ESTIMATING RELATIONSHIPS BETWEEN ARSENIC EXPOSURE THROUGH RICE
CONSUMPTION AND DISEASE

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ABSTRACT

Andrew Ethan Yosim: Estimating Relationships Between Arsenic Exposure Through Rice
Consumption and Disease
(Under the direction of Rebecca C. Fry)

Arsenic (As) is a carcinogen and developmental toxicant with significant detrimental health
outcomes associated with early life exposure. Until recently, the potential for exposure to As via
food in the US was considered minimal. Recent data suggest that rice may contain elevated
levels of inorganic arsenic (iAs) and dimethylarsinic acid (DMA), which are associated with
adverse health outcomes. In order to assess whether rice consumption could introduce exposure
at levels of As associated with disease, As levels in 1,343 rice-based products from the FDA
were analyzed and compared to three constructed daily exposure models. The majority of
samples had elevated levels of As and an average single serving of rice, and many rice-products,
either meets or exceeds a child's daily modeled exposure threshold. Estimates suggest average
lifetime rice consumption equates to exposure levels of As known to be associated with elevated
risk for lung and bladder cancer.

To my grandfather, whose love of science was contagious.

To my family, who have always supported and encouraged my pursuit of knowledge.

To my fiancée, whose love allows me to "…wake at dawn with a winged heart and give thanks for another day of loving…"

To my mentor, who stoked my curiosity and challenged me to continually discover.

ACKNOWLEDGEMENTS

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LIST OF ABBREVIATIONS

ACGIH   American Conference of Industrial Hygienists

As   Arsenic

AS3MT   Arsenic (+3 oxidation state) methyltransferase

ATSDR   Agency for Toxic Substances and Disease Registry

IARC   International Agency for Research on Cancer

iAs   Inorganic arsenic

BEI   Biological exposure index

BW   Body weight

CDC   Centers for Disease Control and Prevention

CSF   Cancer slope factor

DMA   DMA(III) + DMA(V)

DMA(III)   Dimethylarsinous acid

DMA(V)   Dimethylarsinic acid

EPA   Environmental Protection Agency

FDA   Food and Drug Administration

MCL   Maximum contaminant level

MMA   MMA(V)+MMA(III)

MMA(III)   Monomethylarsonous acid

MMA(V)   Monomethylarsonic acid

MOA   Mode of action

MRL   Minimal risk level

NAS   National Academy of Sciences

NHANES	National Health and Nutritional Examination Study
ppb	Parts per billion
RfD	Reference dose
SAM	*S*-adenosyl methionine
SD	Standard deviation
TMA(III)	Trimethylarsine
TMAO(V)	Trimethylarsine oxide
U-tAs	Urinary total arsenic
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

Research assessing the arsenic (As) content of foods consumed within the United States has increased markedly in the last several years (Cleland et al. 2009, Gilbert-Diamond et al. 2011a, Davis et al. 2012, Jackson et al. 2012). Recent articles in the popular media (Bloom 2014, Reports 2014) have raised concern over the potential presence of As, classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC 2012), in commonly consumed food products. In order to reduce chronic exposure to the toxic metalloid, in 2001, the Environmental Protection Agency (EPA) set a maximum contaminant level (MCL) of 10 μg/L for the total As (tAs) content of publically supplied drinking water in the United States. However, while the As content of drinking water is currently regulated, the As levels in solid foods are currently unregulated.

Arsenic has been detected in foods in both organic and inorganic forms. Relative to organic arsenicals, iAs is often regarded as the more toxic species and has been associated with both cancer endpoints (Sohel et al. 2009) as well as non-cancer endpoints such as cardiovascular disease and neurological effects (States et al. 2009). The mode of action (MOA) of iAs is complex and likely multi-factorial. Some of the strongest experimental evidence indicates that mechanisms such as enzyme inhibition, altered DNA repair, the generation of oxidative stress (Alarifi et al. 2013), epigenetic modifications (Ren et al. 2010), and the induction of genotoxic damage such as chromosomal aberrations are major factors contributing to iAs toxicity (as reviewed in Hughes, Beck et al. 2011, Naujokas, Anderson et al. 2013). The varied deleterious effects associated with

iAs exposure are likely the result of the combined effects of mechanisms such as these, which may be interacting in one or more synergistic and/or causal relationships. The most prevalent route of As exposure worldwide is through the consumption of As-laden drinking water, although individuals may also be exposed to As through other sources including food, air, and soil (Meacher et al. 2002).

