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Rahman, Mohammad Mahmudur; Alauddin, Mohammad; Alauddin, Sarah T.; Siddique, Abu Bakkar; Islam, Md. Rashidul; Agosta, Gabriella; Mondal, Debapriya; Naidu, Ravi. "Bioaccessibility and speciation of arsenic in children's diets and health risk assessment of an endemic area in Bangladesh". Journal of Hazardous Materials Vol. 403, Issue 5 February, no. 124064, (2021).

Available from: http://dx.doi.org/10.1016/j.jhazmat.2020.124064

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Accessed from: http://hdl.handle.net/1959.13/1425479

Bioaccessibility and speciation of arsenic in children's diets and health risk assessment of an endemic area in Bangladesh Mohammad Mahmudur Rahman^{1,2*}, Mohammad Alauddin³, Sarah T. Alauddin³, Abu Bakkar

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15 Abstract

This study determines the bioaccessibility of toxic and carcinogenic arsenic (As) in composite food 16 samples and evaluates potential exposure from food intake in Bangladesh children. Total As 17 18 (tAs), inorganic As (iAs) and bioaccessible As (BAs) in food composite samples consumed by children were compared between an exposed and a control group (based on As in drinking 19 water). Total As concentrations in composite food samples of children exposed to mean As 20 21 level of 331 µg/l in drinking and cooking water ranged from 586 to 1975 µg/kg, dry weight over 76 to 90 µg/kg in the unexposed group. Average iAs in food composites was 73.9% (range: 22 49.3 to 90.8%). The fraction of BAs using gastric and gastrointestinal phases was 91±13 % and 23 98±11%, respectively. Daily intake of iAs in exposed group ranged from 0.41 to 6.38 µg per 24 kg body weight (BW), which was much higher than the unexposed group (0.08-0.15 µg per kg 25

BW). High iAs content and BAs in composite food samples indicated elevated risk to exposed
children. Further research should include both adult and children using larger sample size to
determine overall As exposure from food intake in Bangladesh, attention must be given to
lowering of As in food.

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Keywords: Arsenic; Children; Food composites; Arsenic speciation; Arsenic bioaccessibility; Health
 risk.

33 **1. Introduction**

Human health risk assessment of trace elements, specifically arsenic (As) in food has 34 received considerable attention in recent years because of food safety concerns (Antoniadis et 35 al., 2017; Antoniadis et al., 2019). Health risk assessment of trace elements including As and 36 its effects on plants and humans is crucial for effective regulatory guidelines. A recent study 37 explored the transfer of the trace elements from soil to humans, emphasising that the human 38 health risk assessment is a global one. This is due to their possible transfer through the food 39 chain to people and is considered to be the main exposure route (Antoniadis et al., 2019). 40 Arsenic is a carcinogen that has been detected in the groundwaters of Bangladesh, used both 41 42 for drinking and cooking. Chakraborti et al. (2010) reported 27.2% and 42.1% of the 52,202 43 water samples analysed in Bangladesh had concentrations above 50 and 10 µg/l, respectively. In addition to drinking water, people from the As-endemic areas in Bangladesh are significantly 44 exposed to As through their daily diet (Rahman et al., 2011; Rahman et al., 2013; Rahman et 45 46 al., 2009). The risk posed by rice based diet has been well reported (Carbonell-Barrachina et al., 2012; Islam et al., 2017c; Signes-Pastor et al., 2016) since rice is consumed in large 47 quantities in Bangladesh. Its people usually consume more than 170 kg per capita per annum 48 compared to the world average of 57 kg per capita per annum (Shew et al., 2019). 49

