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Speciated arsenic concentrations, exposure, and associated health risks for rice and bulgur

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ABSTRACT

Arsenic species were determined in rice and bulgur samples that were collected from 50 participants who also supplied exposure related information through a questionnaire survey. Speciation analysis was conducted using an HPLC–ICP-MS system. Ingestion exposure to arsenic and associated health risks were assessed by combining the concentration and questionnaire data both for individual participants and the subject population. Inorganic arsenic dominated both in rice and bulgur but concentrations were about an order of magnitude higher in rice (160 ± 38 ng/g) than in bulgur. Because participants also consumed more rice than bulgur, exposures were significantly higher for rice resulting in carcinogenic risks above acceptable level for 53% and 93% of the participants when the in-effect and the proposed potencies were used, respectively, compared to 0% and 5% for bulgur. An inorganic arsenic standard for rice would be useful to lower the risks while public awareness about the relation between excessive rice consumption and health risks is built, and bulgur consumption is promoted.

1. Introduction

Rice is a staple food around the world, however, it has been shown to contain higher levels of As compared to other cereal grains (Adomako et al., 2011; Schoof et al., 1999; Williams et al., 2007a; Zhu et al., 2008). Total arsenic (tAs) content of rice varies geographically depending on the As content of soil and irrigation water; concentrations of up to 1830 ng/g in a sample from a region of Bangladesh irrigated with contaminated water have been reported (Meharg and Rahman, 2002). Zavala and Duxbury (2008) suggested a global "normal" concentration range between 82 and 202 ng/g. Higher levels were reported for rice grown in the US, France, Italy compared to rice from Bangladesh, Venezuela, Egypt, India, Thailand, and Pakistan (Meharg et al., 2009; Zavala and Duxbury, 2008). Inorganic arsenic (iAs) and dimethylarsenic acid (DMA) dominates in raw rice (Batista et al., 2011; Meharg et al., 2009; Mihucz et al., 2007; Torres-Escribano et al., 2008; Williams et al., 2005; Zavala et al., 2008). Proportion of iAs in tAs in rice is also geographically variable ranging from 11% to 93% (Torres-Escribano et al., 2008). iAs concentrations are generally >50 ng/g (Zhu et al., 2008), and may reach up to about 800 ng/g (Zavala et al., 2008). Arsenic accumulation in rice was reported to be dependent on genetic factors and soil type (Ye et al., 2012), and occurs at higher rates due to anaerobic systems under flooded conditions in growing rice plant compared to aerobic conditions for other crops such as wheat, barley, and maize (Williams et al., 2007a). tAs content of wheat range from 10 ng/g to 500 ng/g with mean levels of <100 ng/g (Adomako et al., 2011; Williams et al., 2007a).

Studies have been pointing out the role of rice in people's exposure to arsenic (Meacher et al., 2002; Meharg et al., 2009; Meliker et al., 2006; Schoof et al., 1999; Sun et al., 2008; Tsuji et al., 2007; Williams et al., 2007b; Xue et al., 2010; Yost et al., 2004; Zavala and Duxbury, 2008; Zhu et al., 2008), and consumer groups have been calling for a Maximum Contaminant Level (MCL) (Jalonick, 2012). While no MCL is in place in the US and the EU, China promulgates an MCL of 150 ng/g for iAs (Qian et al., 2010), and Hungary and China have 300 ng/g and 700 ng/g, respectively for tAs (Mihucz et al., 2007; Qian et al., 2010). iAs (As^{3+} and As^{5+}) has been classified as a human carcinogen (IARC, USEPA) for which a steeper slope factor (SF) has been proposed (Adomako et al., 2011; Tsuji et al., 2007) for the in-effect SF value (IRIS, 2013). Organic arsenic compounds are considered less toxic than iAs, however, they cannot be assumed as completely benign (Schoof et al., 1999; Williams et al., 2007b), e.g., DMA may act as a cancer promoter (Brown et al., 1997). Population studies have shown that rice is a major source of tAs and iAs with drinking water among other food stuffs and other sources such as air pollution, cigarette smoking, soil,

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chromated copper arsenate – treated wood (Meacher et al., 2002; Schoof et al., 1999; Tsuji et al., 2007; Xue et al., 2010; Yost et al., 2004). Contaminated drinking water is the largest source of exposure to As in regions where contamination is a problem even at moderate levels (Meliker et al., 2006), whereas rice is the largest iAs source in regions where drinking water As exposure is low (Fontcuberta et al., 2011; Meacher et al., 2002; Meliker et al., 2006; Tsuji et al., 2007; Xue et al., 2010).

Analyses have shown that the most influential variable on exposure to As in rice is the amount of rice consumption (Jorhem et al., 2008; Williams et al., 2007b). Average daily per capita rice consumption ranges from 9 g/d in Europe to 278 g/d in Asia (Jorhem et al., 2008). Method of cooking the rice and As content of water used in cooking may also play a significant role. Increasing water As concentration has been shown to increase the As level in the cooked rice; cooking with aliquots of water (steaming) or until dryness do not affect the As level in cooked rice, while cooking with abundant water and discarding the excess water has been shown to decrease As level compared to raw rice (Fontcuberta et al., 2011; Laparra et al., 2005; Mihucz et al., 2010, 2007; Raab et al., 2009; Rahman et al., 2006; Sengupta et al., 2006; Torres-Escribano et al., 2008). Rinsing with water prior to cooking also reduces the As content (Mihucz et al., 2007; Raab et al., 2009; Sengupta et al., 2006) because rice contains considerable portion of tAs in its bran and outer grain than its bulk (Sun et al., 2008). As a result, brown rice is generally found to have higher levels of As compared to white (polished) rice. Rice bran and its products, especially solubles, were reported to contain 10–20 folds of bulk grain concentrations, which would have more detrimental effects because of their use in food aid programs to undernourished children (Sun et al., 2008).

