Arsenic Bioaccessibility in Cooked Rice as Affected by Arsenic in Cooking Water

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Abstract: Rice can easily accumulate arsenic (As) into its grain and is known to be the highest As-containing cereal. In addition, the As burden in rice may increase during its processing (such as when cooking using As-polluted water). The health risk posed by the presence of As in cooked rice depends on its release from the matrix along the digestive system (bioaccessibility). Two types of white polished long-grain rice, namely, nonparboiled and parboiled (total As: 202 and 190 μg As kg^{-1}, respectively), were cooked in excess of water with different levels of As (0, 10, 47, 222, and 450 μg As L^{-1}). The bioaccessibility of As from these cooked rice batches was evaluated with an *in vitro* dynamic digestion process. Rice cooked with water containing 0 and 10 μg As L^{-1} showed lower As concentrations than the raw (uncooked) rice. However, cooking water with relatively high As content ($\geq$47 μg As L^{-1}) significantly increased the As concentration in the cooked rice up to 8- and 9-fold for the nonparboiled and parboiled rice, respectively. Parboiled rice, which is most widely consumed in South Asia, showed a higher percentage of As bioaccessibility (59% to 99%) than nonparboiled rice (36% to 69%) and most of the As bioaccessible in the cooked rice (80% to 99%) was released easily during the first 2 h of digestion. The estimation of the As intake through cooked rice based on the As bioaccessibility highlights that a few grams of cooked rice (less than 25 g dry weight per day) cooked with highly As contaminated water is equivalent to the amount of As from 2 L water containing the maximum permissible limit (10 μg As L^{-1}).

Keywords: bioavailability, rice, toxicity, trace elements

Practical Application: Studies on As bioaccessibility are needed for determining human As intake from rice for use in accurate risk assessments to establish updated legislation regarding maximum level of As in food. High As bioaccessibility from parboiled rice (consumed by the majority of the people in South Asia), and the findings of high As levels in discarded rice gruel (fed to livestock), has implications for human and animal health.

Introduction

Arsenic (As), especially inorganic arsenic (i-As), is classified as a nonthreshold, class 1 carcinogen (IARC 1987; WHO 2001). Human exposure to As causes diverse health problems such as various cancers and skin disorders (Rahman and others 2001; Mandal and Suzuki 2002), but also interferes with children's intelligence and growth (Wasserman and others 2004; Wang and others 2006). The predominant route for As exposure to humans is via oral ingestion (EFSA 2009). Studies have indicated that in addition to As-contaminated drinking water, dietary As exposure is dominated by rice consumption in many countries (Tsuji and others 2007; Williams and others 2007a). In fact, rice is the staple food for half of the world's population and shows high levels of total As (t-As) with 30% to 90% being i-As (Roychowdury 2008; Signes-Pastor and others 2008c; Norton and others 2009). Rice is highly efficient in accumulating As compared with other cereal crops (wheat, barley, and corn) (Su and others 2010). Arsenic concentration in rice is typically 10 times higher than that in wheat and barley (Williams and others 2007c). In addition, the burden of As in rice can significantly increase when As-contaminated water is used during the dehusking, parboiling, and cooking processes (Sengupta and others 2006; Signes-Pastor and others 2008a, b; Raab and others 2009).

The toxic effect of As on human health depends on the level of dietary intake. However, for a more accurate assessment of the health risks from ingesting As-contaminated rice, it is important to take into account the effect of the cooking process on the As bioaccessibility. Arsenic bioaccessibility refers to the fraction of As that dissolves during the gastrointestinal digestion and is available for absorption during transit through the small intestine to enter the blood stream, and thus becomes harmful to the human body (Ruby and others 1996).

Arsenic bioaccessibility can be estimated by *in vivo* or *in vitro* methods. Due to ethical reasons, alternative approaches to *in vivo* methods have been developed and show wide acceptance. *In vitro* methods provide an effective approximation of *in vivo* situations and offer the advantages of simplicity, speed, easy

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control, and low cost, high precision, and good reproducibility (Moreda-Piñeiro and others 2011).