Average As content in the uncooked and cooked rice samples collected from households 50 of Nawabganj district in Bangladesh was found to be 340 µg/kg and 460 µg/kg, respectively 51 52 (Ohno et al., 2007) indicating a rising As concentration in cooking whereas average As concentration in cooked rice (139 µg/kg) was lower than uncooked rice (153 µg/kg) in paired 53 54 samples collected from households in the Noakhali district of Bangladesh (Rahman et al., 55 2011). Concentration of As may vary between uncooked and cooked rice, depending on the rice variety, As in raw rice, As in cooking water and process of cooking (Bae et al., 2002; Laparra 56 et al., 2005; Mwale et al., 2018). A recent study reported that transfer of As from water to rice 57 grains was influenced by the increasing concentration of As in water and rice type; 58 concentrations of 84-105 µg/L in cooking water significantly increased As concentration (24-59 337%, and 114% from sunned and parboiled rice, respectively) in cooked rice (Chowdhury et 60 al., 2020). (Sengupta et al., 2006) found that the traditional cooking procedure commonly used 61 62 in Bangladesh (whereby rice is washed with water until clear and excess water is discarded after cooking) can remove up to 57% of rice As. In a study conducted in the Monohordi and 63 Munshiganj districts of Bangladesh, the average As content reported in cooked rice and cooked 64 vegetables were 358 µg/kg and 333 µg/kg, respectively (Smith et al., 2006). Hence, having 65 66 cooked rice as a mainstay of the diet can be an important route of As exposure.

Infant and young children are most susceptible to As toxicity, although arsenical 67 symptoms in children are rare except when they are exposed to very high concentrations of As 68 69 or suffer from malnutrition (Rahman et al., 2001). Chronic exposure to As pose high health risks including neurobehavioural problems and decreased intellectual function in children (von 70 71 Ehrenstein et al., 2007; Wasserman et al., 2004). In a study from Mexico the total As (tAs) and inorganic As (iAs) concentrations in children's diets ranged from 50 to 1150 µg/kg, and 23 to 72 88 µg/kg, dry weight (DW), respectively and daily intake of tAs and iAs ranged from 0.15 to 73 74 10.49 µg per kg BW and from 0.06 to 1.11 µg per kg BW, respectively (García-Rico et al., 2012). In one study from Bangladesh, 2–5 yrs and 6–10 yrs age groups were more exposed to
As due to rice consumption (Islam et al., 2017b). These results are alarming considering the
higher risk of children being exposed to As through the food they eat.

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In characterisation of As exposure and risk from food intake both in adults and children, 78 one aspect that has received increased attention is the bioaccessibility of As (BAs) in consumed 79 food (Laparra et al., 2005). Various studies have estimated the BAs in uncooked food (Signes-80 Pastor et al., 2012; Trenary et al., 2012) including shrimp, radish, mushroom, etc. (Chi et al., 81 2018; Hu et al., 2019; Koch et al., 2013) while other studies concentrated on raw and cooked 82 rice (Laparra et al., 2005; Zhuang et al., 2016). A few studies investigated the BAs through 83 84 simulated gastric phase (GP) and gastrointestinal phase (GIP) digestions (Llorente-Mirandes et al., 2016; Zhuang et al., 2016). The BAs of GP and GIP in raw rice and cooked rice varied from 85 36-102% and 72-96% respectively (He et al., 2012; Signes-Pastor et al., 2012; Zhuang et al., 86 2016). While health risk assessments based on the evaluation of BAs using different in-vitro 87 assay for individual food items, whether raw or cooked have been reported (Laparra et al., 88 2005; Llorente-Mirandes et al., 2016; Zhuang et al., 2016), As speciation and bioaccessibility 89 in food composites using both GP and GIP are limited. Furthermore, estimates of children's 90 91 exposure to As in based on bioavailability of As in a composite diet are rare. To the best of our 92 knowledge, no study has determined the concentrations of tAs, iAs and BAs in cooked food composites consumed by children in endemic areas which can provide an accurate estimate of 93 As intake, exposure, and risks in children. 94

In this communication, for the first time we report As exposure in children from diet (lunch and dinner) comprising cooked rice, vegetables and pulses (which are the most commonly consumed foods by Bangladeshi people) from two As-contaminated villages in Bangladesh. The aim of this study is to determine tAs, iAs and BAs in the children's diet in Asendemic areas of Bangladesh to estimate the health risks for children.

