ARSENIC EXPOSURE RISK FROM RICE AND OTHER DIETARY COMPONENTS IN RURAL BENGAL

Dipti Halder

September 2013
Cover illustration:
Rice field in rural Bengal
(Photograph: Dipti Halder©, 2011)
Dedicated to My Parents…
Foreword

Rice is the staple food for about a third of the global population with most of the world's supply coming mainly from South and Southeast Asia. As a result of the Green Revolution, this region has become heavily dependent on groundwater irrigation for economic development and food security; this region is the world's largest user of groundwater, accounting for withdrawal of over 200 km³ every year. In many parts of this region, the groundwater happens to be contaminated with arsenic and the irrigation-based farming practices has led to high deposition of arsenic in top soils and preferential bioaccumulation of the arsenic in rice compared to other cereal grains. The effects of the massive redistribution of arsenic on sustainable agricultural production in many Asian countries and the impacts on food security at the local and global levels have been unappreciated and under-studied.

In areas where the groundwater is contaminated with arsenic, the primary routes of exposure (to arsenic) are the ingestion of the water, cooking with the water and consumption of locally grown food. In West Bengal and some parts of Bangladesh (so-called arsenic hot spots in the world), communities are currently being provided with water containing low levels of arsenic and the relative contribution of dietary sources to daily intake of arsenic by the local population is expected to have gone up. Reliable data to assess the changing health risks associated with the dietary exposure to arsenic in the local food chain does not exist, however. Miss Halder's thesis is a timely piece of work that has gone a long way to fill this critical gap in data and knowledge. The thesis focuses on rice consumption and has made fundamental contributions in three important areas: (a) concentration and forms (physical and chemical) of As in different type of rice generally consumed in Bengali villages and the exposure risk associated with rice consumption; (b) validation of biomarkers of arsenic exposure (specifically saliva and urine) in the local population; and (c) evaluation of the effects of traditional rice cooking methods on the levels and forms of arsenic in foods.

The research presented in the thesis shows that rice constitutes 76% of the total diet (by weight) of the local population, a predisposing factor in arsenic exposure from this route. The accumulation of arsenic in rice grain was found to be inversely related to the grain size with the highest concentrations in the short bold (SB) type, a variety that is preferred by the villagers because of the its lower cost. Elevated levels of arsenic were also found in locally grown vegetables and represent a significant route of exposure especially for vegans. Over 90% of the arsenic in the rice samples was shown to be inorganic form, a critical observation which portends to the toxicity of rice-borne arsenic in West Bengal. It has be remarked that this was the first study to quantify, meaningfully, the bioavailability of arsenic in the locally grown rice. Few people had thought of what happens to the forms of arsenic when the rice is cooked. In a very interesting and perceptive experiment, the Candidate measured the species of arsenic in rice that was cooked according to tradition local method and showed convincingly that the distribution of the different forms of arsenic in cooked rice is very much similar to that of raw rice. It was shown that inorganic species of arsenic represent about 90% (range: 70% - 100%) of the extractable arsenic and the rest is made up of DMA with the occurrence of MMA being rare. Water extracts of the cooked rice samples showed that trivalent arsenic was the predominating form (~90%) of the inorganic arsenic.
species. These first-of-its-kind data have contributed significantly to our understanding of the effects of cooking on bioavailability of arsenic in local rice meals.

The thesis documents the fact that provision of drinking water from community supplies (deep tube wells) has been successful as a risk management strategy in West Bengal – most villagers now get their water from this source. Nevertheless, the symptoms of toxic exposure to arsenic remain very much in evidence among the local population. The high concentration of arsenic in the saliva and urine of villagers found during the study point to other significant sources of arsenic exposure in the communities. The study thus has a unique perspective in that it deals with arsenic exposure in endemic areas where the ingestion of contaminated water by communities has been minimized. The results are interesting and have far reaching consequences. About one-third of the participants using community water supplies were found to be exposed to unsafe levels of arsenic through dietary sources, mainly from rice consumption. Although cooking of the rice with arsenic-safe water following the traditional cooking method practiced in rural Bengal substantially reduces both total as well as inorganic As content in the cooked rice, consumption of the rice nevertheless still poses a significant health risk to the local population.

The results of all these studies lead to one unmistakable message: in areas where the groundwater is contaminated with arsenic, simply supplying As-safe drinking water to local communities alone is not enough to eliminate the risk of arsenic poisoning. Arsenic in irrigation water permeates the local environment readily and builds up in the human food chain, and this toxic legacy is only now being realized. The research presented in the thesis represents a milepost for future research on this issue.

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Dipti Halder
Stockholm, September 2013
List of Appendix Papers and My Contributions

This thesis is based on the following four papers, referred as corresponding Roman numerals in the text. These papers are attached in the Appendix.

Paper I


I participated in project designing, performed questionnaire survey, collection and laboratory analysis of sample, data interpretation and main part of writing.

Paper II


I participated in project designing, performed questionnaire survey, samples collection, laboratory analysis of the samples for total arsenic, data interpretation and main part of writing.

Paper III


I performed questionnaire survey and participated in laboratory analysis of urine samples for arsenic, data analysis and finalization of manuscript.

Paper IV


I performed questionnaire survey, sample collection and laboratory experiment of rice cooking, interpreted the data and wrote main part of the manuscript.

List of Papers Not Appended in the Thesis

Paper V

Paper VI

Paper VII

Paper VIII

Paper IX

Paper X

Paper XI
### NOMENCLATURE AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>As</td>
<td>Arsenic</td>
</tr>
<tr>
<td>iAs</td>
<td>Inorganic As</td>
</tr>
<tr>
<td>AT</td>
<td>Averaging Time</td>
</tr>
<tr>
<td>ATSDR</td>
<td>Agency for Toxic Substance and Disease Registry</td>
</tr>
<tr>
<td>B</td>
<td>Breadth</td>
</tr>
<tr>
<td>BDL</td>
<td>Below Detection Limit</td>
</tr>
<tr>
<td>BMDI&lt;sub&gt;0.5&lt;/sub&gt;</td>
<td>Bench Mark Dose Level for 0.5% increased prevalence of lung cancer</td>
</tr>
<tr>
<td>BW</td>
<td>Body Weight</td>
</tr>
<tr>
<td>CCCF</td>
<td>Codex Committee on Contaminants in Foods</td>
</tr>
<tr>
<td>CERCLA</td>
<td>Comprehensive, Environmental, Response, Compensation and Liability Act</td>
</tr>
<tr>
<td>Ci</td>
<td>Concentration of Total As in the Exposure Medium</td>
</tr>
<tr>
<td>CR</td>
<td>Cancer Risk</td>
</tr>
<tr>
<td>CR&lt;sub&gt;R&lt;/sub&gt;</td>
<td>Concentration of Total As in Raw Rice</td>
</tr>
<tr>
<td>CSF</td>
<td>Cancer Slope Factor</td>
</tr>
<tr>
<td>C&lt;sub&gt;weq&lt;/sub&gt;</td>
<td>Arsenic Concentration in Drinking Water Equivalent to Inorganic Arsenic Intake from Rice Consumption</td>
</tr>
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<td>DI-iAs</td>
<td>Daily Intake of inorganic Arsenic</td>
</tr>
<tr>
<td>DI-iAS-R</td>
<td>Daily Intake of Inorganic Arsenic from Raw Rice</td>
</tr>
<tr>
<td>DI-iAs-CR</td>
<td>Daily Intake of Inorganic Arsenic from Cooked Rice</td>
</tr>
<tr>
<td>DI-iAs-DC</td>
<td>Daily Intake of Inorganic Arsenic from Dietary Components</td>
</tr>
<tr>
<td>DI-iAs-DW</td>
<td>Daily Intake of Inorganic Arsenic from Drinking Water</td>
</tr>
<tr>
<td>DI-iAs-V</td>
<td>Daily Intake of Inorganic Arsenic from Vegetables</td>
</tr>
<tr>
<td>DMA</td>
<td>Dimethyl Arsinic Acid</td>
</tr>
<tr>
<td>ED</td>
<td>Exposure Duration</td>
</tr>
<tr>
<td>EF</td>
<td>Exposure Frequency</td>
</tr>
<tr>
<td>ELS</td>
<td>Extra Long Slender</td>
</tr>
<tr>
<td>FCR</td>
<td>Field Cooked Rice</td>
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<tr>
<td>HG-AAS</td>
<td>Hydride Generation Atomic Absorption Spectrometer</td>
</tr>
<tr>
<td>HG-AFS</td>
<td>Hydride Generation Atomic Fluorescence Spectrometer</td>
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<tr>
<td>HQ</td>
<td>Hazard Quotient</td>
</tr>
<tr>
<td>IARC</td>
<td>International Agency for Research on Cancer</td>
</tr>
<tr>
<td>ICP-AES</td>
<td>Inductively Coupled Plasma Atomic Emission Spectrometer</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>Inductively Coupled Plasma Mass Spectrometer</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>LS</td>
<td>Internal Standard</td>
</tr>
<tr>
<td>JECFA</td>
<td>Joint FAO/WHO Expert Committee on Food Additives</td>
</tr>
<tr>
<td>L</td>
<td>Length</td>
</tr>
<tr>
<td>LS</td>
<td>Long Slender</td>
</tr>
<tr>
<td>L_{S_{As}}</td>
<td>Log Transformed Arsenic Concentration in Saliva</td>
</tr>
<tr>
<td>L_{TDI}</td>
<td>Log Transformed Value of Total Daily Ingestion of Inorganic Arsenic</td>
</tr>
<tr>
<td>L_{U_{As}}</td>
<td>Log Transformed Arsenic Concentration in Urine</td>
</tr>
<tr>
<td>MMA</td>
<td>Monomethyl Arsonic Acid</td>
</tr>
<tr>
<td>MOA</td>
<td>Mode of Action</td>
</tr>
<tr>
<td>MS</td>
<td>Medium Slender</td>
</tr>
<tr>
<td>NIST</td>
<td>National Institute of Standards and Technology</td>
</tr>
<tr>
<td>PHED</td>
<td>Public Health Engineering Department</td>
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<tr>
<td>PTDI</td>
<td>Provisional Tolerable Daily Intake</td>
</tr>
<tr>
<td>Rfd</td>
<td>Reference Dose</td>
</tr>
<tr>
<td>RNAA</td>
<td>Radiochemical Neutron Activation Analysis</td>
</tr>
<tr>
<td>RR</td>
<td>Raw Rice</td>
</tr>
<tr>
<td>SB</td>
<td>Short Bold</td>
</tr>
<tr>
<td>SRM</td>
<td>Standard Reference Material</td>
</tr>
<tr>
<td>SI</td>
<td>Supporting Information</td>
</tr>
<tr>
<td>TDI-iAs</td>
<td>Total Daily Intake of Inorganic Arsenic</td>
</tr>
<tr>
<td>USEPA</td>
<td>US Environmental Protection Agency</td>
</tr>
<tr>
<td>V</td>
<td>Volume of Water</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>Wi</td>
<td>Amount of Daily Consumption of the Exposure Medium by the Participant</td>
</tr>
<tr>
<td>Xi</td>
<td>Fraction of Inorganic Arsenic Content in the exposure Medium</td>
</tr>
</tbody>
</table>
# Table of Content

