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Arsenic species in wheat, raw and cooked rice: exposure and associated health implications

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Highlights

- Chronic non-cancer health risks among 97% of study participants due to inorganic arsenic intake from wheat.
- Wheat grown in arsenic affected area poses higher risk than rice as a exposure source.
- Total daily intake of inorganic arsenic above the limit of 2.1 µg kg^{-1} day^{-1} body weight in 74% of participants.
- Above 95% of the children at significantly higher risk due to inorganic arsenic exposure from cooked rice.

Abstract

Arsenic concentrations above 10 µg L^{-1} were previously found in 89% of ground water sources in six villages of Pakistan. The present study has ascertained the health risks associated with exposure to total arsenic (tAs) and its species in most frequently consumed foods. Inorganic arsenic (iAs) concentrations were found to be 92.5±41.88 µg kg^{-1}, 79.21±76.42 µg kg^{-1}, and 116.38±51.38 µg kg^{-1} for raw rice, cooked rice and wheat respectively. The mean tAs concentrations were 47.47±30.72 µg kg^{-1}, 71.65±74.7 µg kg^{-1}, 105±61.47 µg kg^{-1}. Wheat is therefore demonstrated to be a significant source of arsenic exposure. Dimethylarsinic acid was the main organic species detected in rice, whilst monomethylarsonic acid was only found at trace levels. Total daily intake of iAs exceeded the provisional tolerable daily intake of 2.1 µg kg^{-1} day^{-1} body weight in 74% of study participants due to concurrent intake from water (94%), wheat (5%) and raw rice (1%). A significant association between tAs in cooked rice and cooking water resulted in tAs intake 43% higher in cooked rice compared to raw rice. The study suggests that arsenic intake from food, particularly from wheat consumption, holds particular significance where iAs is relatively low in water. Chronic health risks were found to be significantly higher from wheat intake than rice, whilst the risk in terms of acute effects was below the USEPA's limit of 1.0. Children were at significantly higher health risk than adults due to iAs exposure from rice and/or wheat. The dietary exposure of participants to tAs was attributable to staple food intake with ground water iAs <10 µg L^{-1}, however the preliminary advisory level (200 µg kg^{-1}) was achievable with rice consumption of ≤200 g day^{-1} and compliance with ≤10 µg L^{-1} iAs in drinking water. Although the daily iAs intake from food was lower than total water intake, the potential health risk from exposure to arsenic and its species still exists and requires exposure control measures.

Key Words: dietary exposure, dimethylarsinic acid, daily intake, cooked rice arsenic, wheat grains.

1. Introduction

Arsenic (As), a naturally occurring metalloid, is widely present as an environmental contaminant and enters the food chain mainly from contaminated water (European Food Safety Agency, 2009) and several widely consumed foodstuffs (Feldmann and Krupp, 2011; Jiang et al., 2015). Seafood has been identified as the main source of organic arsenic (e.g., arsenobetaine and arsenosugars) and is believed to be non-toxic (Taylor et al., 2017; International Agency for Research on Cancer, 2012b). Most exposure and toxicological assessments have focused on inorganic arsenic (iAs) in drinking water. It is yet not fully understood whether exposure to arsenic via most frequently consumed food (e.g. rice and wheat) causes the same health implications as exposure through drinking water.

Exposure from rice has been assessed in a number of studies (U.S. Food and Drug Administration, 2016; Sand et al., 2015; Chen et al., 2016; Davis et al., 2017; Sun et al., 2012). These studies indicate that rice is the most common exposure source for food stuffs. Rice crops have a comparatively higher tendency to take up iAs as they are grown in submerged soil conditions. Among populations not exposed to iAs via drinking water, rice contributes significantly to the iAs intake (Davis et al., 2017).

Wheat is also an important staple food with a worldwide consumption of 730.9 million tonnes, greater than the 506.5 million tonnes of rice consumed annually (Food and Agriculture Organization, 2017). Past studies have reported lower arsenic levels in wheat than rice (Williams et al., 2007b; Su et al., 2010; Bhattacharya et al., 2010) and provided an impetus to further investigate the health risks due to consumption of wheat grown in arsenic affected regions.

Inorganic arsenic is a recognized carcinogen and its chronic exposure has been reported to result in increased risk of bladder, lung, and skin cancer, type 2 diabetes, and cardiovascular disease (International Agency for Research on Cancer, 2012b). Organic arsenic compounds are considered less toxic than iAs but should still be included in exposure assessments. Since toxicity depends on the chemical forms of arsenic, arsenic speciation in rice and wheat can provide useful information for risk assessment and management. The Joint Food and Agriculture Organization and the World Health Organization (FAO/WHO) Expert Committee on Food Additives has set, in 2014, advisory levels of 200 µg kg^{-1} iAs in polished rice grains (Codex Alimentarius Commission, 2014). Apart from the EU regulations (EU) 2015/1006)

(European Commission, 2015) on adopting this limit, several countries have still not implemented this limit and are in the process of setting regulatory limits for rice based products. Adoption of this advisory limit in different geographical regions requires exposure assessment via rice. Considering these facts, this study has determined the concentrations of total arsenic (tAs) and As species in wheat, raw and cooked rice to assess the relative contribution of dietary arsenic to aggregate daily exposure. Human health hazards associated with daily consumption of rice, wheat and household groundwater by children (age ≤16 years) and adults (age >16 years) was calculated based on these exposures to provide an indication of hazard of each exposure source.