Bulgur is an ancient food that is still popular in Turkey and other eastern Mediterranean, Middle East, and eastern European countries, for which information regarding its history, functional characteristics, and nutritional value can be found in the literature (Bayram, 2000; Bayram and Öner, 2002). Briefly, it is a quick cooking (Kadakal et al., 2007), parboiled, dry and partially debranned whole wheat product obtained by soaking, cooking, drying, milling, and cracking (Özboy and Köksel, 2001). Wheat is processed for stability and long shelf-life in hot and humid environments, and resistance against mold, mites, and insects (Bayram, 2000; Bayram and Öner, 2002). Due to the processing, bulgur may be expected to contain toxic element levels of even less than wheat. Bulgur is considered as a "health food" in Europe and USA where its consumption has been increasing (Bayram and Öner, 2002). tAs was not detectable (<1 ng/g) in a small sample ($n = 10$) of wheat grains from Tekirdag, Turkey (Koc et al., 2009). Although many food stuffs consumed in Turkey, including rice (Gunduz and Akman, 2013; Uluozlu et al., 2010), have been analyzed for As content, the studies were generally analytical method development studies with small sample sizes; none of them was based on an exposure assessment approach except for drinking water (Kavcar et al., 2009) and tea (Sofuoglu and Kavcar, 2008). Bulgur has not yet been studied at all. This study aimed to investigate tAs and iAs levels in rice and bulgur, estimate exposure and human health risk levels. Rice and bulgur samples were collected from 50 participants living in Izmir, the third most populated city in Turkey. The participants supplied demographic and rice – bulgur consumption information through a questionnaire. The concentration and questionnaire data were used to conduct both individual and population exposure and risk assessments.

2. Material and methods

2.1. Questionnaire

İzmir is the third largest city in Turkey with a population of about 3.5 million. It is located on the Aegean Sea shore in western Turkey. A questionnaire was self administered by 50 randomly selected participants living in İzmir to determine their daily rice and bulgur intake, preferences, and personal characteristics. The questionnaire also inquired about information like brand of rice and bulgur, rice cooking method, education level, body weight, gender, age, etc. Rice cooking method was requested from the person who cooked in the household. Participants were asked to record portions of rice and bulgur consumed daily for 7 days.

2.2. Sampling and sample preparation

The participants were agreed to provide a sample of the rice and bulgur that they cook at home. A total of 50 rice and bulgur samples were collected. All samples were white (polished) rice received in the supplied plastic zip bags and transferred into 60-mL HDPE bottles in the laboratory. All HDPE bottles, pipette tips, and falcon tubes were kept in 20% nitric acid (Merck) bath for at least 3 h, rinsed with ultrapure distilled water (Millipore Elix5) three times, and dried in a hood before use.

Samples were analyzed for As^{3+}, As^{5+}, DMA, and MMA (monomethylarsonic acid) using a method reported by Huang et al. (2010). The HPLC–ICP-MS system was calibrated before each batch of analysis using five point calibration curves ($R^2 > 0.9975$). Stock standard solutions of As^{5+} and As^{3+}, 2000.0 mg/L, were prepared by dissolving As_2O_5 (Merck, 1.09939) and As_2O_3 (Fischer, 1327-53-3), respectively, in ultrapure water. DMA (1000.0 mg/L) and MMA (553.0 mg/L) were prepared by dissolving dimethylarsinic acid sodium salt trihydrate (Merck, 8.20670) and disodium methyl arsenate hexahydrate (Supelco, PS 281), respectively, in ultrapure water.

Five grams of each sample (wet weight) were extracted with 15 mL 0.28 M HNO_3 (69%, Merck) by heating in a 95 °C water bath for 90 min. Then, the samples were cooled at room temperature, and centrifuged at 5000 rpm for 35 min at 18 °C before filtering with 0.45 μm Teflon filter.

2.3. Arsenic speciation analysis

An HPLC (Agilent 1200 series) coupled with an ICP-MS (Agilent 7500ce) was used for chemical speciation analysis. An anion-exchange column (Hamilton PRP-X100) was used in the LC system. Samples were loaded with a syringe into a 100-μL sample loop. The effluent from the LC column was connected to the concentric nebulizer with PEEK tubing and a low dead volume PEEK connector. The buffer solution was 10 mM ammonium carbonate ($(NH_4)_2CO_3$) and 30 mM ammonium carbonate, at pH 8.5. Separations were performed by a 15 min gradient program at room temperature. The flow rate was 1 mL/min. For the first 4 min 10 mM ammonium carbonate was used and then changed to 30 mM ammonium carbonate in 1.5 min and stayed for 5.5 min. After that it turned to 10 mM ammonium carbonate in a minute and finally stabilized in 3 min. The operating conditions of the ICP-MS were as follows; 27 MHz of RF generator frequency, 1500 W power output, argon flow rate with plasma 15 L/min, auxiliary 1 L/min, carrier 1 L/min and, nebulizer 0.08 rps.

