Journal of Exposure Science and Environmental Epidemiology (2012) 1 – 7

www.nature.com/jes

ORIGINAL ARTICLE

An *in vitro* assessment of bioaccessibility of arsenicals in rice and the use of this estimate within a probabilistic exposure model

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In this study, an *in vitro* synthetic gastrointestinal extraction protocol was used to estimate bioaccessibility of different arsenicals present in 17 rice samples of various grain types that were collected across the United States. The across matrix average for total arsenic was 209 ng/g ± 153 ($\bar{x} \pm 2\sigma$). The bioaccessibility estimate produced an across matrix average of 61% ± 19 ($\bar{x} \pm 2\sigma$). The across matrix average concentrations of inorganic arsenic (iAs) and dimethylarsinic acid (DMA) were 81 ng/g ± 67.7 and 41 ng/g ± 58.1 ($\bar{x} \pm 2\sigma$), respectively. This distribution of iAs concentrations in rice was combined with the distribution of consumption patterns (from WWEIA) in a Stochastic Human Exposure and Dose Simulator model to estimate population-based exposures. The mean consumption rate for the population as a whole was 15.7 g per day resulting in a 0.98 μg iAs per day exposure. The mean consumption rate for children 1–2 years old was 7 g per day resulting in a 0.48 μg iAs per day exposure. Presystemic biotransformation of DMA in rice was examined using an *in vitro* assay containing the anaerobic microbiota of mouse cecum. This assay indicated that DMA extracted from the rice was converted to dimethylthioarsinic acid, although a second oxygen–sulfur exchange to produce DMDTA was not observed.

Journal of Exposure Science and Environmental Epidemiology advance online publication, 2 May 2012; doi:10.1038/jes.2012.24

Keywords: rice; arsenic; bioaccessiblity; exposure assessment

INTRODUCTION

The International Agency for Research on Cancer (IARC) has classified inorganic arsenic (iAs) as a class 1 carcinogen,[1] reflecting strong epidemiological evidence that exposure to iAs increases cancer risk. Because of this classification, the World Health Organization (WHO) has established an exposure guideline of 10 μg/l in drinking water[2] and the US Environmental Protection Agency (EPA) has established an enforceable maximum contaminant level (MCL) for drinking water of 10 μg/l.[3] For non-occupationally exposed individuals, the two predominant exposure routes for arsenic are drinking water and diet. Drinking water exposures contain almost exclusively iAs but dietary sources of exposure contain various arsenicals with markedly different ($>10^3$ difference in a 50% lethal dose (LD_{50})) toxicities.[4] Therefore, chemical-form-specific dietary exposure assessments potentially provide a more accurate basis to characterize risks posed by the dietary component of aggregate arsenic (water + diet) exposure. Considering these aggregate exposures, the WHO established 2 μg iAs/kg body weight per day as the provisional maximum tolerable daily intake.[2]

Rice is a target food for arsenic speciation. Rice is a dietary staple for over one-half of the world's population and it accounts for 70% of the caloric intake in developing countries.[5] A growing number of researchers are producing chemical-form-specific (0.01–0.271 μg/g iAs and 0.004–0.9 μg/g dimethylarsinic acid (DMA)) data sets for arsenic in rice.[6–15] The majority of arsenic speciation studies use chemical-based extraction techniques (i.e., hot water, trifluoroacetic acid, hydrochloric acid) to liberate arsenicals from the (cooked or uncooked, dried and milled) rice. These extraction approaches fall short of providing a biological relevance to the exposure, which is essential to risk characterization. The bioavailability of arsenic exposure can be estimated using a swine feeding study; however, such studies are expensive and difficult to conduct.[16] The high cost of swine-based assessments has led to a search for *in vitro* techniques. The beginning of a more physiologically based approach in arsenic speciation of rice samples is marked by the use of enzymes as a means of improving the extraction efficiencies.[7,11,17] Sanz et al.[11] suggested that improved extraction efficiencies for arsenicals in rice were due to α-amylase-catalyzed hydrolysis of α-1-4 linkages in rice starch and protease-catalyzed digestion of proteins in rice that bind arsenicals. *In vitro* techniques that estimate the solubility or bioaccessibility of the arsenicals in rice by mimicking the chemical and enzymatic conditions in the gastrointestinal tract have been reported.[6] Laparra et al.[10] have attempted to include the uptake of the arsenicals from rice and in doing so have included a bioavailability estimate by using cultured Caco 2 cells (derived from human colonic adenocarcinoma) within an *in vitro* assay. He and Zheng[18] recently used a mass balance approach to estimate *in vivo* bioaccessibility of arsenic in rice using human subjects. Their 60% estimate represents a lower limit for the bioavailability of arsenic in rice. Juhasz et al.[19] in 2006 conducted a

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Received 25 July 2011; accepted 12 December 2011

swine feeding study on two rice samples and determined a relative bioavailability of 33.1 and 89.4%.