Adverse human health effects of long-term exposure to high levels of iAs in drinking water are well documented in areas such as Bangladesh and West Bengal, India (Chowdhury et al. 2000). Populations in areas such as these are routinely exposed to iAs in drinking water at levels much higher than national or World Health Organization (WHO) guidelines (WHO 2008). For example, iAs levels up to 2500 µg/L have been detected in the groundwater of Bangladesh, which exceed Bangladeshi national guidelines of 50 µg/L by 50-fold (Nordstrom 2002). Although levels of exposure to As in drinking water are generally not as high as those observed in Bangladesh, high level exposure is observed in the United States, as many private wells used for drinking water have iAs levels that exceed EPA limits (Sanders et al. 2012).

Through a series of reduction and oxidative methylation reactions, iAs can be metabolized to mono-, di-, and trimethylated forms such as the pentavalent species, dimethylarsinic acid [DMA(V)], monomethylarsonic acid [MMA(V)], trimethylarsine oxide [TMAO(V)] and the trivalent species dimethylarsinous acid [DMA(III)], and monomethylarsonous acid [MMA(III)]. The pharmacokinetics of human As metabolism are not fully understood (NRC 1999). The metabolism of iAs into mono- and dimethylated forms occurs primarily in the liver as well as the kidneys, lung, and testes (Healy et al. 1997), although the site of methylation is heavily dependent on the conversion rate of arsenate to arsenite (EPA 2010). At these sites, the primary enzyme arsenic-(+3 oxidation state)-methyltransferase (AS3MT) is responsible for the transfer of a methyl

group from S-adenosyl methionine (SAM) to As substrates. In humans, the pentavalent species MMA(V) and DMA(V) are readily excreted through the urine or may be further metabolized to form the trivalent species (NRC 1999).

Arsenic metabolism was widely believed to act as a detoxifying process, such that each successive metabolite product would be less toxic than iAs. However, in the last fifteen years, evidence has emerged that the toxicity of some of the metabolites may be of increasing concern, as forms of the trivalent arsenicals DMA(III) and MMA (III) have been shown to be more cytotoxic in primary human hepatocyte, epidermal keratinocytes, bronchial epithelial, and UROtsa cells compared to iAs treatment (Styblo et al. 2000). In addition, while research into the health effects associated with As metabolites is sparse, DMA(V) and MMA(V) are classified as Group 2B carcinogens (IARC 2012), and recent work suggests that individuals with higher levels of MMA(III) or DMA(III) in exfoliated urothelial cells have increased risk for diabetes (Currier et al. 2014). Although the majority of the present work focuses on forms of As whose risk is well characterized such as tAs or iAs, levels of the metabolites DMA and MMA in rice products are presented and may significantly contribute to an individual's later-life health outcomes.

In areas where exposure via water, soil, or air is low, food becomes the largest source of As exposure. Foods potentially high in As include shellfish, grains, fruit and their juices (Xue et al. 2010). For example, following growing concerns of excessive As exposure in children, the Food and Drug Administration (FDA) proposed an action level of 10 μg/L for the iAs content of apple juice (FDA 2013b). While shellfish is a major source of As, much of the As in shellfish is found in organic forms such as arsenobetaine and is comprised of relatively little iAs (as reviewed in (Schoof et al. 1999, Adair et al. 2005).

Rice has been implicated as a major dietary source of As due to its uptake of As from contaminated soil, ground, and surface water (Meharg 2004, Norton et al. 2012). Unlike many other food staples that are grown under aerobic conditions, flooded rice paddy fields provide anaerobic conditions in which reduced forms of As may be incorporated into the growing rice (Xu et al. 2008, Su et al. 2010). Under these conditions silicon transporters readily transport iAs and organic metabolites, which act as silicic acid analogues (Ma et al. 2008, Li et al. 2009), while the phosphate analogues, arsenate and deprotonated DMA are transported via phosphate transporters (Meharg et al. 2012). The incorporated arsenicals predominantly accumulate in the hull of rice through a variety of mechanisms such as xylem transport, phloem transport, and sequestration of As-thiol complexes (Zhao et al. 2009, Tuli et al. 2010). The rate and extent to which arsenicals are incorporated into rice plants are influenced by both inter-species and inter-subspecies differences in As transport and binding (Abedin et al. 2002a, Abedin et al. 2002b, Williams et al. 2005, Liu et al. 2006, Ma et al. 2008), as well as concentrations of silicon, phosphate, heavy metals, and other minerals (Abedin et al. 2002b, Chen et al. 2005, Guo et al. 2007, Lihong et al. 2009, Zhang et al. 2011). While the mineral content of the soil and redox conditions of the rice paddy govern arsenical transport, rice predominately accumulates iAs based on its proclivity to bind iAs at higher rates than organic arsenicals (Abedin et al. 2002b) and the lack of affinity in binding to iron oxyhydroxides to form immobile forms (Meharg et al. 2012).

