Wheat is an emerging exposure route for arsenic in Bihar, India

Sidharth Suman[1,2,3], Pushpa Kumari Sharma[2,3,4], Abu Bakkar Siddique[5], Md. Aminur Rahman[5], Ranjit Kumar[2], Mohammad Mahmudur Rahman[5], Nupur Bose[6], Shatrunjay Kumar Singh[3], Ashok Ghosh[2], Helen Matthews[1] and Debapriya Mondal[1]*

[1] School of Science, Engineering & Environment, University of Salford, Salford, M5 4WT, UK

[2] Mahavir Cancer Institute and Research Center, Patna, India

[3] Department of Environment and Water Management, A.N. College, Patna, India

[4] Aryabhatta Centre for Nanoscience and Nanotechnology, Aryabhatta Knowledge University, Patna, India

[5] Global Centre for Environmental Remediation (GCER), Faculty of Science, The University of Newcastle, Callaghan, NSW 2308, Australia

[6] Department of Geography, A.N. College, Patna, India

Corresponding author * d.mondal@salford.ac.uk

Abstract:

In arsenic (As) endemic areas of south-east Asia, where a subsistence rice-based diet is prevalent, As exposure from food is mainly focused on rice intake. However, consumption of wheat is substantial and increasing. We present a probabilistic assessment of increased cancer risk from wheat-based food intake in a study population of rural Bihar, India where As exposure is endemic. Total As in wheat grains (43.64±48.19 µg/kg, n=72) collected from 77 households across 19 villages was found to be lower than reported As in wheat grains from other south-east Asian countries but higher than a previous study from Bihar. This is the first study where

As concentration in wheat flour was used for risk estimation, bearing in mind that it was the flour obtained after indigenous household processing of the grains that was used for making the home-made bread (*chapati*) which contributed 95% of wheat intake for the studied population. Interestingly, while 78% of the surveyed participants (n=154) consumed rice every day, *chapati* was consumed every day by 99.5% of the participants. In contrast to previous studies, where As concentration in wheat grain was found to be lower than the flour due to the removal of the bran on grinding, we did not find any appreciable lowering of arsenic in the wheat flour (49.80±74.08 µg/kg, n=58), most likely due to external contamination during processing and grinding. Estimated gender adjusted excess lifetime cancer risk of 1.23×10^{-4} for the studied rural population of Bihar indicated risk higher than the 10^{-4}-10^{-6} range, typically used by the USEPA as a threshold to guide regulatory values. Hence, our findings suggest As exposure from wheat-based food intake to be of concern not only in As endemic areas of rural Bihar but also in non-endemic areas with similar wheat-based diet due to public distribution of the wheat across India.

Key words: arsenic; wheat flour; probabilistic risk; wheat intake; Bihar

1. Introduction:

Arsenic (As) contamination in groundwater of Bihar, India was first reported in 2003 (Chakraborti et al., 2003), with more than 9 million people currently facing health risks due to As exposure (Chakraborti et al. 2017). In a 2007 study, 10.8% (7,164 out of 66,623) of drinking water samples, covering 11 districts of Bihar, were reported to have As concentration higher than the Indian permissible limit of 50 µg/l (Nickson et al., 2007). But in 2017, Chakraborti et al., reported that 17.8% of the drinking water samples (out of 19,961) had As above 50 µg/l.

Moreover, in a recent study 22 out of 38 districts of Bihar were reported to have As in drinking water above the WHO permissible limit of 10 µg/l (Chakraborti et al., 2018).

Like all other As affected areas in south-east Asia, exposure is no longer restricted to drinking water and food is becoming a significant route. While exposure from rice is well explored (Mondal and Polya 2008; Mondal et al., 2010; Mwale et al., 2018) and health effects (Banerjee et al., 2013) along with perception of risk (Mondal et al, 2019) studied, little is known about exposure from wheat-based food intakes especially in India and only few studies have reported As concentrations in Indian wheat samples. This is mostly because, unlike rice which is cultivated in submerged soil conditions resulting in high As uptake, wheat is not reported to have As as high as in rice (Bhattacharya et al., 2010). Kumar et al. (2016) reported mean As concentration of 27 ± 24 µg/kg (n=35, range=7.7-108 µg/kg) in wheat grain samples collected from households in the Samastipur district of Bihar, India, while Bhattacharya et al. (2010) previously reported much higher mean As concentration of 129 µg/kg (n=8, range= 90-200 µg/kg) in wheat samples collected from fields of West Bengal, India. Arsenic was also reported in wheat samples collected from fields of Ropar wetland, Punjab (mean= 110 ± 20 µg/kg; range= 30-210 µg/kg; n=9), an area not known to have geogenic As exposure, and authors largely attributed the As contamination to addition of industrial wastes, excessive use of pesticides and fertilizers in agricultural fields, and settlement of fly ash on soil (Sharma et al., 2016).

With greater annual consumption than rice, wheat is the important staple food worldwide (Rasheed et al., 2017). It is also the most important food-grain of India, next to rice but the exposure of As from wheat is sparsely explored. Along with variation in As concentrations in samples collected from different regions of India there exists variation in the pattern of dietary consumption among different sub-populations of the different As exposed areas of the country. These variations were rarely considered for As health risk assessment.

In this study, whole wheat grain and wheat flour samples were collected from households of As affected areas in Bihar and the total As was determined in both samples. We focused on wheat-based food intake and determined the overall increased cancer risk due to As in wheat using a probabilistic method. To best of our knowledge, this is the first study where detailed dietary assessment was done to estimate the As exposure from wheat-based food intake and furthermore As in wheat flour was considered over wheat grain for risk assessment.

2. Materials and methods

2.1 Survey area

As a part of an on-going study "Nature and nurture in arsenic induced toxicity of Bihar, India", wheat samples were collected from 77 households across 19 villages in 10 out of 22 As affected districts of Bihar (Figure 1) in 2017-18. Study locations were selected based on the previous data on arsenic affected areas in Bihar, India. In each village, the survey team met with either the village head or local elderly available resident to gather information on the village demography and then randomly selected households stratified by low and high economic status based on initial observation including type of house. From each household, an adult male and female took part in a questionnaire survey (n=154) administered by the research team which included a detailed food frequency questionnaire (FFQ) combined with 24-hour recalls (24HRs) (Freedman et al., 2018) to determine the total wheat consumption (g/day). The percentage contribution of each wheat-based food consumed towards total wheat consumption was determined using 24HRs and the information on composite mean nutrient in each food as per the latest Indian food composition database (Longvah et al., 2017). This study which is a part of the an on-going research project that includes further sample and data collection, for example, self-recorded health status, lifestyle, demographic and socio-economic conditions along with anthropometric measurements was conducted in accordance with national and international guidelines for the protection of human subjects and was approved by both the University of Salford Ethics Committee and Mahavir Cancer Sansthan Institutional Ethics Committee.