2. Materials and Methods

2.1 Study area and study participants

The study villages were located within four districts of Pakistan (Kasur, Sahiwal, Bahawalpur and Rahim Yar Khan), where arsenic concentrations above 10 µg L^{-1} were previously found in 89% of household ground water sources. The sampling frame consisted of 223 households comprising 398 volunteers enrolled and interviewed in our previous studies aimed to assess household ground water arsenic concentrations (Rasheed et al., 2017a) and dietary consumption patterns (Rasheed et al., 2017b). Thus, data on age (3-80 years, mean 36±17 years), gender (246 men and 149 women), body weight (56.6±19.9 Kg), occupation (n=186 farmers and agriculture labour), cooked rice (469 ± 202 g day^{-1} $person^{-1}$) and wheat intake (372 ± 119 g day^{-1} $person^{-1}$) were obtained by questionnaire from 398 participants in the 223 households enrolled in our earlier study (Rasheed et al., 2017b). The households ground water sources (n=228) used both for the drinking and food preparation were found to have geometric mean (GM) iAs concentration as 55.33 µg L^{-1} (range: 0.48-3090 µg L^{-1}) and associated daily total water intake of 15.4 µg day^{-1} (0.02-262.57 µg day^{-1}) (Rasheed et al., 2017a).

Wheat and rice was sampled from the households. Raw rice samples were collected from 105 households of villages (Chak-46/12-L, Chak-48/12-I and Chak 49/12-I, Badarpur, Basti Balochan and Kotla Arab), while cooked rice samples could be obtained from 24 households. Twelve households provided paired rice samples (both raw and cooked). The main occupation in the study villages was wheat farming with 47% of 398 study participants engaged in this work (Rasheed et al., 2017b), thus, wheat consumed in the villages was cultivated locally. Following the sampling strategy described by Cubadda et al. (2010), wheat grain samples (n = 189) from two of the most cultivated wheat varieties were collected from the households of six villages. Individual samples (150 g each) were pooled into 8 composite samples weighing in the range of 0.9-7.5 kg.

2.2 Samples collection procedure

For raw rice and wheat samples, sterile re-sealable airtight polyethylene zip lock bags were used, whereas for cooked rice (100 grams) 2 oz polyethylene sterile containers were used. After collection, raw rice (250 grams) and wheat samples (150 grams) were stored at room temperature, while cooked rice samples were kept in an insulated cooler containing ice in the field and later stored at -20 °C. Cooked rice samples were shipped to Brooks Applied laboratory, USA by FedEx courier with dry ice under strict quarantine regulations and stored at -20 °C prior to analyses. Raw rice and wheat samples were shipped and stored at ambient temperature (20°C) until analysis in National water quality laboratory Pakistan and Brooks Applied laboratory (BAL), USA.

2.3 Treatment of rice and wheat samples for total arsenic

Rice and wheat samples were rinsed with deionized water (DIW) to remove dust and then dried by air flow at room temperature. Dried samples were milled to powder in a pre-cleaned commercial blender with stainless steel blades. Following USEPA method 3050b (United States Environmental Protection Agency, 1996), a representative 1-2 gram (wet weight) or 1 gram (dry weight) sample was digested with repeated additions of nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). The resultant digest was reduced in volume while heating at 95°C ± 5°C and then diluted with ultrapure water to a final volume of 100 mL and subjected to analysis.

2.4 Treatment of rice and wheat samples for arsenic speciation

Microwave-assisted HNO_3 digestion for arsenic speciation involved adding 0.35 g of ground raw or cooked rice and wheat samples separately into 15 mL sample tubes. 10 ml of 0.16 M suprapure HNO_3 was added to the tube and left to stand overnight.

Microwave irradiation was performed with the temperature profile as: 3 min ramp to 55 °C, 10 min at 55 °C, 2 min ramp to 75 °C, 10 min at 75 °C, 2 min ramp to 95 °C, 30 min at 95 °C. The extracts were centrifuged (10 min, 8000 rpm, 4 °C) and the supernatants filtered through a 0.22 μm filter. The filtrate was stored at 4 °C and analyzed within 24 hours to minimize any species inter-conversion. For final analysis, 0.1 mL of the filtered solution was combined with 0.9 mL of DIW in a 1.5 mL vial and mixed for 10 seconds with a vortex mixer (D'Amato et al., 2011; Raab et al., 2009b).