Evaluating differences in bioavailability among arsenicals must also account for the presystemic biotransformation of arsenicals that is mediated by the microbiota of the gastrointestinal tract. Biotransformation of arsenicals is significant because the formation of methylated metabolites of iAs produces intermediates that are more reactive and toxic than iAs. These reactive metabolites play important roles in the toxic and carcinogenic actions of arsenic.[20,21] In addition, the microbiota of the gastrointestinal tract produces sulfur analogs of a variety of oxyarsenicals.[22,23] The conversion of oxyarsenicals to thioarsenicals produces more reactive species that are cytotoxic and genotoxic.[24,25] Hence, evaluating presystemic metabolism of DMA in rice to dimethylthioarsinic acid (DMTA) by gastrointestinal anaerobic microbiota is relevant to risk characterization.

From a risk assessment perspective, the species-specific bioaccessibility estimates for arsenic address the uncertainties associated with the concentration of arsenicals in rice; however, ingestion patterns are also a source of uncertainty that need to be addressed in estimating an exposure. In the United States, Meacher combined arsenic speciation data from Schoff[12] with the consumption rate data from the Continuing Survey of Food Intakes by Individuals, US Department of Agriculture 1992–94, within a Monte Carlo modeling framework to estimate the dietary arsenic exposure in the US population. Mean iAs exposure from diet was estimated as 2.75 and 3.56 μg per day for females and males, respectively. Using an exposure model, Yost et al.[26] estimated iAs exposures in children aged 1–6 years old to be 3.2 μg per day. Because of the limited speciation data, Meacher et al.[27] and Yost et al.[26] were both forced to assume that the food commodities consumed by the population contained a constant iAs concentration. Both authors identified the absence of a more stratified sample and bioavailability data, as sources of uncertainty within their estimates.

Here, we profile concentrations of arsenicals in rice samples collected across the United State using a synthetic gastrointestinal-based extraction procedure. We combined the profile information with US rice consumption data[28] using the Stochastic Human Exposure and Dose Simulator (SHEDS), a probabilistic exposure model.[29,30] The goal of this research was to incorporate bioaccessibility estimates for iAs in rice into an exposure model and identify research efforts needed to target sources of uncertainty associated with the modeled exposure estimates.

MATERIALS AND METHODS

Figure 1 is a flowchart summary of the experimental conditions used to generate the bioaccessibility estimates of iAs in rice. It also provides an