100 2. Materials and methods

101 *2.1. Sample collection and preparation*

All the reagents used in this study were of analytical grade. The details of chemicals and 102 reagents are given in the Supplementray Information (SI). For this study, a total of 14 diet 103 104 samples from lunch and dinner menus were collected in 2018 from 14 households in two Ascontaminated villages (Shahpur and Sursoi), which are located in Chandpur district of 105 106 Bangladesh. It is worth noting that Chandpur was reported to be a severely As-contaminated area with 95.7% and 92.6% groundwater samples (n=1165) having As above 10 µg/L, the WHO 107 provisional guideline value and 50 µg/L, the Bangladesh standard value of As in drinking water, 108 109 respectively (Chakraborti et al., 2010). Usually in these areas, lunch and dinner comprise of cooked rice, fish curry with different vegetables and lentil soup (locally known as dal). 110 Households with at least two children were selected at random. Details of ethical approval are 111 presented in SI. Altogether 31 children were selected from these two contaminated villages (9 112 boys and 8 girls from Shahpur and 7 boys and 7 girls from Sursoi) for this study (denoted as 113 exposed group) and their food portion sizes were weighted for lunch and dinner to determine 114 their daily dietary intake rates. For these 31 exposed children (16 boys and 15 girls) age, body 115 116 weight as well as daily amount of food consumption (rice, curry and dal) was determined. The 117 average age and weight of these children were 8.3 yrs (range 2 - 15 yrs) and 26 kg (range 10 -56 kg), respectively. The average daily food consumption (fresh wt.) was 304 g (range: 85 -118 563 g). For the sake of comparison, diet samples of 4 children were also collected from 119 120 Bhelanagar (denoted as unexposed group) which is situated in the Narsingdi Municipality where As-safe drinking water supply was available through a pipeline. 121

Food samples were collected from a plate (duplicate portion) when it was being served to children. The separately cooked rice, curry and dal were mixed and homogenised properly to create a composite sample. The mixed food samples were stored in zip-lock bags and stored in an ice box with ice and transported to the laboratory and then kept refrigerated until processing. These samples were dried in an oven at 65° C for 72h. The dried samples were again homogenised by grinding them. The samples were stored in zip-log bags. We also collected cooking and drinking water samples from both exposed and unexposed groups. Water samples from both these groups were collected in plastic bottle (pre-washed with 1:1 nitric acid) and preserved with 0.1% (v/v) nitric acid. The samples were subsequently transported to the University of Newcastle by courier under strict biosecurity protocol.

For tAs determination, the samples were digested using microwave acid digestion
system that was employed by Islam et al. (2017c). The digests were diluted to 10 mL using
0.1% HNO₃ and passed through a 0.45 μm syringe filter for the determination of tAs in the diet
samples and water samples using inductively coupled plasma mass spectroscopy (ICP-MS,
PerkinElmer, NexION 350, USA).

Arsenic speciation analysis for inorganic As – sum of arsenite (AsIII), and arsenate (AsV), monomethylarsonic acid (MMA) and dimethyl arsinic acid (DMA) was carried out following the method of Signes-Pastor et al. (2016). Details of the procedure have been discussed in our previous publication (Islam et al., 2017c). High performance liquid chromatography (HPLC, Agilent 1200) coupled with ICP-MS (Agilent 7900) was used for As speciation analysis.

143 2.2. In-vitro BAs assay

Physiologically-based extraction test (PBET) is one of the most practical and feasible *in-vitro* methods to determine metal bioaccessibility. The method was adopted from the
previously described studies (Kafaoglu et al., 2016; Llorente-Mirandes et al., 2016; Zhuang et
al., 2016). Details procedure are given in SI. The prepared samples were anayzed using ICPMS for bioaccessible As.