2.4. QA/QC

Five duplicates were analyzed as individual samples. The mean difference between the duplicates were 10%, 21%, 34%, and 43% for As^{3+}, DMA, As^{5+}, and MMA, respectively, for which the average concentration of the five-duplicates were 51, 7.1, 1.9, and 1.1 ng/g, respectively. Limit of Detection (LOD) was calculated as the mean concentration plus three standard deviations ($n = 5$) for As^{3+} and As^{5+} as 2.4 and 2.7 ng/g, respectively. DMA and MMA were not detected in the blanks, therefore, instrument detection limits were determined (0.15 ng/g). All the reported concentrations in this study were blank corrected, and based on wet weight. A standard reference material (Rice Flour, EU-JRC, IRMM-904) was put through the sample processing and HPLC–ICP-MS analysis. Recoveries were calculated based on the certified tAs concentration of the SRM and tAs concentration calculated by summing the concentrations of the analyzed four species. The mean (±standard deviation) recovery was 66.1 ± 0.5%.

2.5. Exposure–risk assessment

Ingestion exposure to arsenic in rice and bulgur was estimated by calculating a chronic daily intake using

$$CDI = \frac{C \times DI}{BW} \times \frac{EF \times ED}{AT} \quad (1)$$

where *CDI* is the chronic daily intake (μg/kg d), *C* is the contaminant concentration (μg/g); *DI* is the average daily intake rate of rice or bulgur (g/d) estimated as the 7-day average from the questionnaire data; *BW* is body weight (kg) reported by the participant in the questionnaire; *EF* is the exposure frequency (d/yr), *ED* is the exposure duration (yr), *AT* is the averaging time (d). The second term in the equation is unity for chronic–toxic risk assessment, while *EF*, *ED*, and *AT* are assumed as 365 d/yr, 70 yr, and 70 × 365 d, respectively in this study, making the second term unity for carcinogenic risk assessment as well. The selected values of these three input variables and participant specific values of the remaining three input variables (*C*, *DI*, and *BW*) were used to estimate the subject's lifetime chronic daily exposure.

Lifetime cancer risk associated with ingestion exposure was calculated using

$$R = CDI \times SF \quad (2)$$

where R is the probability of excess lifetime cancer risk (or simply risk), and SF is the slope factor of the chemical $(\mu g/kg\ d)^{-1}$.

The hazard quotient (HQ) was calculated to estimate chronic–toxic risk using

$$HQ = \frac{CDI}{RfD} \quad (3)$$

where RfD is the reference dose (μg/kg d).

SF and RfD values employed in this study were 1.5 $(\mu g/kg\ d)^{-1}$ and 0.3 (μg/kg d), respectively, obtained from the USEPA (IRIS, 2013). However, a proposed, steeper SF value of 3.67 $(\mu g/kg\ d)^{-1}$ that has been used in the literature (Adomako et al., 2011; Tsuji et al., 2007) was also considered. In addition to the individual assessment for the study participants, population exposure–risk assessment was carried out using Monte-Carlo simulation.

2.6. Monte-Carlo simulation and statistical analyses

Concentration data were censored for non-detects to avoid overestimation of exposure and risk. Since the number of below detection limit (BDL) concentrations were small, half the detection limit values were used for censoring. Statistical analyses were performed using SPSS (Release 12.0); Monte-Carlo simulations were performed using Crystal Ball (v 4.0e) software. Monte-Carlo simulation is a computer-based method of analysis that uses statistical sampling techniques in obtaining a probabilistic approximation to the solution of a mathematical equation or a model. Exposure and risk distributions of İzmir population were estimated using the simulated values (n = 10,000).

Kruskal–Wallis and Mann–Whitney tests were used to determine whether the concentrations and risks associated with exposure to these concentrations differed across different participant groups. However, the samples sizes of the subgroups were small in some instances; so further analyses were conducted with subgroups of only sufficient sample size. In this study, p-values <0.05 were considered to point a significant difference between the compared groups.

3. Results and discussion

3.1. Rice and bulgur consumptions and other questionnaire data

Randomly recruited 50 people participated in this study. The mean age of the participants was 33.5 years (range: 14–75 yrs). Body weights of the participants ranged from 46 to 95 kg with a mean value of 66 kg. Characteristics of the participants are presented in Table 1. Medium grain rice was preferred in the majority of the participant households, and it was kept in hot water before cooking, which is discarded afterwards. Daily rice and bulgur consumption rate ranges were 5–75 g/d and 0–113 g/d with average values of 38 g/d and 22 g/d, respectively. The median values, 35 and 22 g/d, respectively, were close to the mean values. The mean daily rice consumption estimated in this study is about 80% higher than the value (21 g/d) reported by International Rice Research Institute (Manners, 2013) but closer to the value (27 g/d in 2009) reported by Turkish Cereal Council (Sade et al., 2011). Nevertheless, it is much less than the consumption rates in Asian diets, but higher than European diets (Jorhem et al., 2008). Globally, majority of bulgur is produced and consumed in Turkey (Yıldırım et al., 2008). The majority is consumed in the eastern and southern regions (up to 68 g/d) with a national average of about 33 g/d, whereas consumption in western regions is roughly down to 21 g/d (Bayram and Öner, 2002; Yıldırım et al., 2008). This study was conducted in İzmir, located on the Aegean Sea shore in western Turkey. The mean consumption rate determined in this study (22 g/d) agrees well with the previously reported values. The consumption rises to about 96 g/d in eastern Mediterranean countries such as Iraq, Israel, Lebanon, and Syria (Yıldırım et al., 2008).