2.5 Analytical procedures

tAs was measured using inductively coupled-plasma dynamic reaction cell-mass spectrometry (ICP-DRC-MS) on an ELAN DRC II ICPMS (Perkin Elmer SCIEX, Concord, Ontario, Canada). Following the methods of D'Amato et al. (2011) and Alava et al. (2012), all sample extracts were analyzed for iAs (defined as the sum of arsenate (AsV) and arsenite (AsIII)), MMA, and DMA employing an Agilent 7700 CRC ICP-MS with a Dionex GP40 HPLC (IC) System. An aliquot of filtered sample was injected using Dionex HPLC onto an anion-exchange column and mobilized isocratically using an alkaline (pH >7) eluent. The mass-to-charge ratio (m/z) of As at mass 75 was monitored using an Agilent 7700 and the area under the arsenic peaks was used for quantitation. Selenium at m/z 82 was monitored as an internal standard. Retention times for each eluting species were compared to known standards for species identification.

2.6 Quality Assurance

For quality control, method blanks, blank spikes, standard reference materials (SRMs) and duplicates were treated in the same way as the samples and incorporated into each digestion batch and analytical run. SRMs include NIST Rice flour (SRM 1568a) for cooked and uncooked rice and NIST Wheat flour (SRM 1567a). Data quality in terms of precision, accuracy, method reporting limits (MRLs) and method detection limits (MDLs) met the criteria established in BAL's quality assurance project plan, i.e. relative percent difference (RPD) of <25%, percent recovery of 75 to 125%.

2.7 Arsenic Exposure Assessment

Daily intake of tAs and As species for wheat and rice was calculated using Eq. (1) (Agency for Toxic Substances and Disease Registry, 2005).

$$EDI = \frac{C \times IR}{BW} \tag{1}$$

EDI is the estimated daily intake (µg day^{-1} body weight), C represents the average arsenic concentration of rice or wheat (µg g^{-1}), IR is the rice or wheat intake rate (g day^{-1}), and BW is the body weight (kg) of the study individuals. EDI is calculated on the basis of previously published body weights, IR of rice and wheat (Rasheed et al., 2017b), wheat and rice tAs and arsenic species measured in this study. Raw rice intake was derived from cooked rice by applying a raw-to-cooked rice equivalence factor (Bae et al., 2002).

Total water intake already includes direct drinking water and indirect water intake through food such as cooked rice, wheat bread/chappati, pulses, vegetables, milk, yoghurt and chicken (Rasheed et al., 2017b). Therefore, raw rice intake of iAs instead of cooked rice was taken into account for exposure and risk assessment. EDI values were compared with the World Health Organization's (WHO) provisional tolerable daily intake (PTDI) of 2.1 µg kg^{-1} day^{-1} (World Health Organization, 1989) to assess exceedance. Since the PTDI of 2.1 µg kg^{-1} bw day^{-1} was withdrawn by JECFA in 2010, the ratio between EDI and minimum risk levels set by ATSDR for iAs (Agency for Toxic Substances and Disease Registry, 2017) were calculated for each study participant using Eq. (2 and 3).

$$\mathrm{HQ} = EDI/MRL_{chronic} \tag{2}$$

$$\mathrm{HQ} = EDI/MRL_{acute} \tag{3}$$

Where;

HQ	Hazard quotient
EDI	Estimated daily intake
MRL	Minimum risk level (chronic exposure 0.0003 mg $kg^{-1}day^{-1}$, acute exposure 0.005 mg $kg^{-1}day^{-1}$) (Agency for Toxic Substances and Disease Registry, 2017)

The Hazard index (HI) was calculated as total non-cancer health hazard posed by iAs through combined daily intake of raw rice and wheat grains using Eq. (4 & 5) (United States Environmental Protection Agency, 1989).

$$\mathrm{HI} = EDI_{raw\ rice+wheat}/MRL_{chronic} \tag{4}$$

$$\mathrm{HI} = EDI_{raw\ rice+wheat}/MRL_{acute} \tag{5}$$

A calculated HQ or HI greater than 1 suggests that there may be health concerns (United States Environmental Protection Agency, 1989).

2.8 Evaluation of margins of safety (MoS) for iAs in rice

The Current Codex Alimentarius (CCA), or 'food code' was set in 2014 and sets an advisory level of 200 µg kg^{-1} of iAs in white rice (Codex Alimentarius Commission, 2014), although this limit is still debated and the process of setting legal standards for iAs in rice or rice based products is still incomplete. Modification of the formula used by Shibata et al. (2016) in Eq. (6), integrating input variables from this study, was used to assess the suitability of CCA's advisory limit for adoption by regulatory agencies in arsenic affected regions.

$$MTL_{rice} = \left(\sum_{3}^{80}((MRL\,.\,BW - (PGV_{water}\,.IR_{water} + C_{wheat}\,.IR_{wheat})).IR_{rice}^{-1}\right).398^{-1} \tag{6}$$

MTL_{rice} is the maximum tolerable levels of rice, MRL is the minimum risk level defined by Agency for Toxic Substances and Disease Registry (2017) as 0.005 mg $kg^{-1}day^{-1}$ for acute and 0.0003 mg $kg^{-1}day^{-1}$ for chronic arsenic exposure, PGV_{water} is the WHO's Provisional Guideline Value for arsenic (0.01 mg L^{-1} or 10 µg L^{-1}) in drinking water, and IR is abbreviated for the daily intake for water, wheat or rice and C_{wheat} wheat iAs concentration (Table 1).