The aims of this study were to investigate the effect of varying As concentration in cooking water on total As in 2 types of rice (parboiled and nonparboiled) and the As bioaccessibility in the cooked rice. We also estimated As daily intake through cooked rice based on the observed As bioaccessibility.

Material and Methods

Instrumentation

A microwave digester with a power of 1000 W and a maximum temperature of 170 °C (CEM, Microwave digestion MAR Xpress, Mathews, NC, U.S.A.) was used for the digestion of samples (raw rice, cooked rice, and cooking water). Concentrations of As in the digested solution samples were determined by inductively coupled plasma mass spectrometry (ICP-MS). A Thermo-Fisher Scientific X-Series II instrument was used; scandium (50 μg/L), rhodium (10 μg/L), and iridium (5 μg/L) in a 2% HNO_3 matrix were used as internal standards for ICP-MS. The instrument parameters used were those previously published by Al-Rmalli and others (2010).

Reagents

Deionized water (Romil-UpS, Ultra Purity water) was used for the preparation of reagents and standards. All material were treated with 10% v/v nitric acid (HNO_3) for 24 h and then rinsed 3 times with deionized water before being used. The pepsin enzyme was purchased from Sigma Chemical Co. (Catalogue No. P-7000; Poole, Dorset, U.K.). Analytical grade HNO_3 and hydrochloric acid (HCl) were from Fisher Scientific (Loughborough, U.K.). A commercial (Fisher) standard solution of arsenate (1000 mg/L) was used to prepare the standards and to spike the cooking water.

Sample preparation

Two types of polished (white) rice were used. Long-nonparboiled and parboiled rice from the same manufacturer and produced in the United States were purchased in the U.K. Visual inspection of the rice lots indicated that they are from the same variety with the exception of one being parboiled and the other nonparboiled. Rice used in the current study is known to be widely consumed by the Bangladeshi community in the U.K., who have recently been shown to have the highest As exposure compared to other ethnic communities thus far studied in Europe (Cascio and others 2011). This was attributed to their much higher rice intake than other ethnic groups.

Rice was cooked using deionized water spiked with different levels of As (arsenate, As^{V}): 0, 10, 47, 222, and 450 μg As L^{-1}. These levels are in agreement with previous studies and simulate the scenario of both As-endemic and noncontaminated areas (Laparra and others 2005; Signes-Pastor and others 2008b; Raab and others 2009). For cooking purposes, rice samples of 50 g were cooked by the traditional method in excess water (total amount of cooking water equal to 250 mL) (Signes-Pastor and others 2008b). In this method of cooking, the water remaining after cooking (gruel) was discarded. A portion of this gruel was kept for As determination. The cooked rice samples were dried to constant weight, milled to a fine powder, and then stored at 4 °C until analysis. As concentrations in raw rice and cooking water were also determined.

In vitro gastrointestinal digestion

The digestion process carried out in this study was based on the static digestion process previously reported by Laparra and others (2005). In order to simulate more accurately the human digestion, this study performed a simple dynamic *in vitro* digestion process. Cooked rice (10 g wet weight, w.w.) was diluted in 90 mL deionized water adjusted to pH 2.0 with 6 mol/L HCl. Freshly prepared pepsin solution (1 g pepsin in 10 mL of 0.1 mol/L HCl) was added into the solution at a rate of 0.02 g of pepsin per 10 g rice. The solution was made up to 100 mL with deionized water and incubated in a shaking water bath (120 rpm) at 37 °C. Three aliquots of 20 mL each were withdrawn from the solution every 2 h. The solution was refilled with deionized water with pepsin in order to maintain the same condition at all times. The aliquots were transferred into polypropylene centrifuge tubes and centrifuged at 50310 g for 30 min at 4 °C to separate the soluble fraction from the precipitate. Total As was analyzed in the soluble fractions in order to determine the As bioaccessibility in cooked rice.

