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Consumption of Brown Rice: a Potential Pathway for Arsenic Exposure in Rural Bengal

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Brown Rice Consumption Pattern in Rural Bengal

Brown Rice Classification

SB: Short Bold
MS: Medium Slender
LS: Long Slender

Arsenic (As) distribution pattern in Brown Rice

Concentration of As (mg kg\(^{-1}\))

Type of Brown Rice

SB: Short Bold
MS: Medium Slender
LS: Long Slender
ABSTRACT

This study assesses the arsenic (As) accumulation in different varieties of rice grain, that people in rural Bengal mostly prefer for daily consumption, to estimate the potential risk of dietary As exposure through rice intake. The rice samples have been classified according to their average length (L) and L to breadth (B) ratio into four categories, such as short-bold (SB), medium-slender (MS), long-slender (LS) and extra-long slender (ELS). The brown colored rice samples fall into the SB, MS or LS categories; while all Indian Basmati (white colored) are classified as ELS. The study indicates that average accumulation of As in rice grain increases with decrease of grain size (ELS: 0.04; LS: 0.10; MS: 0.16 and SB: 0.33 mg kg\(^{-1}\)), however people living in the rural villages mostly prefer brown colored SB type of rice because of its lower cost. For the participant consuming SB type of brown rice, the total daily intake of inorganic As (TDI-iAs) in 29% of the cases exceeds the previous WHO recommended provisional tolerable daily intake (PTDI) value (2.1 µg day\(^{-1}\) kg\(^{-1}\) BW), and in more than 90% cases the As content in the drinking water equivalent to the inorganic As intake from rice consumption (C\(_W,\text{eqv}\)) exceeds the WHO drinking water guideline of 10 µg L\(^{-1}\). This study further demonstrates that participants in age groups 18-30 and 51-65 yrs are the most vulnerable to potential health threat of dietary As exposure compared to participants of age group 31-50 yrs, because of higher amount of brown rice consumption pattern and lower BMI.

Key Words: Arsenic exposure; brown rice grain; Provisional Tolerable Daily Intake (PTDI); Body Mass Index (BMI)
INTRODUCTION

Globally, arsenic (As) in drinking water has been identified as a serious public health issue due to its potential risk to human health. The identifiable health problem is most acute in Southeast Asia, notably in West Bengal (Eastern-India) and adjoining area of Bangladesh where people are heavily dependent on groundwater for domestic purposes like drinking, cooking, bathing and washing. Since the first reporting of elevated level of dissolved As in drinking water of West Bengal, extensive research has been undertaken regarding well screening, source characterization and mobilization along with possible mitigation processes. The initiatives have also led to development of strategies to reduce As exposure from drinking water. Both national and international agencies are now working to provide safe drinking water to the affected rural population, by remediation of the As contaminated groundwater, changing the sources of drinking water by targeting deeper safe aquifer, or supplying treated surface water. However not much attention has been given to crop irrigation as a pathway of As exposure. People are still irrigating their lands with groundwater that contains elevated level of As. Consequently, average As concentration in the soils of the irrigated land continues to increase, which can lead to significant bioaccumulation of As in the crops.

The general practice of rice (*Oryza sativa* L.) cultivation involves continuous flooding of the irrigated land. This often leads to soils becoming reduced with time during cultivation which increases the bioavailability of As in the soils and consequently the accumulation of As in rice grains. The accumulation of As in rice grains is highest during dry season rice (Boro) cultivation, which solely relies on groundwater irrigation. Meharg and Rahman, reported that for boro rice cultivation, about 1000 mm irrigation water is required per hectare and they estimated that if irrigation water contains 100 µg L⁻¹ of As, the annual accumulation of As in the paddy soil would be as high as 100 mg m⁻². In Bangladesh, the concentration of As in rice grains positively correlates with As concentration in irrigation water.