Hypothesis testing results showed that the differences in bulgur and rice consumption between females and males were not significant. Both rice and bulgur consumption did not correlate with participant age. Both rice and bulgur consumed at a higher rate by high school graduates than primary school graduates. High school graduates also consumed rice at a higher rate than university graduates. Individuals in families of ⩾3 consumed more bulgur and rice than those of in ⩽2.

Table 1
Participant characteristics.

		N		%
Gender				
Female		19		38
Male		31		62
Age				
14–24		16		32
25–32		15		30
33–49		12		24
50–75		7		14
Education				
Primary school		9		18
High school		23		46
Undergraduate		13		26
Graduate		5		10
Rice type				
Baldo (medium grain)		33		66
Osmancık (medium grain)		13		26
Calrose (medium grain)		1		2
Jasmine (long grain)		1		2
Other		2		4
Water source for cooking rice				
Tap water		36		72
Bottle water		8		16
Purified tap water		6		12
Type of cooking for rice				
First keeping in hot water		32		64
After washing with water		12		24
Directly		6		12
Brand	Rice		Bulgur	
	N	%	N	%
A	12	24	11	22
B	8	16	6	12
No Name	7	14	10	20
D	6	12	3	6
E	4	8	–	
F	–		4	8
G	–		3	6
Others	11	22	9	18

3.2. Concentrations of arsenic species

As^{3+} and DMA were detected in all 50 rice samples, whereas BDLs were 6% and 16% for As^{5+} and MMA, respectively. Majority of the arsenic in rice was iAs. Ratio of iAs in tAs ranged from 42% to 97% with a mean value of 80%. Variation in the concentrations of arsenic species in rice is presented in Fig. 1. All concentrations reported in this study are wet weight based. The mean concentrations were 151, 40, 8.7, and 2.7 ng/g for As^{3+}, DMA, As^{5+}, and MMA, respectively. The maximum concentrations were 276, 157, 26, and 10 ng/g for As^{3+}, DMA, As^{5+}, and MMA, respectively. The median, mean, and 95th percentile tAs concentrations were 199, 202, and 284 ng/g, respectively. None of the tAs concentrations were above the Chinese tAs standard (Qian et al., 2010), however even the median value was higher than its iAs standard (Qian et al., 2010). Thirty-four percent of the tAs concentrations were within the "global range" of 82–202 ng/g estimated by Zavala and Duxbury (2008) assuming 12.5% moisture content (Williams et al., 2007a). tAs concentrations measured in this study were higher than those measured in rice sold in Turkey in analytical method development studies (Gunduz and Akman, 2013; Uluozlu et al., 2010); similar to those measured in Brazilian, Japanese, Spanish, and Vietnamese rice (Batista et al., 2011; Dong Phuong et al., 1999; Meharg et al., 2009); lower than those of American, Australian, and French rice

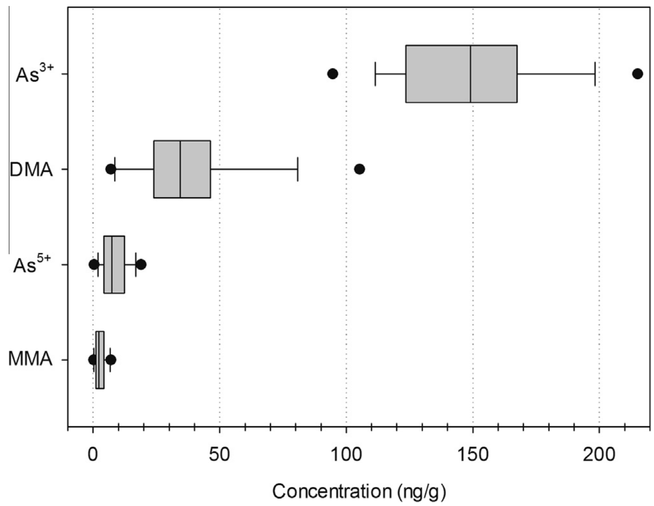

Fig. 1. Concentrations of arsenic species in rice (wet weight, black dots depict 5th and 95th percentiles).

(Dong Phuong et al., 1999; Meharg et al., 2009); but higher than those of Bangladeshi, Chinese, Egyptan, Indian, Italan, and Thai (Meharg et al., 2009) rice not considering rice grown in As-contaminated areas. On the other hand, iAs concentrations measured in this study were higher than those measured in American, Bangladeshi, Brazilian, Italian, Indian, Spanish, and Thai rice (Batista et al., 2011; Meharg et al., 2009; Torres-Escribano et al., 2008; Zavala et al., 2008); similar to those of American and Chinese rice (Lamont, 2003; Meharg et al., 2009); not considering rice grown in As-contaminated areas.