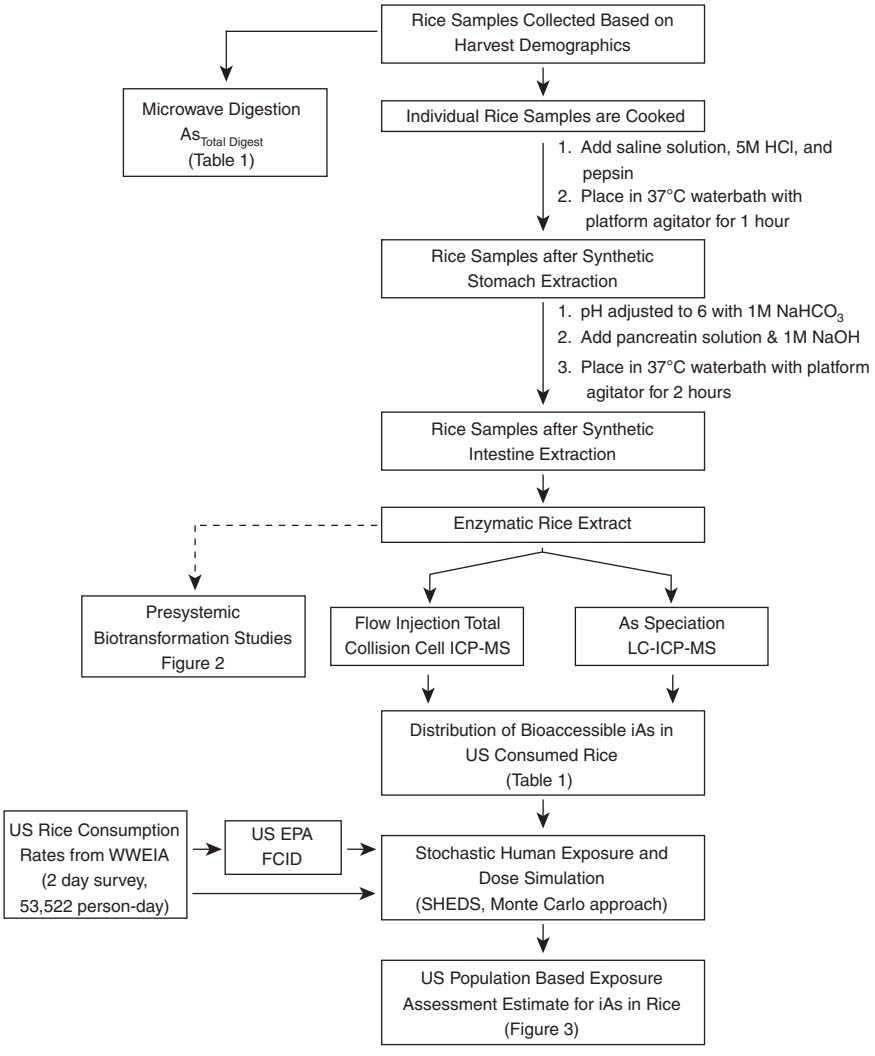

Figure 1. Summary of the experimental conditions associated with the laboratory analysis and an outline of the population-based exposure modeling for inorganic arsenic in rice.

overview of how this distribution estimate was combined with ingestion patterns from What We Eat In America (WWEIA, dietary component of National Health and Nutrition Examination Survey (NHANES)) within a SHEDS model to produce a population-based exposure assessment for iAs from rice. General experimental information is provided below. For detailed information, see Supplementary Text S1.

Rice Sample Collection and Cooking

Seventeen rice samples were purchased from retail sources in the United States between August 2007 and July 2008. Sixteen of these rice samples were grown in the United States and one was imported from Thailand. This domestic/import ratio crudely reflects the production-based distribution for domestic (85%) *vs* imported (15%) rice in the United States[31] with Thailand providing 67% of imported rice.[32] Rice samples included 10 white (five long grain, one medium grain, two short grain and two parboiled) and 7 brown (four long grain, two medium grain and one instant). In most cases, the grain length was classified by the manufacturer on the product labeling; however, if this classification was not provided, an in laboratory classification was made based on grain length. Williams et al.[33] have reported a statistical difference in As_{Tot} concentration in rice grown in different regions of the United States (i.e., California *vs* the southeastern United States). This regional variation in the arsenic levels in US rice may be important from an exposure assessment perspective because poor geographical stratification may bias the sample set toward high estimates of rice arsenic levels.[8,33] To minimize the impact of poor geographic stratification, the data set in Table 1 was collected using US rice harvest demographics as a guide; however, the complete sampling shown in Table 1 did overweigh California-grown rice. Rice samples were cooked before the *in vitro* gastrointestinal extraction following the water to rice ratio specified by the manufacturer. In all cases, 18 MΩ (Millipore, MA, USA) water used in the cooking process was absorbed by rice.

Total Arsenic Determination

The microwave digestion (CEM Mars 5 Microwave Oven, Matthews, NC, USA) was completed as previously described.[7] Samples were analyzed by inductively coupled plasma mass spectrometry (ICP-MS; Agilent 7500ce, Santa Clara, CA, USA). All microwave sample digestions were conducted in triplicate along with a laboratory-fortified matrix (LFM), a rice flour standard reference material (SRM, NIST 1568 or NIST 1568a), two method blanks and a laboratory-fortified blank (LFB). The method detection limit was 0.122 ng/g as determined by seven replicate analyses of a fortified blank.[34]

Enzyme Extraction and Analysis of Arsenic in Enzymatic Extracts

A simulated two component (stomach then intestine) gastrointestinal extraction was used to evaluate bioaccessibility of arsenic in rice samples. This *in vitro* technique was initially developed for measurement of iron

Table 1. Total arsenic and speciation-based bioaccessibility estimates for arsenic from US purchased rice samples.