In addition to rice plants directly transporting and accumulating iAs and organic As species, arsenicals can be directly methylated at the rhizosphere and *in planta*. which may result in higher levels of iAs and DMA in the grain or husk (Williams et al. 2005, Tuli et al. 2010). Arsenate present in the soil or contaminated groundwater may be reduced via arsenate reductase by a number of soil-based microbes proximal to growing rice (Xu et al. 2007), and *in vitro* studies

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suggest reduction *in planta* via the actions of homologues of the protein ACR2 (OsACR2;1 and OsACR2;2) in both the roots and shoots of the plant (Duan et al. 2007). In addition, genome-wide analysis from two different rice varieties found up regulation of three genes annotated as methyltransferases in the roots of the plant (Norton et al. 2008). At present, additional studies are needed to further elucidate the locations of As methylation in rice.

While the average US citizen consumes approximately 12 kilograms of rice a year (Batres-Marquez et al. 2009, Rice 2009), certain sub-populations may be more vulnerable to iAs exposure; ethnicities who routinely consume large amounts of rice or individuals who consume rice as a dietary alternative. For example, many individuals on low gluten diets or those suffering from celiac disease routinely consume large quantities of rice or rice-based products (Munera-Picazo et al. 2014a, Munera-Picazo et al. 2014b). Rice with a high iAs content can act as an exposure vehicle, as iAs remains largely unmodified and bioavailable to absorption even after rice has been harvested and cooked (Laparra et al. 2005). Children and infants may be at particular risk for iAs exposure (Xu et al. 2008) as in comparison to adults, they often consume larger quantities of fruits and rice products proportional to their body weight (Yost et al. 2004). In addition, recent work has revealed that infants may be exposed to high levels of iAs through As-rich formulas and infant foods (Carbonell-Barrachina et al. 2012, Jackson et al. 2012).

Exposure to elevated levels of iAs can be particularly harmful during early childhood development (Steinmaus et al. 2013) and several adverse health effects in adults are associated with early childhood exposure, including both increased incidence of cancers, diabetes, and cardiovascular disease, as well as increase mortality associated with cancers, bronchiectasis, and cardiovascular disease (Tokar et al. 2011, Smith et al. 2012). Our lab along with others has shown that iAs exposure is associated with epigenetic alterations (Smeester et al. 2011, Bailey et al. 2013,

Bailey et al. 2014, Rager et al. 2014). Importantly, alterations to the genome and epigenome during critical developmental periods have been proposed as a plausible link between environmental toxicant exposure and later-life health complications (Barouki et al. 2012).

In 2011, and again in 2013, the FDA released the results of testing performed on various rice-containing food products available for purchase within the United States. Currently, this is the largest dataset available on the speciated As content in food to date. In the present work, the results of these tests were analyzed for statistical trends and patterns. A total of 1,343 rice-based food samples were analyzed, including 487 samples of commercially available rice. All of the tested rice samples with quantifiable data exceed the EPA's limit of 10 µg/L for As in drinking water, the only available standard against which to judge the samples.

While the EPA enforces a drinking water limit of 10 µg/L of As, there are currently no federal guidelines that regulate As levels in food. In July of 2013, the FDA proposed an action level of 10 µg/L iAs for apple juice, although no current recommendations have been made for other juices or foods (FDA 2013b). In order to assess the possibility of elevated As exposure associated with rice or food products containing rice, daily exposure thresholds extrapolated from the EPA water MCL were constructed resulting in estimates of 0.68-1.00 µg As/kg bodyweight per day. Results from the analysis of the FDA records were compared against these models to determine the potential for As exposure across different age groups. The results were also compared against current risk estimates of As in drinking water to determine if chronic consumption of rice or rice-based products could contribute to As exposure at levels associated with the development of disease. These data highlight that for children, consumption of a single serving of rice or of many rice-based products could exceed their estimated daily exposure threshold and significantly contribute to As exposure at levels that may be associated with later-

life adverse health effects. Additionally, chronic consumption of rice consistent with national averages is associated with increased risks for lung and bladder cancer and may significantly contribute to the nation's burden of disease.