149 The BAs (%) was calculated according to the following equation.

150
$$BAs (\%) = \frac{Bioaccessible \ As \ concentration \ in \ food \ composite}{Total \ As \ concentration \ in \ food \ composite} \times 100$$

151 *2.3. Quality control*

Standard reference material (SRM 1568b rice flour) obtained from the National Institute 152 of Standard and Technology (NIST), USA was used to validate the analysis. Concentration of 153 total As in SRM rice flour (1568b) was 266±11 (n=6) µg/kg, indicating 93% recovery (certified 154 value of 285±14 µg/kg). Blanks, duplicates and calibration check verification (CCV) samples 155 were included. The mean variation between duplicate samples (n=12) was 2.8% (0.5-7.1%) and 156 the recoveries for CCVs (n=5) amounted to 103% (99 -105%). In addition, we determined the 157 158 accuracy of the As speciation method using SRM rice flour. The certified values for DMA, MMA and iAs in SRM rice flour were $180 \pm 12 \ \mu g/kg$, $11.6 \pm 3.5 \ \mu g/kg$ and $92 \pm 10 \ \mu g/kg$, 159 respectively. The analytical results (n=5) for As speciation indicated that the values for DMA, 160 MMA and iAs were 162 ±14 µg/kg, 8.2 ± 3.1 µg/kg and 83 ± 8 µg/kg, respectively. Thus, 161 recoveries for DMA, MMA and iAs were 90%, 70.7% and 90.2%, respectively. 162

163 *2.4. Statistical analysis*

Data were analysed and represented graphically using statistical software JMP version 14, IBM SPSS version 25, Microsoft Excel 2013, and Graph Pad Prism 8. Confidence level from 95% was considered for all statistical analyses.

167 2.5. Risk assessment

To evaluate the potential exposure of children to As, we evaluated the established daily dietary intake (EDDI) of As, hazard quotient (HQ) and cancer risk (CR) from cooked food using the following equations:

171
$$EDDI = \frac{FC \times iAs \times \%BAs \times ED \times EF}{BW \times AT}$$

172
$$HQ = \frac{EDDI}{RfD} and$$

173
$$CR = EDDI \times CSF$$

Where, FC is cooked food consumption (g/day, fresh weight, FW); iAs is the concentration of 174 175 the inorganic As in food component (µg/kg using FW); BAs is bioaccessibility of As through GIP tract (%); BW stands for body weight, (kg) of respective children; ED represents exposure 176 duration (years) of the children taking into consideration their respective ages; EF is exposure 177 frequency (365 days per year); AT represents average lifetime (365 days per year × number of 178 exposure years); CSF is cancer slope factor (1.5 mg/kg per day); and RfD is oral reference dose 179 $(3 \times 10^{-4} \text{ mg/kg per day for As})$, as suggested by USEPA (IRIS 2013). In the case of HQ <1, 180 non-carcinogenic risks are not considered but for HQ >1, there may be adverse health effects 181 arising from exposure (Abtahi et al., 2017; Shibata et al., 2016; Zhuang et al., 2016). In terms 182 of carcinogenic risk assessment, if CR <10⁻⁶, the increased cancer risk is deemed to be 183 negligible, and $>10^{-6}$ a departure from negligible risk, CR $> 10^{-4}$ is considered to be an 184 unacceptable increased cancer risk (Fakhri et al., 2018; Shibata et al., 2016). 185

186 **3. Results and discussion**

187 *3.1. Total As in diets*

Mean As in cooking and drinking water (n=5) for the exposed group was $331 \mu g/l$ 188 (range: 88 - 720 µg/l) whereas As concentration in cooking and drinking water (supply tap 189 190 water, n=2) of the control group was $<1 \mu g/l$. The concentration of tAs, iAs and BAs (%) in 191 the composite food samples are summarized in Table 1. The mean and range of As in food (dry wt.) were 1072 μ g/kg and 586 – 1975 μ g/kg for the exposed group, respectively. Considering 192 that on average the moisture content was 80% in cooked composite food in this study, the mean 193 and range of As in food (fresh wt.) were 214 μ g/kg and 117 – 395 μ g/kg, respectively. The tAs 194 195 concentrations (mean and range) in composite food samples (dry wt.) for the control group were 85 µg/kg and 76-90 µg/kg, respectively (Table 1), which were equivalent to 17.1 µg/kg and 196 15.3 - 18.1 µg/kg, fresh wt., respectively. The results (Fig. 1) revealed that there was a 197 significant difference between the mean As concentrations in food samples between the 198

exposed and control groups (p<0.001), while the mean As concentration in food samples collected from exposed group was 12.5 times higher than in the unexposed group. The highest concentrations of tAs were 1975 μ g/kg (DW) and 395 μ g/kg (FW) from sample RC1. The tAs concentration in the diets of children in Sonora, Mexico ranged from 50 to 1150 μ g/kg, dry wt (García-Rico et al., 2012), which was much lower than reported in this study.