	Sample[a]	*Total arsenic*[b]			As^{b}_{Chrom}			*Chromatographic recovery (%)*[c]
		Total digest As_{Tot} *(ng/g)*[d]	*Gastrointestinal total* As_{GT} *(ng/g)*[e]	*Bioaccessibility (%)*[f]	*iAs (ng/g)*[g]	*DMA (ng/g)*[g]	Σ_{Chrom} *(ng/g)*	
1	W-LG	230 ± 14.7	126 ± 15.4	55 ± 6.7	72 ± 15.4	37 ± 11.1	109	87
2	B	131 ± 56.0	76 ± 3.2	58 ± 2.5	71 ± 16.3	11 ± 2.0	82.1	108
3	B	237 ± 24.3	152 ± 14.4	64 ± 6.1	119 ± 18.5	36 ± 15.0	155	102
4	W-SG	81 ± 19.4	48 ± 10.7	59 ± 13.2	37 ± 7.8	16 ± 3.7	52.8	110
5	B	279 ± 17.9	165 ± 23.4	59 ± 8.4	117 ± 13.9	40 ± 8.3	157	95
6	B	282 ± 38.6	165 ± 10.1	59 ± 3.6	128 ± 5.1	26 ± 2.1	154	93
7	W-SG	103 ± 31.7	47 ± 2.9	45 ± 2.8	44 ± 7.0	10 ± 1.3	53.9	116
8	B	264 ± 29.6	162 ± 12.4	61 ± 4.7	132 ± 21.2	29 ± 2.0	161	99
9	W-LG	313 ± 3.6	180 ± 26.6	58 ± 8.5	56 ± 4.8	116 ± 12.3	172	96
10	B	107 ± 21.1	64 ± 17.9	60 ± 16.8	48 ± 7.4	11 ± 5.5	58.7	92
11	INST	120 ± 29.6	103 ± 34.4	86 ± 28.6	42 ± 4.7	27 ± 1.6	69.0	67
12	W-LG	248 ± 2.8	133 ± 5.9	54 ± 2.4	67 ± 5.3	53 ± 3.0	120	90
13	W-LG	213 ± 25.2	136 ± 31.3	64 ± 14.7	81.3 ± 10.7	46 ± 9.2	127	93
14	W-MG	259 ± 54.9	172 ± 7.5	66 ± 2.9	63 ± 12.8	60 ± 34.8	123	72
15	PAR[h]	294 ± 98.0	233 ± 28.9	79 ± 9.8	131 ± 15.0	84 ± 16.7	215	92
16	W-LG[i]	144 ± 30.7	74 ± 17.0	51 ± 11.8	60 ± 1.0	25 ± 10.4	84.4	114
17	PAR[h]	242 ± 22.0	159 ± 43.8	66 ± 18.1	106 ± 18.9	76 ± 7.6	182	114
	Across matrix avg[j]	209 ± 153	129 ± 105	61 ± 19	81 ± 67.7	41 ± 58.1		96 ± 28
18	SRM 1568	348 ± 52.3	308 ± 68.0	88 ± 20	80 ± 18.8	229 ± 46.4	310	101 ± 15
19	SRM 1568a	272 ± 47.2	257 ± 30.0	95 ± 11	74 ± 18.5	167 ± 19.2	241	94 ± 14

[a]Samples were categorized according to US Rice Federation Production classifications. W-LG is white, long grain; W-MG is white, medium grain; W-SG is white, short grain; B is brown; INST is instant; PAR is parboiled.
[b]All values reported as $\bar{x} \pm 2\sigma$, $n = 3$. All concentrations are reported on oven dried (OD) weight. The average gram of oven dried/gram of cooked rice = 0.30 ± 0.14 for the data in the table. Inorganic arsenic (iAs = As^{III}+As^{V}) and DMA were the only arsenicals detected.
[c]Chromatographic recovery calculated as $(\Sigma_{chrom}/As_{GT}) \times 100$.
[d]A LFM was run with every rice microwave total digest, recoveries averaged 102% ± 17. A LFB was run with every two rice microwave total digest, recoveries averaged 102% ± 31.
[e]A LFM and LFB were run with each gastrointestinal extraction. LFM recoveries averaged 92% ± 28 and LFB recoveries averaged 98% ± 19.
[f]Bioaccessibility is calculated as $(As_{GT}/As_{Tot}) \times 100$.
[g]Species-specific LFM and LFB were run with each gastrointestinal extraction. iAs LFM average recoveries were 84% ± 19 and LFB average recoveries were 92% ± 25. DMA LFM average recoveries were 95% ± 10 and LFB average recoveries were 104% ± 20.
[h]Boxed mix contained separate seasoning packet. Analysis pertains to only the rice component of the packet.
[i]Rice purchased in US imported from Thailand.
[j]Across matrix average equals the average of the mean reported for that rice in each column. Across matrix averages do not contain SRM data and is calculated using non-rounded values.