Rice is considered as one of the major staple food, particularly in the Asian countries, where per person daily rice intake may be up to 0.5 kg (dry weight). In West Bengal and Bangladesh, rice consumption provides an average 72.8% of the daily caloric intake per capita. In rice, As is mostly present in inorganic and methylated forms, but their distribution varies genetically. Therefore rice is considered as one of the potential route of dietary As exposure in many parts of the world. Several studies done in last few years indicate that despite of higher average dissolved As content in irrigation water of Asia, the average As content in Asian rice (0.16 mg kg⁻¹) is comparatively lower than global mean
value of As content in rice (0.20 mg kg\(^{-1}\)) as well as those of American (0.20 mg kg\(^{-1}\)) and European rice (0.22 mg kg\(^{-1}\)) (see Supporting Information, SI Table 1).\(^{19}\) The higher As content in American rice has been attributed to the legacy of previous extensive use of arsenical pesticides in the country.\(^{29}\) It has been shown that in American rice, As is present mostly in organic form (DMA), which is less toxic compared to its inorganic As species found in Asian and European rice.\(^{24}\) Zavala and Duxbury,\(^{19}\) have calculated the global normal distribution range of As in rice grain (0.08-0.20 mg kg\(^{-1}\)) and Williams et al.\(^{30}\) have estimated that consumption of rice with As concentration of 0.08 mg kg\(^{-1}\) is equivalent to WHO guideline value of 10 µg L\(^{-1}\) in drinking water. However, most of these studies are based on market-basket survey. So far negligible attempt has been made to systematically estimate the attendant health risk of dietary As exposure by quantifying As content in households rice that are consumed by the at-risk inhabitants in rural villages of India or Bangladesh.

The goal of this study is to combine a questionnaire based survey with collection and analysis of rice samples consumed in rural areas of West Bengal, India. Although the local inhabitants have been provided with safe drinking water during the last 3-4 yrs, skin lesions remain quite common in the study area. We hypothesize that consumption of As-enriched rice is an important contributor to the high incidence of skin lesions that still exist in the region. A risk quotient approach is used to assess the potential health risks of As exposure due to consumption of rice.

**MATERIALS AND METHODS**

**Field Site Description.** The field survey was conducted in three neighboring villages, namely Chhoto-Itna, Debogram and Tehatta of Nadia District, West Bengal, India (Figure 1). The study areas are surrounded by agricultural lands. The major agricultural practices are the cultivation of jute (May-September) and boro rice (December-April). Farming is the common occupation of the habitants. The educational and socio-economic statuses of the villages are very poor.

**Sampling and Classification of Rice Grain.** A total of 157 rice samples were collected from randomly selected households. The sample collection was followed by a detailed questionnaire survey which includes information about the rice color, cost, source of rice (buys from market or cultivated in their own field). Further, number of consumers of each rice sample, amount of rice consumption by the participants and con-founding factors like age, height and body weight of each participant was also collected during survey. After collection, rice samples were stored in airtight polyethylene zipper bags at room temperature. During
survey, few varieties of Indian Basmati rice (Kohinoor®, India Gate®) (n = 7) were also
collected from local market to use as control. Five rice grains were picked randomly from
each sample packet to measure length (L) and breadth (B) by micrometer screw gauge. The
rice samples (n = 164) were classified according to grain size and shape by taking their
average L and L to B ratio into four categories viz short-bold (SB, L < 5.50 mm, L/B < 1.1-2 ),
medium-slim (MS, L= 5.51-6.60 mm, L/B >3 ), long-slim (LS, L= 6.61-7.50 mm, L/B
>3 ) and extra-long slender (ELS, L >7.50, L/B >3). Thirty rice samples (18%) were chosen
randomly to further measure L and B by image analysis (for details see Supporting
Information) for testing the accuracy of rice grain classification (SB, MS, LS and ELS).
However none of the rice samples changed its classification after measurement by image
analysis (see Supporting Information, SI Table 2).

Analysis of As in Rice Samples. Rice grains were carefully washed with Millipore water
(18MΩ) and then dried in a hot air oven (65°C for 48 h). Dried rice grains were grounded
using mechanical grinder. The grounded rice samples were acid digested following the
procedure reported by Meharg and Rahman,15 (see Supporting Information for detail digestion
procedure). The acid digested samples were then analyzed for total As using hydride
generation atomic absorption spectrometer (HG-AAS, Varian AA240) at the Department of
Chemistry, University of Kalyani. The HG-mode was preferred because of its lower detection
limit (<1μg L⁻¹) for As. Two reagent blank and one standard reference materials (SRM
1568a), prepared by National Institute of Standards and Technology (NIST) were included in
every batch of 30 samples to ensure accuracy of the analysis. In each batch the recovery of the
SRM sample was within 96-104%. Thirty five rice samples (21%) were selected randomly to
reanalyze to check the precision of the analysis (for details quality assurance, see the
Supporting Information).