*Foreword*........................................................................................................................................... v  
*Acknowledgements*.............................................................................................................................. vii  
*List of Appended Papers and my contributions*.................................................................................... ix  
*List of Papers Not appended in the Thesis*.............................................................................................. ix  
*Nomenclature and abbreviations*.......................................................................................................... xi  
*Table of Content*................................................................................................................................... xiii  
*Abstract*.................................................................................................................................................. 1  

1. **Introduction**...................................................................................................................................... 1  
   1.1. Background of arsenic toxicity .................................................................................................... 1  
   1.2. Arsenic in groundwater of West Bengal, India and Bangladesh and ongoing mitigation measures .................................................................................................................. 2  
   1.3. Potentiality of staple diet as an alternative As exposure pathway ............................................. 3  
   1.4. Total and different species content of As in cooked rice ............................................................ 5  

2. **Research objectives**........................................................................................................................ 5  

3. **Material and Methods**.................................................................................................................... 6  
   3.1. Study area (Paper I, II, III & IV) ................................................................................................. 6  
   3.2. Questionnaire survey (Paper I, II, III & IV) ............................................................................... 7  
   3.3. Collection and analysis of rice, vegetables and drinking water (Paper I, II & III) ..................... 7  
   3.4. Collection and analysis of urine and saliva as biomarker of current As exposure (Paper II & III) ........................................................................................................................................ 8  
   3.5. Effect of traditional rice cooking, practiced in the villages of As affected regions, on the concentration of total and different species of As in rice (Paper IV) .................................................................................................................. 9  
   3.6. Assessment of As exposure through consumption of drinking water, raw rice and vegetables (Paper I & II) and cooked rice (Paper IV) ................................................................. 10  

4. **Results and discussion**.................................................................................................................... 12  
   4.1. Outcomes of the questionnaire surveys (Paper I, II, III & IV) .................................................... 12  
   4.2. Variability and distribution of As in different types of rice consumed in rural Bengal (Paper I) ................................................................................................................................. 12  
   4.3. Distribution of As in different types of vegetables consumed in rural Bengal (Paper II) .......... 13  
   4.4. Distribution of As in drinking and cooking water (Paper II & IV) ............................................. 14  
   4.5. Major As species in the dietary components (Paper II) ............................................................. 14  
   4.6. Human exposure to As through consumption of rice, drinking water and vegetables (Paper I & II) ......................................................................................................................... 17  
   4.7. Arsenic in urine and saliva: current status of As exposure (Paper II & III) ................................ 19  
   4.8. Variation of total As concentration in raw and cooked rice and its relation to As concentration in the cooking water (Paper IV) ................................................................. 20  
   4.9. Evaluating the effect of cooking on As content in rice (Paper IV) ......................................... 22  
   4.10. Variation of As species in raw and cooked rice (Paper IV) ..................................................... 23
4.11. Human exposure to As through consumption of cooked rice (Paper IV) 24

5. Conclusions .................................................................................................................. 25

6. Recommendations and future scope of research ....................................................... 25

References ....................................................................................................................... 27

Other References .......................................................................................................... 33
**Abstract**

This study investigates the risk of arsenic (As) exposure from staple diet to the communities in rural Bengal, even when they have been supplied with As safe drinking water. The results indicate that average accumulation of As in rice grain increases with decrease of grain size [extra-long slender (ELS): 0.04 mg kg⁻¹; long slender (LS): 0.10 mg kg⁻¹; medium slender (MS): 0.16 mg kg⁻¹ and short bold (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. Among the vegetables generally consumed in rural villages, the accumulation of As is highest in the leafy type of vegetables (0.21 mg kg⁻¹), compared to non-leafy (0.07 mg kg⁻¹) and root vegetables (0.10 mg kg⁻¹). Arsenic predominantly accumulates in rice (>90%) and vegetables (almost 100%) in inorganic species [As(III & V)]. The estimates of exposure via dietary and drinking water routes show that when people are consuming water with As concentration <10 µg L⁻¹, the total daily intake of inorganic As (TDI-iAs) exceeds the previous provisional tolerable daily intake (PTDI) value of 2.1 µg day⁻¹ kg⁻¹ BW, recommended by World Health Organization (WHO) in 35% of the cases due to consumption of rice. Considerably high concentration of As in urine and saliva despite drinking of As safe water (<10 µg L⁻¹) further supports that dietary intake of As, mainly through consumption of rice could be alternative pathway of As exposure among the population. When the level of As concentration in drinking water is above 10 µg L⁻¹, the TDI-iAs exceeds the previous PTDI for all the participants. These results imply that when rice consumption is a significant contributor to the TDI-iAs, supplying water with As concentration at current national drinking water standard for India and Bangladesh (50 µg L⁻¹) would place many people above the safety threshold of PTDI. When As concentration in drinking water exceeds 50 µg L⁻¹ As exposure through drinking water largely predominates over the exposure through dietary intake. It is found that the consumption of vegetables in rural Bengal does not pose significant health threat to the population independently. It is also revealed that cooking of rice with high volume of As safe (<10 µg L⁻¹) water can decrease both total and inorganic As content in cooked rice. However, the assessment of As exposure risk indicates that despite such lowering in As concentrations, still consumption of cooked rice is a significant pathway of As exposure to the population in rural Bengal. This study suggests that any effort to mitigate the As exposure of the villagers in Bengal must consider the risk of As exposure from rice consumption together with drinking water.

**Keywords:** Rural Bengal; Arsenic; Rice and other dietary components; Total daily intake; Biomarkers; Risk assessment

1. **Introduction**

1.1. **Background of arsenic toxicity**

Arsenic (As) is a metalloid, naturally and anthropogenically occurs in the environment (Smedley and Kinniburgh, 2002). In nature together with its elemental form (0 oxidation state), it exists in (+III), (+V) and occasionally (-III) oxidation state by forming various inorganic and organic compounds (Cullen, 2008; Hughes et al., 2011). Throughout the history As is known as carcinogen to human (Centeno et al., 2002; Cullen, 2008). Until early 1900s, As was mainly known for acute toxicity because of its involvement in numbers of well-known
murrars, such as death of Napoleon Bonaparte in 1821 (Cullen, 2008; Hughes et al., 2011). The chronic exposure of As at low level and its potentiality to develop cancer was first studied in the mid of 1950s among orchard workers, who were chronically exposed to As containing pesticide residue in soils (Hughes et al., 2011). Later, a number of landmark epidemiological studies (for e.g. Tseng et al., 1968; Chen et al., 1988) have highlighted the susceptibility of the population to develop cancer due to chronic As exposure through drinking water and established the dose-response relationship between As concentration and As toxicity (Hughes et al., 2011). Consequently, in 1992, World Health Organization (WHO) decreased the provisional drinking water guideline for As from 50 µg L\(^{-1}\) to 10 µg L\(^{-1}\) (WHO, 1993). Though initially inorganic As species were considered to be more toxic than organic counterparts, recent investigation indicates that methylated As species in (+III) oxidation state are even more toxic than inorganic species (Smith and Steinmaus, 2009). The exposure (ingestion, inhalation and dermal) to As may cause both non-carcinogenic and carcinogenic health effect to human body (Kapaj et al., 2006). The extent of toxicity vary among individuals depending on rate of ingestion, duration of exposure and methylation capacity of individuals determined by co-factors such as genetic factor, gender, age, ongoing medication, nutritional status, smoking habit and intake of alcohol, coffee and tea (Tseng, 2009). The mode of action (MOA) of As toxicity includes biochemical interactions with sulfur and phosphorous containing groups, formation of reactive oxygen and nitrogen intermediates, altered DNA repair and methylation, genotoxicity and neurotoxicity (Hughes et al., 2011). The most common health outcomes are development of hypertension, diabetics and cardiovascular diseases; neurobehavioral change in adolescence and neuropathic effects; diminished verbal IQ and long-term memory loss; abnormal pregnancy outcomes among women; development of skin lessons (e.g. melanosis, leucomelanos, keratosis, hyperkeratosis etc.); incidence of cancer in gastrointestinal, liver and respiratory tract etc. (Bates et al., 1992; Hopenhayn-Rich et al., 2000; Vahter and Concha, 2001; Kapaj et al., 2006; Smith and Steinmaus, 2009; Chatterjee et al., 2010; Hughes et al., 2011). Consequently, As has been specified as a Group I carcinogen by International Agency for Research on Cancer (IARC) (Chan and Huff, 1997; Centeno et al., 2002; Smith and Steinmaus, 2009) and placed in first position according to its frequency to occur and potentiality to human toxicity, in the latest Comprehensive, Environmental, Response, Compensation and Liability Act (CERCLA) priority list of hazardous substances, published by the Agency for Toxic Substances and Disease Registry (ATSDR) (Hughes et al., 2011).

1.2. Arsenic in groundwater of West Bengal, India and Bangladesh and ongoing mitigation measures

Over the last few decades, the contamination of groundwater by As has been highlighted as an environmental disaster in many regions of the world, including in the countries of Europe, North America and Australia (Nordstrom, 2002; Nriagu et al., 2007). The problem is most acute in South and Southeast Asia, particularly in eastern to northeastern part of India and adjoining Bangladesh, jointly called Bengal Basin (Chakraborti et al., 2004, 2008; Bhattacharyya et al., 2011). In early of 1970s people living in this region were compelled to shift their drinking water source from surface water to groundwater to avoid various water borne diseases like diarrhea and cholera (Mukherjee et al., 2007). Millions of hand pumped tubewells were installed mostly by private initiatives in the shallow aquifers (<50 m) (Escamilla et al., 2011). At present, about 95% people living in this region are heavily dependent on groundwater for domestic purposes like drinking, cooking, bathing and washing (Chatterjee et al., 2010; Fendorf et al., 2010). Consequently, the occurrences of high As in shallow groundwater has caused severe mass poisoning putting more than 60 million people at risk in this region (Smith et al., 2000; Chakraborti...
et al., 2004, 2008, 2010; Chatterjee et al., 2010).

Since the first reporting of elevated level of dissolved As in drinking water of West Bengal (Saha, 1984), extensive research has been undertaken regarding well screening, source characterization and mobilization along with possible mitigation processes (Bhattacharya et al., 1997; Nickson et al., 1998; Harvey et al., 2002; van Geen et al., 2003; Islam et al., 2004; McArthur et al., 2004; Charlet and Polya, 2006; von Brömssen et al., 2007; Nath et al., 2008; Biswas et al., 2011, 2012a, b). The initiatives have also led to development of strategies to reduce As exposure from drinking water. Both national and international agencies are currently working to provide safe drinking water to the affected rural population, either by remediation of As contaminated groundwater, changing the sources of drinking water by targeting deeper safe aquifer, or supplying treated surface water (Ahmed et al., 2006; Jakariya et al., 2007). Despite the implementation of these mitigation measures, the success to reduce the extent of As exposure is still limited.