bioaccessiblity in rice[35,36] and has been previously applied to arsenic bioaccessibility.[6] A sample set consisted of three replicates, three reagent blanks, one LFM and one LFB. In addition, the rice flour SRMs (NIST 1568 and NIST 1568a) were analyzed as samples. Flow injection analysis of total and speciation-based arsenic analysis of enzymatic extracts were conducted using an Agilent 1100 liquid chromatograph (LC; Santa Clara, CA, USA) with ICP-MS (Agilent 7500ce) as a detector. Supplementary Table S1 summarizes the chromatographic conditions used to analyze the enzymatic extracts.

Exposure Modeling

The SHEDS model used in this study has been described previously.[37] In this study, the SHEDS model used dietary surveys that were collected as part of the WWEIA to estimate rice consumption patterns in the United States. Food codes used in the Food and Nutrient Database for Dietary Studies (FNDDS) were used to identify dietary sources of rice. In some cases, rice was a component in a food code of FNDDS and recipe files associated with EPA's Food Consumption Intake Database were used to estimate rice consumption for that food code. Combined NHANES 2001–2006 (FNDDS 1.0–3.0) data that represented 53,522 person-day of dietary consumption were used.

Cecal Contents Analysis

Cecal contents were prepared as previously described.[38] Cecal contents were fortified with 200 μl of the enzyme extract of rice and brought to a final volume of 0.6 ml. These reaction mixtures were then anaerobically incubated for 0 or 24 h and flash frozen using liquid nitrogen. Samples were stored at $-80\,^{\circ}C$ until analyzed. Stability of thiolated arsenicals can be enhanced by storing them in high pH solutions that have been deoxygenated.[39] Therefore, all samples were prepared in deoxygenated buffers at pH 9 and chromatographed using separation 2 (Supplementary Table S1). To produce an adequate arsenic concentration in cecal samples, a rice flour naturally containing approximately 1000 ng As_{Tot}/g was extracted by the enzymatic procedure outlined above using rehydrated rice flour. This rice sample was obtained from Douglas Heitkemper (Food and Drug Administration (FDA), Cincinnati, OH, USA). Samples found to contain DMTA based on separation 2 (Supplementary Table S1) were also analyzed by separation 3 (Supplementary Table S1) to confirm the presence of DMTA based on elemental detection using ICP-MS.

RESULTS AND DISCUSSION

The most common method of estimating arsenic exposure in rice depends on a determination of total arsenic concentration after acid digestion (As_{Tot}) of rice. One of the best samplings of US rice for As_{Tot} from an exposure assessment perspective is the US FDA Market Basket Survey, which is comprised of samples collected from 1991 to 2005. The As_{Tot} concentration reported in these combined surveys is 228 ng/g $\pm$ 173.[40] Supplementary Table S2 contains a comparison of this market basket estimate to other US-based literature estimates for As_{Tot} in rice. The across matrix mean for As_{Tot} reported in Table 1 for this study is 209 ng/g $\pm$ 153. Although the mean of data in Table 1 is comparable to other studies of US rice, 11 of the 17 samples have As_{Tot} concentrations that exceed the "global normal range" of 82–202 ng/g calculated by Zavala et al.[41] from pooled global data.[7,14,42–45] However, As_{Tot} data may be inappropriate for estimating risk from consumption of US grown rice because US rice that contains elevated As_{Tot} concentrations has been shown to contain a disproportionate percentage of DMA, rather than iAs.[8,15] Indeed, variability of As_{Tot} content in US and Asian rice has been reported.[41] Finally, consistency with As_{Tot} in rice should not be overemphasized in this discussion because of the toxicological importance of iAs concentrations and As_{Tot} is poorly correlated with iAs in US grown rice.[8,15]