Quantification of t-As

For total As measurements 0.5 g (dry weight (d.w.)), raw rice, cooked rice, discarded gruel, and the withdrawn aliquots were digested in 2 mL concentrated HNO_3 using published methodologies (Williams and others 2007b).

NIES CRM rice flour nr 10-b was used as certified reference material (CRM). The experimentally found content was 0.10 mg t-As kg^{-1}, representing 92% of the certified value (0.11 mg/kg). Quality controls of CRM and blanks were run in each digestion set.

Results and Discussions

Effects of cooking on rice arsenic contents

Table 1 (nonparboiled rice) and Table 2 (parboiled rice) show the As mass balance throughout the rice cooking process when cooking water was spiked with different As concentrations (0, 10, 47, 222, or 450 μg As L^{-1}). In both cases, As concentration in cooked rice decreased when the cooking was done with As-free water or with water containing 10 μg As L^{-1}. Under these conditions, the ratio between "As in cooked rice (μg)"/"As in raw rice (μg)" was approximately 0.7. This value was in agreement with those from previous studies that reported significant reductions of As in rice cooked by the traditional method and using low-As cooking water; the As removed during the cooking process was associated with the washing and discarded waters (Sengupta and others 2006; Signes-Pastor and others 2008b; Raab and others 2009). On the other hand, when water with a high-As level was used (47, 222, or 450 μg As L^{-1}), the As concentration in the cooked rice increased significantly compared to the raw rice. Thus, the ratio between "As in cooked rice"/"As in raw rice" increased up to 8.9 and 7.3 for long-nonparboiled and long-parboiled rice samples, respectively, for the highest As content in the cooking water (450 μg As L^{-1}). In addition, the low value (around 0.4) of the ratio between "As in discarded gruel"/"As in cooking water" highlighted the large burden of As transferred from the contaminated cooking water to the cooked rice. These results reinforce conclusions reported by previous studies (Ackerman and others 2005; Laparra and others 2005; Signes-Pastor and others 2008a; Torres-Escribano and others 2008; Rahman and Hasegawa 2011). Significant levels of As were found in the discarded gruel. The average concentration in the discarded gruel was 38, 37, 66, 282, and 499 μg As L^{-1} when cooking water with 0, 10, 47, 222, and 450 μg As L^{-1} was used, respectively. This could worsen the scenario of As exposure in As-endemic areas since discarded

Table 1–Arsenic mass balance in long-nonparboiled rice throughout its cooking process using water containing As over the range 0–450 μg As L^{-1}.

As in nonparboiled rice (μg As kg^{-1})	Raw rice (g)	As in raw (μg)	As in washings (μg/L)	Washings (g)	As in washings (μg)	As in cooking water (μg/L)	Cooking water (g)	As in cooking water (μg)
i	*ii*	*iii* = (*i*×*ii*)/1000	*iv*	*v*	*vi* = (*iv*×*v*)/1000	*vii*	*viii*	*ix* = (*vii*×*viii*)/1000
202[a]	50	10.1	0	256[b]	0	0	260	0
202	50	10.1	10	260	2.6	10	260	2.6
202	50	10.1	47	270	12.7	47	260	12.2
202	50	10.1	222	265	58.8	222	260	57.7
202	50	10.1	450	262	118	450	263	118
As in washing (μg/L)	**Washings (g)**	**As in washings (μg)**	**As in cooked rice (μg As kg^{-1})**	**Cooked rice (g)**	**As in cooked rice (μg)**	**As in discarded gruel (μg/L)**	**Discarded gruel (g)**	**As in discarded gruel (μg)**
x	*xi*	*xii* = (*x*×*xi*)/1000	*xiii*	*xiv*	*Xv* = (*xiii*×*xiv*)/1000	*xvi*	*xvii*	*xviii* = (*xvi*×*xvii*)/1000
11	144	1.6	135	50	6.8	33	95	3.1
20	250	5.0	140	50	7.0	30	98	2.9
79	248	19.6	260	50	13.0	46	69	3.2
223	252	56.2	981	50	49.1	264	80	21.1
446	249	111	1780	50	89.0	471	98	46.2

[a]All values are expressed in dry weight.
[b]It has been considered that 1 mL of water weight is 1 g.