1.3. Potentiality of staple diet as an alternative As exposure pathway

As a consequence of “Green Revolution” farmers in India and Bangladesh are now cultivating their land three to four times per year, which has led these countries to become self-reliant for food. These cultivations are largely dependent on groundwater irrigation (Dey et al., 1996). In last few decades thousands of high capacity, large diameters motorized pumps have been installed to meet this irrigation requirement (Norra et al., 2005; Neidhardt et al., 2012). Currently, about 85% of the total groundwater abstraction is used for agricultural irrigation purpose (BGS and DPHE, 2001). The groundwater irrigation is highest during the dry season rice (Oryza sativa L.) (Boro) cultivation, which solely relies on groundwater irrigation (Dey et al., 1996). Meharg and Rahman (2003) have reported that for Boro rice cultivation, about 1000 mm irrigation water is required per hectare. These pumps are mostly abstracting groundwater from shallow aquifers (Meharg and Rahman, 2003), which are heavily contaminated with dissolved As. Consequently, As is increasing on the top soil of the irrigated lands. Meharg and Rahman (2003) have 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. By monitoring As concentration in the Bangladesh paddy field soil over a period of 3 years, Dittmar et al. (2010a) have further predicted that continuation of current irrigation practice would increase the As concentration in top 40 cm of the paddy field soil by a factor of 1.5 to 2 by the year 2050. The increased As concentration in the irrigated lands ultimately results in the accumulation of As in the food crops cultivated on these lands (Meharg and Rahman, 2003; Roberts et al., 2007; Dittmar et al., 2010b; Roberts et al., 2010; Spanu et al., 2012). In Bangladesh, the concentration of As in rice grains positively correlates with As concentration in irrigation water (Zavala and Duxbury, 2008). General cultivation practices such as continuous flooding of the irrigation land for the cultivation of rice also facilitates As mobilization in the rice fields. Continuous flooding of the lands leads to soils becoming reduced with time during rice cultivation, which increases the bioavailability of As in the soil pore water by reductive dissolution of As hosting mineral phases such Fe oxyhydroxides in the soils (Marin et al., 1993; Abedin et al., 2002; Meharg and Rahman, 2003; Roberts et al., 2011; Spanu et al., 2012). As a result, the accumulation of As in rice is comparatively 10 fold higher than other cereals (Williams et al., 2007; Raab et al., 2009). Several studies have already reported the accumulation of As in rice grains cultivated in this regions (Roychowdhury et al., 2002; Duxbury et al., 2003; Meharg and Rahman, 2003; Roychowdhury et al., 2003; Meharg, 2004; Williams et al., 2005, 2006, 2007; Mondal and Polya, 2008; Roychowdhury, 2008; Zavala and Duxbury, 2008; Panaullah et al., 2009; Bhattacharya et al., 2010).
The accumulation of As in rice grain is not only limited to the regions, where As concentration in groundwater is high. A detail literature survey indicates that the accumulation of As in rice grains has been reported from six continents out of seven, except Antarctica (Fig. 1) (Zavala and Duxbury, 2008; Meharg et al., 2009; Rahman and Hasegawa, 2011; Sahoo and Kim, 2013). It is revealed 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\(^{-1}\)) (Zavala and Duxbury, 2008) (Supporting Information (SI) of Paper I). The higher As content in American rice has been attributed to the legacy of previous extensive use of arsenical pesticides in the country (Williams et al., 2007). It has been shown that in rice As is predominantly present in inorganic and methylated forms, but their distribution varies genetically and geographically (Williams et al., 2005; Liu et al., 2006; Mondal and Polya, 2008; Signes et al., 2008; Norton et al., 2009; Ahmed et al., 2011; Bhattacharya et al., 2013). In American rice, As is present mostly in organic form (DMA), which is less toxic compared to its inorganic As species predominantly found in Asian and European rice (Williams et al., 2005). Zavala and Duxbury (2008) have calculated the global normal distribution range of As in rice grain (0.08 - 0.20 mg kg\(^{-1}\)) and Williams et al. (2006) 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.

Rice is presently considered as one of the major staple foods throughout the world, being consumed as high as 400 million tons globally per year, representing 50% of the total cereal consumption (Ricestat, 2007). Particularly in the Asian countries per person daily rice intake may be up to 0.5 kg (dry weight) (Zavala and Duxbury, 2008; FAO, 2002). In West Bengal and Bangladesh, rice consumption provides on average 72.8% of the daily caloric intake per capita (Ninno and Dorosh, 2001; Mondal and Polya, 2008). Therefore rice has the
potentiality to be an alternative route of As exposure in many parts of the world, especially in India and Bangladesh (Williams et al., 2006, 2007; Mondal and Polya, 2008; Signes et al., 2008; Zavala and Duxbury, 2008; Gilbert-Diamond et al., 2011; Banerjee et al., 2013). The recent findings indicate that the accumulation of As is also high in other dietary components such as vegetables, which are commonly consumed with rice in the As affected regions of rural Bengal (Roychowdhury et al., 2002; Alam et al., 2003; Rahman et al., 2003; Williams et al., 2006; Roychowdhury, 2008; Signes-Pastor et al., 2008).

The above presented review indicates that it is highly imperative to estimate the attendant health risk of dietary As exposure by quantifying the As content in household rice and other dietary components, commonly consumed in rural Bengal. It is also of great interest to investigate the risk of As exposure through dietary intake to the community, where people have been supplying with As safe drinking water for last few years. The findings can have important consequences on the formulation of strategies towards sustainable As mitigation management in rural Bengal.

1.4. Total and different species content of As in cooked rice

Although rice is generally consumed in cooked form, most of the studies conducted to assess rice consumption as an alternative route of As exposure considered only the total as well as inorganic As contents in the raw (uncooked) rice. A few recent investigations have reported that the total concentration as well as the content of different species of As in the cooked rice can be different from that in the corresponding raw rice, depending on the As concentration in cooking water and the processes of rice cooking (Ackerman et al., 2005; Laparra et al., 2005; Rahman et al., 2006; Sengupta et al., 2006; Mihucz et al., 2007; Signes et al., 2008; Raab et al., 2009). This suggests that the assessment of As exposure by only quantifying As content in the raw rice could be potentially biased (Ackerman et al., 2005). However, these investigations were merely based on the laboratory cooking of small number of rice samples, mostly collected from the supermarket. To date, only Bae et al. (2002), Mondal and Polya (2008), Ohno et al. (2009) and Pal et al. (2009) have investigated the effect of indigenous cooking habit of the rural villagers by quantifying the As content in the pair of raw and cooked rice, collected from the affected villages. However, these studies were limited to investigations on the change in total As content only. Smith et al. (2006) have quantified the inorganic As content in the cooked rice collected from the villages, but did not report the speciation of As in the corresponding raw rice. So far, no significant attempt has been made to assess the changes in speciation of As in rice due to indigenous cooking practice of the villagers, which is important for meaningful assessment of As exposure risk from rice consumption in rural Bengal.

2. RESEARCH OBJECTIVES

The overall objective of the research presented in the thesis is to assess the potentiality of consumption of rice and other dietary components as an alternative pathway of As exposure among the population in rural Bengal. The specific objectives are:

i) Assessment of variability and distribution of As in different type of rice generally consumed in As affected villages and estimation of risk of As exposure to the population from rice consumption (Paper I);

ii) Quantification of total dietary intake of As to assess the risk of As exposure to the communities of rural Bengal, where people have been supplied with As safe drinking water for the past few years (Paper II);

iii) To investigate the current status of As exposure among population by determining the As concentration in biomarkers such as urine and saliva (Paper II & III) and

iv) Assessment of change in concentration of total and different species of As in rice because of traditional rice
cooking, practiced in the villages of As affected regions (Paper IV).

3. MATERIAL AND METHODS

3.1. Study area (Paper I, II, III & IV)

The study was conducted in three neighboring villages, namely Chhoto Itna, Debogram and Tehatta of Tehatta-II Block in Nadia District of West Bengal, India (Fig. 2). The area was selected based on a previous (2006 - 2007) cross-sectional survey carried out by Guha Mazumder et al. (2010) in Nadia District. The study areas are surrounded by agricultural lands. The major agricultural practices in the area include 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 villagers are very poor. Though, the background As concentration in groundwater is very high in these villages, most of the people are drinking Public Health Engineering Department (PHED), Government of West Bengal supplied As safe water (<10 µg L⁻¹) for last 3 – 4 years (Guha Mazumder et al., 2010; Bhowmick et al., 2013). Thus, these three villages have

Fig. 2. Map of the study area: (a) India; (b) West Bengal, the red circle indicates the block of the study area in the Nadia District (modified from Public Health Engineering Department, PHED, Govt. of West Bengal, Web site http://www.wbphed.gov.in/); and parts (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.
provided the opportunity to investigate the risk of As exposure from dietary consumption to the population, when they are supplied with As safe drinking water.

The study presented in the thesis combines questionnaire-based survey among the population in the study areas to collection and quantification of total and different As species in drinking water and different components of local staple diet, assessment of As exposure from different routes and determination of As concentration in biomarkers. The specific methods adopted to address each objective have been summarized in the following section.

3.2. Questionnaire survey (Paper I, II, III & IV)

Two questionnaire surveys were conducted to estimate the risk of As exposure from local staple diet and current status of As exposure among the population (Paper I, II & III) and to assess how does indigenous rice cooking in the rural villages affect As concentration and its species distribution in rice (Paper IV) respectively. The first survey includes a cohort of 157 participants (male: 68 and female: 89), selected randomly from the villages. The only selection criterion was that they must have lived in their villages at the time of the study for at least 10 years. Such a selection criteria was imposed only to ensure that the study would provide a real exposure scenario of the population in the area. The participants were interviewed face to face in local language of Bengali following a structured questionnaire, which included demographic information (e.g. age, height, body weight, level of education, occupation, marital status, smoking habit, ongoing medication etc.), dietary habit (frequency, amount of consumption and source of the components) and pattern of drinking water intake (Nriagu et al., 2012) (Paper I, II & III). The second survey includes a total of 59 participants from 29 households in Debogram village. These 59 participants include 29 female, who cooked rice in the surveyed households. Here also the participants were selected and interviewed similarly to the first survey. Additionally, the women were asked about the procedure of rice cooking and source of cooking water (Paper IV).

3.3. Collection and analysis of rice, vegetables and drinking water (Paper I, II & III)

Following the first questionnaire survey, rice samples (n = 157) were collected from the household of each participant. 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. Since, in the rural households of Bengal, vegetables are not always available in the household’s basket like rice; vegetables were collected directly from the sources. The questionnaire survey revealed that although some participants had home garden within their premises, major portion of daily vegetables for all the participants was collected from the local market. Accordingly, the available vegetable samples were collected both from the home gardens (n = 28) and vegetable shops in the local market (n = 52). Vegetable samples were collected two times over the year (summer and winter) to include all available types of seasonal vegetables that people consume. Drinking water samples (n = 24) were collected in 15 mL prewashed polyethylene bottles from the sources, which supply water to the participants and then acidified onsite with 0.15 mL HNO₃ (14N, Suprapur, Merck). After being returned to the laboratory vegetables and drinking water samples were preserved at -20°C and 4°C respectively until they were analyzed.