Estimation of Dietary As Exposure. Total Daily Intake of inorganic As (TDI-iAs) due to
consumption of rice was calculated for each participant using the equation:

\[ TDI-iAs \ (mg \ day^{-1} \ kg^{-1} \ BW) = \frac{(C_R \times X \times W)}{BW} \]  \hspace{1cm} (1)

where \(C_R\), \(X\), \(W\) and \(BW\) represent the total As concentration in rice sample (mg kg⁻¹),
percentage of inorganic As content in rice sample, the daily consumption of rice (kg day⁻¹)
and body weight of the participant (kg) respectively. Previous study by Williams et al.24
reported that in Indian rice As is mostly (81 ± 4%) present in inorganic form [both as As(III)
and As(V)], which was further supported by Mondal et al.22. Thus in this study, to
approximate the inorganic As content in rice, \(C_R\) for each sample was multiplied by X of 0.81.
It should be mentioned here that as \(TDI-iAs = f (X) \ (from \ equation \ 1)\), a change in X would
affect the value of TDI-iAs in similar fashion with same extent. Though, currently there is no
WHO recommended Provisional Tolerable Daily Intake (PTDI) value for inorganic As,\textsuperscript{32} the
potential risk of dietary As exposure was evaluated by comparing TDI-iAs values with
previous WHO recommended PTDI value of 2.1 \( \mu \text{g day}^{-1} \text{ kg}^{-1} \) BW. Furthermore, assuming
percentage of inorganic As in drinking water to be 100\%, the As content in the drinking water
equivalent to inorganic As intake from rice consumption \((C_{W,\text{equiv}})\) was also predicted for each
participant using the equation:

\[
(C_{W,\text{equiv}} \times V) / BW = (C_R \times X \times W) / BW \quad (2)
\]

or \( C_{W,\text{equiv}} (\mu \text{g L}^{-1}) = (C_R \times X \times W) / V \quad (3) \)

where \( V \) represents the amount of daily water consumption (L day\(^{-1}\)) of the participant,
collected during questioner survey. The Body Mass Index \( [\text{BMI} = BW/H^2] \quad (4) \), where \( H \)
represents height of the participant in m] value of the participants was also calculated to
evaluate the effect of confounding factors on dietary exposure of As due to rice consumption.

RESULT AND DISCUSSION

Variability and Distribution of As in Different Types of Brown Rice Grain. In this
study, one important observation is that all the surveyed household rice samples \((n = 157)\)
were of brown color, whereas Indian Basmati rice samples \((n = 7)\) were of white color. The
subsequent grain size and shape determination indicates that out of these total household
samples, 73 samples (47\%) were SB, whereas 43 (27\%) and 41 (26\%) samples were MS and
LS respectively and all the Basmati rice samples were ELS. People in rural Bengal prefer
brown colored rice (particularly SB brown rice) because of its lower cost and they think it
takes more time to digest, thus they do not feel hungry for long time after taking a meal.
Variability and distribution of total As in the classified brown rice grain and Indian Basmati
has shown by Box and Whisker plot (Figure 2). The figure represents that the concentration of
As in rice grain varies largely according to grain size. Both the variation and median As
concentration is highest (range: 0.09-0.64 mg kg\(^{-1}\), median: 0.32 mg kg\(^{-1}\)) in SB type of rice,
compared to MS (range: 0.06-0.33 mg kg\(^{-1}\), median: 0.16 mg kg\(^{-1}\)) and LS (range: 0.01-0.24
mg kg\(^{-1}\), median: 0.10 mg kg\(^{-1}\)) type of rice, which means that As concentration decreases
with increasing grain size. The As levels in SB and MS types of rice are clustered in middle
position, but for LS type of rice, the values have some positive skewness. This indicates that
for SB and MS types of rice, the middle 50\% (from 25\textsuperscript{th} percentile to 75\textsuperscript{th} percentile) of the
observed values are spread around the median value, while for LS type of rice, the upper part
of the box has wider spread of As levels. Furthermore the 100\textsuperscript{th} percentile As concentration
values of MS and LS rice are nearly equal to 50th and 25th percentile value of SB rice respectively. This point outs that 50% and 75% samples of SB rice have As concentrations above highest value observed for MS and LS type of rice respectively. Considering the global normal range of As concentration in rice, about 90% of SB and 20% of MS type of rice samples exceed this range, whereas for LS type of rice, 100% samples are within this global range. The previous studies made by Meharg et al.33 and Smith et al.34 have reported the higher accumulation of As in the outer bran layer of rice grain. Thus higher As concentration in SB brown rice compared to MS and LS brown rice, might be due to the development of thicker outer bran layer by complex interaction between environmental and genetic controls.26,27 From this discussion it is clear that more As will be ingested into the human body by consuming equal amount of SB type of rice than MS and LS types of rice.