Table 2–Arsenic mass balance in long-parboiled rice throughout its cooking process using water containing As over the range 0 to 450 μg As L^{-1}.

As in parboiled rice (μg As kg^{-1})	Raw rice (g)	As in raw rice (μg)	As in washings (μg/L)	Washings (g)	As in washings (μg)	As in cooking water (μg/L)	Cooking water (g)	As in cooking water (μg)
i	*ii*	*iii* = (*i*×*ii*)/1000	*iv*	*v*	*vi* = (*iv*×*v*)/1000	*vii*	*viii*	*ix* = (*vii*×*viii*)/1000
190[a]	50	9.5	0	253[b]	0	0	258	0
190	50	9.5	10	253	2.5	10	258	2.58
190	50	9.5	47	252	11.8	47	254	11.9
190	50	9.5	222	253	56.2	222	253	56.2
190	50	9.5	450	254	114	450	257	116
As in washing (μg/L)	**Washings (g)**	**As in washings (μg)**	**As in cooked rice (μg As kg^{-1})**	**Cooked rice (g)**	**As in cooked rice (μg)**	**As in discarded gruel (μg/L^{-1})**	**Discarded gruel (g)**	**As in discarded gruel (μg)**
x	*Xi*	*xii* = (*x*×*xi*)/1000	*xiii*	*xiv*	*Xv* = (*xiii*×*xiv*)/1000	*xvi*	*xvii*	*xviii* = (*xvi*×*xvii*)/1000
14	240	3.4	140	50	7.0	43	100	4.3
12	240	2.9	165	50	8.3	44	91	4.0
47	239	11.2	282	50	14.1	86	92	7.9
224	240	53.8	837	50	41.9	300	80	24.0
446	241	107	1461	50	73.0	528	96	50.7

[a]All values are expressed in dry weight.
[b]It has been considered that 1 mL of water weight is 1 g.

gruel is widely used to feed livestock and it is also often consumed by humans, especially from poverty stricken families (Roy and others 2007; Ahiduzzaman and Sadrul-Islam 2009; Rahman and Hasegawa 2011). Livestock are fed rice straw, rice bran, rice gruel, and they also drink As contaminated water. Thus, their level of As exposure may be rather high and this requires further study for the benefit of animal health. It has implications for human health and the food chain in general since these As-exposed animals or their products are often consumed as food by humans or used as fertilizers (such as cow dung).

Table 3– Arsenic bioaccessibility in cooked long-nonparboiled and long-parboiled rice as affected by the As concentration in the cooking water.

As in cooking water (μg As L^{-1})	Arsenic bioaccessibility % ± SE[a] (μg As kg^{-1} in cooked rice) Nonparboiled rice	Parboiled rice
0	40 ± 1 (135)	59 ± 2 (140)
10	36 ± 1 (140)	61 ± 6 (165)
47	67 ± 3 (260)	85 ± 4 (282)
222	69 ± 7 (981)	99 ± 8 (837)
450	64 ± 5 (1, 780)	97 ± 9 (1, 462)

[a]SE = standard error based on 3 replicates.