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: household rice plus Basmati rice collected from local market) 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-slender (MS, L= 5.51-6.60 mm,
of As would increase the extraction yield, but at the same time might cause some decomposition of the organically bound As and falsely indicate higher inorganic As content (Šlejkovec et al., 1999). The chromatographic separation of the species in the extract was performed with Hamilton PRP-X100 anion exchange column and subsequently quantified with hydride generation atomic fluorescence spectrometer (HG-AFS, PS Analytical, Kent, UK) (Šlejkovec et al., 1999). The total As concentration in the samples, in which speciation was performed, was reanalyzed by Radiochemical Neutron Activation Analysis (RNAA) (Byrne and Vakselj, 1974) to estimate the fraction of As recovery with the extractant (see SI Paper II for detailed methodology of As speciation and total As determination with RNAA in rice and vegetables samples). No attempt was made to speciate As in drinking water, as it was reported that As in groundwater is present primarily in inorganic form (Chowdhury et al., 2000; Shraim et al., 2002).

### 3.4. Collection and analysis of urine and saliva as biomarker of current As exposure (Paper II & III)

In order to assess the current status of As exposure among the population urine and saliva samples were collected and analyzed for As. In Paper II a total of 80 urine samples were collected from the group of participants, who were drinking PHED supplied tap water, presumed to be safe for As (<10 µg L\(^{-1}\)). While paper III reports about 101 urine samples, among which 78 samples represent the participants who were drinking tap water supplied by PHED. A total of 45 urine samples were common in two papers (Paper II & III). Additionally for Paper III a total of 101 saliva samples were collected from the corresponding participants, from where urine samples were collected. After collection urine and saliva samples were preserved in a salt-ice mixture in the field and after being returning to the laboratory preserved at -20 °C until analysis. For further details of collection and preservation of urine and saliva samples readers are referred to Paper III.
After filtering through 0.45 μm membrane the urine samples were digested with HNO₃ and H₂O₂ for the quantification of total As with HG-AAS, specified above (see SI of Paper II for details of the digestion procedure). Reagent blanks and SRM samples of 2670a were included in every batch of urine analysis to maintain accuracy. The recovery of SRM was always >97%. To check precision of the analysis one-fifth of the samples were selected randomly and reanalyzed (see SI of Paper II for details of the quality assurance). The measured As concentration in urine samples was corrected to the mean specific gravity of the samples (1.015 g mL⁻¹). Total As concentration in saliva samples was analyzed at Department of Chemistry, University of Girona with inductively coupled plasma - mass spectrometer (ICP-MS) (Agilent 7500c). Before analysis with ICP-MS each samples were centrifuged and mixed with appropriate amount of HNO₃ (2% v/v), ethanol (2% v/v), and internal standard (I.S) (10 μg L⁻¹ Rhodium standard). The accuracy of the analysis was maintained by analyzing the in house secondary standard, prepared by spiking different concentration of As to non-contaminated saliva samples, collected from volunteers of different age and gender (Paper III). The recovery of As was varied between 99% - 101%. For further details of the saliva analysis, readers are referred to Paper III.

3.5. Effect of traditional rice cooking, practiced in the villages of As affected regions, on the concentration of total and different species of As in rice (Paper IV)

Following the second questionnaire survey, as mentioned in the section 3.1, a total of 29 pairs of raw and cooked rice samples were collected from the surveyed households. After collection, raw rice samples were stored at room temperature until processed for the analysis, while cooked rice samples were stored in an ice box at the field and after being returned to the laboratory preserved at -20 °C. Collected raw rice samples were classified into the categories of SB, MS and LS as described in the section 3.3. Cooking waters (n = 4) were collected directly from the sources following the same procedure of drinking water collection, as mentioned in the previous section.

Since water from rice washing and discarded water after cooking (starch water) were not available in the households during sample collection, mass balance of As in the cooking processes to account for the change in As concentration in cooked rice, was conducted based on the laboratory experiment. In the laboratory, rice samples were cooked following the traditional procedure practiced in the rural Bengal. A total of 15 raw rice samples (52%) were selected randomly and 10 g of each sample was washed twice with 60 mL of Milli-Q water (18 MΩ) by continuous stirring for 2 minutes at each step. After washing, 3 samples (10%) were boiled with another 60 mL of Milli-Q water until the rice grain became soft like the collected cooked rice samples. After cooking, starch water was collected in a beaker and the weight was determined. Equivalent weight of dry cooked rice to the corresponding raw rice sample was determined by drying the cooked rice samples at hot air oven at 65 °C until the weight became constant. The rice wash water (n = 15) and starch water (n = 3) were also stored in polyethylene bottles at 4 °C for future processing for As quantification.

Collected raw rice (n = 29) and cooked rice samples (n = 32; households cooked and laboratory cooked) were analyzed for the concentration of total As and different As species following the same procedure at the Department of Environmental Sciences, Jožef Stefan Institute. The concentration of total As was quantified by RNAA following the methodology described in Paper II. To estimate precision of the analysis, 6 raw rice (21%) and 3 cooked rice samples (10%) were randomly selected and reanalyzed. The cooking water (n = 4) collected from the field, were analyzed directly by inductively coupled plasma atomic emission spectrometer (ICP-AES, Thermo Scientific iCAP 6500). Water from rice washing (n = 15) and
starch water (n = 3) were analyzed for total As after digestion with HNO₃ (see SI of Paper IV for detail digestion procedure). All the cooking water, rice washing water and starch water samples were analyzed at the Department of Chemistry, KTH Royal Institute of Technology. For the purpose of mass balance, the concentration of As in rice-wash water and starch water have been expressed in terms of dry weight of the raw rice before cooking.

The speciation of As in raw and cooked rice was performed in two batches: 14 in the first batch and 15 in the second batch. To make the risk assessment more relevant, present study has utilized water as extractant to provide a better approximation of the bioavailable form of As in rice (see SI of Paper IV for detail extraction procedure). The chromatographic separation and subsequent quantification of different As species in the extracts were performed following the same procedure as mentioned in the section 3.3. Typical positions of the peaks in the chromatographic separation, corresponding to different As species present in the standard and extracts of raw and cooked rice samples are shown in Fig. 3. Other than the cooked rice samples of the second batch, samples were extracted in duplicate and As species were separated and quantified in duplicate for each extraction (total of 4 (2 × 2) measurements). Arsenic species were not quantified in the parallel extraction of cooked rice samples in the second batch because of difficulties with the clogging of chromatographic column. To compare the extraction recovery, 3 raw (#RR-5, 8 & 13) and 1 cooked rice (#FCR-12) samples were selected randomly and As species were quantified by extraction with a mixture of methanol and water (1:1).

3.6. Assessment of As exposure through consumption of drinking water, raw rice and vegetables (Paper I & II) and cooked rice (Paper IV)

Daily intake of inorganic As due to consumption of a particular exposure medium (e.g. drinking water, raw and cooked rice and vegetables) (DI-iAs) for a participant was computed using the equation as follows (EPA, 2001):

\[
DI-i\text{As (µg day}^{-1} \text{ kg}^{-1} \text{ BW}) = (C_i \times X_i \times W_i) / BW
\]

(1)

where \(C_i\), \(X_i\), \(W_i\) and BW represents the concentration of total As in the exposure medium (µg L⁻¹ for drinking water and
µg kg⁻¹ for raw and cooked rice and vegetables), fraction of inorganic As content in the medium, amount of daily consumption of the exposure medium by the participant (L day⁻¹ for drinking water and kg day⁻¹ for raw and cooked rice and vegetables) and body weight of the participant (kg) respectively. The daily intake of inorganic As due to consumption of raw rice (DI-iAs-R) was calculated in Paper I considering the average fraction of inorganic As content in raw rice as 0.81, as reported by Williams et al. (2005). To make an exact estimation, here the values of DI-iAs-R have been recalculated by quantifying the average fraction of inorganic As content in the rice samples consumed in the study area as 0.92 (Paper II). The average fraction of inorganic As content in the collected vegetables samples was quantified as 1 (Paper II). Since, As in groundwater is present primarily in inorganic form (Chowdhury et al., 2000; Shraim et al., 2002), total As concentration was used for calculation of daily intake of As from drinking water (Paper II). By combining DI-iAs-R, daily intake from drinking water (DI-iAs-DW) and daily intake from vegetables (DI-iAs-V), the total daily intake of inorganic As (TDI-iAs) for each participant was calculated (Paper II). For the calculation of daily intake of inorganic As through consumption of cooked rice (DI-iAs-CR) the fraction of inorganic As content in individual cooked rice sample was used for the corresponding participants, instead of taking similar average fraction for all participants (Paper IV). It should be mentioned here that at the recent 72nd meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the previous provisional tolerable daily intake (PTDI) value for inorganic As intake (2.1 µg day⁻¹ kg⁻¹ BW) has been withdrawn, because the value was in the lower range of BMDL₀.₅ (bench mark dose level for 0.5% increased prevalence of lung cancer) (FAO/WHO, 2010). Thus currently there is no established guideline to assess the health risk due to dietary intake of inorganic As. However, the Codex Committee on Contaminants in Foods (CCCF) has argued that daily intake of inorganic As below BMDL₀.₅ does not necessarily indicates that there is no risk and cannot be regarded as a safety standard (Codex, 2011). This argument motivated to compare daily intake of inorganic As of the participants with the previous PTDI value of 2.1 µg day⁻¹ kg⁻¹ BW to assess health risk of inorganic As exposure (Paper I, II & IV).