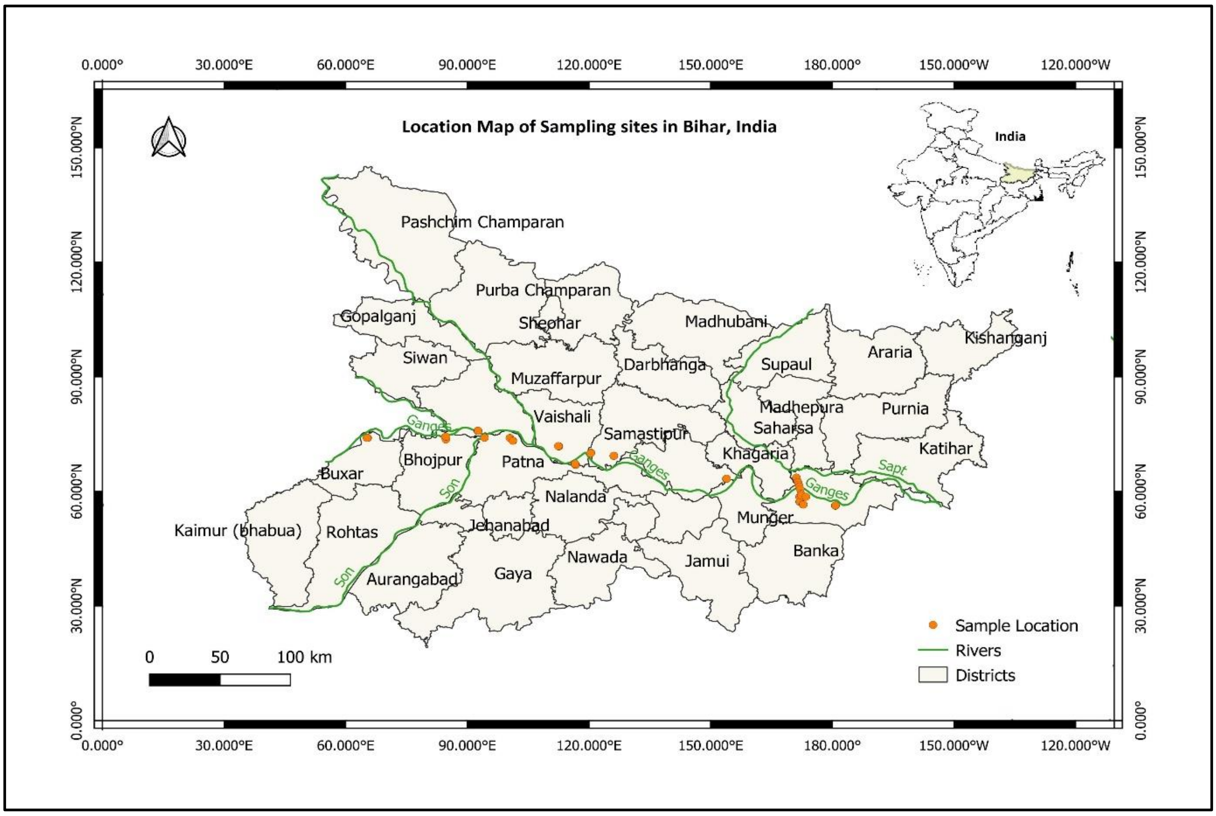

Figure 1: Sampling location of households surveyed in Bihar, India

2.2 Sample collection and analysis

A total of 72 wheat grain and 58 wheat flour samples (53 paired samples) were collected from the 77 households. Wheat grain and wheat flour samples were collected in plastic zip-lock bags and transported to the laboratory in Patna, stored at room temperature until the grains were washed, dried, and ground to powder. Powdered grain and flour samples were shipped to the University of Newcastle, Australia for analysis.

Both powdered wheat grain and flour samples were digested for the analysis of total As and other elements (cadmium (Cd), calcium (Ca), copper (Cu), lead (Pb), magnesium (Mg), manganese (Mn), selenium (Se), and zinc (Zn)), based on the protocol of Roychowdhury et al. (2002). The determination of As and other trace elements was carried out with an Agilent 7900 (Tokyo, Japan) inductively coupled plasma mass spectrometer (ICP-MS). Major elements such as Ca, Mg, Zn were analysed using the dual view (Axial and radial) inductively coupled plasma emission spectrometer (ICP-OES, PerkinElmer Avio 200). CRM, blanks, duplicates, and continuing calibration verification (CCV) were included in each batch throughout the elemental analysis.

2.3 Risk assessment

Overall increased cancer risk was calculated using the following equation (USEPA, 1989)

$$TR = CSF \times \frac{(IR \times C \times Cing \times BCF) \times ED}{BW \times LT}$$

Where TR is the excess lifetime cancer risk; CSF is the oral cancer slope factor for inorganic As (per mg/kg/day); IR is the ingestion rate of the wheat based food intake (g/day) estimated by combining information from 24HRs and FFQ for every participant (n=154); C is the total As concentration in the wheat (µg/kg); Cingi is the proportion of inorganic As in the wheat; BCF is the bioconcentration factor; ED is the exposure duration (years); BW is body weight of the exposed person (kg); LT is the life expectancy of the exposed person.

We used both point estimates and a probabilistic approach for increased lifetime cancer risk estimation. For this later risk assessment approach, a Monte Carlo simulation of 10,000 iterations was carried out. Of the input variables in the above-mentioned equation, C, IR and BW were determined in this study, the remaining parameters were estimated from analogous studies.

Data was analysed using Microsoft Excel and Stata 11.2 for descriptive statistics and the probability distributions for input variables were determined with the software @Risk (Trial

Version 7, Palisade Corp., USA) in combination with Microsoft Excel for probabilistic risk estimation.

3. Results and discussion

3.1 Arsenic concentration in wheat grain and flour samples

The percentage recovery for rice flour (SRM 1568a) and mean percentage difference between the duplicates (n=12) are shown in Table 1. Mean total As concentration in wheat grain (43.64 ± 48.19 µg/kg; range 0.96-234.3 µg/kg; Table 2), was lower than concentrations reported in previous studies especially from Bangladesh, China and Pakistan (Table 3, Figure 2) but higher than the reported concentrations in wheat from a previous study in Bihar (27± 24 µg/kg; range 7.7-108 µg/kg) by Kumar et al., (2016). This could be due to the type of survey, for example, household or market-based compared to field study; the wheat variety; and most importantly the study area being highly As contaminated or not. In compiling the studies (Table 3 and Figure 2) attention was paid to the quality assurance of As analysis in individual studies as summarised in the supplementary material.

Wheat could be a decent source of several vitamins and minerals, including Se and Mn. Both in wheat grains and flour, Pb, Se, Fe and Mg were found to be positively correlated with As, while Mn was found to be negatively correlated (Table 2). Further studies on nutritional value of wheat cultivated in As affected areas could help determine the effect of As on essential nutrients in wheat.