CHAPTER 2: METHODS

Database generation

Concentrations of tAs, iAs, MMA [MMA(V)+(MMA(III)], and DMA [DMA(V)+(DMA(III)] in 1,343 rice and rice-products were obtained from the FDA website (FDA 2011). Of the 1,343 rice and rice-product records, 1,275 contained quantifiable data (94.9%). Of the 487 records that dealt with rice, one lacked a numerical value and was excluded from further analysis. Subcategories of rice for study were designated as "basmati rice", "white, short grain rice", "white, medium grain rice", "white, long grain rice", "brown rice", "jasmine rice", "parboiled rice", and "instant rice" by the inclusion of the aforementioned words in their description. Categories for rice products were designated as "mixes and pudding", "beverages", "cereals", "cookies", "dietary supplements", "grain-based bars", "pasta", and "snacks", with subcategories of these products designated as "rice cakes", "hot/ready to eat cereal", and "infant and toddler cereal".

Determination of extrapolated upper As exposure models

Using the results from the previous analysis concerning the tAs content of rice and rice products, three exposure models were constructed to determine whether a single serving of rice could significantly contribute to or exceed a modeled threshold of As in children.

The first model was based on an extrapolated calculation of As exposure consistent with the EPA's limit of As in drinking water   The limit of 10 μg/L As was multiplied by an individual's assumed water intake (2L) to determine an upper daily intake of As due to water consumption. This upper limit of 20 μg As was then extrapolated to all sources of As exposure based on the average daily proportion of As exposure associated with water consumption. Estimates suggest that water contributes 42.2% to 45.3% of exposure in men and women, respectively (Meacher et al. 2002). Using the more conservative figure of 42.2% for women, the total potential exposure to As consistent with the current water guidelines suggests that an individual's upper threshold of tAs exposure is 47.39 μg/day. This value was divided by the body weight of an adult to determine the upper threshold of As per kg of body weight per day. Utilizing the EPA's own weight assumptions of 70 kg for an adult male, an upper threshold of 0.68 μg As/kg per day was determined. This model assumes consistent proportions of exposure from water and food for different age groups, which is supported by previous findings that the contribution of tAs by food group does not change significantly after age six (Yost et al. 2004). Age-specific As thresholds were calculated by multiplying the lower derived threshold of 0.68μg As/kg/day by the average weight of both male and female children at each time point as determined by childhood anthropometric reference data collected from the National Health and Nutrition Examination Survey (NHANES) (CDC 2012).

Additional models were created to reflect values of As exposure associated with food consumption that had been utilized by the EPA's risk assessment process for sensitivity analysis. As with the first model, consumption of two liters of water per day was assumed (one liter from cooking and one liter from drinking). Values for dietary intake of As were estimated by the EPA at both 30 and 50 μg/day, which were combined with the 20 μg of As in water to produce modeled

daily thresholds of 50 and 70 µg As. The model did not consider any significant exposure from other sources or uncertainty factors, so such values were not included in the three analyses. These values were once again divided by the average weight of an individual (70kg) to produce thresholds of 0.71 and 1.00 µg As/kg bodyweight per day.

Estimation of cancer incidence and mortality

To determine whether levels of As found in rice may be associated with increased incidence of cancer, As levels associated with average daily rice consumption were multiplied by an incremental lifetime cancer risk derived from an EPA oral cancer slope value for As.

Equation 1. Incremental cancer risk (per µg/kg) = CSF (mg/kg-day) × 0.001 ÷ BW

where: CSF = oral cancer slope factor; 16.9 and 25.7 for males and females, respectively

     0.001 = conversion from milligrams to micrograms

     BW = body-weight standard of 70 kg utilized by the EPA

The average American consumes 11.8 kg of dry rice per year, which is equivalent to a daily average consumption of 32.3 grams. The 32.3 g of rice was multiplied by the values for the average tAs (217 µg/kg) and highest tAs content (854 µg/kg), henceforth referred to as "mean rice" and "high rice," respectively. Based on the calculations, an average daily single serving of mean rice yields an exposure of 7.0 µg As, while high rice yields a daily exposure of 27.6 µg As. The As values of 7.0 and 27.6 µg were then multiplied by the calculated incremental cancer incidence risk values for men (4.8×10^{-4} µg/kg) and women (7.3×10^{-4} µg/kg) to determine the excess incidence of cancer associated with an average lifetime-consumption of rice containing either calculated mean or high As levels (NRC 1999, EPA 2010). Results from this analysis were

compared to lifetime cancer risks for various chemicals regulated in drinking water by the EPA (Appendix 4).