Bioaccessibility Estimate for Arsenic in Rice

Table 1 also reports gastrointestinal totals (As_{GT}) and speciated arsenic concentrations associated with the 17 rice samples subjected to simulated gastrointestinal tract extraction. After gastrointestinal treatment of rice, As_{GT} concentrations ranged from 47 to 233 ng/g with an across matrix average of 129 ng/g $\pm$ 105 ($\bar{x} \pm 2\sigma$). The extraction efficiency of the simulated gastrointestinal tract (bioaccessibility) ranged from 45 to 86% with an across matrix performance of 61% $\pm$ 19 ($\bar{x} \pm 2\sigma$). These data were consistent with other enzymatic extraction efficiencies reported for rice.[7,11,17] Sanz et al.[11] have postulated that the differences in extraction efficiencies may be caused by differences in binding of arsenicals to starches and proteins in different rice samples. Comparison of the enzymatic extraction of NIST 1568 (88% $\pm$ 20, $\bar{x} \pm 2\sigma$) and NIST 1568a (95% $\pm$ 11, $\bar{x} \pm 2\sigma$) with the data in Table 1 indicated more complete arsenic extraction in rice flour SRMs. The particle size associated with SRMs from an earlier data set[6] are best characterized as a rice flour rather than whole grain. To evaluate the effect of particle size, sample four in Table 1 was milled to a fine powder and gastrointestinally extracted. An extraction efficiency of 76% $\pm$ 12 ($\bar{x} \pm 2\sigma$) for this powder suggests that particle size alone does not account for differences in extraction efficiency between the samples and the SRMs. The lower bioaccessibility associated with non-SRM samples was consistently within the 63–100% range for recovery of iAs from unmilled rice samples prepared in arsenic-contaminated water.[10] The low bioaccessibility estimates were also consistent with the 60% minimum bioavailibility of arsenic from rice reported by He et al.[18] In summary, the bioaccessibility data are limited and the need to correlate these data with bioavailability is essential; however, the authors see an inherent risk assessment need to move toward gastrointestinal *in vitro* assays as a means of estimating the species specific biologically relevant arsenic concentration in rice.

Speciation of the Bioaccessible Fraction of Arsenic in US Consumed Rice

Chemical-form-specific data for enzymatically extracted rice samples (using separation 1, Supplementary Table S1) are presented in columns 6 and 7 in Table 1. Results for NIST 1568 and NIST 1568a are included in the last two rows of Table 1. The high variability of the iAs to DMA ratio across samples is consistent with earlier reports.[8,15] However, these results differ from earlier reports in that DMA concentration is generally lower than iAs concentration.[6–8,14,15] This observation is supported by the across matrix average for iAs and DMA of 81 ng/g $\pm$ 67.7 and 41 ng/g $\pm$ 58.1 ($\bar{x} \pm 2\sigma$), respectively. The predominance of iAs in these samples cannot be attributed to analytical error as pre-extraction species-specific LFM data does not support a species-specific bias in the extraction or chromatographic components of the analyses. This ratio difference may reflect region-specific differences in iAs and DMA.[8,15,33] Despite this ratio difference, the across matrix iAs concentration of 81 ng/g $\pm$ 67.7 ($\bar{x} \pm 2\sigma$) was in good agreement with the literature values (Supplementary Table S2). Finally, from a As_{Tot} perspective, 11 of the 17 samples in Table 1 exceed the "global normal range"; however, from a bioaccessibility/speciation perspective, none of the samples exceeded the Ministry of Health in China (GB 2762-2005) limit of 0.150 μg iAs/g for rice.[46]

Biotransformation of DMA in Rice to DMTA by an *In Vitro* Cecal Content Assay

Besides iAs, the other major arsenic species present in US rice is DMA. Heitkemper[8] and Zavala[15] have described a trend for an increase in DMA concentrations with an increase in As_{Tot} in rice. Although DMA is less acutely toxic than iAs and possibly less bioavailable,[19] the DMA content may be toxicologically significant because it can be biotransformed to DMTA by the anaerobic microbiota of the mouse cecum.[22,23] One goal of this study was to

investigate whether DMA released from rice by gastrointestinal extraction also underwent metabolic conversion to DMTA. A milled rice flour containing 900 ng/g DMA was used to assure suitable arsenic concentrations for detection of metabolites produced in the *in vitro* cecal content assay. Figure 2 shows three LC-ICP-MS mass chromatograms (*m/z* 75) from the rice enzymatic extract/cecal content incubation experiments. Each mass chromatogram indicates that DMA extracted from the rice was converted to DMTA by the anaerobic microbiota. Significant conversion of DMA to DMTA occurred at the beginning of the incubation period (Figure 2), which is consistent with other time course data.[22] The second mass chromatogram in Figure 2 shows that additional conversion was observed after 24 h of incubation. The third mass chromatogram verifies a retention time match between a standard and the DMTA in the sample. Because the DMTA present in the *in vitro* assay was well below the detection limit associated with ESI-MS, a second chromatographic separation (Supplementary Figure S1) was used to confirm the presence of DMTA using elemental-based detection. Presence of the dithiolated arsenical (dimethyldithioarsinic acid) DMDTA was not observed, although this could be due to the low concentration of the precursor. These data indicate that some of the DMA ingested in rice is likely converted to DMTA before systemic uptake.

