calcutta, inorganic arsenic, North 24-Parganas, total arsenic.

INTRODUCTION

Arsenic-contaminated groundwater from tube wells is the main source for drinking, cooking and other household purposes in West Bengal (India) and for that reason groundwater is the most important source of arsenic (As) intake. However, recently it has been considered that foods are responsible for an important part of As intake too, and studies on total As (t-As) in food obtained from As-endemic areas have increased in recent years (Roychowdhury *et al.* 2003). Furthermore Signes *et al.* (2008a) suggested that rice cooked with As-contaminated water can be an important As source, especially inorganic As (i-As), and that the conditions of the cooking process could affect the amount and/or speciation of this element in cooked rice.

Most of the population of West Bengal depends heavily on rice for their caloric intake, suggesting that if their rice is contaminated with As it will become an important dietary source of this metalloid (Watanabe *et al.* 2001). Koichi *et al.* (2007) studied arsenic intake via water and food in a population living in an As-affected area of Bangladesh and reported that the average As intake from cooked rice represented about 56% of the daily arsenic intake (Ohno *et al.* 2007).

In West Bengal the agricultural system is mostly groundwater-dependent producing a large amount of arsenic deposit on the irrigated lands (Signes-Pastor *et al.* 2007). Besides, rice is cultivated in As-contaminated soils under anaerobic conditions (at which As is highly available for plant uptake) increasing its As concentration to levels higher than other crops (Meharg *et al.* 2004).

The As concentration of cooked rice is known to increase with the As concentration of cooking water but the effects of cooking methods have not been defined in detail. It is assumed that if cooking As-free water could be provided to villagers, cooked items will have an As concentration lower than expected due to migration of

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2
3 some As to discarded washing and cooking waters (Signes *et al.* 2008b). In fact,
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5 Sengupta *et al.* (2006) tested the three major rice cooking procedures from this
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7 area. Using low-As containing water ($\text{As} < 3 \mu\text{g l}^{-1}$) in all three methods, they
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9 concluded that the traditional method, in which rice is washed until clear, cooked
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11 with a rice:water ratio of about 1:6 and with extra water being discarded, removed
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13 up to 57% of the As from rice containing As $203\text{-}540 \mu\text{g kg}^{-1}$. The second method,
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15 in which rice is washed as in the traditional method but is cooked with a lower
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17 rice:water ratio (1:1.5-2) and no water is discarded, removed about 28% of rice
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19 As. Finally, the contemporary method, in which the rice was unwashed and no
20
21 water was discarded, did not modify the rice As content (Sengupta *et al.* 2006).
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26 In this paper the effects of: a) rice cooking methods and b) rice type on t-As
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28 concentration in cooked rice are reported. The two most popular rice types, atab
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30 and boiled (Signes *et al.* 2008b), were cooked using the three most popular cooking
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32 methods in the Indian subcontinent (Sengupta *et al.* 2006). The two types of rice
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34 under study were from the block of Barasat in North 24-Parganas, where 70% of its
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36 tube wells have As concentrations above $50 \mu\text{g l}^{-1}$, maximum permissible limit of As
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38 in drinking water established by the World Health Organization in 1984 (Chowdhury
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40 *et al.* 2000), although the current provisional guideline by WHO was established in
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42 $10 \mu\text{g l}^{-1}$ (WHO 2006). The cooking experiments were carried out as previously
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44 described by Sengupta *et al.* (2006) but using cooking water with an As
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46 concentration of $40 \mu\text{g l}^{-1}$, which is more representative of this specific district of
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48 West Bengal. Finally, an estimation of the comparative importance of cooked rice
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50 (obtained by three different cooking methods) and drinking water (containing $40 \mu\text{g}$
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52 As l^{-1}) to the total As daily intake was studied.
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MATERIALS AND METHODS

Instrumentation

Determination of total arsenic (t-As) was performed with a Unicam atomic absorption spectrometer, model Solaar 969, equipped with a continuous hydride generation Unicam Solaar VP90 (AAS-HG).

Other equipment used included a sand bath (Falc, Treviglio, Italy) model BS 70 with a maximum temperature of 200°C, a muffle furnace (Horbesal, Barcelona, Spain) model 12 PR/300 series 8B with a maximum temperature 1200°C, a grinder (Moulinex, Valencia, Spain), and a hot air oven (Selecta, Barcelona, Spain) with maximum temperature of 250°C.