It is important ot note here that although children from the control group live in 204 municipal areas and use tap water (As<1 µg/L) for drinking and cooking, we do not know 205 whether they use food items that are low in As. Generally in the municipal and city areas of 206 207 Bangladesh, food crops including rice, vegetables and pulses are sourced from As-contaminated villages which are available in local markets. In this study, we were not sure about the sources 208 of food crops for both the exposed and control groups, whether they originated from 209 contaminated or uncontaminated areas or mixed agro-ecological zones. However, since As 210 concentrations in composite food samples for the exposed group were much higher than those 211 212 in the control group, we expect that As concentration in cooking water contributed to the increase in As in the cooked food composites. It is also expected that the cooking procedure 213 would have affected the concentration of As in food samples (Bae et al., 2002; Laparra et al., 214 215 2005).

216 *3.2. Inorganic As content and speciation*

In this study, inorganic As was the major species present in the food samples (Table 2) with an average of 74% (range 49-91%). This is similar to the study conducted by Laparra et al. (2005) who reported 77 % (range: 32-103%) of iAs in cooked rice. Smith et al. (2006) reported iAs content of 87% and 96% in cooked rice and vegetables, respectively, in samples from Bangladesh. Ohno et al. (2007) found up to 100% of iAs in cooked rice from Bangladesh. Based on their duplicate diet survey conducted in Pabna, Bangladesh, Kile et al. (2007) reported that on average 82% of As present in food samples was iAs (n=35). We could not detect any
MMA (V) in the food samples but DMA (V) was present in all samples except RC 8.

The iAs concentration in the diets of children in Sonora, Mexico was 23 to 88 µg/kg, 225 dry wt (García-Rico et al., 2012), which is much lower than the present study. The higher iAs 226 (289 to 1624 µg/kg, dry wt) detected in this study could be attributed to both cooking process 227 and As-contaminated water used for cooking (Bae et al., 2002; Laparra et al., 2005; Zhuang et 228 al., 2016). Laparra et al. (2005) reported a 5-17 fold increase in iAs content in the rice, after 229 cooking with simulated As-contaminated water. This to a great extent reflects the reality of the 230 situation concerning As-endemic areas throughout Asia. A recent study reported that cooking 231 water (84-105 µg/l) significantly increased As concentration in sunned (24-337%) and 232 parboiled rice (114%) (Chowdhury et al., 2020). 233

Maximum tolerance level of iAs in the rice (uncooked) for infants and young children is 100 µg/kg as recommended by the European Union (EU) (Ashmore et al., 2019). Out of 14 samples in the exposed group, all exceeded the EU safe level for infants and young children. Simulating the cooking practices followed in Asian As-endemic areas, Laparra et al. (2005) reported that both tAs and iAs increased when cooking with As-contaminated water and BAs depends on toxic iAs content. However, iAs in cooked rice could be more harmful due to the high bioaccessibility of As (>90%) compared to raw rice (Laparra et al., 2005).

241 *3.3. BAs in cooked food composite*

The bioaccessible fractions of As (mean \pm SD) determined in both *in-vitro* GP and GIP digestion were 91 \pm 13 % (range 68 -106%) and 98 \pm 11% (range 72 -117%), respectively (Table 1). No significant difference were observed between the two phases (GP and GIP) although slightly more BAs is found in the GIP, which could be due to the effect of time. There was no noticeable difference of BAs between the samples collected from the control and exposed group. In Sonora, Mexico, BAs ranged from 4 to 97% (mean 44%) (García-Rico et al.,