Table 1. Concentrations of As and other elements in the CRM and percentage variation in replicates analysed

	NIST SRM 1568b (Rice flour) (n=5)			Variation in duplicates (n=12)
Element	Certified value (µg/kg)	Observed value (µg/kg)	Recovery (%)	Mean ± SD (%)

As	285 ± 14	254 ± 15	89.2	14.36 ± 13.22
Cd	22.4 ± 1.3	17.9 ± 2	80.1	9.42 ± 9.25
Pb	8* ± 3	6.3 ± 0.8	78.7	29.04 ± 22.21
Se	365 ± 29	300 ± 27	82.4	38.69 ± 32.41
Mn	19200 ± 1800	18966 ± 665	98.8	4.73 ± 3.33
Cu	2350 ± 160	2890 ± 284	92.9	9.08 ± 9.37
Zn	19400 ± 260	14210± 293	73.2	4.15 ± 3.08
Ca	118.4 ± 3.1	126.2 ± 3.7	106.6	5.44 ± 3.07
Fe	7.42 ± 0.44	7.22 ± 0.76	97.2	11.46 ± 8.05
Mg	559 ± 10	470.3 ± 12	84.1	3.80 ± 3.32

*reference value only

Table 2: Total As and concentration of other elements in wheat grain and wheat flour samples collected from households in Bihar, India

	Wheat grain (n=72)		**Wheat flour (n= 58)**	
	mean ± SD	Range	mean ± SD	Range
As (µg/kg)	**43.64 ± 48.19**	**0.96 - 234.3**	**49.80±74.08**	**3.59-448.25**
Cd (µg/kg)	19.70 ± 9.78	4.35-51.41	21.57±8.60	5.03-51.61
Pb (µg/kg)	61.82 ± 59.54*	10.31-384.73	62.82±32.66*	19.35-178.53
Se (µg/kg)	138.41±168.53*	0.67-953.67	113.52±111.91*	0.67-432.66
Mn (mg/kg)	33.71± 6.7**	16.73-58.51	32.84±7.39**	13.90- 51.70
Cu (mg/kg)	4.54 ± 0.92	2.98-9.24	4.84±0.80	3.61-8.37
Zn (mg/kg)	23.44 ± 4.55	14.82-34.44	23.01±4.57	14.77-36.11
Ca (mg/kg)	425.40 ± 77.21	305.91-870.04	425.60±72.28	229.52-596.22
Fe (mg/kg)	83.65 ±113.52*	25.10-779.83	67.70±33.32*	33.20-180.34
Mg (mg/kg)	1085.98±102.39*	896.22-1456.18	1100.37±131.01*	555.03-1379.19

*elements that were positively correlated with As; **elements that were negatively correlated with As (using Spearman's rho ($p <0.05$)

Table 3: Total As concentration in whole wheat grains in different studies

Country	Area or site or type	Year (published)	Sample Size (n)	Mean total As (µg /kg)	(Range) SD (µg /kg)	Reference
India	Gangetic, WB	2005	3	94.52	22.79	Samal (2005)

Scotland	Field	2007	29	30	10-210	Williams et al. (2007)
China	Kunshan	2008	40	38(median)	29-86	Huang et al. (2008)
England	Field	2008	37	70	10-500	Williams et al. (2007)
Pakistan	Manchar (water As > 10 ppb)	2008	150	317	96	Arain et al. (2009)
Pakistan	Manchar (water As <10 ppb)	2008	150	175	56	Arain et al. (2009)
China	Zhengzhou, Henan	2009	40	136.66	70-210	Liu et al. (2009)
India	Nadia, WB	2010	8	129	10-190	Bhattacharya et al. (2010)
Europe	Multinational	2010	8	7.7	5.4	Zhao et al. (2010)
Hungary	Martonvasar, 2006	2010	2	6	3	Zhao et al. (2010)
Hungary	Martonvasar, 2007	2010	2	3	2	Zhao et al. (2010)
Poland	Choryn, 2007	2010	2	13	3	Zhao et al. (2010)
UK	Woolpit, 2007	2010	2	9	4	Zhao et al. (2010)
Ghana	Market based (origin China)	2011	19	50	10	Adomako et al. (2011)
Italy	Field	2010	141	8.62	4.6-14.8	Cubadda et al. (2010)
Italy	Field	2011	3	29.8	0.7	D'Amato et al. (2011)
Chaina	Huaibei, Coal Mining Area	2013	75	33.3	(6.5-54.9) 8.05	Shi et al. (2013)
China	Mangolia (low As)	2014	25	66.9	22.8-154	Tong et al. (2014)
China	Mangolia (high As)	2014	25	238	138-365	Tong et al. (2014)
Bangladesh	All Agroclimatic Zone	2015	30	281	1	Ahmed et al. (2015)
Brazil	Sao Gotardo	2015	3	19	-	Corguinha et al. (2015)
Bangladesh	Faridpur	2016	5	864	490-1150	Kamrozzaman et al. (2016)
Pakistan	Field of 12 districts	2016	12	90	63	Al-Othman et al. (2016)
India	Field of Ropar wetland, Punjab	2016	9	110	20	Sharma et al. (2016)
India	Bihar	2016	35	27	(7.7 – 108) 24	Kumar et al. (2016)
Argentina	Santa Fe	2016	8	36	<LOD - 73	Sigrist et al. (2016)

Italy	North West Italy	2018	18	10	(06-22)	Giordano et al. (2018)
China	Henan	2018	48	270	130-340	Guo et al. (2018)
Pakistan	Four Region	2018	8	105	61.47	Rasheed et al. (2018)
China	Field Dongdagou	2018	22	417	155	Zhang et al. (2018)
China	Field Xidagou	2018	14	224	279	Zhang et al. (2018)
Belgium	Field	2018	9	22	6	Ruttens et al. (2018)
Bihar	Household based study		72	43.64	(0.96-234.3) 48.19	This study