2.9 Statistical Analysis

Microsoft Excel and SPSS 24.0 (IBM, New York, NY, USA) were used for statistical analyses. Descriptive analysis was performed for As test data, EDI and HQ of wheat, raw and cooked rice to determine the mean±SD. The data was subjected to bivariate analysis using correlation (Pearson) analysis between different variables to understand their interrelationships. ANOVA was used to test for differences in arsenic between different subgroups with respect to age. A statistical significance level of $P \leq 0.05$ was used.

3. Results & Discussion

The present study estimated the arsenic content of wheat, raw and cooked rice grains and the associated health risk posed by exposure to arsenic and its species in the human population of rural settings in Pakistan. Data so obtained has been presented and discussed in subsequent sections.

3.1 Arsenic speciation and quality control

Mean tAs measured in SRM NIST rice flour (SRM 1568a for cooked and uncooked rice) was 270±10 µg kg^{-1} (n=4), within the certified range of 285 ± 14 µg kg^{-1}, yielding a recovery of 97%. tAs concentration 5.60 µg kg^{-1} measured in SRM NIST wheat flour (1567a) (n=2) was found within the certified range of 4.8 ± 0.3 As µg kg^{-1} yielding a mean recovery of 83%. As no SRM with certified values of arsenic species was available, therefore SRM 1568a was used for quality control in speciation analysis for both rice and wheat. The results indicated 104 ± 1 µg kg^{-1} of iAs (certified value 92 ± 10 µg kg^{-1}), 179.5 ± 0.5 µg kg^{-1} of DMA (certified value 180 ± 12 µg kg^{-1}), 14.5 ± 0.5 of MMA µg kg^{-1} (certified value 11.6 ± 3.5 µg kg^{-1}) and yielded recoveries of 97%, 100% and 75% respectively. These results were also in agreement with earlier reported results of arsenic species in SRM 1568a as 80-110 µg kg^{-1} (iAs), 160-174 µg kg^{-1} (DMA) and 2-14 µg kg^{-1} (MMA) (D'Amato et al., 2011; Carbonell-Barrachina et al., 2012; Antoni, 2016). Overall, the spike recoveries of tAs, iAs, DMA and MMA in digests of matrix spikes (n=3), matrix spike duplicate (n=3), duplicate (n=3), blank spikes (n=3), post spikes (n=3) were 83-93% for wheat and 86-102% for raw and cooked rice.

3.2 Arsenic in raw and cooked rice

The mean concentration of tAs in raw rice (47.47±30.72 µg kg^{-1}) was found to be lower than in cooked rice i.e. 71.65±74.71 µg kg^{-1} (Table 1).

Table-1: Summary statistics of As and its species concentrations in raw rice, cooked rice and wheat (μg kg^{-1}) on wet weight basis

Analyte	Statistics	Raw Rice	Cooked Rice*	Wheat
		n=105 *(for tAs)* ***n=10*** *(for As species)*	***n=24***	***n=8***
tAs	Mean±SD	47.47±30.72	71.65±74.7	105±61.47
	min-max	<LOD-186	24-270	49-241
iAs	Mean±SD	92.5±41.9	79.21±76.42	116.38±51.38
	min-max	63-200	18-300	64-228
DMA	Mean±SD	13±7.38	8.72±13.75	≤LOD
	min-max	LOD-23	≤LOD-48	≤LOD
MMA	Mean±SD	≤LOD	≤LOD	≤LOD
	min-max	≤LOD	≤LOD	≤LOD
As (organic:DMA+MMA)	Mean±SD	13.5±7.38	9.23±13.75	1±0.0
	min-max	1-23.5	1-48.5	1-1
SumAs	Mean±SD	106±47	88.44±82.91	117.38±51.38
	min-max	66.02-223.5	19-309.5	65-229
iAs percentage	Mean±SD	87.53±6.38	91.13±8.78	99.02±0.36
	min-max	80-98.53	69.9-99.67	98.46-99.56
As percentage (organic)	Mean±SD	12.47±6.38	8.87±8.78	0.98±0.36
	min-max	1.47-2	0.33-30.1	0.44-1.54

SD: Standard deviation, n= number of samples
LOD: 5 μg kg^{-1} for tAs and 0.5 μg kg^{-1} for iAs, DMA and MMA
** Cooked rice MMA of 83 μg kg^{-1} excluded as a single outlier as they exceeded other samples by more than ten times, and, inclusion in data set, would result in twice the current reported mean for the whole sub-group.*

The mean tAs concentration in raw rice (n=105) was lower than (108-383 μg kg^{-1}) reported in white polished rice grown in Bangladesh, India, China, Taiwan, Thailand, Vietnam, Spain, Brazil, Turkey and USA (Table 2). Our results were higher than the mean tAs of 30-40 μg kg^{-1} for rice grown in Malawi (Joy et al., 2017) and Egypt (Meharg et al., 2009) and comparable to the findings of Rahman et al. (2009) reporting tAs concentrations of 61 μg kg^{-1} in Pakistani Basmati rice available in Australian supermarkets.