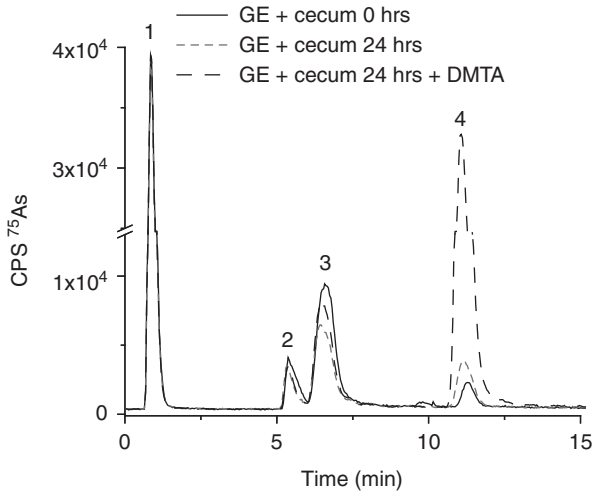

Figure 2. Mass chromatograms of *m/z* 75, which confirm the conversion of DMA in a gastrointestinal rice extract to DMTA using an *in vitro* cecum content assay. Peak identification: 1-flow injection marker; 2-iAs; 3-DMA; 4-DMTA. Chromatographic conditions for separation 2 are outlined in Table S1. The DMTA concentration in the GE + cecum 0 hrs sample was 1.1ng/g DMTA using DMA as the primary standard. GE, gastrointestinal extract.

Exposure Modeling of Arsenic in Rice

An overarching goal of this study was to advance exposure assessment for arsenic in rice by combining speciation-based bioaccessibility estimates for iAs in US consumed rice with data on the distribution of consumption patterns for rice within the context of an exposure assessment model. The speciation-based bioaccessible iAs distribution for US consumed rice used in the model was estimated by the across matrix average (81 ng/g $\pm$ 67.7, $\bar{x} \pm 2\sigma$) reported in Table 1. Distribution of consumption patterns was estimated using the data from WWEIA two-day dietary surveys. Supplementary Figure S2 is a plot of rice consumption from WWEIA as a function of population percentiles. The SHEDS dietary module then utilizes rice consumption from WWEIA together with a distribution of iAs concentrations in rice with Monte Carlo simulation to generate the arsenic exposure profiles.[29] Figure 3 summarizes the exposure estimates obtained from the SHEDS model as a function of population percentiles. Figure 3 indicates that 75% of the population was exposed to $<0.95\ \mu$g iAs per day from consuming ≤ 14.9 g of rice per day (Supplementary Table S3a), whereas 95% of the population was exposed to $<5.2\ \mu$giAs per day (Supplementary Table S3b) from consuming ≤ 84.6 g (Supplementary Table S3a) of rice per day. Similarly, 75% of the 1- to 2-year age group was exposed to $<0.34\ \mu$g iAs per day (Supplementary Table S3b) from consuming ≤ 5.4 g of rice per day (Supplementary Table S3a). The 1- to 2-year age groups have been included in Figure 3 to provide an exposure estimate for a more highly exposed population. Supplementary Table S3a and b provides consumption patterns and estimated exposures to iAs, respectively, from rice for other age-based subpopulations within the United States.

This species-specific exposure assessment modeling yielded improved assessment of the iAs concentration variability, which Meacher[27] and Yost[26] could not evaluate. How well this distribution reflected the actual distribution in the United States was a source of uncertainty in the model given the limited sampling, although most of the literature-based data summarized in Supplementary Table S2 support an iAs range in rice of 30–200 ng/g ($n = 148$). This range represents a modest increase in variability relative to the 81 ng/g $\pm$ 67.7 ($\bar{x} \pm 2\sigma$) reported in Table 1. Indeed, some of the negative bias observed between literature values and data in Table 1 can be accounted for by the inclusion of the bioaccessible iAs estimate. Therefore, the smaller sample set ($n = 17$) used in this study adequately approximates the range of concentrations available to the US consumer when compared with the larger literature-based estimates ($n = 148$, Supplementary Table S2). Ultimately, the quality of this distribution estimate needs to be balanced with the value added by estimating the bioaccessible component of the exposure. The *in vitro* bioaccesssibility assay adopted by the authors adds this