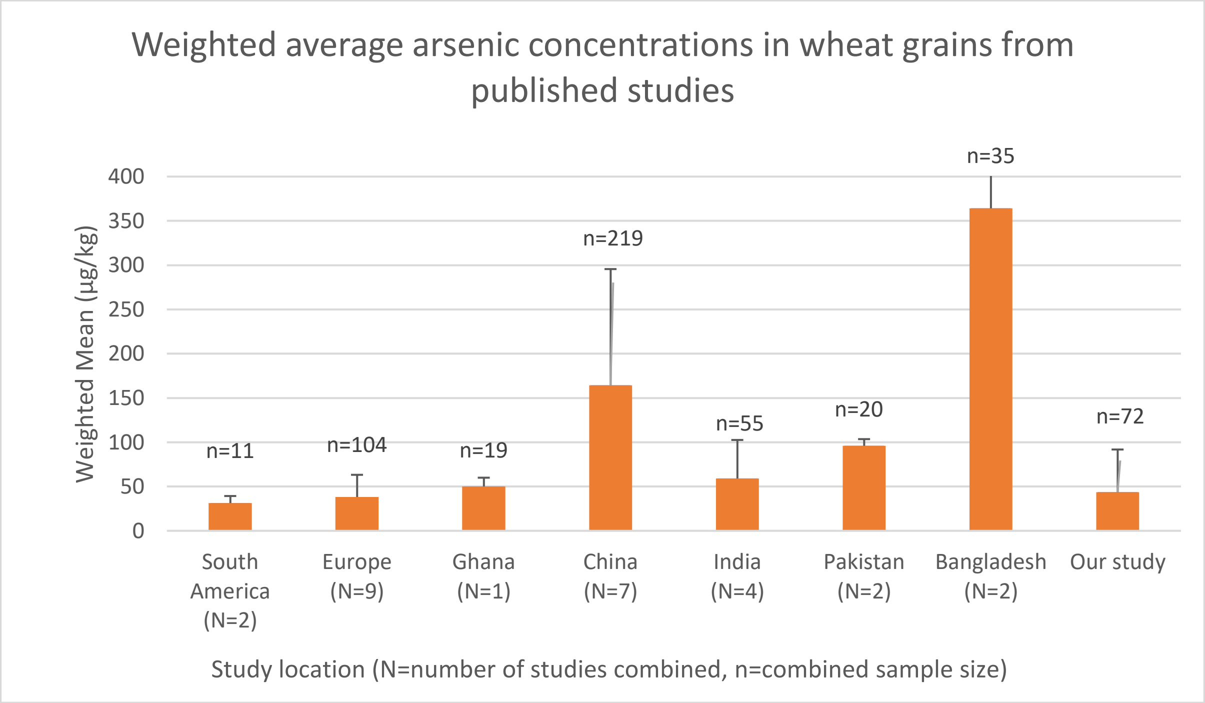

Figure 2: Weighted mean and SD of As concentrations in wheat grains using 27 published studies

Though the average As concentration in the wheat flour (49.80 ± 74.08 μg/kg) was higher than in wheat grains (Table 2) and correlated (Figure 3a) but based on an independent t-test run on 53 paired samples, there was no significant difference in As concentration between wheat grain and wheat flour (t (52) = 1.03, p = 0.31) samples.

In previous studies, As concentration in flour was found to be lower. For example, by 40% (grain: 69.0 ± 17.0; flour: 41.2 ± 13.7 µg/kg) in the study by Zhao et al. (2010); by 56% (grain: 29.8 ± 0.7; flour: 13.2 ± 1.3 µg/kg) in the study by D'Amato et al. (2011); and by 52% (grain: 33.1 ± 7.9; flour: 15.8 ± 3.7 µg/kg) in the study by Zhang et al. (2009). This is because milling of wheat grain into bran and white flour fractions results in a three to four-fold higher As concentration in bran than in the white flour (Zhao et al., 2010), thus most of the whole grain As remains in the bran. But such significant decrease was not observed in this study and in fact an overall increase based on average concentrations in wheat grain and wheat flour was noted. This could be attributed to either soaking of the grains in As contaminated water (as shown in Figure 4) or external contamination during grinding. Though further studies can confirm the effect of soaking on arsenic uptake by wheat grain if any, we found a little stronger relationship (Figure 3b) between wheat flour and drinking water (Spearman's Rho= 0.3174, P<0.05), often the same water used in the household for soaking compared to wheat grain (Spearman's Rho=0.2492, P<0.05).

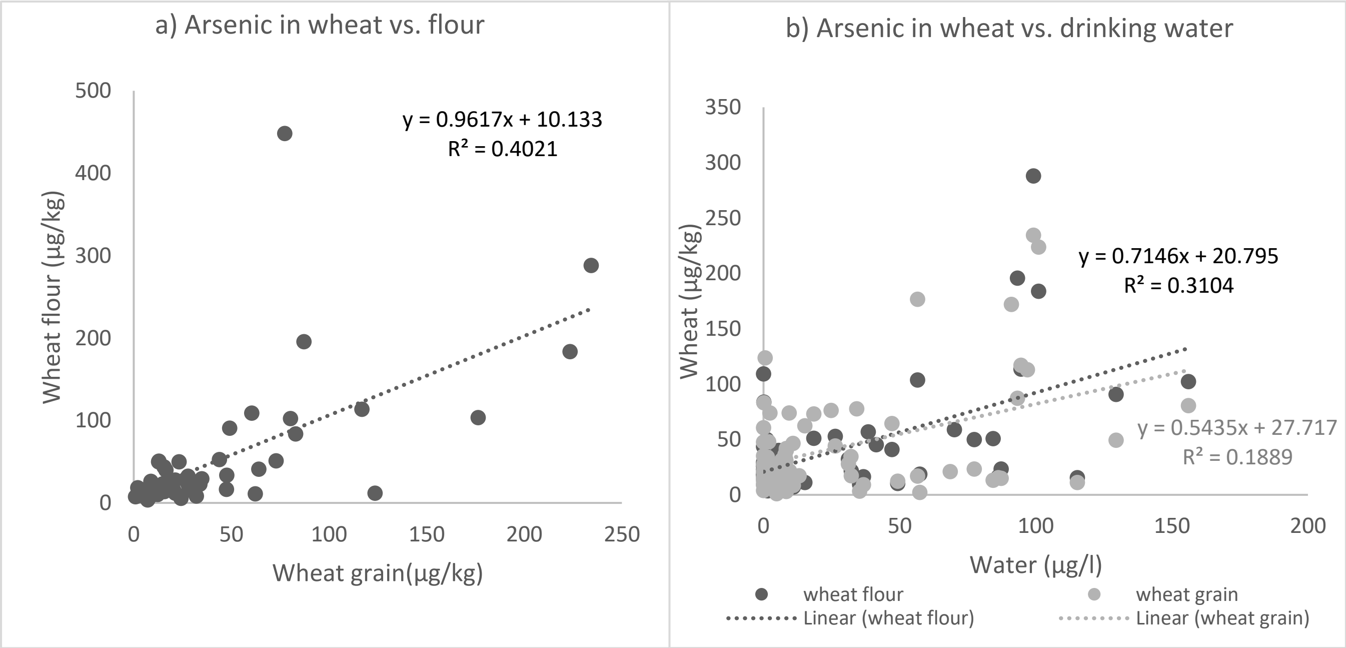

Figure 3: a) Arsenic concentration in wheat flour compared to wheat grains; b) Arsenic concentration in wheat flour and grain in relation to As in drinking water

2.2 Estimation of wheat intake from wheat-based food consumption

Consumption of wheat was mainly from home-made bread (*chapati*), which is mostly cooked in a traditional way in rural Bihar, as shown in Figure 4. The other wheat-based products consumed by the surveyed population include roasted wheat-flour drink (*Sattu*), some bakery products like biscuits and refined wheat products such as vermicelli, noodles etc. (Table 4). Compared to a similar study in Pakistan (Rasheed et al., 2017), considering all potential dietary sources of wheat, consumption (based on this study) in rural Bihar was found to be lower (Table 5).