Additionally, in Paper I the As concentration in the drinking water equivalent to inorganic As intake from rice consumption (Cᵦₑⱼᵦₑⱼᵦₑⱼₑ) was also predicted for each participant using the equation:

\[ (Cᵦₑⱼₑ) / BW = (Cᵦᵦₑ × X × W) / BW \] (2)

or \( Cᵦₑⱼₑ = (Cᵦᵦₑ × X × W) / V \) (3)

where V represents the amount of daily water consumption (L day⁻¹) of the participant, collected during questionnaire survey. In Paper IV the non-carcinogenic and carcinogenic health effects of As exposure to the population through consumption of cooked rice were also assessed by calculating hazard quotient (HQ) and cancer risk (CR) as follows (EPA, 2001):

HQ = DI-iAs-CR / Rfd \( \text{and} \) CR = (DI-iAs × EF × ED × CSF) / AT \( \text{where} \) Rfd, EF, ED, AT and CSF stand for the reference dose of As for assessing non-carcinogenic health effect (0.304 µg kg⁻¹ day⁻¹), exposure frequency (365 days yr⁻¹), exposure duration, averaging time (USEPA value of 70 × 365 days yr⁻¹) and cancer slope factor for As (1.5 per mg kg⁻¹ day⁻¹) respectively (USEPA, 1998). A fixed value of 10 years was considered for ED, as the criteria for the participant selection was to have lived in the current place for last 10 years. According to US Environmental Protection Agency (USEPA), a value of HQ >1 indicates potential risk of adverse non-carcinogenic health effect of As to the participant, while CR >1 per 10⁴ people suggests that As exposure is significant to develop cancer among the population during lifetime (USEPA, 1998).
4. RESULTS AND DISCUSSION

4.1. Outcomes of the questionnaire surveys (Paper I, II, III & IV)

Similar to previous studies conducted in other parts of India and Bangladesh (Roychowdhury et al., 2002; Alam et al., 2003; Signes-Pastor et al., 2008), the food frequency questionnaire survey adopted in the Paper I, II & III reveals that rice with vegetables is the main staple food in rural Bengal. People mostly consume rice three times per day with vegetables. Daily laborers and farmers even carry homemade food and drinking water to the working places. The consumption of wheat, fruits, and animal protein (in the forms of egg, fish, chicken, mutton, and beef) is very occasional, estimated to be 2 - 4 times per month; therefore these routes are not considered for the assessment of dietary As intake in these studies. By weight rice contributes almost 76% of the total diet, while the rest is comprised of vegetables. Since, the villagers either do not have land for rice cultivation or do not grow enough rice to last a whole year, the market place is the major source of rice to the villagers. The consumption pattern of different dietary components and drinking water shows that amount of rice consumption varies according to the age of the participants (Table 1). The older (51 – 65 years) participants consume higher amount of rice compared to the younger (18 – 30 years) and middle age group (31 – 50 years) participants. However, the amount of vegetable consumption and drinking water intake is similar for the participants in all age groups (Table 1) (Paper I & II).

The questionnaire survey conducted as a part of Paper IV further reveals that market place is the major source of rice for daily consumption in the study area. In all the surveyed households, rice grains are washed at least 2 - 4 times with excess amount of water before cooking. After washing, rice grains are boiled with 3 – 6 times the volume of water usually in an aluminum cookware. When the rice grains become soft, the excess starch water is discarded (Paper IV).

Previous studies have also reported similar rice cooking method from other parts of Bengal (Bae et al., 2002; Sengupta et al., 2006; Roychowdhury 2008; Pal et al., 2009).

4.2. Variability and distribution of As in different types of rice consumed in rural Bengal (Paper I)

In this study, one important observation is that all the surveyed household rice samples (n = 157) were of brown color, whereas

Table 1. Consumption pattern of drinking water and dietary components (rice and vegetables) among the participants of different age groups.

<table>
<thead>
<tr>
<th>Dietary Component</th>
<th>18 - 30 yrs (n = 13)</th>
<th>31 - 50 yrs (n = 112)</th>
<th>51 - 65 yrs (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice (g day⁻¹ dry wt)</td>
<td>200 350 400</td>
<td>100 350 500</td>
<td>150 400 450</td>
</tr>
<tr>
<td>Vegetables (g day⁻¹ dry wt)</td>
<td>86.3 118 131</td>
<td>22.7 121 147</td>
<td>78.3 119 137</td>
</tr>
<tr>
<td>Drinking Water (L day⁻¹)</td>
<td>2.5 3.5 6.0</td>
<td>2.0 3.5 6.0</td>
<td>2.5 3.5 5.0</td>
</tr>
</tbody>
</table>
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 (Fig. 4). 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 figure further indicates that the 100th percentile As concentration values of MS and LS rice are nearly equal to 50th and 25th percentile value of SB rice respectively. This points out 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 Mehart et al. (2008) and Smith et al. (2009) 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 (Liu et al., 2006; Norton et al., 2009; Ahmed et al., 2011). 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 low level of As concentration for Indian Long Basmati samples (ELS) (Fig. 4). 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 (Norton et al., 2009; Ahmed et al., 2011). It is interesting that the mean As concentration in American white Basmati rice from Texas (0.26 ± 0.08 mg kg⁻¹) (Zavala and Duxbury, 2008), is nearly six times higher than Indian Basmati rice samples (0.04 ± 0.02 mg kg⁻¹), collected from the present study area.

4.3. Distribution of As in different types of vegetables consumed in rural Bengal (Paper II)

Vegetables that are generally consumed in the villages can be classified into three categories based on the edible part: leafy (n = 16), non-leafy (n = 44) and root (n = 20) vegetables (Fig. 5). For all age groups, leafy and root vegetables represent the major portion of vegetable intake in the study area (Fig. 5). The types of daily leafy vegetable consumption depend on the seasonal availability and amount of individual leafy vegetable intake over the year is roughly same. Potato is available in the market throughout the year and constitutes a significant portion of the daily intake of vegetables.

The comparison of mean As concentrations in the different groups of vegetable samples collected from the study area shows higher
As accumulation in the leafy vegetables (mean: 0.21 mg kg\(^{-1}\)) compared to the non-leafy and root vegetables (mean: 0.07 and 0.1 mg kg\(^{-1}\) respectively) (Fig. 5). Though the study conducted by Alam et al. (2003) at Samta village of Jessore district in Bangladesh did not find significant difference in As accumulation among the different groups of vegetables, studies by Williams et al. (2006) from different parts of Bangladesh and Roychowdhury et al. (2002) from As affected regions of Murshidabad, West Bengal also reported similar higher As accumulation in leafy vegetables. If As accumulation is compared among the individual vegetables of different groups, higher As concentrations are observed in spinach and amaranth leaf for leafy vegetables, amaranth steam for non-leafy vegetables and giant taro, arum tuber and elephant foot for root vegetables (Fig. 5). The high accumulation of As in taro, arum tuber and elephant foot has also been reported in Bangladesh (Alam et al., 2003; Huq and Naidu, 2005; Williams et al., 2006). In Bangladesh, Das et al. (2004) and Huq and Naidu (2005) have also reported high concentration of As in potato. However in West Bengal, Roychowdhury et al. (2002) have found high concentration of As in potato skin compared to potato flesh. Since the consumption of potato skin is not very common in our study area, present study attempted to quantify As concentration in potato flesh only and has found that it shows the lowest As accumulation (mean: 0.07 mg kg\(^{-1}\)) amongst the root vegetables (Fig. 5). The comparison between As concentrations in vegetables collected from the households and market places did not show any significant difference.

**4.4. Distribution of As in drinking and cooking water (Paper II & IV)**

The first questionnaire survey indicates that people in the study area drink mostly...
groundwater. The drinking water sources include PHED supplied tap water, government installed deep tube wells and shallow private tube wells. The concentration of As in drinking water varies largely from below detection limit (BDL, <1 µg L⁻¹) to 875 µg L⁻¹. Out of the total surveyed drinking water sources (n = 24), only 9 (37.5%) sources are safe compared to WHO provisional drinking water guideline of 10 µg L⁻¹ for As. In 6 (25%) and 9 (37.5%) tube wells the level of As concentration is >10 – 50 and >50 µg L⁻¹ respectively. The PHED supplied tap water and government installed deep tube wells are safe, while privately owned household shallow tube wells are mostly contaminated with As. The survey data reveals that though the number of drinking water sources with As concentration <10 µg L⁻¹ is small, the majority of the participants (n = 116, 73.9%) collect drinking water from these sources for the last 3 – 4 years. Only 17.8% (n = 28) and 8.28% (n = 13) of the participants are consuming drinking water with As concentration of >10 - 50 and >50 µg L⁻¹ respectively. Presently, people in the study area are sharing common low As drinking water sources due to increased social awareness about As exposure from drinking water according to a recent study by Guha Mazumder et al. (2010) (Paper II).

The second questionnaire survey reveals that all the studied households (n = 29) collect drinking and cooking water from the common sources. Out of the 29 surveyed households, 24 use PHED supplied tap water, while other 5 households share 3 privately installed tubewells. The determination of As concentration in the collected water samples (n = 4) again indicates that the PHED supplied tap water is safe from As (BDL), while the privately owned tubewells are severely contaminated (>50 µg L⁻¹) (SI Table SI 1 of Paper IV) (Paper IV).

4.5. Major As species in the dietary components (Paper II)

Speciation of As (organic and inorganic species) accumulated in the foods is necessary to accurately estimate the potential dietary As exposure (Mondal and Polya, 2008). Few previous studies (e.g. Rahman et al., 2003; Williams et al., 2005; Smith et al., 2006; Williams et al., 2006; Mondal and Polya, 2008; Signes-Pastor et al., 2008; Roychowdhury, 2008) have attempted to quantify inorganic As accumulation in food components collected from the different As affected regions of West Bengal and Bangladesh. The present study tried to verify these trends by determining inorganic As content in rice and different groups of vegetables that generally people prefer to consume in the study area (Table 2). Although, a significant amount of As was not extracted by the mixture of methanol and water, the present study further supports the predominant accumulation of inorganic As in the rice and vegetables grown in West Bengal and Bangladesh. This study indicates that 91.7% and 100% of the extractable As is present as inorganic species in rice and most of the studied vegetables respectively (Table 2). It is further revealed that the percentage of inorganic As content
in rice varies to some extent according to location (Table 2), which may be because of complex interaction between edaphic and environmental factors (Liu et al., 2006; Norton et al., 2009; Ahmed et al., 2011).

However, in most types of the vegetables As is entirely present (100%) in inorganic form throughout the Bengal region. This prompted us to assume total As concentration for vegetables and 0.92 times of the total As

### Table 2. Accumulation of inorganic As in food stuffs collected from study area and its comparison to other studies conducted in different parts of West Bengal and Bangladesh.