Because arsenic levels were low in bulgur, only tAs concentration was determined in all 50 samples using ICP-MS analysis after microwave digestion. The five highest concentration samples were analyzed for arsenic species to infer on the composition. tAs concentrations in bulgur ranged from BDL to 75 ng/g, with a mean value of 21 ng/g. However, skewness of the data was high (2.52); the median value was 16 ng/g. The 95th percentile concentration was 64 ng/g. Arsenic in bulgur was dominated by As^{3+} (86 ± 7%, $n = 5$) and As^{5+} (12 ± 5%, $n = 5$). Arsenic concentrations in bulgur were 10 and 12 times lower than rice when mean and median concentrations are considered, respectively. Fig. 2 shows the difference in tAs concentrations in rice and bulgur. The mean As concentrations in wheat grain reported in the literature are in the range of 20–70 ng/g (Adomako et al., 2011; Williams et al., 2007a), which could go up to 740 ng/g when wheat is grown in As-contaminated areas (Norra et al., 2005). tAs was BDL in all wheat ($n = 10$) and barley ($n = 10$) samples from Tekirdag, Turkey (Koc et al., 2009); but it was detectable in boiled wheat samples from Turkey used to test an analytical method with an average of 110 ng/g ($n = 4$) (Uluozlu et al., 2010). Transfer of As from soil to grain was reported to be an order of magnitude higher in rice than wheat or barley due to As being more mobile in aneorobic paddy soil systems for rice than in aerobic soil systems for wheat and barley (Williams et al., 2007a).

There were 11 different brands of rice consumed in households of the participants, however, four brands (A, B, D, and E) and no-name (NN) rice were preferred by the majority (79%). iAs median concentration ranked among the five as 167 (D), 165 (A), 150 (E), 148 (NN), and 136 (B) ng/g, for which the differences were not significant. Two types of medium grain rice, baldo and osmancık, were the choice in 92% of the participant households. iAs content of the two rice types were not significantly different with median values of 155 and 161 ng/g, respectively. There were 13 different brands of bulgur consumed in households of the participants, however, five brands (A, B, D, F, and G) and no-name (NN) bulgur were preferred by the majority (82%). tAs median concentration ranked among the six as 40 (F), 37 (G), 19 (NN), 13 (B), 8.7 (D), and 8.1 (A) ng/g, for which the differences were not significant.

3.3. Individual exposure assessment

Arsenic exposures from ingestion of rice and bulgur were calculated for the 50 participants by using the 7-day average rice and bulgur consumption rates and body weights obtained from the questionnaires, and the measured concentrations. Descriptive statistics for the estimated exposures are presented in Table 2. Provisional tolerable weekly intake (PTWI) of 15 μg/kg body weight for iAs has been withdrawn and replaced with a benchmark dose lower confidence limit for a 0.5% increased incidence of lung cancer in human ($BMDL_{0.5}$) of 3 μg/kg d by the Joint Food and Agriculture Organization of the United Nations/WHO Expert Committee on Food Additives (JECFA) in 2010 (JEFCA, 2010). We present a carcinogenic risk assessment in the next section based on the excess lifetime cancer risk approach using SF which is similar to the margin of exposure approach using BMDL. The $BMDL_{0.5}$ of 3 μg/kg d determined by JEFCA is in the range of the two SF values we used in the assessment: 1.5 and 3.67 $(\mu g/kg\ d)^{-1}$, the in-effect and a proposed value, respectively. Therefore, here we compared the estimated iAs exposures in this study to the tolerable daily intake (TDI) of 2 μg/kg d, which is calculated by dividing the withdrawn PTWI by seven, because there are many assessments based on PTWI in the literature. All estimated exposures for rice were less than 1/5 of the TDI including the maximum value for tAs. The median and mean iAs exposures corresponded to 4% and 5% of the TDI, respectively. These ratios were similar to those for Brazil (Batista et al., 2011);

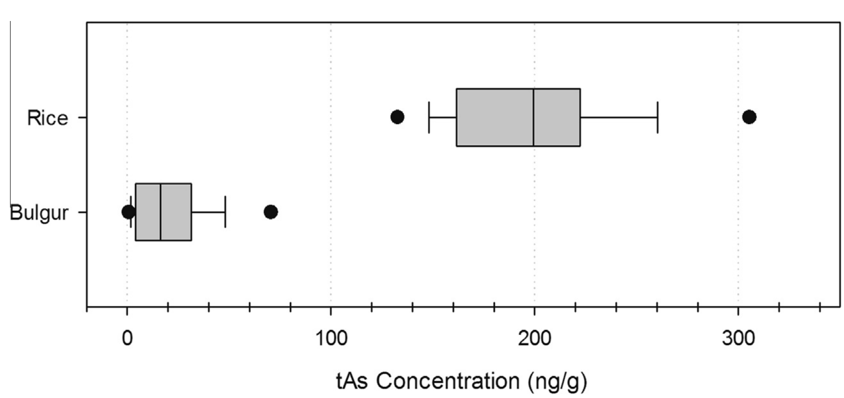

Fig. 2. Total arsenic concentrations in rice and bulgur ($n = 50$) (wet weight, black dots depict 5th and 95th percentiles).

Table 2
Ingestion exposure to arsenic through rice and bulgur ($n = 50$).