In rural India, the percentage share of total daily energy intake from cereal grains is reported to be 57.4% (NSS, 2014a), hence the diet is mainly dominated by cereals, which was found to be true for rural Bihar with 67% of energy intake coming from cereals (NSS, 2014a). Next to rice, wheat is the most important food-grain in India and is the staple food of millions of Indians, particularly in the northern and north-western parts of the country. Per capita consumption of wheat in Bihar (186 g/day) was reported to be higher than that for India (143 g/day) as shown in Table 5 (NSS, 2014b). While overall rural per capita consumption of wheat in India has increased by 0.1 kg per person per month between 2004-05 and 2011-12, the consumption via PDS (PDS stands for Public Distribution System, which means the distribution of some essential commodities by the government at subsidised rates through ration shops, fair price shops and control shops and more widely used in rural areas) has increased considerably from 0.31 kg per person per month in 2004-05 to 0.74 kg per person per month in 2011-12 (NSS, 2014b). Hence, there is an increase in wheat consumption in rural

India and furthermore in rural Bihar. Compared to other As affected areas of India, like West Bengal with subsistent rice-based diet, consumption of wheat-based food was found to be significant in rural Bihar. In fact, based on this study we found that 78% of the studied population consumed rice everyday while wheat-based home-made bread (*chapati*) was consumed everyday by all the participants (99.5%).

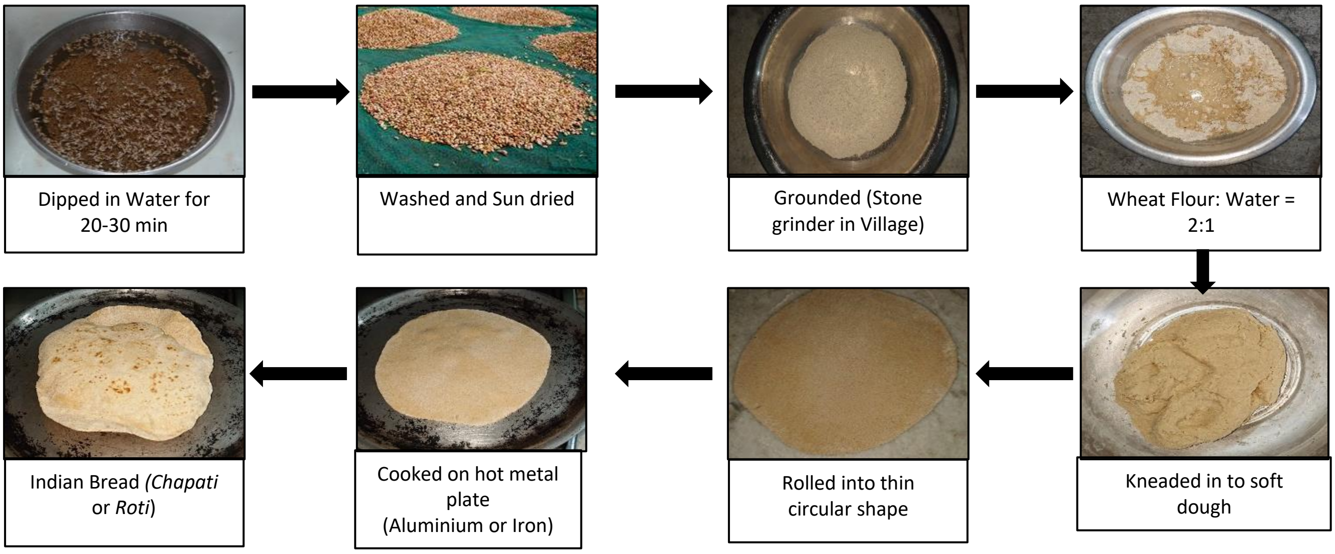

Figure 4: Household processing for preparation of Indian Bread (*Chapati or Roti*)

Table 4: Wheat-based food consumption in rural Bihar (mean ± SD)

	Total wheat intake	Indian bread (*Chapati)*	Bakery	Wheat flour drink *(Sattu)*	Refined Wheat Product
Intake (g/day)	223.78 ±107.59	215.07±109.29	2.48 ±10.00	1.59±9.90	3.61±13.40
Percentage contribution to total wheat intake		**94.90 ±16.45**	1.15 ± 4.65	0.68 ± 4.20	2.87±13.23

Table 5: Comparison of mean wheat intake in different studies

Country/Area	Study Year	Age group/ Gender	Mean Wheat Intake (g/Day)	Reference
Pakistan	2017	3-6 Years 6-16 Years >16 Years Male >16 Years Female	149 227 426 358	Rasheed et al. (2017)
Bangladesh	2004	male female	179 131	Watanabe et al. (2004)
China	2015	children adult	13 44	Zeng et al. (2015)
Europe	2013	mean	182	FAO (2013)
India Bihar	2011-12 2011-12	per capita per capita	143 186	NSS (2014b)
Bihar	2017-19	male female	272 165	This Study

3.3 Estimated increased cancer risk from wheat-based diet

The input parameters defined as probability distributions are given in Table 6. In this studied population, 95% of wheat intake was from home-made bread (*chapati*) which is made from the wheat flour, hence total As concentration in the wheat flour was used over wheat grains in estimating the cancer risk from wheat intake (Table 6). Published literature on inorganic As content in wheat flour is sparse hence we have combined the results for both wheat grain and flour (Table 7) to determine the parameter for probabilistic risk estimation. Though we have used the bioaccessible fraction based on the study by Alhobiti and Beauchemin (2018), the bioaccessible fraction of As in wheat depends on the variety (Alhobiti and Beauchemin, 2018), hence it should be further explored for Indian varieties. Thus, while the probabilistic risk assessment is limited by the accuracy and representativeness of the input data, it constitutes a framework to which improved data can readily be added to for improved risk estimation (Mondal and Polya, 2008).

Figure 5 presents the cumulative distribution of gender adjusted and separately for male and female, the excess lifetime cancer risk from wheat-based food intake. The point estimated mean risk of 1.08 x 10^{-4} was similar to probabilistic estimate of 1.23 x 10^{-4}, but the probabilistic approach to As risk assessment can take into account for the variabilities and parameter uncertainties (Mondal and Polya, 2008). Besides, the 5th percentile (9.36 x 10^{-6}) and 95th percentile (3.67 x 10^{-4}) could be relevant to determining the sub group of the studied population with low and high increased cancer risk from wheat-based food intake. Estimated risk in female (9.96 x 10^{-5}) was lower than in male (1.44 x 10^{-4}). Previously, for Pakistan, where wheat intake is comparatively higher (as shown in table 4), cumulative cancer risk was found to be 2 persons in a population of 10,000 in the highest exposed group (Rasheed et al., 2017) compared to our estimate of 1 in 10,000.