It is worthwhile to note the remarkably narrow whisker band along with low level of As concentration for Indian Long Basmati samples (ELS) (Figure 2). The maximum As concentration observed for ELS rice samples (0.07 mg kg\(^{-1}\)) is lower than the observed minimum As concentration for SB (0.09 mg kg\(^{-1}\)), 10th percentile for MS (0.09 mg kg\(^{-1}\)) and 25th percentile value for LS (0.08 mg kg\(^{-1}\)) type of rice grain. The mean As concentration in ELS rice (0.04 mg kg\(^{-1}\)) is nearly 8.5 times lower than the mean As value for SB brown rice (0.33 mg kg\(^{-1}\)) and 5.6 times lower than the mean value of all types of brown rice grains (0.23 mg kg\(^{-1}\)) collected from the study area. The lower As concentration in white rice compared to brown rice is possibly due to the removal of outer bran layer of rice grain during polishing to make the grain color white.26,27 It is interesting that the mean As concentration in American white Basmati rice from Texas (0.26 ± 0.08 mg kg\(^{-1}\)),19 is nearly six times higher than Indian Basmati rice samples (0.04 ± 0.02 mg kg\(^{-1}\)), collected from our study area.

**Human Exposure to Dietary As through Consumption of Brown Rice.** According to the type of brown rice consumption, the TDI-iAs values of individual participants were grouped into three categories to estimate the effect of different type of brown rice grain to dietary As exposure. The range of TDI-iAs values for SB, MS and LS rice consumers was 0.48-4.34, 0.20-1.56 and 0.05-1.22 µg day\(^{-1}\) kg\(^{-1}\) BW respectively with median value of 1.59, 0.71 and 0.53 µg day\(^{-1}\) kg\(^{-1}\) BW (Figure 3). The comparison of these three groups of TDI-iAs values with the previous WHO recommended PTDI value (2.1 µg day\(^{-1}\) kg\(^{-1}\) BW) indicates that for 29% of the participants consuming SB type of rice, TDI-iAs values exceed the threshold value, while for none of the participants consuming MS and LS type of rice, the TDI-iAs values exceed this threshold value (Figure 3). It should be mentioned here that JECFA (Joint FAO/WHO Expert Committee on Food Additives) withdrawn the previous PTDI value.
because it was in the lower range of confidence interval of the BMDL$_{0.5}$ (benchmark dose limit for 0.5% response), calculated from epidemiological studies.\textsuperscript{32} Consequently, in near future if WHO decreases the threshold value of PTDI, the number of participants, potentially exposed to dietary As due to consumption of brown rice will likely increase significantly. Furthermore the calculation of $C_{W, \text{eq}}$ indicates that for more than 90% SB type of rice consumers, the ingestion rate exceeds the WHO recommended drinking water guideline value of 10 $\mu$g L\textsuperscript{-1} (range: 4.1-83.1 $\mu$g L\textsuperscript{-1}, median: 22.2 $\mu$g L\textsuperscript{-1}). For MS and LS type of rice consumers, in 50% (range: 2.34-25.2 $\mu$g L\textsuperscript{-1}, median: 11.1 $\mu$g L\textsuperscript{-1}) and 25% (range: 0.64-33.5 $\mu$g L\textsuperscript{-1}, median: 8.43 $\mu$g L\textsuperscript{-1}) cases, the ingestion rate exceeds the threshold value (Figure 4). This study suggests that in rural Bengal, consumption of SB type of brown rice is a significant risk factor in terms of dietary exposure to As, whereas people consuming MS and LS types of brown rice are comparatively at lower risk.

To find out the vulnerable age group, participants who consumed SB type of brown rice were classified into three categories (younger: 18-30, middle aged: 31-50 and older: 51-65 yrs) according to their age. The distribution of TDI-iAs values among these age groups indicates that for almost 50% participants of younger and older age groups, the intake rate exceeds the WHO recommended threshold value, while 80% participants of middle age group are at much lower risk from dietary As exposure due to SB type of rice consumption (Figure 5). Nevertheless, for none of participant consuming MS and LS type of rice the TDI-iAs value exceed the WHO recommended PTDI value, it would be of great interest from the point of view of future lowering of threshold value, to investigate the distribution of TDI-iAs among different age groups of the participants. However, because of uneven distribution of number of participants consuming MS and LS rice in different age groups (for e.g. number of younger consumers for MS and LS rice are 2 and 4 out of total number of participants 43 and 41 respectively), the comparison was only limited to the SB type of rice consumers in this study.