Arsenic bioaccessibility in cooked rice

The As bioaccessibility in cooked rice represents the amount of As in the cooked rice available for uptake into the blood stream (Laparra and others 2005). Table 3 shows that the percentages of As bioaccessibility were 36% to 69% and 59% to 99% for cooked long-nonparboiled and parboiled rice, respectively. Laparra and others (2005) reported that the bioaccessibility of As in cooked rice was 63% to 99% when highly As-contaminated water (200 to 1000 μg/L) was used. The results of our study were in agreement with those previous studies; however, lower percentages of As bioaccessibility were found when free or low-As (10 μg/L) cooking water was used. The level of As bioaccessibility in cooked rice with free or low-As (40% to 61%, respectively) was similar to those previously reported in white rice cooked with deionized water (38% to 57%) (Sun and others 2012). We can explain these findings in the following manner: at low As concentration in the cooking water, the As atoms are likely to be tightly bound to binding sites with the biological macromolecules (carbohydrates, proteins, and lipids) throughout the rice grain, including the interior core. However, as the concentration of As increases, there is likely to be 2 populations of As (tightly bound and weakly bound). When the tightly bound sites are saturated, the excess As atoms are weakly bound (adsorbed) to the rice structure. The tightly bound As atoms are not readily biosaccessible, whereas the loosely adsorbed As are readily bioaccessible. This explains the As bioaccessibility pattern for cooking using low and high As-containing water.

Arsenic bioaccessibility in rice is associated with As speciation. Juhasz and others (2006) reported that dimethylarsinic acid (DMA) in rice was poorly absorbed by oral administration and showed low absolute bioavailability values (only 33% of the total rice-bound As bioaccessible). Conversely, i-As in rice showed high bioaccessibility values (89%). In general, i-As concentration in rice ranges from 40% to 100% of the t-As (Signes-Pastor and others 2008c; Meharg and others 2009; Rahman and Hasegawa 2011). Rice produced in the U.S.A., although generally containing high levels of t-As, has a lower slope of i-As compared with t-As than rice produced in Asian countries, indicating a greater proportion of DMA (Meharg and others 2009). Rice used to carry out our experiment contained 55% of the t-As as i-As and 45% as DMA; this As-speciation pattern was expected since the rice was produced in the U.S.A. An average percentage of 50% of As bioaccessibility in cooked rice was found when cooking water free of As or low-As water (10 μg/L) was used. This points out that most of the As bioaccessibility in rice may come from i-As since 50% of the t-As is in inorganic forms. This statement is in agreement with previous studies (Sun and others 2012). On the other hand, the As bioaccessibility in cooked rice was much higher when water with high-As concentration was used (average percentage of 80%), which is correlated with the high concentration of i-As in the cooking water. Long-nonparboiled rice showed lower values of As bioaccessibility (range 36% to 69%, mean of 55%) than long-parboiled rice (range 59% to 99%, mean of 80%). This is in agreement with previous studies that have reported that the bioaccessibility of As in rice decreases in the general order of extra long-grain, long-grain, and parboiled, to brown rice (He and others 2012). This might be related to the rice matrix (Moreda-Piñeiro and others 2011) and the dehusking process. Parboiled rice undergoes a wet dehusking procedure, which includes 2 steps before mechanically dehusking that may modify its matrix (Signes-Pastor and others 2008c). The modification of the matrix might affect the efficiency of the rice cooking process, which would enhance As discharge and increase the As bioaccessibility (Moreda-Piñeiro and others 2011). Further studies are necessary to explain the higher As bioaccessibility for parboiled rice, compared to nonparboiled rice. Here, we propose the following explanations:

(1) Parboiling involves hydrothermal processing (soaking, heating, and drying) of rice that leads to changes in the molecular structure of parboiled rice (Luh and Mickus