Reagents

Deionized water was used for preparation of the reagents and standards. All glassware was treated with 10% v/v HNO₃ for 24 h and then rinsed three times with deionized water before use.

All chemicals were of, at the least, pro analysis quality. Commercial standard salt from NaAsO₂ (Panreac, Barcelona, Spain) was used for the preparation of standards. Magnesium nitrate, MgNO₃ and MgO (Panreac, Barcelona, Spain) were used in the ashing solution and HNO₃ 65% (Merck, Germany) during the digestion of samples. Hydrochloric acid, HCL 37% (Merck, Germany), IK 99% (Retapur, Leuven, Belgium) and ascorbic acid 99% (Panreac, Barcelona, Spain) were used as reducing agents. As a reducing solution for AsH₃ generation, NaBH₄ (Panreac, Barcelona, Spain) solutions were prepared daily.

Study area

The study was conducted in a village of North 24-Parganas district, under the block Barasat, approximately 25 km from Calcutta (India). The total area of the village is

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3 5.0 km², with 22270 people living in residential area of the village with the
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5 remaining area being cultivated land. The average annual income of the villagers is
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7 350 US\$ annum⁻¹. The main source of water for the village is 100, mainly shallow,
8
9 wells and tube wells, for irrigation purposes. The village was chosen as the model
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11 village in our study because it was known that 70 % of its tube wells had As
12
13 concentrations above 50 µg l⁻¹ and it is highly affected by As contamination in the
14
15 groundwater.
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17 18 19 20 **Rice samples**

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22 Two different rice (*Oryza sativa* L.) types (according to their dehusk processing)
23
24 were selected for this study, atab and boiled rice, which are obtained by dry-
25
26 dehusking (only mechanical dehusking) or wet-dehusking (includes soaking in
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28 water, light boiling, and mechanical dehusking), respectively (Signes et al. 2008b).
29
30 Both of rice types were from the same boro rice variety, khitish rice (Initial
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32 Evolution Trial IET-4094); authors decided to use this variety because boro rice
33
34 varieties represent the highest potential health risks for humans (plants are
35
36 irrigated with As contaminated water during the dry season).
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38 39 40 **Cooking methods**

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42 Worldwide there are three common methods of cooking rice (Sengupta et al.
43
44 2006):

- 45
46 1) The traditional method (A) still used by more than 90% of the villagers
47
48 in the Bengal delta: raw rice is washed until the washings become clear
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50 (five to six times), the washings are discarded and then the rice is boiled
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52 in excess water (five to six times the weight of the raw rice) until
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54 cooked, finally discarding the remaining water (discarded water) by
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56 tilting the pan against the lid before serving the rice.
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3 2) The intermediate method (B): The rice is washed as above and boiled
4 with water of a volume 1.5 times to twice the weight of rice until no
5 water is left to discard.
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9 3) The contemporary method (C): unwashed rice is boiled with a volume of
10 water 1.5 to twice the weight of rice; the wash and discard steps are
11 both omitted.
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18 Thirty six samples of rice were analyzed for t-As (18 of atab rice and 18 of boiled
19 rice), and 12 samples (6 of atab rice and 6 of boiled rice) were cooked following
20 each one of the three cooking methods under study (traditional, intermediate, and
21 contemporary). In all cooking steps, water containing $40 \mu\text{g As l}^{-1}$ was used.
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28 **Quantification of t-As**

29 Raw and cooked rice samples were dried in an air-oven (Selecta) for 72 h at 70 °C.
30 Then, samples were ground in a domestic grinder (Moulinex) and the resulting
31 powder was vacuum-packed and kept in the freezer at -18 °C until analysis. Total
32 As in washing and discarded waters was analyzed immediately after carrying out
33 the cooking simulations.
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43 Raw rice, washing water, discarded water, and cooked rice were analyzed for t-As
44 by Hydride Generation Atomic Absorption Spectrometry (HG-AAS). A 1-g portion of
45 homogenized dried and ground rice sample or a 5-ml water (washed or discarded)
46 sample was weighed and digested using the method previously described by Muñoz
47 et al. (2000). Calibration standards were prepared using the same HCl
48 concentration of the samples and certified materials. The instrumental conditions
49 used for As determination by HG-AAS were as follows. Reducing agent: 1.4% (m/v)
50 NaBH_4 in 0.4% NaOH, 5 ml min^{-1} ; HCL solution: 10 % (v/v), 10 ml min^{-1} ; carrier
51 gas: argon, 250 ml min^{-1} flow rate; and for atomic absorption spectrometry,
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3 wavelength: 183.7 nm; spectral bandpass: 0,5 nm; hollow cathode lamp current
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5 setting 8 mA; air/acetylene flame with a fuel flow rate of 0.8 l min⁻¹.
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9 The certified reference materials (rice flour=NIST SRM 1568a; and bush, branches
10 and leaves=GBW07603) used for testing this analytical method were provided by
11 CYMIT Química S.L. (Barcelona, Spain) and produced by the US National Institute
12 of Standards and Technology and the Institute of Geophysical and Geochemical
13 Exploration of China, respectively. These two materials were already dried and
14 powdered, so they were subjected to the same exact digestion process than rice or
15 water samples without any previous preparation.
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24 25 26 **Statistical analyses**