Exposure	Rice							Bulgur		
(μg/kg d)	As^{3+}	DMA	MMA	As^{5+}	tAs	iAs	%TDI[b]	tAs	iAs[a]	%TDI[b]
Minimum	0.010	0.002	<0.001	<0.001	0.013	0.010	1	<0.001	<0.001	–
Median	0.073	0.018	0.001	0.004	0.091	0.074	4	0.003	0.002	0.1
Mean	0.096	0.024	0.002	0.005	0.127	0.101	5	0.008	0.007	0.4
Std. Deviation	0.071	0.021	0.002	0.005	0.093	0.075		0.015	0.013	
95th percentile	0.244	0.060	0.005	0.017	0.316	0.259	13	0.049	0.043	2.1
Maximum	0.301	0.096	0.009	0.024	0.393	0.307	15	0.082	0.072	3.6

[a] Estimated by assuming 88% iAs in tAs.
[b] TDI: Tolerable daily intake for iAs.

higher than for WHO European diet; and lower than for WHO Far East diet (Jorhem et al., 2008), China (Qian et al., 2010), and arsenic contaminated areas (Williams et al., 2005). Contribution of rice to the TDI reaches >10% for 10% of the participants. Contribution of bulgur to the TDI was 35-folds and 14-folds lower compared to rice based on median and mean exposures, respectively. Contribution of bulgur did not exceed 4% even at the maximum exposure level.

iAs exposure through rice and bulgur for high school graduates were significantly higher than those for primary school and university graduates. There were also significant differences in the exposure among the preferred brands for both rice and bulgur. Since concentrations did not differ significantly among rice brands, the differences were mainly due to differences in consumption rates per body weight.

3.4. Individual risk assessment

Chronic–toxic and carcinogenic risks associated with ingestion of iAs through rice and bulgur ingestion pathways were calculated for the 50 participants. Carcinogenic risk was estimated using two SF values; the published, in-effect value by the USEPA (IRIS, 2013) and a proposed value that is used in the literature (Adomako et al., 2011; Tsuji et al., 2007), 1.5 $(\mu g/kg\,d)^{-1}$ and 3.67 $(\mu g/kg\,d)^{-1}$, respectively. Estimated risks are shown in Fig. 3, along with the threshold value for chronic–toxic risks and acceptable carcinogenic risk levels. All chronic–toxic risk values were below 15% of the threshold (HQ = 1.0) for bulgur, whereas all were less than or equal to the threshold for rice (maximum HQ = 1.02). Although Qian et al. (2010) used PTWI as the reference dose, similarly to this study, chronic–toxic risks estimated for Chinese people were <1. All of the estimated carcinogenic risks using the in-effect SF value for bulgur were below the acceptable risk level of 1.0×10^{-4} (Kavcar et al., 2009). The proportion of the participants with $R > 1.0 \times 10^{-4}$ rose to 5% when the estimation was based on the proposed SF value. Rice, on the other hand, presented a more serious situation with 53% of the participants having risks higher than the acceptable level even if the in-effect SF value was used. The percentage rose to 93% when the proposed SF value was employed. Three percent of the participants had $R > 1.0 \times 10^{-3}$ in this case. As presented in Section 3.2, arsenic concentrations are higher than some of the concentrations reported in the literature, but they are not placed at the top of the range. The determining factor for exposure-risk is probably the relatively higher rice consumption rates compared to the US and the EU. This speculation was tested by conducting a sensitivity analysis for the population risk assessment, and will be presented in the following section. The estimated risk levels are similar to the American risk levels from diet and water (<10 μg/L), which were reported to be at or near the acceptable level of 1 in 10,000 (Tsuji et al., 2007). Risk levels estimated in this study are lower than those estimated for Bangladesh, China, and India with median risks of 22, 15, and 7 in 10,000, respectively; and higher than Italy and similar to the US with median risks of 0.7 and 1.3 in 10,000, respectively (Meharg et al., 2009). Mondal and Polya (2008) also estimated higher risk levels for West Bengal, India.

3.5. Population exposure–risk assessment

Individual risks (Section 3.4) have shown that neither chronic–toxic nor carcinogenic risk levels associated with bulgur ingestion are significant. Therefore, we conducted population assessments for only rice consumption. A Monte Carlo simulation was run for

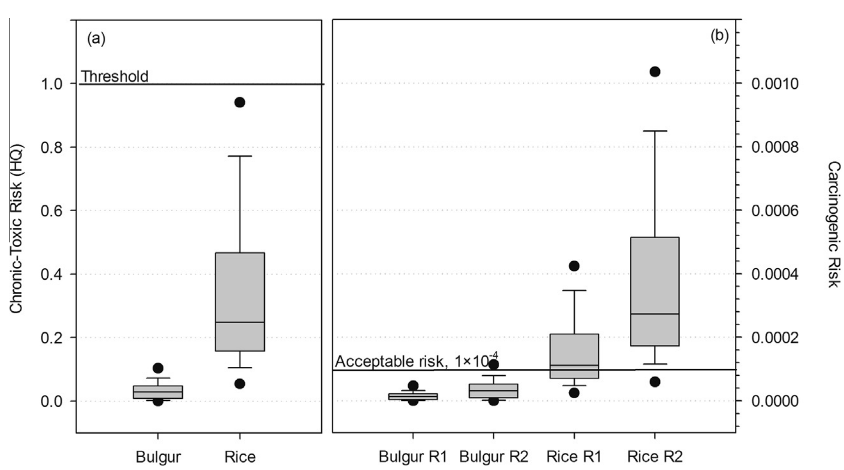

Fig. 3. (a) Chronic–toxic and (b) carcinogenic risks for iAs in rice and bulgur (R1: SF = 1.5 $(\mu g/kg\,d)^{-1}$, R2: SF = 3.67 $(\mu g/kg\,d)^{-1}$, Black dots depict the 5th and 95th percentiles).