Table-2: Comparison of arsenic and its species in raw polished white rice (μg kg^{-1}) with past studies

Sampling location	n	tAs	iAs	MMA	DMA	Reference
Bangladesh	11	131 (30–300)	83 (10–210)		19 (0–50)	Williams et al. (2005)
India	15	46 (30–50)	27 (20–40)	0.7	66	Williams et al. (2005)
India	29	283 ± 13	194	2.0±0.0	14 ±1	Halder et al. (2014)
China	248	116.5	90.9	-	-	Huang et al. (2013)
China	33	230 (19–586)	154 (71–386)	1.30 (7–13)	40 (9-147)	Zhu et al. (2008)
Spain	39	188 ± 78	114 ± 46	-	-	Torres-Escribano et al. (2008)
Spain	7	170	80	<LOD	50	Williams et al. (2005)
Turkey	50	202	159.7	2.70	40	Sofuoglu et al. (2014)
Pakistan	10	47.47 (0.5-186)*	92.5 (63-200)	0.5	13 (0.5-23)	This study
Taiwan	nd	383 (190–760)	247 (110–510)	32 (15–60)	37(30–50)	Williams et al. (2005)
Korea	30	135	85	20	30	Kim et al. (2013)
Thailand	79	139.48 ± 5.94	81.58	<2.0 (<2.0–6.4)	29 (2.42–85.95)	Nookabkaew et al. (2013)
Vietnam	12	136.31 ± 11.42	91.2	<2.0 (<2.0–4.14)	16.25 (5.94–25.08)	Nookabkaew et al. (2013)
USA	24	265 (162–383)	103 (52-217)	0.6(0–6)	155 (40–302)	Zavala et al. (2008)
USA	34	108	65	3	40	Kim et al. (2013)
Brazilian	44	222.9	112 (56-218)	8 (0–29)	93 (39–258)	Batista et al. (2011)

**n=105*

The mean tAs concentration of 71.6 μg kg^{-1} (24-270 μg kg^{-1}) in cooked rice (n=24) was lower than mean concentrations (170-370 μg kg^{-1}) previously reported for cooked rice consumed in Bangladesh and West Bengal (Mondal and Polya, 2008;

Rahman et al., 2006; Smith et al., 2006; Bae et al., 2002; Roychowdhury et al., 2002). The maximum concentrations in cooked rice were 270 µg kg^{-1} (tAs), 300 µg kg^{-1} (iAs), 48 µg kg^{-1} (DMA), whilst MMAs were detected in raw or cooked rice as ≤LOD.

The mean iAs of 92.50±41.88 µg kg^{-1} in raw rice and 79.21±76.42 µg kg^{-1} in all cooked rice samples (Table 1) revealed only one raw rice (200 µg kg^{-1}) and two cooked rice (290 µg kg^{-1}, 300 µg kg^{-1}) samples which exceeded the preliminary advisory limit of 200 µg kg^{-1} iAs in rice (Codex Alimentarius Commission, 2014). Rice distributed in several areas of Pakistan is mainly produced in the primary rice growing region of Punjab (Rasheed et al., 2016) using ground water and/or surface water irrigation. However, even with low As in irrigation water, rice can accumulate 10-fold higher iAs than other grains (Davis et al., 2017) and may require exposure control measures.

In line with the earlier studies (Williams et al., 2005; Ma et al., 2016; Mondal and Polya, 2008; Rahman et al., 2011), arsenic concentrations in raw rice comprised of >80% of iAs, whilst cooked rice was found to have 69-100% of iAs (Table 1). The mean DMA concentration in raw rice (13±7.38 µg kg^{-1}) was higher than in cooked rice (8.72±13.75 µg kg^{-1}) and comparable to the raw rice of south Asian origin, but much lower than rice grown in Brazil and USA (Table 2). The higher proportion of iAs and stronger linear relationship with tAs (R^2 = 0.97) than DMA (R^2 = 0.4) has categorized raw rice into “iAs type” as per criteria set by Zavala et al. (2008), whereas, demethylation of DMA and MMA in rice also increase the iAs contents as reported by Chavez-Capilla et al. (2016). Proportion of iAs in raw rice varies geographically depending on the crop variety and uptake of iAs and other arsenic species by crop plants from soil and irrigation water (Santra et al., 2013; Fu et al., 2014; Phan et al., 2014; Talukder et al., 2012). This suggests that arsenic absorption in cooked rice varies with the arsenic concentration in cooking water and with cooking method.

3.3 Impact of cooking

The tAs concentration in paired raw and cooked rice samples (*n* = 12) was found to be 8-186 μg kg^{-1} (mean 83.1 μg kg^{-1}) in the raw samples and 26-260 μg kg^{-1} (mean 55.29 μg kg^{-1}) in cooked rice respectively (Figure 1).