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28 All data were subjected to analysis of variance (ANOVA) and the Tukey least-
29 significant difference multi-comparison test to determine significant differences
30 among samples (cooking method and type of rice). The statistical analyses were
31 performed using SPSS 14.0 (SPSS Science, Chicago, USA).
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38 **RESULTS**

39 **Analytical quality assurance**

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41 The analytical characteristics for the t-As methodology were as follows: detection
42 limit, 4 µg kg⁻¹ (calculated according to the European Standard prEN 13804, which
43 establishes that the detection limit is numerically equal to three times the standard
44 deviation of the mean of blank determination); precision 2 % (calculated as mean
45 relative standard deviation obtained from six independent analyses of certified
46 material NIST SRM 1568a); accuracy for rice flour (NIST SRM 1568a), found value
47 = 0.29 ± 0.04 mg kg⁻¹ (certified value = 0.29 ± 0.03 mg kg⁻¹); accuracy for bush,
48 branches and leaves (GBW07603), found value = 1.18 ± 0.03 mg kg⁻¹ (certified
49 value = 1.25 ± 0.10 mg kg⁻¹).
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t-As in cooked rice

Table I shows the results from a mass balance experiment dealing with the effects of the cooking method on t-As concentration in atab rice. The raw atab rice increased its t-As content by 27.6%, 37.8% and 42.2%, when it was cooked with As contaminated water ($40 \mu\text{g l}^{-1}$) using traditional, intermediate or contemporary methods, respectively. Rice washing reduced t-As content in atab rice by 4.5-11.6%.

Table II shows the results from a mass balance experiment studying the effects of the cooking method on t-As concentration in boiled rice. The raw boiled rice decreased its t-As by 12.7% when it was cooked by the traditional method but increased its t-As by 15.9% or 23.5%, when it was cooked by the intermediate or contemporary methods, respectively. Rice washing reduced t-As by 9.1-16.4%.

Cooked boiled rice had higher t-As concentration than cooked atab rice ($390 \pm 12 \mu\text{g kg}^{-1}$ and $283 \pm 11 \mu\text{g kg}^{-1}$), independently of the cooking method used (Table III). No significant differences were found in t-As in the water coming from washing atab or boiled rices; however, t-As was higher in water discarded at the end of the cooking method when boiled rice was used.

Rice cooked by the traditional method had significantly lower t-As ($258 \mu\text{g kg}^{-1}$) than rice samples cooked by the intermediate or contemporary methods; no significant differences were found between rice samples cooked by these two methods (mean of $376 \mu\text{g kg}^{-1}$) (Table IV).

DISCUSSION***t-As in cooked rice***

The results reported here have shown that there is a strong dependence of the t-As content in the cooked rice on the cooking protocol and on the type of rice being