2012), hence, the average value of BAs was much higher in this study compared to Sonora. In 248 our previous study, we determined in-vivo BAs in various rice genotypes ranging from 25-94% 249 and we reported that the BAs varied based on rice varieties (Islam et al., 2017a). Cooking 250 process affects the BAs both in GP and GIP extraction as Zhuang et al. (2016) reported that 251 BAs in raw rice using GP and GIP extraction were 62-93% and 75-96%, respectively, whereas 252 38-67% and 72-80% were evident in cooked rice. Several studies have investigated the BAs, 253 254 which ranged from 20% to 99% considering rice, seaweed, mushroom, radish and shrimp using different in-vitro methods (Table 2). Based on the limited data of BAs regarding cooked and 255 composite food samples further analysis is recommended and particularly for children's diets 256 257 from other As-contaminated areas.

The concentrations of tAs, iAs and BAs in both GP and GIP in food composite samples of the exposed group were significantly higher (p < 0.001) than the control group (Figs. 1A and B). There was, however, no noticeable difference in BAs fraction (shown as percentage) between the control and exposed groups. No correlation was observed between tAs, iAs and BAs with cooking water. This could be attributed to the food composites, including types of rice, vegetables, fish and pulses used in this study.

Bioaccessibility is generally influenced by the level of contamination in food samples (Zhuang et al., 2016). The linear regression illustrated in Fig. 2 displays statistically significant relationships ($R^2 = 0.964 - 0.983$, p < 0.001) between tAs with iAs and BAs (both GP and GIP) in composite food samples, which confirmed that BAs does rely on As concentration (Zhuang et al., 2016). A similar strong relationship ($R^2=0.928$, p<0.01) has been found between contamination level and bioaccessibility of As in raw rice, and this dose's proportional relationship was considered for the purposes of risk assessment (Zhuang et al., 2016).

271 *3.4. Potential health risk assessment*

Different risk assessment indices such as EDDI, HQ, CR have been calculated using the 272 273 generated data of different cooked food composites for 31 children, which is presented in Table 3. Daily intake of tAs and iAs ranged from 0.84-7.75 (mean: 2.7 ± 1.8 and median: 2.1) µg per 274 kg/ BW and 0.41-6.38 (mean: 2.0 ± 1.5 and median: 1.7) µg per kg/ BW, respectively. In this 275 study, the exposed group had unusually higher values of As intake than the control group. Daily 276 intake of tAs was 2.7 (0.15-10.49) µg per kg/BW in García-Rico et al. (2012) study, similar to 277 our findings, yet daily intake of iAs 0.52 (0.06-1.11) µg per kg/BW was much lower than this 278 study. A few studies reported higher iAs intake than our findings (Díaz et al., 2004; Martí-Cid 279 et al., 2007). The recommended upper limit for iAs exposure by the Joint FAO/WHO Expert 280 281 Committee on Food Additives (JECFA) using the benchmark dose lower confidence limit for a 0.5% (BMDL0.5) increased incidence of lung cancer is 3 µg/kg BW per day (Cubadda et al., 282 2017). The mean iAs exposure in this study was below the upper recommended limit of $3 \mu g/kg$ 283 284 BW per day although the maximum exposure is more than double the limit. Overall, 32% of the children in our study exceeded the above tolerance level, which is also consistent with the 285 32% reported by (Kile et al., 2007) based on a duplicate dietary survey at Pabna district in 286 Bangladesh. The USEPA has stated that there is no "safe" level of exposure to iAs because it is 287 very toxic. Inorganic As is directly related to BAs, so there is a need to elucidate the risk 288 289 assessment. A recent study showed that regulation limits in most countries do not take into account of the environmental interfaces such as mobility of trace elements in plants. It 290 concluded that there were reduced limits of trace elements and consequently health risk 291 292 associated with As were underestimated (Antoniadis et al., 2019).