Table 6: The input parameters used in calculation of As attributable cancer risks

Input variable	Point estimate	Probabilistic estimates	Data source
As in wheat flour (µg/kg)	49.8±74.08	Pearsons (shape parameter =1.5, scale parameter=30.8)	This study
Inorganic As	79%	Uniform (*a*=29.48, *b*=106.75)	Based on previous 6 studies (Table 7)
Bioaccessibility	80%	Constant	Alhobiti and Beauchemin (2018)

Wheat intake (g/day)	male: 272	Triang (Min=51.80, continuous mode=240, Max=556.81)	This study
	female: 165	Normal (mean=170.58, SD=77.97)	
Body weight (kg)	male: 60	Triang (Min=34.94, continuous mode=48.3, Max=96.26)	This study
	female: 52	Weibull (scale parameter= 1.95, shape parameter= 23.45)	
Exposure duration (years)	equal to age if age < 40	Constant	Mondal & Polya (2008)
	equal to 40 of age > 40		
Life expectancy (years)	male: 67.8 (Bihar)	Constant	https://niti.gov.in/content/life-expectancy
	female: 68.4 (Bihar)		
cancer potency slope factor $((mg/kg)/d)^{-1}$	1.5	Constant	USEPA
gender distribution (%)	male: 52.2% (Bihar)	Constant	Census of India (2011)
	female: 47.8% (Bihar)	Constant	

Table 7: Inorganic As content in wheat

Country	Year (published)	Sample	Sample Size	% inorganic As	Reference
Italy	2010	Wheat Grains	20	95.1	Cubadda et al. (2010)
Italy	2011	Wheat Grains	3	97	D'Amato et al. (2011)
Italy	2011	Wheat flour	3	95	D'Amato et al. (2011)

China	2014	Wheat Grains (Grown in low As)	25	37.2	Tong et al. (2014)
China	2014	Wheat Grains (Grown in High As)	25	42.8	Tong et al. (2014)
Argentina	2016	Wheat flour	8	69.44	Sigrist et al. (2016)
Pakistan	2018	Whole Wheat	8	99.02	Rasheed et al. (2018)
Belgium	2018	Wheat Grains	9	79-94	Ruttens et al. (2018)

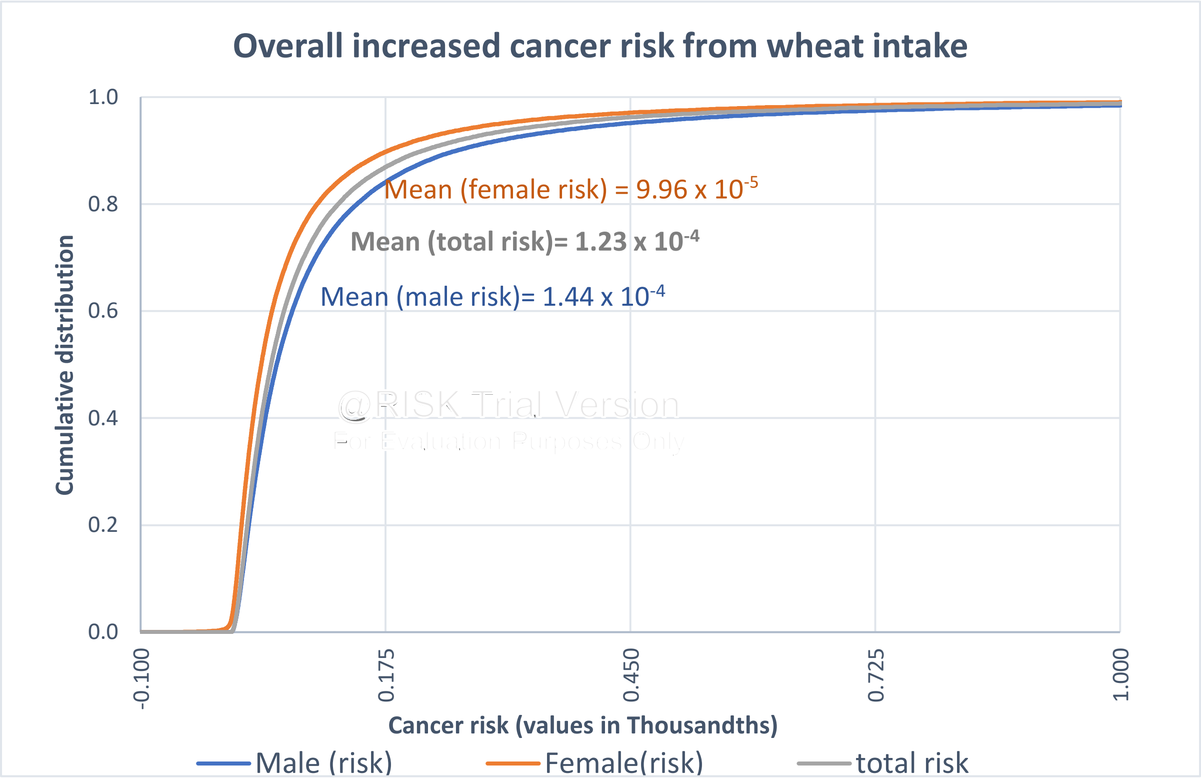

Figure 5: Cumulative probability distributions of gender adjusted excess lifetime cancer risk from wheat intake for the studied population.

Conclusion

Next to rice, wheat is the most important food-grain of India and As in wheat is an emerging concern. To the best of our knowledge, this is the first study conducted using detailed dietary assessment to determine As exposure from wheat-based food intake. The overall aim of this study was to determine the increased cancer risk due to As from wheat-based diet in rural Bihar, where As exposure is endemic.

Though total As estimated in wheat grains collected from the households in this study (43.64 µg/kg) was lower than reported As in wheat from other contaminated areas but it was higher than a previous study from Bihar reporting a mean of 27 µg/kg of total As. The estimated excess lifetime cancer risk of 1.23 x 10^{-4} for the studied rural population of Bihar indicated risk higher than the 10^{-4}-10^{-6} range, typically used by the USEPA as a threshold to guide regulatory values. Hence As exposure from wheat-based food intake is of concern in rural Bihar. In this studied population, 95% of wheat was found to be consumed in the form of home-made bread (*chapati*) prepared from the wheat flour which in turn was prepared from the wheat grains after soaking them in water followed by drying in the open sun and grinding them to powder. In contrast to previous studies, we did not find a significant decrease in total As in wheat flour compared to wheat grains and rather a mean increase was observed, though not significant. While the possible influence of the water used for soaking the wheat grain before grinding it to flour and/or external contamination during grinding needs further investigation, our observation confirms that modification of household-based wheat processing method may reduce As exposure.