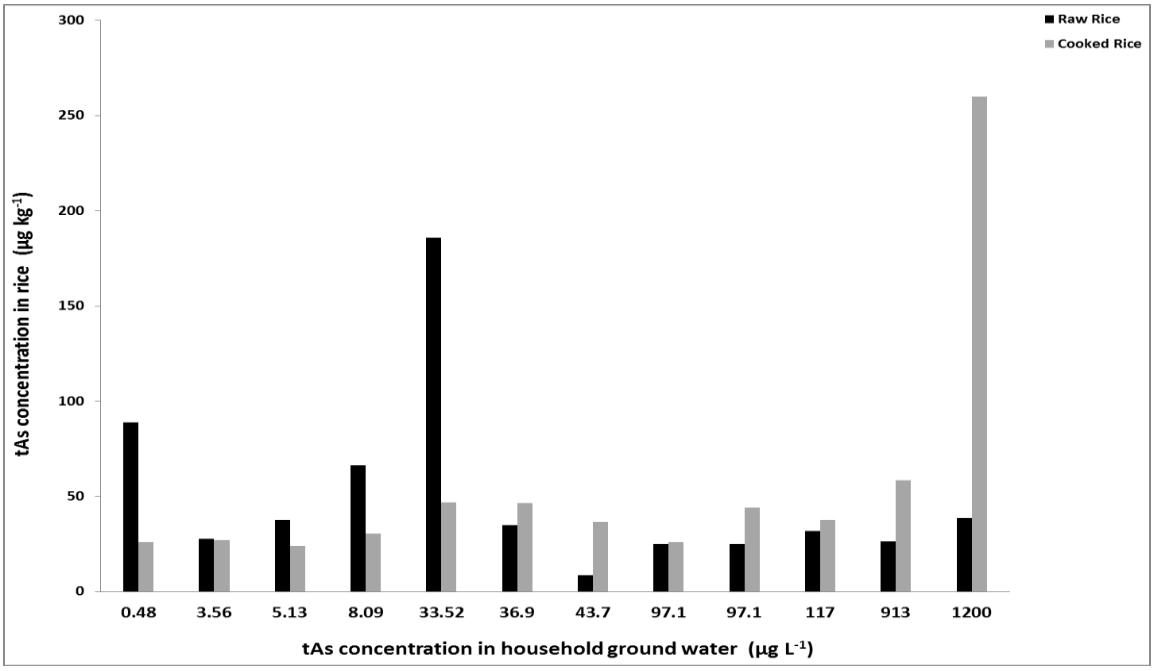

Figure-1: The concentration of tAs in raw and corresponding cooked rice samples (n=12)

A significant association (r=0.85, p<0.001) was found between tAs in cooked rice (n=24, mean 71.65 μg kg^{-1}) and tAs of corresponding cooking water (n=24, mean 382.56 μg kg^{-1}). Seven households out of twelve showed an increase of up to 43% in tAs of rice after cooking (Figure 1). The five households which cooked in low arsenic water (0.48-33.52 μg L^{-1}) showed a significant decrease of up to 48% (r=0.92, p=0.02) in tAs. An increased tAs in cooked rice is in agreement with Ohno et al. (2009) (raw 220±110 *vs* cooked 260±150 μg kg^{-1}), whilst reduced tAs after cooking in low arsenic water is comparable to other studies (Rahman et al., 2011; Sengupta et al., 2006; Raab et al., 2009a) which showed up to a 57% decrease in cooked rice. As per information inquired from householders, two main cooking methods were used; the Traditional method (A) and the Intermediate method (B) categorized by Signes et al. (2008) but the impact of cooking method on arsenic concentrations in rice requires further investigation.

3.4 Arsenic in wheat grains

The mean tAs concentration of 105±61.47 µg kg^{-1} in wheat grains grown in the study area was higher than the mean tAs concentration of 47.47±30.72 µg kg^{-1} in raw rice (Table 1). Wheat is grown locally in this study area for household consumption using mainly ground water irrigation, whilst rice is also purchased from local shops indicating the supply of rice from sources beyond the study area. Rice has a greater capacity for As uptake from soil water than wheat. (Williams et al., 2007b; Norra et al., 2005). In this study, higher levels of As in wheat suggests a direct relationship to the use of highly As contaminated irrigation water and it is likely that if rice were grown in this area, As levels in rice might have been higher due to the relatively greater uptake capacity of rice compared to wheat.

The mean tAs concentration in locally cultivated wheat grains (Table 1) was higher than the range of 20-129 µg kg^{-1} found in wheat grown in the USA, Netherlands, and India (Gartrell et al., 1986; Wiersma et al., 1986; Sharma et al., 2016; Bhattacharya et al., 2010) but lower than the wheat grown (362 µg kg^{-1}) in West Bengal, India (Roychowdhury et al., 2002). The maximum tAs concentration (241 µg kg^{-1}) was lower than that found in Cornwall, Southwest England (500 µg kg^{-1}) (Williams et al., 2007a) and 317-400 µg kg^{-1} in Pakistan (Baig et al., 2011; Arain et al., 2009). Arsenic determined in wheat was mainly iAs with mean and maximum concentrations of 116.38±51.38 µg kg^{-1} and 228 µg kg^{-1} respectively.

Milling of wheat grains to separate bran from wheat flour may result in a 23−29% reduction of tAs (Zhao et al., 2010). By applying this factor to this study, the mean tAs concentration of wheat grains might be reduced from 105 µg kg^{-1} to 75-81 µg kg^{-1} after milling. However, wheat flour conventionally kneaded in the study area (for chapatti/bread making) with arsenic rich water combined with its high levels of consumption is expected to result in high levels of arsenic exposure.