The rice consumption pattern and BMI of the participants consuming SB type of rice are compared according to the age groups in Table 1, to shed light on possible reason why younger and older age groups are more vulnerable. The table shows that the amount of rice consumption by the participants of younger and older age groups (median: 350 g and 400 g respectively) are considerably higher than that of the middle aged participant (median: 250 g). However, the BMI's of the participants of younger (median: 17.4 kg m\textsuperscript{-2}) and older age groups (median: 18.4 kg m\textsuperscript{-2}) are lower than that of middle aged participants (median: 18.8 kg m\textsuperscript{-2}). This signifies that despite higher amount of rice consumption, the participants of younger and older age groups seem to be underweight (normal BMI range: 18.5-24.9 kg m\textsuperscript{-2}).
High amount of rice consumption together with lower BMI are considered to be the predisposing factors for adverse effects of higher TDI-iAs values among participants in the younger and older age groups. This study indicates that dietary As exposure does not only depend upon concentration of As in brown rice but also depends on the amount of rice consumption and nutritional status (related to the BMI) of the rice consumer.

Implications. This study shows that consumption of brown rice, particularly of SB type, in rural Bengal may be a potential alternative pathway of As exposure. Consequently, the remediation of As from drinking water only may not be enough to mitigate the As risk to the local population. Increased attention needs to be paid to this exposure pathway which should be linked to any policies on sustainable use of irrigation water. Otherwise, the number of health outcomes and geographical area coverage of As exposure and poisoning will be compounded more in near future. The economical constraint in the rural villages is also an important burden that is closely linked to the As exposure. The reduction of poverty may also help to mitigate this problem by increasing the living standard (consumption of long grain rice, proper nutrition etc.) of the rural populations. Thus As mitigation in West Bengal and Bangladesh demands an integration of both scientific and social policies.

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SUPPORTING INFORMATION AVAILABLE

Inter and intra-regional comparison of As content in rice grains, details about image analysis and reclassification of rice grains, digestion procedure for quantification of total As and quality assurance for As measurement in rice grains have given in the Supporting Information. This information is available free of charge via the internet at http://pubs.acs.org.

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(32) FAO/WHO; *Summary and conclusions of 72nd meeting, JECFA*: Rome, 2010


TABLE CAPTIONS.

Table 1. Age group wise distribution of body weight (BW), amount of rice consumption and body mass index (BMI) of the participant, consuming SB type of rice.
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<table>
<thead>
<tr>
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<th>18-30 yr</th>
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<tr>
<td></td>
<td>BW (kg)</td>
<td>Rice consumption (g)</td>
<td>BMI (kg m⁻²)</td>
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<td>35.5</td>
<td>250</td>
<td>15.0</td>
</tr>
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<td>47.5</td>
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<tr>
<td>Max</td>
<td>61.5</td>
<td>400</td>
<td>23.4</td>
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FIGURE CAPTIONS.

Figure 1. Map of the study area: a, India; b, West Bengal, marked with red circle indicates the block of the study area in Nadia District (modified from Public Health Engineering Department, PHED, Govt. of West Bengal, web site http://www.wbphed.gov.in/); c, d and e represent three villages Chhoto Itna, Debogram and Tehatta with sampling locations. Satellite images of the three villages acquired from Google Earth 6.0.2.

Figure 2. Variation of As Concentration in different type of brown rice. The length of the box represents 25th to 75th percentile. The median value is represented by middle triangle inside the box. The lower and upper solid squares indicate the 10th and 90th percentile and lower and upper whiskers represent the minimum and maximum value respectively. The red lines represent the global normal range of As in rice (0.08-2.0 mg kg\(^{-1}\)).

Figure 3. Variation of TDI-iAs for the participants consuming different type of brown rice. The red line represents the previous WHO recommended PTDI value of 2.1 µg day\(^{-1}\) kg\(^{-1}\) BW.

Figure 4. Distribution of C\(_{W,\text{eqv}}\) for the participants consuming different type brown rice. The red line indicates WHO drinking water guideline of 10 µg L\(^{-1}\).

Figure 5. Distribution of TDI-iAs for the participants consuming SB brown rice, according to different age groups. The red line represents the previous WHO recommended PTDI value of 2.1 µg day\(^{-1}\) kg\(^{-1}\) BW.
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