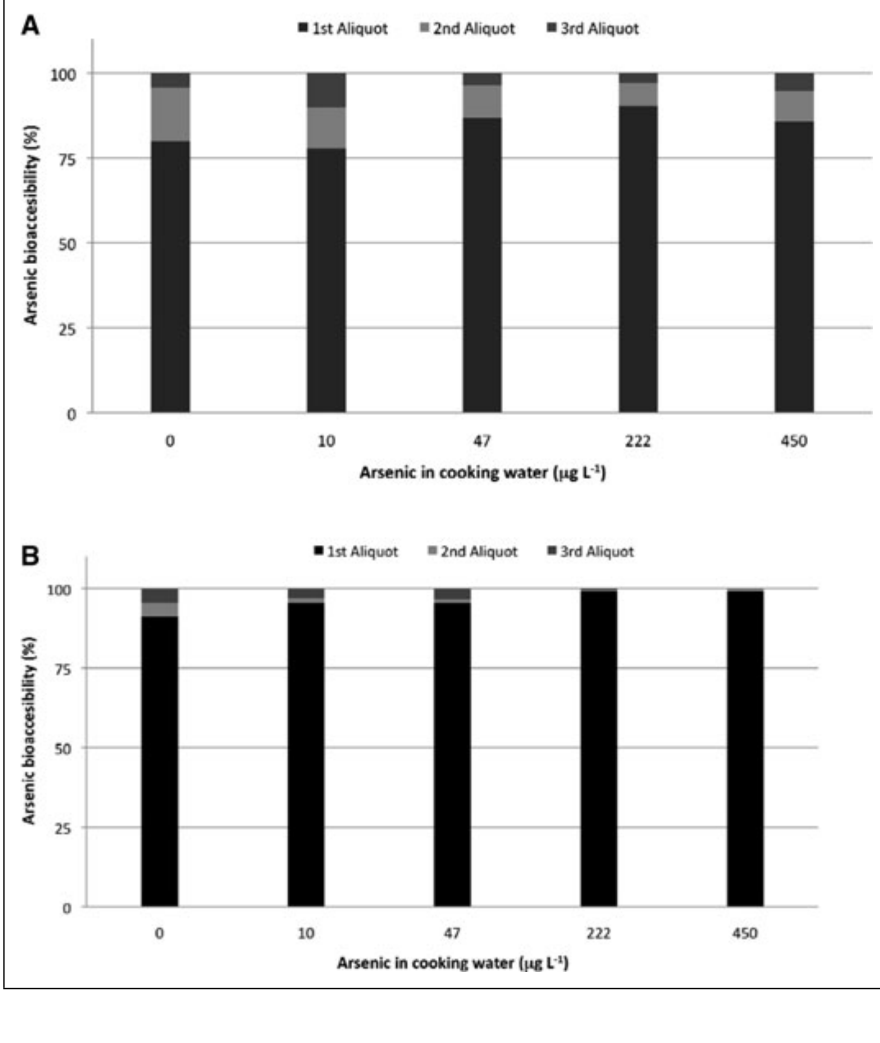

Figure 1–Arsenic bioaccessibility quantified using dynamic *in vitro* gastric digestion as affected by the rice type ("A" = long-nonparboiled and "B" = long-parboiled grain) and the arsenic concentration in the cooking water. The aliquots refer to the sample of the resulting solution of the *in vitro* gastrointestinal digestion withdrawn at intervals of 2 h.

1991), especially in its macromolecules such as starch, amylose, proteins, and lipids. The high temperature of the parboiling process causes the starch to undergo gelatinization and some of the protein molecules denature and aggregate. Due to greater intermolecular interaction between the macromolecules (associated with gelatinization, crystallization, aggregation, and so on), the scope for strong interaction with external substances (such as metals) that are present in the cooking water is reduced. The As in the cooked parboiled rice is likely to be loosely adsorbed onto the rigid molecular structures (gelatinized starch/aggregated proteins and other), and therefore can be easily released during the digestion process. In contrast, with nonparboiled rice, As can interact with the less rigid, native molecular structure of the nonparboiled rice where the interaction between As and the individual macromolecules can occur prior to irreversible heat-induced changes in the structure of the protein and carbohydrate molecules. Thus, for the latter case, the As is likely to be found highly permeated throughout the rice grain including its interior core, incorporated within its macromolecules, therefore reducing its bioaccessibility.

(2) Since the parboiled rice grains are hard/rigid and do not stick to each other, As adsorbed on the surface can easily dissolve in the aqueous medium during the dynamic digestion process. Parboiled rice grains do not stick to each other; hence, there is greater surface area for interaction with water and digestive enzymes that will enable the As to become bioaccessible much more readily compared to nonparboiled rice grains which stick to each other and thereby prevent or reduce the accessibility of water and enzymes to interact with As-bound rice and to facilitate its release.