the estimation of CDI, HQ, and R. Fitted distributions of the input variables to the CDI (HQ and R) model are presented in the Supporting Information (SI). The simulation was run for 10,000 trials. Frequency histogram of the resulting model outputs and their fitted distributions are presented as Fig. 4. Descriptive statistics are also shown in the figure. The mean and median chronic–toxic risks were less than the threshold value (HQ < 1.0), whereas the mean and median carcinogenic risks estimated using the in-effect and the proposed SF values were higher than the acceptable risk (R1 and R2 > 1.0×10^{-4}). Proportion of the population with chronic–toxic risks higher than the threshold value was 1%, whereas proportion of the population with greater than the acceptable carcinogenic risk were 59% and 92% when the in-effect and the proposed SF values were used, respectively. Proportion of the population with carcinogenic risks >1.0×10^{-3} were 0.0% and 1.5% with the in-effect and the proposed SF values, respectively.

A sensitivity analysis was conducted during the simulation to determine the influence of each input variable on exposure-risk. The input variables were iAs concentration, rice consumption, and body weight. Sensitivity measured by contribution to variance revealed that the most influential input variable was rice intake with 82% contribution. iAs concentration and *BW* contributed with 11% and 7%, respectively. Similarly, previous risk assessments in the literature (Torres-Escribano et al., 2008; Yost et al., 2004) reported rice consumption as the determining factor. An uncertainty analysis was conducted for the estimated population CDI, HQ, and R1. Results of the analysis are presented in Table 3 as 5th to 95th percentile range along with the median for the median, and 90th and 95th percentile values. In addition, Quartile Coefficient of Dispersion (QCD), which is a non-parametric analogous to coefficient of variation for normal distribution, defined as interquartile range divided by the median was calculated to estimate the extent of uncertainty in the median and 95th percentile population risk values. QCD values were calculated as 4.9% and 7.1% for the median and the 95th percentile risks, respectively, indicating low uncertainty due to the Monte Carlo simulation process.

3.6. Limitations and research needs

This study was conducted with rice and bulgur samples, and personal and consumption information obtained from 50 randomly selected participants. This is a rather small sample size therefore Monte Carlo simulation was carried out to infer on the city population. However, a large size, preferably a market basket study that would study arsenic speciation in rice sold in Turkey is needed. The future study should be able to differentiate home produced and imported rice, to reflect geographic variation in home produced rice, and to track the producing farm and the cultivar. Geographic variation, the producing farm, and the cultivar information could be utilized in agricultural management to reduce iAs in the produced rice. The use of poultry litter as fertilizer should also be checked as it may be As contaminated depending on roxarson addition into poultry feed against intestinal parasites (Liu et al., 2009).

Bioavailability of iAs has been shown to be high (63–99%) (Juhasz et al., 2006; Laparra et al., 2005). In this study we assumed a 100% bioavailability for iAs, as it has been assumed by others (Adomako et al., 2011; Batista et al., 2011; Jorhem et al., 2008; Meharg et al., 2009; Meliker et al., 2006; Mondal and Polya, 2008; Qian et al., 2010; Torres-Escribano et al., 2008; Tsuji et al., 2007; Ye et al., 2012). However, Laparra et al. (2005) have shown that only 3.9–17.8% of the bioavailable iAs may be bioaccessible. This is a critical issue for estimating human health effects that needs to be studied (Meliker et al., 2006; Torres-Escribano et al., 2008; Tsuji et al., 2007). Speciation is affected by the method of cooking the rice. It has been shown that pre-rinsing, cooking with abundant water, and discarding the extra at the end reduces As concentration (mainly iAs) in rice, whereas cooking with contaminated water results in an increase (Mihucz et al., 2007; Raab et al., 2009; Sengupta et al., 2006). Nonetheless, iAs dominates in the cooked rice (Mihucz et al., 2007; Raab et al., 2009). Majority of participants in this study preferred to keep the rice in hot water before cooking, effect of which was not investigated in this study but it should reduce the estimated exposure and risks by 8 to 28% based on the results of previous studies in the literature (Mihucz et al., 2007; Raab et al., 2009; Sengupta et al., 2006).

It is known that brown rice contains more As than white rice because a considerable portion is in its bran and outer grain, therefore As (mainly iAs) is removed during processing for white rice (Sun et al., 2008). As a result, rice bran and solubles products are more concentrated with regards to As than rice grain. Thus, their consumption is of importance because they are promoted as