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2
3 used. The dependence of t-As content in the cooked rice on the cooking method
4 was previously reported by Sengupta *et al.* (2006). These researchers, however,
5 used almost As-free cooking water in their experiments ($< 3 \mu\text{g As l}^{-1}$) and
6 concluded that the traditional and intermediate methods removed 57% and 28% of
7 the t-As content initially present in raw rice; the contemporary method did not
8 remove any arsenic from raw rice (Sengupta *et al.* 2006). In the present
9 experiment the t-As concentration in cooking water was higher ($40 \mu\text{g l}^{-1}$) than in
10 Sengupta's experiment and under these experimental conditions all cooking
11 methods increased significantly the t-As content in cooked rice compared to raw
12 rice. However, cooking rice by the traditional method always implied the lowest As
13 concentration in the final rice. Sengupta *et al.* (2006) showed that when rice was
14 cooked by this traditional method, the washing water was able to remove an
15 important amount of the metalloid, about 25-27%; a similar pattern was reported
16 for the discarded water, which caused an As reduction of about 29.9-30.1%.
17 Consequently, it seems that the traditional method of cooking rice is the best one
18 to reduce As intake, because the rice washing and cooking with a high volume of
19 water reduces the As concentration in the final cooked rice compared to other
20 methods; this is even true when As contaminated water is still used ($40 \mu\text{g As l}^{-1}$).
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43 As previously described by Signes *et al.* (2008b) t-As concentrations were always
44 higher in boiled rice samples than in atab rice; this is without any doubt link to
45 usage of As-contaminated water in the dehusking operations of the wet method.
46 The atab rice is obtained from paddy rice exclusively by mechanical dehusking while
47 boiled rice is obtained after heating wet paddy rice before its mechanical hulling.
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55 **Daily arsenic intake**

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57 The parameter most commonly used for the evaluation of As risk assessment is the
58 Provisional Tolerable Weekly Intake (PTWI) established by FAO/WHO at the level of
59 $15 \mu\text{g i-As week}^{-1} \text{ kg}^{-1}$ body weight (WHO 1989). This parameter can be easily
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3 transformed into the tolerable daily intake (TDI) as follows: $TDI = PTWI/7 \text{ days} =$
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5 $2.14 \mu\text{g i-As day}^{-1} \text{ kg}^{-1}$ body weight, and if we assumed that adults in West Bengal
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7 have a mean body weight of approximately 70 kg, the TDI can be established at
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9 $150 \mu\text{g i-As day}^{-1}$. According to Roychowdhury *et al.* (2002), the mean average
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11 daily intakes for an adult in West Bengal are 712.5 g of cooked rice and 4 l of water
12
13 (Roychowdhury *et al.* 2002). According to Signes *et al.* (2008a) approximately 95%
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15 of the As present in cooked rice will be present as i-As; so t-As can be considered
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17 equal to i-As for the below described estimations of As intakes.
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22 Table V summarizes the estimation made in this study for the daily intake of t-As
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24 from rice cooked using the three studied cooking methods. Initial As concentrations
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26 in raw atab rice and cooking water were $185 \mu\text{g kg}^{-1}$ and $40 \mu\text{g l}^{-1}$; after cooking
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28 this type of rice following the traditional, intermediate and contemporary methods,
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30 the daily intakes could be estimated in 168, 182, and $187 \mu\text{g day}^{-1}$, respectively. If
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32 these amounts are added to the As intake coming from the drinking water, total
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34 daily i-As intakes could be established at 328, 342, and $347 \mu\text{g day}^{-1}$, for
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36 traditional, intermediate and contemporary cooking methods. Similar patterns but
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38 higher figures were obtained for boiled rice (initial concentration of $315 \mu\text{g kg}^{-1}$),
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40 $356, 420, 437 \mu\text{g day}^{-1}$, respectively.
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45 In both cases (atab and boiled rice) daily As intakes were higher than the WHO TDI
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47 of $150 \mu\text{g i-As day}^{-1}$. In fact, considering just cooked rice will provide figures higher
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49 than this recommended maximum intake. When atab rice was used, the daily As
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51 intake was 2.1-2.3 times higher than TDI and when boiled rice was used the daily
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53 As intake was 2.3-2.9 times higher than TDI.
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Table I. Mass balance table in atab rice as affected by the cooking method.

Method	As in boiled rice ($\mu\text{g}/\text{kg}$) i	Weight of raw rice (g) ii	t-As in raw rice (μg) iii = (i \times ii)/1000	As in washings ($\mu\text{g kg}^{-1}$) iv	Weight of washings (g) v	t-As in washings (μg) vi = (iv \times v)/1000	As in cooking water ($\mu\text{g kg}^{-1}$) vii	Weight of cooking water (g) viii	t-As in cooking water (μg) ix = (vii \times viii)/1000
A	185	12	2.220	40	60	2.400	40	60	2.400
B	185	12	2.220	40	60	2.400	40	24	0.960
C	185	12	2.220	0	0	0.000	40	24	0.960

As in washings ($\mu\text{g}/\text{kg}$) x	Weight of washings (g) xi	t-As in washings (μg) xii = (x \times xi)/1000	As in cooked rice ($\mu\text{g}/\text{kg}$) xiii	Weight of cooked rice (dry) (g) xiv	t-As in cooked rice (μg) xv = (xiii \times xiv)/1000	As in discarded water ($\mu\text{g}/\text{kg}$) xvi	Weight of discarded water (g) xvii	t-As in discarded water (μg) xviii = (xvi \times xvii)/1000
47	57	2.679	236	12	2.832	108	14	1.512
44	57	2.508	255	12	3.060	0	0	0
0	0	0.000	263	12	3.156	0	0	0

Table II. Mass balance table in boiled rice as affected by the cooking method.