293 Considering BAs as the input parameter for As, EDDI value was 0.35-6.2 μ g/kg BW 294 per day which was substantially higher than that of the control group in this study (Table 3) and 295 higher than the US-based study of Shibata et al. (2016) who reported 0.82 -1.1 μ g/kg BW per 296 day and the value of 0.53-0.74 μ g/kg BW per day as reported by Zhuang et al. (2016). In this

study, the HQ ranged from 1.2-20.5 (mean: 6.8 and median: 5.6) and the highest value was for 297 298 Sh-B1 in the RC 1 group (Table 3, Fig. 3). All participants in the study area exceeded the tolerance level of HQ (>1) that could induce adverse health effects, and HQ was less than 1 in 299 the control group. Zhuang et al. (2016) found HQ value of 2.3-5.8 for cooked rice while (Shibata 300 et al., 2016) stated values of 0.02-0.37 and 0.19-5.17 for acute and chronic doses of As from 301 rice cereal and other dietary sources for infants and toddlers in the USA, respectively, which 302 were all much lower than this study. Based on the CR assessment, all children in this study had 303 a risk level greater than 10^{-4} and Sh-G1 in the group showed the highest risk of 9.2×10^{-3} . The 304 CR value in this study was notably higher than what Fakhri et al. (2018) found (0.2-5.5) $\times 10^{-5}$ 305 for shrimp but much lower than the value of $(4.5-5.5) \times 10^{-2}$ reported for rice in Iran. 306

To the best of our knowledge this is the first study on bioaccessibility of As in children's 307 composite diet over single food item comparing exposed and non-exposed participants in 308 309 Bangladesh. It is important to note that all soluble fractions of metals are not bioavailable/absorbable in the human body (Laparra et al., 2005). Furthermore the in-vitro 310 metal bioavailability technique has many problems and limitations, for example, human 311 physiology of food digestion is quite complex, involves many biochemical reactions and varies 312 313 from person to person. Also, the amount of soluble/digested metals is not fully accessible or 314 absorbable to animal organs (Van Campen and Glahn, 1999). Therefore, further research is recommended and both in-vitro and in-vivo bioaccessibility models using a wide range of 315 samples must be considered. 316

317 4. Conclusion

This study evaluated the tAs, iAs and BAs in food composites consumed by children in Bangladesh, comparing samples from As exposed and unexposed (based on As in drinking water) groups. Results indicated that exposure to iAs from food composite is one of the major risks to health due to very high bioaccessibility and consequently should be considered a high

priority public health issue in Bangladesh where major mitigation measures are focused on 322 drinking water. This study revealed that the mean As concentration in food composite samples 323 from the exposed group was much higher than those of the unexposed group. It also appears 324 that BAs was higher in GIP digestion than GP digestion in food composites. Based on the BAs 325 326 results, the mean EDDI of As from food composite was just below the JECFA's recommended upper limit of 3 µg/kg BW per day. The higher values of HQ and CR observed for the exposed 327 group indicated high risk to children in As-endemic areas. As a part of routine As monitoring 328 in Bangladesh, further research is required with larger sample sizes along with other food 329 components to estimate the actual risk of As from food intake. Furthermore, considerable 330 331 attention must be given to lowering of As in food to curtail exposure and health risk in Asendemic populations, especially children. Certain practices such as use of As-safe cooking 332 water for food preparation and appropriate cooking methods to reduce As content should be 333 advocated in As-endemic areas to ensure consumption of food, especially rice in a protective 334 way. This study highlights the importance of BAs estimation in food and provides a framework 335 for better exposure and risk assessment. 336

337 Acknowledgements

We are grateful to the authority of the GCER, The University of Newcastle for laboratory support. Financial support from CRC CARE is highly appreciated. This study was also partially supported by a Fox Fellowship grant from Wagner College, New York, USA. Authors are grateful to Mr Sanjit Kumer Shaha, Diabetic Association of Bangladesh for his help in sampling the food samples.

343 Declaration of interests

344 Authors declare there are no conflicts of interest.

345 Authors contributions

MMR, concept, design of the study, analysed the data and wrote the first draft of the manuscript, final approval of the manuscript; ABS and MRI, data analysis and interpretation and drafted the manuscript; MA, STA and GA, participated in data collection, analysis and edited the manuscript; DM and RN revised and edited the manuscript critically, provided technical oversight to the manuscript. All authors read and commented on drafts of the manuscript.

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