The As concentrations determined in the wheat samples collected from the households in this study may not be representative of As in wheat cultivated in Bihar, since previous studies have reported higher As concentrations in wheat cultivated in endemic areas. But this reinforces the fact that due to the public distribution system in India, chances are there that the As contaminated wheat is consumed in areas where there is no contamination in drinking water, making it the most significant route of exposure, especially for sub-populations with similar wheat-based diets as observed in this studied rural areas of Bihar. Moreover, since wheat-based products are readily distributed, further studies should not just be restricted to As endemic areas and should include As estimation in all wheat-based food products.

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Competing interests

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

References:

Adomako, E.E., Williams, P.N., Deacon, C. and Meharg, A.A., 2011. Inorganic arsenic and trace elements in Ghanaian grain staples. *Environmental pollution*, 159(10), pp.2435-2442.

Ahmed, M. K., Shaheen, N., Islam, M. S., Habibullah-Al-Mamun, M., Islam, S., & Banu, C. P., 2015. Trace elements in two staple cereals (rice and wheat) and associated health risk implications in Bangladesh. *Environmental monitoring and assessment*, 187(6), 326.

Al-Othman, Z.A., Ali, R., Al-Othman, A.M., Ali, J. and Habila, M.A., 2016. Assessment of toxic metals in wheat crops grown on selected soils, irrigated by different water sources. *Arabian Journal of Chemistry*, 9, pp.S1555-S1562.

Althobiti, R.A. and Beauchemin, D., 2018. Inductively coupled plasma mass spectrometry with on-line leaching to assess the maximum bio-accessibility of toxic and essential elements in wheat from Saudi Arabia. *Journal of Analytical Atomic Spectrometry*, *33*(4), pp.642-648.

Arain, M.B., Kazi, T.G., Baig, J.A., Jamali, M.K., Afridi, H.I., Shah, A.Q., Jalbani, N. and Sarfraz, R.A., 2009. Determination of arsenic levels in lake water, sediment, and foodstuff from selected area of Sindh, Pakistan: estimation of daily dietary intake. *Food and Chemical Toxicology*, *47*(1), pp.242-248.

Banerjee, M., Banerjee, N., Bhattacharjee, P., Mondal, D., Lythgoe, P.R., Martínez, M., Pan, J., Polya, D.A. and Giri, A.K., 2013. High arsenic in rice is associated with elevated genotoxic effects in humans. *Scientific reports*, *3*, p.2195.

Bhattacharya, P., Samal, A. C., Majumdar, J., & Santra, S. C., 2010. Arsenic contamination in rice, wheat, pulses, and vegetables: a study in an arsenic affected area of West Bengal, India. *Water, Air, & Soil Pollution*, 213(1-4), 3-13.

Census of India, 2011. http://censusindia.gov.in/. Accessed on 09 June 2019.

Chakraborti, D., Mukherjee, S.C., Pati, S., Sengupta, M.K., Rahman, M.M., Chowdhury, U.K., Lodh, D., Chanda, C.R., Chakraborti, A.K. and Basu, G.K., 2003. Arsenic groundwater contamination in Middle Ganga Plain, Bihar, India: a future danger?. *Environmental health perspectives*, *111*(9), pp.1194-1201.

Chakraborti, D., Rahman, M.M., Das, B., Chatterjee, A., Das, D., Nayak, B., Pal, A., Chowdhury, U.K., Ahmed, S., Biswas, B.K. and Sengupta, M.K., 2017. Groundwater arsenic contamination and its health effects in India. *Hydrogeology Journal*, 25(4), pp.1165-1181.

Chakraborti, D., Singh, S., Rahman, M., Dutta, R., Mukherjee, S., Pati, S. and Kar, P., 2018. Groundwater arsenic contamination in the Ganga River Basin: a future health danger. *International journal of environmental research and public health*, *15*(2), p.180.

Corguinha, A. P. B., de Souza, G. A., Gonçalves, V. C., de Andrade Carvalho, C., de Lima, W. E. A., Martins, F. A. D., ... & Guilherme, L. R. G., 2015. Assessing arsenic, cadmium, and lead contents in major crops in Brazil for food safety purposes. *Journal of food composition and analysis*, 37, 143-150.

Cubadda, F., Ciardullo, S., D'Amato, M., Raggi, A., Aureli, F., & Carcea, M., 2010. Arsenic contamination of the environment− food chain: A survey on wheat as a test plant to investigate phytoavailable arsenic in Italian agricultural soils and as a source of inorganic arsenic in the diet. *Journal of agricultural and food chemistry*, 58(18), 10176-10183.

D'Amato, M., Aureli, F., Ciardullo, S., Raggi, A. and Cubadda, F., 2011. Arsenic speciation in wheat and wheat products using ultrasound-and microwave-assisted extraction and anion exchange chromatography-inductively coupled plasma mass spectrometry. *Journal of Analytical Atomic Spectrometry*, *26*(1), pp.207-213.

Food and Agriculture Organization (FAO), 2013. FAO Statistical Yearbook-2013. Food and Agriculture Organization of the United Nations (http://faostat.fao.org/site/567/DesktopDefault.aspx#ancor).

Freedman, L.S., Midthune, D., Arab, L., Prentice, R.L., Subar, A.F., Willett, W., Neuhouser, M.L., Tinker, L.F. and Kipnis, V., 2018. Combining a Food Frequency Questionnaire With 24-Hour Recalls to Increase the Precision of Estimation of Usual Dietary Intakes - Evidence From the Validation Studies Pooling Project. *American journal of epidemiology*, *187*(10), pp.2227-2232.

Giordano, D., & Blandino, M., 2018. Arsenic, lead and cadmium distribution in the pearled fractions of different winter wheat cultivars (Triticum aestivum L.). *Journal of cereal science*, 80, 94-101.

Guo, G., Lei, M., Wang, Y., Song, B., & Yang, J. 2018. Accumulation of As, Cd, and Pb in Sixteen Wheat Cultivars Grown in Contaminated Soils and Associated Health Risk Assessment. *International journal of environmental research and public health*, 15(11), 2601.

Huang, M., Zhou, S., Sun, B. and Zhao, Q., 2008. Heavy metals in wheat grain: assessment of potential health risk for inhabitants in Kunshan, China. *Science of the Total Environment*, 405(1-3), pp.54-61.

Kamrozzaman, M. M., Khan, M. A. H., Ahmed, S., Sultana, N., & Quddus, A. R., 2016. Evaluation of Five Wheat Varieties on Arsenic Contaminated Soils of Bangladesh. *International Journal of Applied Sciences and Biotechnology*, 4(3), 330-337.

Kumar, M., Rahman, M. M., Ramanathan, A. L., & Naidu, R. (2016). Arsenic and other elements in drinking water and dietary components from the middle Gangetic plain of Bihar, India: health risk index. *Science of the Total Environment*, 539, 125-134.