The fact that parboiled rice showed higher As bioaccessibility is of particular concern since this type of rice is the most commonly consumed by people in Bangladesh and India (Roy and others 2007; Ahiduzzaman and Sadrul-Islam 2009; Rahman and Hasegawa 2011).

Several *in vitro* approaches have been cited to assess the As bioaccessibility and bioavailability in food samples (Moreda-Piñeiro and others 2011). Most of them include an enzymatic extraction with artificial gastrointestinal fluid (Ackerman and others 2005; Laparra and others 2005). Figure 1 shows the results of the dynamic gastric digestion and illustrates the percentages of As bioaccessibility obtained throughout the digestion. Most of the cooked rice-bound As was released within the first 2 h of the simulated dynamic gastric digestion. In fact, up to 99% of the total As bioaccessibility in the parboiled rice, cooked with high-As (450 μg/L) water, was obtained within the first 2 h of the dynamic process.

Arsenic intake

The shortage of bioavailability data and the difficulty in performing bioaccessibility studies led to a conservative or simplified approach in human risk assessment, assuming that 100% of the As or i-As in rice is bioavailable. Our findings now confirm that this approach causes overestimation of As intake from rice. Figure 2 shows the estimation of As intake (μg As) through rice cooked (nonparboiled and parboiled polished rice) with As-free and As-contaminated water based on As bioaccessibility. It also reports the amount of cooked rice equivalent to drinking 2 L water (the