3.5 Estimated daily intake of arsenic from dietary sources

A significantly higher iAs intake from raw rice (0.3±0.1 µg kg^{-1} bw day^{-1}) or cooked rice (0.8±0.4 µg kg^{-1} bw day^{-1}) was found for the 6-16 age group compared to the 3-6 years and >16 years, whilst exposure from wheat intake was significantly higher among children of 3-6 years than other age groups (Table 3). The cooked rice iAs exposure for children (<16 years) is comparable to the mean exposure of 0.7 µg day^{-1} for children of 1-2 years old reported by Mantha et al. (2017) and 1-6 years by Yost et al. (2004). Mean iAs exposure from raw rice (0.3±0.1 µg kg^{-1} bw day^{-1}) was higher than for an average 70 kg body weight person in the US (0.02 µg kg^{-1} bw day^{-1}) as reported by Mantha et al. (2017). The mean total daily intake (TDI) of iAs (16±40 µg kg^{-1} bw day^{-1}) comprised 1.5% from raw rice, 4.5% from wheat and 94% from water which was higher than the mean iAs dietary intake (0.1 to 0.4 µg kg^{-1} bw day^{-1}) of the European population (European Food Safety, 2014). Contrary to this study, a maximum cooked rice contribution of 41% was reported by Signes et al. (2008b), suggesting the significance of inter-individual and geographical variations in food safety regulations.

Table-3 Descriptive statistics for the estimated exposures of iAs stratified by study population

Source	Age groups	n	Consumption (g day^{-1}) or (L day^{-1})	iAs intake µg kg^{-1} bw day^{-1}		n(%) >2.1 µg kg^{-1} bw day^{-1}
				(Mean ± SD)	(Min-max)	
Raw Rice	All participants	168	136	0.3±0.1	0.1-0.6	0
	3-6 years	4	27	0.2±0.1	0.1-0.3	0
	6-16 years	34	79	0.3±0.1	0.1-0.6	0
	>16 years	130	154	0.2±0.1	0.1-0.5	0
	P-value		0.0005	0.033		
Cooked Rice	All participants	168	469	0.7±0.3	0.1-2.4	2 (2)
	3-6 years	4	91	0.7±0.1	0.4-0.7	0
	6-16 years	34	272	0.8±0.4	0.3-1.7	0
	>16 years	130	532	0.7±0.3	0.1-2.4	2 (2)
	P-value		0.0005	0.033		
Wheat	All participants	394	372	0.7±0.3	0.2-2.1	1(0.3)
	3-6 years	4	149	1.1±0.3	0.8-1.5	0
	6-16 years	59	227	0.9±0.3	0.4-1.7	0
	>16 years	331	400	0.7±0.3	0.2-2.1	1(0.3)
	P-value		0.0005	0.0005		
Water**	All participants	398	3.5	15±40	0.02-263	255 (65)
	3-6 years	5	1.9	8±6	2.6-17	5 (100)
	6-16 years	61	2.9	16 ±44	0.07-227	48 (79)
	>16 years	332	3.6	15 ±40	0.02-263	202 (61)
	P-value		0.0005	0.2		
Total dietary intake*	All participants	398		16±40	0.4-264	294 (74)
	3-6 years	5		10±6	2.8-18	5 (100)
	6-16 years	61		17±44	1-228	54 (89)
	>16 years	332		16±40	0.4-264	235 (71)
	P-value			0.13		

n.i: not included

**Based on raw rice, wheat and total water intake*

***iAs intake from water obtained from our previous study (Rasheed et al., 2017a)*

Mean iAs exposure from raw rice (0.3±0.1 µg kg^{-1} bw day^{-1}) was comparable (Jorhem et al., 2008) which showed a rice contribution in Sweden of 1.3% of the provisional weekly tolerable intake (PWTI) of 15 µg kg^{-1} bw (2.1 µg kg^{-1} bw day^{-1}). When compared with the provisional tolerable daily intake (PTDI) of 2.1 µg kg^{-1} bw day^{-1}, 2%, 0.3%, 65% and 74% of the study participants exceeded for iAs intake from cooked rice, wheat, water and TDI respectively (Table 3). These finding suggest that the estimated daily intake of iAs from raw rice, cooked rice and wheat grains contributed to a much lesser extent in arsenic exposure, compared to intake from water.

Study participants exposed to iAs (water) <1 µg L^{-1} showed a TDI of 0.5±0.3 µg tAs kg^{-1} bw day^{-1} with approximately 92% of intake from staple food (raw rice and wheat), whereas participants exposed to iAs (water) <10 µg L^{-1} showed a TDI of 0.9±0.3 tAs µg kg^{-1} bw day^{-1} with approximately 60% of intake from food (raw rice and wheat). Study participants exposed to iAs (water) >10 µg L^{-1} had a tAs TDI of 17±39µg kg^{-1} bw day^{-1}, with 4.4% of intake from staple food. These results suggest that the persistent exposure from food should always be taken into account with water for any type of health risk assessment or risk management.