Liu, W. X., Liu, J. W., Wu, M. Z., Li, Y., Zhao, Y., & Li, S. R., 2009. Accumulation and translocation of toxic heavy metals in winter wheat (Triticum aestivum L.) growing in agricultural soil of Zhengzhou, China. *Bulletin of environmental contamination and toxicology*,82(3), 343-347.

Longvah, T., Anantan, I., Bhaskarachary, K., & Venkaiah, K. 2017. Indian food composition tables. Hyderabad: National Institute of Nutrition, Indian Council of Medical Research.

Mondal, D., Mwale, T., Xu, L., Matthews, H., Oyeka, A., Lace-Costigan, G., Polya, D.A., 2019. Risk perception of arsenic exposure from rice intake in a UK population. *Palgrave Communication*, in press.

Mondal, D., Banerjee, M., Kundu, M., Banerjee, N., Bhattacharya, U., Giri, A.K., Ganguli, B., Roy, S.S. and Polya, D.A., 2010. Comparison of drinking water, raw rice and cooking of rice as arsenic exposure routes in three contrasting areas of West Bengal, India. *Environmental geochemistry and health*, *32*(6), pp.463-477.

Mondal, D. and Polya, D.A., 2008. Rice is a major exposure route for arsenic in Chakdaha block, Nadia district, West Bengal, India: A probabilistic risk assessment. *Applied Geochemistry*, *23*(11), pp.2987-2998.

Mwale, T., Rahman, M. and Mondal, D., 2018. Risk and benefit of different cooking methods on essential elements and arsenic in rice. *International journal of environmental research and public health*, 15(6), p.1056.

NSS (2014a) NSS Report No.560: Nutritional Intake in India, 2011-12; http://www.indiaenvironmentportal.org.in/files/file/nutritional%20intake%20in%20India%202011-12.pdf. Accessed on 08 June 2019.

NSS (2014b) NSS Report No. 558 (68/1.0/2) Household Consumption of various Goods and Services in India, 2011-12. Accessed on 08 June 2019. http://mospi.nic.in/sites/default/files/publication_reports/Report_no558_rou68_30june14.pdf

Nickson, R., Sengupta, C., Mitra, P., Dave, S.N., Banerjee, A.K., Bhattacharya, A., Basu, S., Kakoti, N., Moorthy, N.S., Wasuja, M. and Kumar, M., 2007. Current knowledge on the distribution of arsenic in groundwater in five states of India. *Journal of Environmental Science and Health, Part A*, *42*(12), pp.1707-1718.

Rasheed, H., Kay, P., Slack, R., & Gong, Y. Y., 2018. Arsenic species in wheat, raw and cooked rice: Exposure and associated health implications. *Science of the Total Environment*, 634, 366-373.

Rasheed, H., Slack, R., Kay, P. and Gong, Y.Y., 2017. Refinement of arsenic attributable health risks in rural Pakistan using population specific dietary intake values. *Environment international*, 99, pp.331-342.

Roychowdhury, T., Uchino, T., Tokunaga, H., & Ando, M., 2002. Survey of arsenic in food composites from an arsenic-affected area of West Bengal, India. *Food and Chemical Toxicology*, 40(11), 1611-1621.

Ruttens, A., Cheyns, K., Blanpain, A. C., De Temmerman, L., & Waegeneers, N., 2018. Arsenic speciation in food in Belgium. Part 2: Cereals and cereal products. *Food and chemical Toxicology,* 118, 32-41.

Samal, A.C., 2005. An investigation on Accumulation of Arsenic in Ecosystem of Gangetic West Bengal and Assessment of Potential Health Risk, Ph. D. Thesis, University of Kalyani, West Bengal, India.

Sharma, S., Kaur, J., Nagpal, A.K. and Kaur, I., 2016. Quantitative assessment of possible human health risk associated with consumption of arsenic contaminated groundwater and wheat grains from Ropar Wetand and its environs. *Environmental monitoring and assessment*, *188*(9), p.506.

Shi, G.L., Lou, L.Q., Zhang, S., Xia, X.W. and Cai, Q.S., 2013. Arsenic, copper, and zinc contamination in soil and wheat during coal mining, with assessment of health risks for the inhabitants of Huaibei, China. *Environmental Science and Pollution Research*, *20*(12), pp.8435-8445.

Sigrist, M., Hilbe, N., Brusa, L., Campagnoli, D., & Beldoménico, H., 2016. Total arsenic in selected food samples from Argentina: Estimation of their contribution to inorganic arsenic dietary intake. *Food chemistry,* 210, 96-101.

Tong, J., Guo, H., & Wei, C., 2014. Arsenic contamination of the soil–wheat system irrigated with high arsenic groundwater in the Hetao Basin, Inner Mongolia, China. *Science of the Total Environment*, *496*, 479-487.

USEPA, 1989. Risk Assessment Guidance for Superfund, vol. I. Human Health Evaluation Manual (Part A). Interim Final. Washington, DC, US Environmental Protection Agency, Office of Emergency and Remedial Response EPA/540/1-89/002.

USEPA, 2019. Integrated Risk Information System (IRIS): Arsenic, inorganic; CASRN 7440-38-2, *National Center for Environmental Assessment.* Accessed on 12 June 2019 from https://cfpub.epa.gov/ncea/iris2/chemicalLanding.cfm?substance_nmbr=278.

Watanabe, C., Kawata, A., Sudo, N., Sekiyama, M., Inaoka, T., Bae, M. and Ohtsuka, R., 2004. Water intake in an Asian population living in arsenic-contaminated area. *Toxicology and Applied Pharmacology*, 198(3), pp.272-282.

Williams, P.N., Villada, A., Deacon, C., Raab, A., Figuerola, J., Green, A.J., Feldmann, J. and Meharg, A.A., 2007. Greatly enhanced arsenic shoot assimilation in rice leads to elevated grain levels compared to wheat and barley. *Environmental Science & Technology*, *41*(19), pp.6854-6859.

Zeng, X., Wang, Z., Wang, J., Guo, J., Chen, X. and Zhuang, J., 2015. Health risk assessment of heavy metals via dietary intake of wheat grown in Tianjin sewage irrigation area. *Ecotoxicology*, 24(10), pp.2115-2124.

Zhang, Q., Wang, S., Nan, Z., Li, Y. and Zang, F., 2018. Accumulation, fractionation, and risk assessment of mercury and arsenic in the soil-wheat system from the wastewater-irrigated soil in Baiyin, northwest China. *Environmental Science and Pollution Research*, *25*(15), pp.14856-14867.

Zhang, W.D., Liu, D.S., Tian, J.C. and He, F.L., 2009. Toxicity and accumulation of arsenic in wheat (Triticum aestivum L.) varieties of China. *Phyton* (Buenos Aires), 147.

Zhao, F. J., Stroud, J. L., Eagling, T., Dunham, S. J., McGrath, S. P., & Shewry, P. R., 2010. Accumulation, distribution, and speciation of arsenic in wheat grain. *Environmental science & technology*, 44(14), 5464-5468.