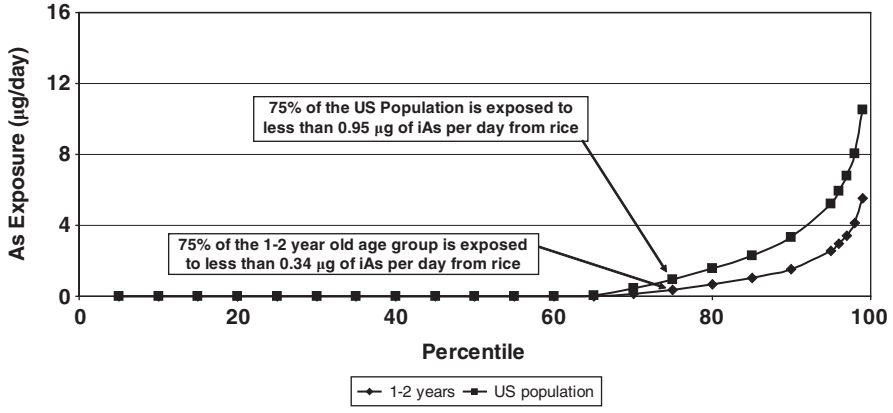

Figure 3. Cumulative density function of iAs exposure for the US population as a whole and for a 1- to 2-year-old sub-population.

value but the higher cost associated with this analysis ultimately limits the sample size that is affordable. Finally, the low-end boundary of the bias was likely better estimated by He et al.[18] as they report a minimum bioavailability of 60% in humans ingesting rice. Certainly, this type of data begins to address the bioavailability uncertainty identified by Yost[26] and Meacher[27] but from a larger modeling perspective, the uncertainties associated with bioavailability are likely secondary to the limitations induced by extrapolating the two-day survey data to estimate long-term (1 month, 1 year, a life time) chronic exposures. The problem is that the two-day survey may capture episodic consumption patterns for non-staple dietary sources, which are not characteristic of long term-exposures; thus, leading to an overestimation of the tails of the consumption rate distribution. For instance, an individual may consume rice on both days of the survey but that individual may not eat rice for the next week or two. Meacher[27] and Yost[26] both acknowledged this limitation especially in the context of the uncertainty in estimating the chronic exposures associated with the upper percentile consumers. Given this inherent limitation, Meacher[27] reported a mean dietary exposure of 2.75 μg iAs per day and 3.56 μg iAs per day for women and men, respectively, while Yost[26] reported an intake of 3.2 μg iAs per day for children 1–6 years of age. The mean estimate for rice in this study for the US population as a whole was 0.98 μg iAs per day and 0.48 μg iAs per day for the 1- to 2-year-old age groups (Supplementary Table S3b). A direct comparison across studies would indicate that between 18 and 33% of the iAs in the US diet comes from rice. This comparison is partly corroborated by earlier work,[26] which estimates that rice represents about 20% of the iAs exposure for the mean consumer in the age group of 1–6 years of age. Xue et al.[37] also estimates that 17% of the total iAs comes from rice resulting in a 0.6 μg per day mean exposure from rice. All of these comparisons have been made based on estimates of the mean because a 2-day survey over estimates the tails in the consumption distribution. This highlights a source of uncertainty and indicates the need for a longitudinal rice consumption study. Finally, if the uncertainties discussed above are all considered acceptable and a mean 0.98 μg iAs per day is used for the US population as a whole, then the risk from this exposure could be placed in a context by comparing it to the risk estimates associated with the 10 ppb drinking water MCL for iAs.[47]

In summary, better estimates of dietary exposure to iAs are needed for many populations worldwide. The challenges in exposure assessment begin with the need to determine the spectrum of arsenicals present in rice and other foods. It extends to include variations in bioavailability, and the susceptibility of arsenic to undergo metabolic transformation. Better estimates of long-term patterns of ingestion of arsenic-containing foods are also needed to improve the predictive value of models. In this context, this manuscript attempts to move toward a risk predictive understanding and from this, to a risk predictive model.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The United States Environmental Protection Agency through its Office of Research and Development funded and managed the research described here. It has been subjected to the Agency's administrative review and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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Supplementary Information accompanies the paper on the Journal of Exposure Science and Environmental Epidemiology website (http://www.nature.com/jes)