3.6 Ratio between combined iAs intake and recommended reference levels

Mean iAs HQ due to chronic exposure from wheat (2.4±1.1) was found to be significantly higher (P=0.0005) than mean HQ for both raw rice consumption (0.8±0.3) and the mean HQ for cooked rice (2.3±1.1) (Table 4). These values were found to be higher than the USEPA advised minimal threshold level of 1.00 (United States Environmental Protection Agency, 1989) in 14% (raw rice), 97% (wheat), 94% (cooked rice) of study participants (Table 4).

Children of age 3-6 and 6-16 years were the most vulnerable groups compared to adults with HQ>1 due to iAs exposure from wheat, raw rice or cooked rice suggesting an increased risk potency probably due to body weight and water/food intakes differences (Table 4). Rice cooked in arsenic rich water (0.48-1270 µg L^{-1}) resulted in higher HQ values in 94% of participants compared to raw rice (14%) and consequently a higher non-cancer health risk (Table 4). Mean HI (2.7±1.1) due to concurrent intake of raw rice and wheat grains (without taking water into account) was found to be higher than the safe limit of 1.0, indicating a moderate health risk in 100% of residents (Table 4). The risk calculated for acute exposure from all exposure sources showed almost no risk.

Table-4: A summary of exposure risks posed to study population due to iAs intake from rice and wheat grains

Age Group (years)	Statistics	HQ (RR)	HQ (Wheat)	HQ (Cooked Rice)	HI
3-6	n	4	4	4	5
	Mean ± SD	0.8±0.1	3.7±1.0	1.9±0.5	3.6±1.7
	n(%) >1	0	4 (100)	4 (100)	4 (80)
6-16	n	34	59	34	61
	Mean ± SD	0.98±0.4	2.9±1.1	2.7±1.2	3.3±1.2
	n(%) >1	15 (44)	59 (100)	33 (97)	60 (99)
>16	n	130	331	130	332
	Mean ± SD	0.8±0.2	2.3±1.1	2.2±1.0	2.6±1.0
	n(%) >1	9 (7)	320 (97)	121 (93)	331 (100)
All participants	n	168	394	168	398
	Mean ± SD	0.8±0.3	2.4±1.1	2.3±1.1	2.7±1.1
	n(%) >1	24 (14)	383 (97)	158 (94)	395 (100)
P-value (between age subgroups)		**0.033**	**0.0005**	**0.033**	**0.006**

**Sum of raw rice and wheat, RR: raw rice*

Study area participants were also eating other food like pulses, vegetables, milk, yoghurt and chicken (Rasheed et al., 2017b) which may also be of concern, but potentially not as great as the concern regarding consumption of staples rice and wheat. Therefore, the exposure data for rice and wheat provided here may prove helpful for regulation of arsenic exposure from the most frequently consumed food. An evaluation of margins of safety for iAs in rice has resulted in the MTL_{rice} of 0.1 mg kg^{-1} due to an average rice consumption of 469 g day^{-1}. The CCA's advisory level of 0.2 mg kg^{-1} iAs in white polished rice is only achievable in a study population with an average rice consumption of 200 g day^{-1} and compliance with 10 µg L^{-1} iAs in drinking/cooking water.

Since As intake from water used for preparation of tea, yoghurt drink (lassi), milk, wheat flour kneading, washing and cooking of rice, chicken, pulses and vegetables (as indirect water intake:Rasheed et al. (2017b)) was taken into account for this exposure assessment, however the future investigation should also consider arsenic speciation of poultry products, locally grown vegetables, and dairy products such as milk, butter and meat of livestock reared with arsenic contaminated water.

4. Conclusions

Inorganic arsenic exposure from consumption of wheat was higher in this study population than rice followed by lower levels of dimethylarsinic acid (DMA) from raw and cooked rice. Raw rice was a moderate source of exposure in the study villages although cooking in arsenic rich, low volumes of cooking water, and higher cooked rice consumption frequency may contribute significantly in producing a potential risk. The prolonged arsenic exposure of study participants from total water intake (including indirect water used for rice cooking and wheat flour kneading), raw rice and locally grown wheat, was demonstrated by a total daily intake of 16±40 µg iAs kg^{-1} bw day^{-1} with relative contributions from food (6%), drinking and cooking water (94%). The chronic non-cancer risks due to aggregated exposure of iAs from wheat and raw rice have indicated somewhat higher mean hazard quotient values (2.7±1.1) than the acceptable limit of 1.0 in 100% of participants. Children were subject to significantly higher exposure and health risks compared to adults**.** Dietary exposure to inorganic arsenic occurs naturally such as in raw rice or wheat grains and is unavoidable; however growing the crops with low arsenic irrigation water, rice cooking and wheat flour kneading in low arsenic water may reduce the dietary exposure. The study findings suggest that an inorganic arsenic maximum tolerable level for the most frequently consumed food such as rice and wheat as well as recommendations on their consumption frequency would be useful to lower the exposure risk. Moreover, arsenic remediation of water used for drinking, irrigation and food preparation is an immediate requirement for populations in arsenic affected regions.

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Competing interests

The authors declare that they have no competing/conflicting interests.

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