<table>
<thead>
<tr>
<th>Dietary component</th>
<th>Type of Component</th>
<th>Reference</th>
<th>Sample location</th>
<th>Range of As conc. (mg kg⁻¹)</th>
<th>% Extracted</th>
<th>% of Inorganic As</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice</td>
<td>Household rice</td>
<td>Present study</td>
<td>West Bengal</td>
<td>0.01-0.64</td>
<td>61</td>
<td>91.7 ± 9</td>
</tr>
<tr>
<td></td>
<td>Household rice</td>
<td>Mondal &amp; Polya, 2008</td>
<td></td>
<td>0.02-0.17</td>
<td>82</td>
<td>74 ± 13</td>
</tr>
<tr>
<td></td>
<td>Market rice</td>
<td>Williams et al., 2005</td>
<td>India</td>
<td>0.03-0.30</td>
<td>75.5</td>
<td>80 ± 3</td>
</tr>
<tr>
<td></td>
<td>Paddy rice</td>
<td>Signes-Pastor et al., 2008</td>
<td>Bangladesh</td>
<td>0.12-0.66</td>
<td>-</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>Atab rice</td>
<td></td>
<td>West Bengal</td>
<td>-</td>
<td>98.4</td>
<td>49.8</td>
</tr>
<tr>
<td></td>
<td>Boiled rice</td>
<td></td>
<td>-</td>
<td>100</td>
<td>80.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Puffed rice</td>
<td></td>
<td>-</td>
<td>100</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rice</td>
<td>Rahman et al., 2003</td>
<td></td>
<td>0.04-1.11</td>
<td>-</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>Cooked rice</td>
<td>Smith et al., 2006</td>
<td>Bangladesh</td>
<td>0.09-0.24</td>
<td>70.5</td>
<td>89.9</td>
</tr>
</tbody>
</table>

| Leafy Vegetables  | Spinach           | Present Study | West Bengal | 0.24-0.35 | 14.7 | 100 |
|                   | Indian spinach    |          | - | 0.11-0.18 | 25 | 100 |
|                   | Vegetables        | Roychowdhury, 2008 |          | 0.13-0.29 | 70 | 89.2 |

| Non-leafy vegetables | Green papaya    | Present study |          | 0.04-0.11 | 51.4 | 74.3 |
|                      | Bottle gourds   | Williams et al., 2006 | Bangladesh | 0.32-0.47 | 90 ± 24 | 100 ± 0 |
|                      | Green banana    |          | - | 0.05-0.50 | 89 ± 37 | 100 ± 0 |
|                      | Long Yard Bean  |          | - | 0.33-0.87 | 87 | 100 ± 0 |
|                      | Tomato          | Signes-Pastor et al., 2008 | West Bengal | - | 96.4 | 100 |
|                      | Cauliflower     |          | - | 85.7 | 100 |
|                      | Brinjal          |          | - | 90.6 | 100 |

| Root vegetables    | Potato           | Present study |          | 0.03-0.12 | 91.3 | 100 |
|                    | Potatoes         | Williams et al., 2006 | Bangladesh | 0.05-0.89 | 128 | 100 ± 0 |
|                    | Arum stolon      |          | - | 0.05-1.93 | 79 ± 33 | 100 ± 0 |
|                    | Arum tuber       |          | - | 0.09-0.31 | 100 | 100 ± 0 |
|                    | Carrot           | Signes-Pastor et al., 2008 | West Bengal | - | 92.5 | 80.3 |
|                    | Radish           |          | - | 92.2 | 61 |
|                    | Onion            |          | - | 102 | 100 |
|                    | Betel nut        |          | - | 76.5 | 100 |
|                    | Potato           |          | - | 101 | 58 |
|                    | Vegetables       | Rahman et al., 2003 |          | 0.01-0.12 | - | 94 |
|                    | Vegetables       | Smith et al., 2006 | Bangladesh | 0.02-2.33 | - | 96 |
concentration for rice during calculation of DI–iAs (Paper II).

4.6. Human exposure to As through consumption of rice, drinking water and vegetables (Paper I & II)

The comparison of DI-iAs-R, DI-iAs-DW and DI-iAs-V has been shown in Fig. 6, which indicates that the consumptions of rice and drinking water are the major sources of inorganic As intake in the study area. If the value of DI-iAs-R for each participant is compared with previous WHO recommended PTDI value, 17% of the participants are above the threshold for risk due to intake of inorganic As from the consumption of rice only (Fig. 6) (Paper II). Further in order to estimate the contribution of different type of brown rice to the dietary As exposure from rice consumption, the DI-iAs-R values of individual participants are grouped into three categories according to the type of rice consumption (Fig. 7). The range of DI-iAs values for SB, MS and LS rice consumers is 0.55 - 4.92, 0.23 - 1.77 and 0.05 - 1.38 µg day⁻¹ kg⁻¹ BW respectively with median value of 1.80, 0.81 and 0.60 µg day⁻¹ kg⁻¹ BW (Fig. 7). The comparison of these three groups of DI-iAs-R values with the previous WHO recommended PTDI value (2.1 µg day⁻¹ kg⁻¹ BW) indicates that for 37% of the participants consuming SB type of rice, DI-iAs-R values exceed the threshold value, while for none of the participants consuming MS and LS type of rice, the DI-iAs-R values exceed this threshold value (Fig. 7). Furthermore the calculation of \( C_{w,eqv} \) indicates that for more than 90% SB type of rice consumers, the ingestion rate exceeds the WHO recommended drinking water guideline value of 10 µg L⁻¹ (range: 4.65 – 94.2 µg L⁻¹, median: 25.3 µg L⁻¹) (Fig. 8). For MS and LS type of rice consumers, in 67% (range: 2.66 - 28.7 µg L⁻¹, median: 12.6 µg L⁻¹) and 49% (range: 0.74 - 38.1 µg L⁻¹, median: 9.58 µg L⁻¹) cases, the ingestion rate exceeds the threshold value (Fig. 8). 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 (Paper I).

The comparison of the values of DI-iAs-DW and DI-iAs-V for individual participants to the previous PTDI value further indicates that similar to rice 17% of the participants are also above the threshold of risk due to intake of inorganic As from consumption of drinking water, while for none of the participant did the DI-iAs-V exceed the threshold value of PTDI (Fig. 6) (Paper II). Although As in most of the vegetables is present exclusively as inorganic species (Table 2), consumption of vegetables alone is not a potential health risk to the population. When DI-iAs-V is considered together with DI-iAs-R to estimate the total dietary intake of inorganic As (DI-iAs-DC: DI-iAs-R + DC-iAs-V) the extent of health risk to the population is increased by 7%
vegetables, particularly in leafy type, consumption of vegetables might have significant effect to decrease the health burden of As toxicity to the rural population in Bengal. However, the critical comparison between the advantage of folate supplement and the disadvantage of increased inorganic As intake with the consumption of vegetables is beyond the scope of the present study (Paper II).

The values of TDI-iAs for each participant were categorized according to the ranges of As concentration in drinking water viz <10, 10 – 50 and >50 µg L⁻¹ to examine the contribution of drinking water and dietary components (rice and vegetables) to the TDI-iAs (Fig. 9). The comparison of TDI-iAs for each category with the previous PTDI value indicates that even when the As concentration in drinking water is <10µg L⁻¹, for 35% (n = 41) of the participants the TDI-iAs value exceeds the threshold value of 2.1 µg day⁻¹ kg⁻¹ BW (Fig. 9). At this concentration level, the relative contribution of DI-iAs-R to TDI-iAs does significantly predominate over the contribution of DI-iAs-DW (Fig. 10a). By assessing the risk of As exposure at the three regions of West Bengal, Mondal et al. (2010) have also reported that the risk of As exposure from rice consumption predominates in the area, where As concentration in drinking water is low. This indicates that people in the rural Bengal, even when are supplied with As safe drinking water, still are at potential risk of

**Fig. 9. Comparison of total daily intake of inorganic As (TDI-iAs) due to consumption of drinking water, rice and vegetables at different concentration ranges of As in drinking water. The red line represents the previous WHO recommended Provisional Tolerable Daily Intake (PTDI) value of 2.1 µg day⁻¹ kg⁻¹ BW.**

(Fig. 6). It is worthwhile to mention here that recently Gamble et al. (2007) have found increased As methylation capacity, which decreased the blood As concentration level in Bangladeshi people with the supplement of marginal level of folate. Thus, because of high folate content in the

**Fig. 10. Percentage of contributions of daily intake of inorganic As from rice (DI-iAs-R), drinking water (DI-iAs-DW) and vegetables (DI-iAs-V) to the total daily intake of inorganic As (TDI-iAs) at As concentration in drinking water of: (a) <10, (b) 10 - 50, (c) >50 µg L⁻¹.**
As exposure due to consumption of rice. When the level of As concentration in drinking water is >10 – 50 µg L\(^{-1}\), for all the participants the TDI-iAs value exceeds the previous PTDI value (Fig. 9) and DI-iAs-R and DI-iAs-DW contributes almost equally to the TDI-iAs of the participants (Fig. 10b). This signifies that when consumption of rice contributes significantly to the TDI-iAs, the current national drinking water standard for As of India and Bangladesh (50 µg L\(^{-1}\)) are no longer protecting the population from As health risk. When the As concentration in drinking water exceeds 50 µg L\(^{-1}\), the relative contribution of DI-iAs-DW becomes so high that the influence of DI-iAs-R on the TDI-iAs becomes negligible (Fig. 10c). At all categories, the contribution of DI-iAs-V to TDI-iAs is very small (Fig. 10a, b, c) (Paper II).

4.7. Arsenic in urine and saliva: current status of As exposure (Paper II & III)

The concentration of As in the collected urine and saliva varies within 0.22 – 883 µg L\(^{-1}\) and 0.22 – 84.3 µg L\(^{-1}\) with a median value of 67.7 µg L\(^{-1}\) and 2.99 µg L\(^{-1}\) respectively (Paper II & III). The concentration of As in saliva supports the findings of Yuan et al. (2008), who have reported a mean concentration of 11.9 µg L\(^{-1}\) for the residents of Inner Mongolia, China who were exposed to As concentrations up to 826 µg L\(^{-1}\) in drinking water (Paper III). It is worth to note here that in the present study most of the urine and saliva samples were collected from the participants, who were drinking PHED supplied As safe water (<10 µg L\(^{-1}\)) (Paper II & III). This indicates that despite consumption of mostly safe water the concentration of As in urine and saliva is still considerably high. Additionally, in Paper III it is found that the Log transformed values of total daily ingestion of inorganic As (L-TDI) from rice and drinking water strongly and positively correlates to Log transformed As concentration in urine (L-U\(_{As}\)) (r = 0.50,
p <0.05) and saliva (L_Sa) (r = 0.68, p <0.05) (Fig. 11). By recalling the fact that when As concentration in drinking water is <10 µg L\(^{-1}\), consumption of rice is the major contributor to the TDI-iAs (Paper II); it can be concluded that the intake of As from rice consumption is possibly responsible for the observed considerably high concentration of As in urine and saliva. This is further supported by the strong positive correlation (r = 0.57, p <0.05) of As concentration in urine to DI-iAs-R only (Fig. 12), as reported in Paper II. It is worthwhile to mention here that Cascio et al. (2011) have compared the concentration of urinary As species between the groups of UK resident Bangladeshi people and their white Caucasians counterpart and found that sum of different As species concentrations in urine for Bangladeshi people is 3 folds higher because of their higher amount of rice consumption. The study by Gilbert-Diamond et al. (2011) has also found positive correlation between As intake through rice consumption and urinary As concentration among the pregnant women in United States. This discussion further strengthens the inference that in As affected regions, even when people are supplied with As safe water, they still are at potential risk of As exposure due to consumption of rice. Recently, the study by Banerjee et al. (2013) has reported the health outcome of genotoxicity because of As exposure from rice consumption among the villagers in West Bengal. Furthermore, the Fig. 12 also indicates that the correlation of urinary As concentration to DI-iAs-V is not significant (r = 0.08), which possibly further supports the inference that As intake through vegetables consumption alone is not a potential health risk to the rural population (Paper II).