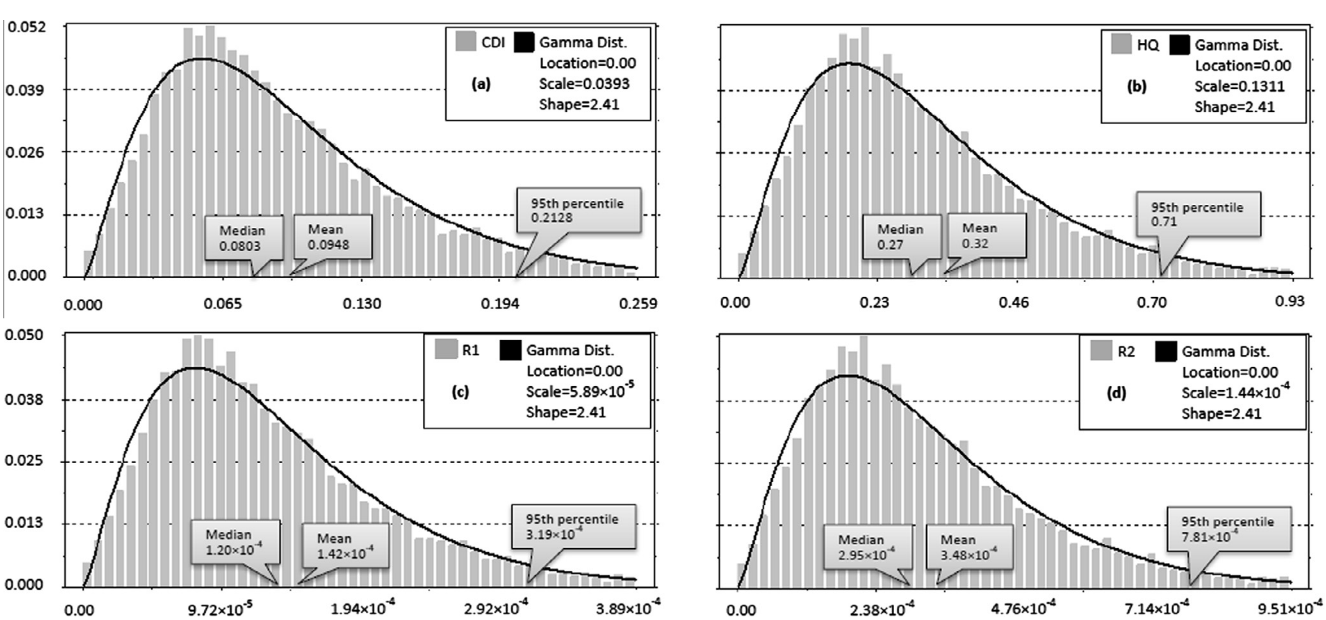

Fig. 4. Estimated population distributions and descriptive statistics (a) chronic daily intake (μg/kg d), (b) chronic–toxic risk (HQ), (c) carcinogenic risk (R1) using SF1, and (d) carcinogenic risk (R2) using SF2 (SF1 = 1.5 (μg/kg d)$^{-1}$, SF2 = 3.67 (μg/kg d)$^{-1}$).

Table 3
Results of the uncertainty analysis for the estimated population exposure and risks.

	CDI			HQ			R1		
	5th	50th	95th	5th	50th	95th	5th	50th	95th
Median	0.077	0.081	0.084	0.259	0.270	0.281	1.16×10^{-4}	1.22×10^{-4}	1.27×10^{-4}
90th percentile	0.166	0.175	0.183	0.556	0.581	0.611	2.48×10^{-4}	2.62×10^{-4}	2.76×10^{-4}
95th percentile	0.201	0.213	0.226	0.670	0.711	0.759	3.02×10^{-4}	3.19×10^{-4}	3.43×10^{-4}

healthy foods (Sun et al., 2008; Zhu et al., 2008). Another rice food that is significant in terms of As content is infant formula products and that of prepared at home by cooking rice flour in cow milk, because infants and children have higher exposure on body mass basis than adults. None of these important sources of As was analyzed in this study. Other foods, including other grains, some vegetables, fruits and fruit juices, dairy products, may be significant sources of iAs intake (Schoof et al., 1999; Yost et al., 2004). Other, less significant sources include exposure to soil and ambient air particles (Meacher et al., 2002), seafood (Schoof et al., 1999), mushrooms, smoking cigarettes, and chromated copper arsenate treated wood (Meliker et al., 2006). An exposure apportionment could be conducted to rank iAs exposure sources (Moschandreas et al., 2002). The most important source other than food, drinking water (depending upon contamination level), had previously been investigated for the subject population (Kavcar et al., 2009), however, concentrations decreased significantly to below the 10 μg/L tAs MCL after a treatment plant have been installed. Small rural communities that still may be using the fairly contaminated aquifers as potable water source should be identified because rice consumption would be more significant for those subpopulations as their risk levels would be considerably higher (Meliker et al., 2006; Mondal and Polya, 2008; Yost et al., 2004). Other population groups at significant risk, those whom consume rice frequently or as subsistence food (Williams et al., 2007b), should be identified.

Rice is found as an important source of inorganic arsenic in this study contributing to the withdrawn tolerable daily intake by 5% and 13% at the median and the 95th percentiles, respectively. Although all estimated chronic–toxic risk values are below the threshold, carcinogenic risks estimated with the in-effect potency factor exceeds the acceptable risk (1.0×10^{-4}) for 59% of the population. The percentage rises to 92% if a proposed potency factor is used. The most influencing variable on exposure-risk is rice consumption rate, and frequent rice consumers are at significant risk. On the contrary, bulgur, a parboiled wheat product known as a healthy food, containes an order of magnitude lower arsenic; contributes with up to 2.1% (95th percentile) to the withdrawn tolerable daily intake; chronic–toxic risks are less than 15% of the threshold; proportion of population with above the acceptable carcinogenic risk is 5% even if the proposed potency factor is used. In conclusion, an iAs MCL for rice would be useful to lower the risks while public awareness about the relation between excessive rice consumption and health risks is built, and bulgur consumption is promoted.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fct.2013.11.029.

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