Method	As in boiled rice ($\mu\text{g}/\text{kg}$) i	Weight of raw rice (g) ii	t-As in raw rice (μg) iii = (i \times ii)/1000	As in washings ($\mu\text{g}/\text{kg}^{-1}$) iv	Weight of washings (g) v	t-As in washings (μg) vi = (iv \times v)/1000	As in cooking water ($\mu\text{g}/\text{kg}^{-1}$) vii	Weight of cooking water (g) viii	t-As in cooking water (μg) ix = (vii \times viii)/1000
A	315	12	3.780	40	60	2.40	40	60	2.400
B	315	12	3.780	40	60	2.40	40	24	0.960
C	315	12	3.780	0	0	0.00	40	24	0.960

As in washings ($\mu\text{g}/\text{kg}$) x	Weight of washings (g) xi	t-As in washings (μg) xii = (x \times xi)/1000	As in cooked rice ($\mu\text{g}/\text{kg}$) xiii	Weight of cooked rice (dry) (g) xiv	t-As in cooked rice (μg) xv = (xiii \times xiv)/1000	As in discarded water ($\mu\text{g}/\text{kg}$) xvi	Weight of discarded water (g) xvii	t-As in discarded water (μg) xviii = (xvi \times xvii)/1000
53	57	3.021	275	12	3.300	133	17	2.261
49	56	2.744	365	12	4.380	0	0	0
0	0	0.000	389	12	4.668	0	0	0

Table III. Effect of rice type (atab or boiled) on As concentrations ($\mu\text{g kg}^{-1}$) in cooked rice, washing water, and discarded water.

Variable	Type of rice	Mean \pm ES
As in cooked rice ($\mu\text{g kg}^{-1}$)	Atab rice	283 \pm 11 b [†]
	Boiled rice	390 \pm 12 a
As in Washings ($\mu\text{g kg}^{-1}$)	Atab rice	46 \pm 1 a
	Boiled rice	50 \pm 2 a
As in discarded water ($\mu\text{g kg}^{-1}$)	Atab rice	109 \pm 9 b
	Boiled rice	146 \pm 9 a

[†]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 cooking method (traditional, intermediate, or contemporary) on As concentrations ($\mu\text{g kg}^{-1}$) in cooked rice, washing water, and discarded water.

Variable	Cooking method	Mean \pm ES
As in Cooked rice ($\mu\text{g kg}^{-1}$)	Traditional	258 \pm 14 b [†]
	Intermediate	365 \pm 14 a
	Contemporary	387 \pm 15 a
As in Washings ($\mu\text{g kg}^{-1}$)	Traditional	49 \pm 1 a
	Intermediate	47 \pm 1 a
	Contemporary	-
As in Discarded Water ($\mu\text{g kg}^{-1}$)	Traditional	127
	Intermediate	-
	Contemporary	-

[†]Values with the same letters were not significantly different at $p < 0.05$ for the variable studied (Tukey multiple range test).

Table V. Daily dietary arsenic intake from water and cooked rice as affected by the cooking method. It is assumed that each person drinks daily about 2.5 L of water and eats daily about 712.5 g of cooked rice. The arsenic concentration in the drinking and cooking water was 40 $\mu\text{g L}^{-1}$.

Rice type	As in raw rice	As in cooked rice			AsICR			AsIDW			DAsI		
	$(\mu\text{g kg}^{-1})$	$(\mu\text{g kg}^{-1})$			$(\mu\text{g day}^{-1})$			$(\mu\text{g day}^{-1})$			$(\mu\text{g day}^{-1})$		
		Mtd A	Mtd B	Mtd C	Mtd A	Mtd B	Mtd C	Mtd A	Mtd B	Mtd C	Mtd A	Mtd B	Mtd C
Atab	185	236	255	263	168	182	187	160	160	160	328	342	347
Boliled	315	275	365	389	196	260	277	160	160	160	356	420	437

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