4.8. Variation of total As concentration in raw and cooked rice and its relation to As concentration in the cooking water (Paper IV)

Total As contents in raw and cooked rice samples are shown in SI of Paper IV (Table SI 2 and Table SI 3). The repeated measurements of As content in the raw (n = 6) and cooked (n = 3) rice samples indicate the respective precision (represented by standard deviation, 1σ) of the analysis to vary within 0.01 – 0.03 mg kg\(^{-1}\) and 0.00 – 0.01 mg kg\(^{-1}\). The total As content in the raw rice samples varies from 0.03 to 0.96 mg kg\(^{-1}\) with a mean value of 0.28 mg kg\(^{-1}\), which is comparable to the findings from household survey in Bangladesh (Figure SI 2 of Paper IV). In 15 samples out of 29, the As concentration exceeds the global normal distribution range of 0.08 – 0.20 mg kg\(^{-1}\) (Zavala and Duxbury, 2008). The determination of grain size and shape has classified 17, 8 and 4 of the collected samples as SB, MS and LS respectively. The major classification of the collected rice samples into the category of SB, again confirms the previous findings that people in the rural Bengal mostly prefer SB type of rice for their daily consumption (Paper I). Similar to the previous study (Paper I), it is also found that the As concentration in raw rice is highest with SB type of rice (range: 0.06 – 0.96 mg kg\(^{-1}\),
mean: 0.36 mg kg\(^{-1}\)) and decreases with increasing grain size to MS (range: 0.07 – 0.33 mg kg\(^{-1}\), mean: 0.17 mg kg\(^{-1}\)) and LS type of rice (range: 0.03 – 0.30 mg kg\(^{-1}\), mean: 0.16 mg kg\(^{-1}\)).

The concentrations of total As in the cooked rice samples vary from 0.03 to 0.74 mg kg\(^{-1}\) with a mean value of 0.19 mg kg\(^{-1}\). This result is comparable to the findings of previous studies conducted in Bengal (Figure SI 3 in SI of Paper IV). The As concentrations in raw and cooked rice samples were compared by the paired T test. The result shows that the mean As concentration in raw rice is significantly different (\(p <0.05\)) from that in cooked rice, indicating that rice cooking definitely changes the As concentration in cooked rice compared to raw rice. A comparison of total As concentration in raw and corresponding cooked rice samples (Fig. 13a) indicates that in 24 households out of 29, the total As concentration has decreased on average by 34.3% (range: 7.5% – 66.3%), while in other 5 households, the As concentration has increased on average by 66% (range: 4.1% - 200%) because of cooking. All the 5 households, where the As concentration has increased, use As contaminated privately installed tubewell water for cooking. The result of the present study is very much in accordance with the findings of previous studies conducted at other parts of Bengal. For example, Pal et al. (2009) also quantified As concentrations in the pair of raw and corresponding cooked rice samples, collected from the As affected villages and reported 8% – 58% decrease in As content because of cooking with As free water. Since, the households of present study, where As concentration in rice has been decreased, use the same PHED supplied water for cooking and follow same rice cooking procedure, the observed variation in As removal is possibly related to the type of raw rice cooked. The regression analysis

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Parameters</th>
<th>#RR-15</th>
<th>#RR-20</th>
<th>#RR-28</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Conc. of As in raw rice (µg kg(^{-1}))</td>
<td>265</td>
<td>402</td>
<td>91.5</td>
</tr>
<tr>
<td>II</td>
<td>Dry weight of raw rice (g)</td>
<td>10.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>III</td>
<td>Total As in raw rice (µg)</td>
<td>2.65</td>
<td>4.02</td>
<td>0.92</td>
</tr>
<tr>
<td>IV</td>
<td>Conc. of As in starch water (µg kg(^{-1}))</td>
<td>32.1</td>
<td>49.6</td>
<td>7.62(^a)</td>
</tr>
<tr>
<td>V</td>
<td>Weight of starch water (g)</td>
<td>33.5</td>
<td>34.4</td>
<td>31.4</td>
</tr>
<tr>
<td>VI</td>
<td>Total As in starch water (µg)</td>
<td>1.08</td>
<td>1.71</td>
<td>0.24(^a)</td>
</tr>
<tr>
<td>VII</td>
<td>Conc. of As in laboratory cooked rice (µg kg(^{-1}))</td>
<td>160</td>
<td>269</td>
<td>72.6</td>
</tr>
<tr>
<td>VIII</td>
<td>Dry weight of cooked rice (g)</td>
<td>9.19</td>
<td>9.34</td>
<td>9.31</td>
</tr>
<tr>
<td>IX</td>
<td>Total As in cooked rice (µg)</td>
<td>1.47</td>
<td>2.51</td>
<td>0.68</td>
</tr>
<tr>
<td>X</td>
<td>Total As in cooked rice and starch water (VI + IX)</td>
<td>2.55</td>
<td>4.22</td>
<td>0.92</td>
</tr>
<tr>
<td>XI</td>
<td>Error in Mass Balance of As: (X-III) / III</td>
<td>-0.04</td>
<td>0.05</td>
<td>0.00</td>
</tr>
<tr>
<td>XII</td>
<td>% Error in Mass Balance of As</td>
<td>-3.92</td>
<td>4.94</td>
<td>0.03</td>
</tr>
<tr>
<td>XIII</td>
<td>Distribution co-efficient (VII / IV)</td>
<td>4.98</td>
<td>5.42</td>
<td>3.90(^b)</td>
</tr>
<tr>
<td>XIV</td>
<td>Change in As conc. due to cooking at laboratory(^c)</td>
<td>-39.6%</td>
<td>-33.1%</td>
<td>-20.7%</td>
</tr>
<tr>
<td>XV</td>
<td>Change in As conc. due to cooking at household(^d)</td>
<td>-32.5%</td>
<td>84.3%(^d)</td>
<td>-15.7%</td>
</tr>
</tbody>
</table>

\(^a\) Values were calculated assuming that there was no procedural loss during rice cooking processes in the laboratory. The measured As concentration in starch water was below instrumental detection limit.

\(^b\) Since, the measured As concentration in starch water for the sample #RR-28 was below instrumental detection limit, distribution co-efficient was not calculated for this sample.

\(^c\) The signs (–) and (+) indicate decrease and increase in As concentration respectively due to cooking of rice.

\(^d\) Arsenic concentration in cooked rice during household cooking was increased because of cooking with As contaminated water.
reveals that the extent of removal of As because of cooking is positively correlated with the corresponding background As concentration in the raw rice \((r = 0.63, p < 0.05)\), indicating that As removal was high when the As concentration in raw rice was also high and vice versa. Conversely, Bae et al. (2002), Mondal and Polya (2008) and Ohno et al. (2009) have reported the increase of As in cooked rice, when rice is prepared with As contaminated water. Ohno et al. (2009) also reported a very strong positive correlation \((R^2 = 0.89)\) of the change in As concentration in rice before and after cooking with As concentration in the cooking water.

### 4.9. Evaluating the effect of cooking on As content in rice (Paper IV)

In all the rice-wash water samples \((n = 15)\), the concentration of As was BDL, indicating a very negligible As removal in the step of washing during rice cooking. This result contradicts the findings of Sengupta et al. (2006), who had reported up to 28% As removal compared to raw rice by washing. However, their washing was more vigorous \((5 – 6 \text{ times washing until wash water became clear})\) compared to the washing step of the present study. The washing processes and result of the present study are comparable to those of the study by Raab et al. (2009), who have reported an As removal of only 1% - 4% by washing for general type of rice; they found the removal of 13% – 15% of As when washing the Basmati rice, however. They suggested that the extent of As removal by washing varies according to the type of rice. The determination of As in the starch water samples \((n = 3)\) indicates that the boiling of washed rice with excessive water has released significant amount of As for the two samples \(#RR-15 & 20\) (Table 3). The mass balance of As in the different cooking steps demonstrates that removal of As through discarded starch water is the only effective form of As removal in the whole rice cooking processes for the two samples (Table 3). At high temperature, used during cooking, the soluble As in the rice grains is presumably dissolved and discarded with starch water (Rahman et al., 2006). Thus, rice cooking can be regarded as another form of extraction of As from rice, where high temperature and high sample to extractant (cooking water) ratio increases the extraction yield (Alava et al., 2012). If it is assumed that there was no procedural loss during laboratory cooking of #RR-28 sample also, the amount of As removed with starch water would be 0.24 µg, which is equivalent to concentration of 7.62 µg kg\(^{-1}\) As in starch water (Table 3). This concentration is close to the As detection limit of ICP-AES \((5 \mu g \, L^{-1})\), used for As quantification in this study. Thus, it could be assumed that for sample #RR-28 also, As was removed through starch water during cooking, though it could not be quantified. The similarity in percentage of As removal by laboratory and field cooking for the 2 samples (#RR-15 & 28) (Table 3) confirms that the rice cooking procedure adopted in the laboratory was comparable to that in the field. Sample #RR-20 was cooked with Milli-Q water in the laboratory, but was cooked with As contaminated water in the field, a disparity that may explain the contrary findings of As concentration in cooked rice by laboratory and field cooking (Table 3). Our results indicate that people should be strongly discouraged from drinking starch water, a habit that is occasionally practiced by the economically poor people in the rural villages. Otherwise it could become another potential pathway of As exposure.

The present study did not attempt to cook rice with As-spiked water to explain how cooking with As contaminated water increases As concentration in cooked rice compared to raw rice. The study of Bae et al. (2002) have reported that probably the chelation of As by rice grains increases the As concentration in cooked rice during cooking with As contaminated water. Ohno et al. (2009) have hypothesized that As is co-adsorbed with water on rice grains during cooking. It is interesting to note the distinct pattern of change in As concentration during laboratory and household cooking of the Sample #RR-20. Laboratory cooking with As free Milli-Q water has decreased the
As concentration in cooked rice by 33.1%, while the household cooking with As contaminated water has increased the As concentration by 84.3% compared to raw rice (Table 3). This indicates the presence of a threshold value of As concentration in cooking water that determines the pattern of change in As concentration in the cooked rice. Previous studies by Mondal and Polya (2008) and Ohno et al. (2009) have suggested the concentration of 10 µg L\(^{-1}\) of As in cooking water as the threshold value, while the concentration of 50 µg L\(^{-1}\) has been suggested by Signes et al. (2008). Here, it is hypothesized that the distribution of As between cooked rice and starch water is perhaps regulated by a distribution co-efficient (ratio of As in cooked rice to that in starch water), such that cooking with water having As concentration of 50 µg L\(^{-1}\) still may sequester As from rice if the As concentration in raw rice is high. On the other hand, cooking with same water may add As to the cooked rice if the As concentration in raw rice is low. The calculation of distribution co-efficient for the rice samples #RR-15 and #RR-20 gives the value of 4.98 and 5.42 respectively (Table 3), which may also vary to different rice variety (Juhasz et al., 2006). This hypothesis needs further validation with larger sample size. The above discussion leads to suggest that people should also be encouraged to use low As cooking water like drinking water.