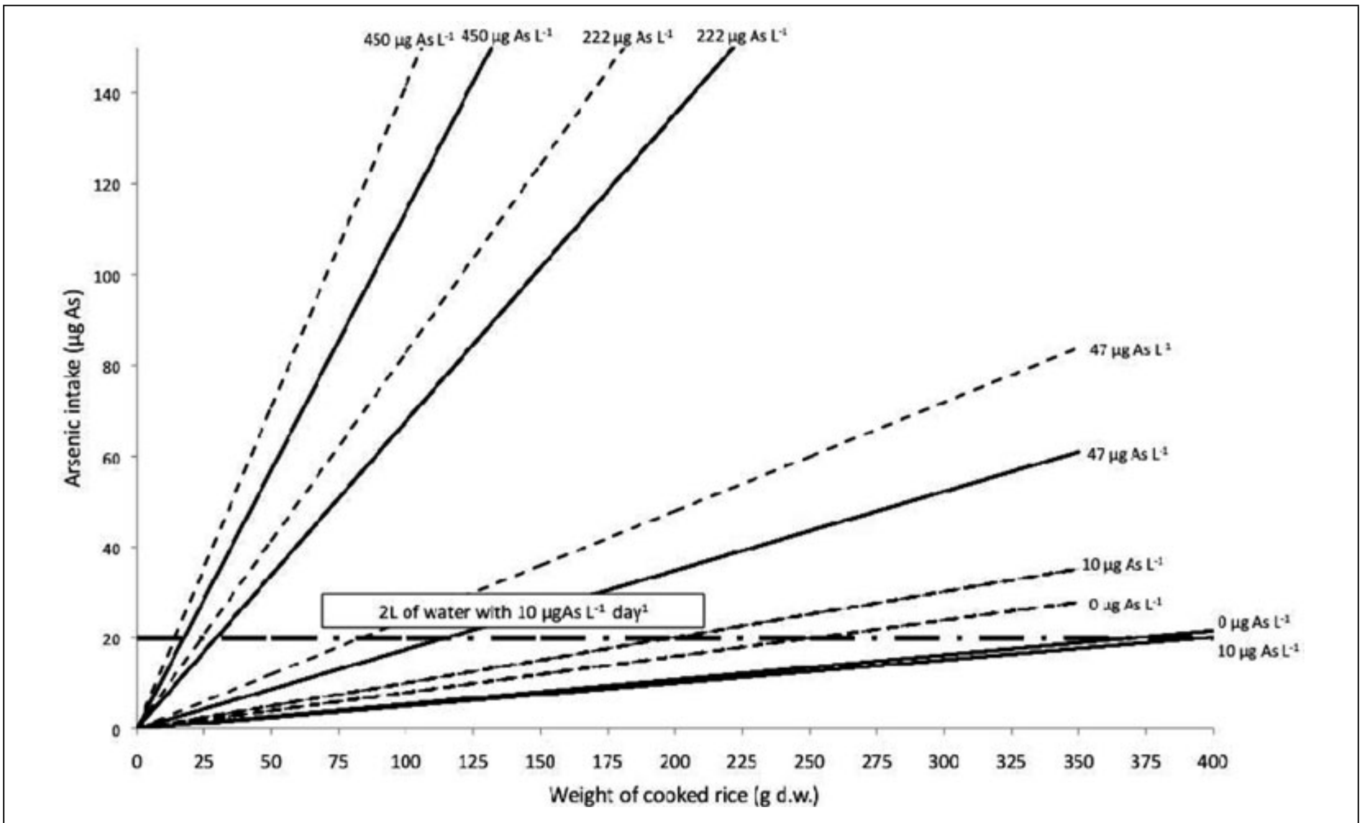

Figure 2–Estimation of arsenic intake (μg As) from cooked rice (nonparboiled rice: solid line; parboiled rice: dashed lines) as affected by the As concentration in the water (0, 10, 47, 222, and 450 μg As L^{-1}).
The horizontal line indicates the amount of cooked rice (g d.w.) equivalent to drinking 2 L water with 10 μg As L^{-1}.

recommended amount of water consumed by the average person per day) containing 10 μg As L^{-1}, which is the maximum permissible limit of As in drinking water (WHO 2006). Since parboiled rice showed higher As bioaccessibility, the amount of parboiled rice equivalent to drinking 2 L of water (containing 10 μg As L^{-1}) is lower than the amount of nonparboiled rice. This is of particular concern since parboiled rice is the most commonly consumed rice type in As endemic areas of Bangladesh and India (Roy and others 2007; Ahiduzzaman and Sadrul-Islam 2009; Rahman and Hasegawa 2011). When cooking water was highly contaminated, a few grams of cooked rice (less than 25 g d.w. d^{-1}) were enough to equal 20 μg As d^{-1} (2 L/d $\times$ 10 μg As L^{-1}). When water with 47 μg As L^{-1} was used, about 100 g of rice d.w. was required. This highlighted how significant rice is as an additional source of As in As-contaminated areas where rice is the staple food. In fact, in Asian As-endemic areas where the population depends heavily on rice for caloric intake (an average adult male consumes 380 g d.w. cooked parboiled rice per day) (Bae and others 2002), the As daily intake through cooked rice could reach up to 539 μg As d^{-1} [1461 μg As kg^{-1} (average of As concentration in parboiled rice cooked with 450 μg As L^{-1}) $\times$ 0.97 (average As bioaccessibility of cooked rice) $\times$ 0.380 kg rice d.w.], which is 3.5 times the tolerable daily intake (TDI) established by FAO/WHO for i-As (150 μg i-As d^{-1} for a person weight 70 kg) (WHO 1989). On the other hand, higher amount of rice (an average of 275 g d.w.) cooked with As-free or As-low (10 μg As L^{-1}) water is necessary to equal the amount of As from drinking 2 L of water with 10 μg As L^{-1}. However, population groups in non-As-endemic areas consuming rice and rice-based products on a daily basis are of concern and the risk cannot be excluded. In fact, the EFSA CONTAM Panel has identified a benchmark dose lower confidence limit ($BMDL_{01}$) value between 0.3 and 8.0 μg As kg^{-1} body weight per day for cancers of the lung, skin, and bladder, as well as skin lesions (EFSA 2009). People consuming rice on a daily basis would be within the range of the $BMDL_{01}$ (EFSA 2009). Further studies on As bioaccessibility in rice-based products are required in order to accurately estimate the As risk assessment.

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