4.10. Variation of As species in raw and cooked rice (Paper IV)

The results of As speciation in raw and cooked rice samples are provided in SI of Paper IV (Table SI 2 and Table SI 3). The recovery of As in the extractions with water varied from 55.7% to 108% with a mean of 78.6% for raw rice and 57.3% to 121% with a mean of 72.8% for cooked rice. The re-extractions of As in 3 raw rice and 1 cooked rice samples with a mixture of methanol and water (1:1) have produced comparable extraction recoveries to that of water only (SI Paper IV, Table SI 4).

The determination of As species in the water extracts indicates that inorganic species of As [namely, As(III) and As(V)] represent on average 93.8 % (range: 84.8% - 100%) of the total extractable As for the raw rice samples and the rest is mainly represented by DMA (range: 0.00% - 15.2%, mean: 6.11%) with very occasional presence of MMA (SI Paper IV, Table SI 2). This speciation result supports the previous findings in Paper II. With the exception of 4 samples (#RR-4, 6, 8 & 13), As(III) is the predominating species contributing on average 70% (range: 0.96% – 95.7%) of the inorganic species; for 4 samples, As(V) predominates over As(III) (SI Paper IV, Table SI 2). Three raw rice samples, which were re-extracted with the mixture of methanol and water includes two of these samples (#RR-8 & 13). In contrast to the water extracts, the determination of As species in the mixture of methanol and water extracts for these two samples also indicates the predominance of As(III) over As(V) (SI Paper IV, Text SI 3 and Table SI 4), suggesting that occasional oxidation of As(III) in the water extraction procedure cannot be ruled out. The distribution of As species in cooked rice is very much similar to that of raw rice. The inorganic species of As again represent on average 88.1% (range: 69.8% - 100%) of the extractable As and the rest is represented by DMA (range: 0.00% - 27.9%, mean: 11.3%) with rare occurrence of MMA (SI Paper IV, Table SI 3). Except for one sample (#FCR-12), all water extracts of the cooked rice samples showed As(III) to be the predominating form of inorganic species (range: 2.93% - 100%, mean: 89.5%) (SI Paper IV, Table SI 3). However, in the extract of methanol and water for sample #FCR-12, As(III) also predominates over As(V) (SI Paper IV, Text SI 3 and Table SI 4), again indicating occasional oxidation during the water extraction. The predominance of As(III) over As(V) in cooked rice samples contradicts the findings of Mihucz et al. (2007) and Signes et al. (2008), who had reported the oxidation of As(III) to As(V) during cooking. However, the overall speciation result in raw and
cooked rice is very much similar to the findings of the previous studies conducted in different parts of Bengal (Rahman et al., 2003; Williams et al., 2005; Smith et al., 2006; Signes-Pastor et al., 2008; Mondal and Polya, 2008). One objective of the present study was to evaluate the effect of rice cooking on the distribution of As species in cooked rice. The contents of As(III), As(V) and DMA in raw and corresponding cooked rice samples have been graphically compared together with the change in total As concentration in Fig. 13. The results indicate that because of cooking with As safe water (n = 24), the inorganic As content in cooked rice has been decreased on average 42.1% (range: 0.3% - 74.4%) compared to raw rice. Conversely, in 3 out of 5 samples, where total As content was increased largely due to cooking with As contaminated water, inorganic As content has been increased on average 78.5%. It is evident that the change (both increase and decrease) in total As concentration because of cooking is very closely followed by similar change in As(III) concentration (Fig. 13a and Fig. 13b) and regression analysis gives a very strong positive correlation \( r = 0.93, p < 0.05 \) between them. The sympathetic behavior of the changes in total As and As(III) concentrations signifies that As concentration in cooked rice is changed mainly in the form of As(III) during cooking. Mihucz et al. (2007) speciated As in the Starch water and also reported that As is lost during cooking with As safe water, mainly in the form of As(III).

Compared to raw rice, the concentration of As (V) in cooked rice has been decreased (Fig. 13c), while the concentration of DMA has been increased (Fig. 13d). However, the regression analysis reveals that unlike As(III), the changes in As(V) and DMA concentrations are independent of the change in total As concentration during cooking. The processes responsible for the observed decrease in As(V) and increase in DMA concentrations in cooked rice is unknown. However, it is worthy to note that the increase in DMA concentration should be interpreted with caution as DMA peaks were poorly resolved from those of As(III) and the measured concentration were low. The experiments discussed here reveal that the cooking of rice with As free water significantly lowers the risk of As exposure from rice consumption by removing some of the As. Thus, meaningful assessment of As exposure risk due to consumption of rice should consider cooked rice rather than raw rice as the critical exposure medium.

4.11. Human exposure to As through consumption of cooked rice (Paper IV)

Further attempt was made to investigate the risk of As exposure to the 59 participants through rice consumption by incorporating cooked rice instead of raw rice in the risk assessment. The estimated DI-iAs-CR of the surveyed participants is in the range of 0.10 to 4.79 µg day\(^{-1}\) kg\(^{-1}\) BW with a median value of 0.92 µg day\(^{-1}\) kg\(^{-1}\) BW. For more than 10% of the participants, the DI-iAs-CR exceeds the previous PTDI value of 2.1 µg day\(^{-1}\) kg\(^{-1}\) BW for inorganic As (Paper IV). The assessment of As exposure risk from the consumption of cooked rice is further supplemented by calculating the values of HQ and CR of the individual participants. The estimated HQ values vary from 0.33 to 15.7 with a median value of 3.04 (Paper IV). A comparison of the HQ value of individual participant to the USEPA specified guideline of 1.0 indicates that almost 90% of the participants are at the risk of adverse non-carcinogenic health effect due to consumption of cooked rice (Paper IV). Furthermore, the calculation of CR indicates that the probability of developing cancer during lifetime among the participants due to consumption of cooked rice varies from 0.21 to 10.3 with a median value of 1.98 per 10\(^{13}\) people (Paper IV). Thus, it can be argued that although cooking of rice with As safe water (<10 µg L\(^{-1}\)) significantly decreases the As content in cooked rice, still consumption of rice is a significant pathway of As exposure to the population in rural Bengal.
5. CONCLUSIONS

The following major conclusions can be drawn from this study:

- The food frequency questionnaire survey adopted in this study indicates that rice with vegetables is the main staple food in rural Bengal, where by weight rice contributes almost 76% of the total diet. Rice cooking procedure is same in all the surveyed households, where rice grains are generally washed at least 2 - 4 times with excess amount of water before cooking and after that, rice grains are boiled with 3 - 6 times of water usually in an aluminum cookware. Amount of daily rice consumption does vary according to the age of the participants, while amount of vegetable consumption and drinking water intake is roughly same among the different age groups of participants (Paper I, II, III & IV).

- The accumulation of As in rice grains decreases with increasing grain size, being highest in SB type of rice and lowest in Basmati rice of ELS type. People in the rural villages mostly prefer SB type of rice for daily consumption because of its lower cost. Among different types of vegetables that are generally consumed in the rural villages higher As accumulation is found in leafy type of vegetables compared to the non-leafy and root vegetables. Following the findings of previous studies, present study also indicates the predominant accumulation of inorganic As in the rice (>90%) and vegetables (almost 100%) grown in West Bengal and Bangladesh (Paper I & II).

- The estimates of As exposure via dietary and drinking water routes show that even when people are drinking As safe water (<10 µg L⁻¹), a significant extent of the participants (35%) are under the risk of chronic As exposure through dietary intake of As, mainly from rice consumption. The consumption of vegetables in rural Bengal does not pose significant health threat to the population independently. When As concentration in drinking water exceeds 50 µg L⁻¹ the exposure to As through drinking water intake largely predominates over the exposure from dietary intake of As. The risk assessments further imply that when rice consumption is a significant contributor to the TDI-iAs, supplying water with As concentration at current national drinking water standard for India and Bangladesh would place many people above the safety threshold of PTDI (Paper I & II).

- Though, people in the study area are now mostly drinking As safe water (<10 µg L⁻¹) for last 3 – 4 yrs, the concentration of As in urine and saliva is considerably high, further supporting that dietary intake of As, mostly through rice consumption could be alternative pathway of As exposure to the rural population (Paper II & III).

- This study reveals that cooking of rice with high volume of As safe water (<10 µg L⁻¹) decrease total as well as inorganic As content in cooked rice considerably. During rice cooking processes As is mainly removed with starch water in the step of boiling. Thus people in the rural villages should be motivated to use safe cooking water as well with drinking water. At the same time people should also be discouraged to drink starch water, which is occasionally practiced in rural Bengal. Otherwise it could become another potential pathway of As exposure. However, the assessment of As exposure risk from consumption of cooked rice indicates that despite significant decrease of As content in cooked rice because of cooking with As safe water (<10 µg L⁻¹), still consumption of cooked rice is a significant pathway of As exposure to the population in rural Bengal (Paper IV).

6. RECOMMENDATIONS AND FUTURE SCOPE OF RESEARCH

This study suggests that though the supply of As safe drinking water (<10 µg L⁻¹) among the population is a top priority in the As affected regions, the supply of safe water alone is not enough to reduce the risk of As poisoning. Although, cooking of rice with As safe water following the traditional cooking method practiced in rural Bengal substantially reduces both total as well as inorganic As content in the cooked rice,
consumption of rice is another potential pathway of As exposure that must also be considered. The attempt should be made to decrease the As content in raw rice grains. The development of cultivars primed to take up limited amounts of As could be an option in this regard and is worth for substantial future research. Another possibility could be the introduction of System of Rice Intensification (SRI) cultivation practice in this region. In SRI cultivation practice, paddy field is dried up between two successive irrigations. Such intermittent irrigation ensures the aeration of the land, which helps to maintain high redox status that essentially hinders the mobilization of As in rice field (Roberts et al., 2011; Spanu et al., 2012). Another great advantage of this cultivation practice is that it requires less irrigation water than the traditional practice (Thiyagarajan and Gujja, 2013), which in turn would reduce the input of As from irrigation water into the rice field (Roberts et al., 2011). Thus, SRI cultivation practice could minimize the transfer of As from groundwater to soils to human food chain and warrants further study in future. Such rice cultivation practice is already popular in many rice producing regions of the world because of its potentiality to increase rice yield, decrease methane emission and controlling rice field malaria (Thiyagarajan and Gujja, 2013). It would be of great interest also to investigate the effect of temporal storing of irrigation water, rather than directly supplying into the irrigation land, on the As loading in the paddy field and subsequent uptake by rice plants. Since the storing of groundwater facilitates the co-precipitation of As with the iron oxides due to oxidation with atmospheric oxygen, it is expected that the input of As from irrigation water to the rice field would be reduced substantially.

It should also be mentioned that when consumption of rice significantly contributes to the TDI-iAs, attempt should be made to decrease inorganic As intake from the drinking water further, so that the safe level of TDI-iAs can be maintained. Otherwise, the incidence of chronic As poisoning would become compounded in future. Furthermore, as mentioned before it is necessary to conduct a critical comparison between the advantage of folate supplement and the disadvantage of increased inorganic As intake with the consumption of vegetables among the population. In conclusion, any mitigation of As poisoning in rural Bengal needs integrated approaches rather than the traditional fragmented strategies.
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