A secondary analysis was carried out to compare these estimated daily exposure values corresponding to mean and high tAs rice (7.0 and 27.6 µg As) to a National Academy of Science's (NAS) estimation of the lifetime risk of dying from cancer due to contaminated drinking water (NRC 1999). The estimates from NAS utilized a linear dose-response with no biological threshold, so levels of As in drinking water were divided by two to account for the NAS's underlying assumption of two liters of water consumed per day.

Estimation of urinary excretion of arsenic

Levels of total urinary arsenic (U-tAs), defined as the sum of the arsenicals As(III), MMA(III), MMA(V), DMA(III), and DMA(V), were estimated for an individual consuming a daily national average quantity of rice (7.0 and 27.6 µg As for mean- and high-As rice, respectively). Due to large inter-individual differences in As metabolism and excretion, several coefficient values were selected to estimate the conversion between As consumption and levels of U-tAs. Coefficient values of 0.4, 0.5, 1, 2, and 3 were selected based on reported and calculated coefficients in populations with low As exposure (Biggs et al. 1997, Kurttio et al. 1998, Meza et al. 2004) (Appendix Table S1).

Estimation of in utero exposure

To determine whether the developing fetus might be exposed to harmful levels of As during pregnancy, previously estimated levels of U-tAs associated with rice consumption of mean or high tAs rice (7.0 and 27.6 µg As) were converted into estimates of maternal blood As levels based on

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simultaneous measurements of As in maternal urine and blood. In order to convert levels of U-tAs to corresponding estimates of maternal blood As levels, conversion factors of 0.03 and 0.09 were utilized based on calculated coefficients from moderately exposed populations (Concha et al. 1998, Hall et al. 2007). Because As readily crosses the placenta, levels of As in cord blood were assumed to be equal to levels in maternal blood based on strong supporting evidence (Concha et al. 1998, Hall et al. 2007, Rudge et al. 2009).

Comparison to Biological Exposure Index, Minimal Risk Level, and Chronic Oral Reference Dose

Estimated levels of U-tAs for rice with mean and high tAs content (7.0 and 27.6 μg As) were compared against the Biological Exposure Index (BEI) level of 35 μg As/L (iAs+MMA+DMA) in urine set by the American Conference of Industrial Hygienists (ACGIH). This value represents an upper level of exposure that is unlikely to be associated with non-cancer health effects among occupationally exposed individuals.

In addition, modeled daily thresholds of As intake from the present work were compared against the minimal risk level (MRL) and chronic oral reference dose (RfD) of 0.0003 mg As/kg/day set by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA), respectively.

CHAPTER 3: RESULTS

As and metabolite levels in rice samples

The FDA dataset included a total of 487 rice samples with tAs ranging from 47 to 854 µg/kg (Table 1). Speciated testing revealed that the majority of the As content was inorganic, with a mean and standard deviation of 106 (49% of tAs) and 44 µg/kg, respectively. All of the tested samples with numerical data exceeded the EPA's 10 µg/kg limit for As in drinking water, with the highest sample representing more than eighty-five times the EPA limit. The rice sample with the lowest As content had 47 µg/kg, over four times the EPA's limit for drinking water. In addition, the rice contained relatively high levels of DMA, with a mean and standard deviation of 104 (48% of tAs) and 91 µg/kg, respectively. The majority of the samples contained low levels of MMA, as many were either below the limit of detection (3 µg/kg) or between the limit of detection and the limit of quantification (23 µg/kg). For those rice samples with quantifiable MMA values (21%), the mean MMA content and standard deviation were 6.2 and 4.7 µg/kg, respectively.

Table 1. Arsenic levels in 487 rice samples. Non numerical values, as those below the limit of detection (3 µg/kg) as well as between the limit of detection and the limit of quantification (23 µg/kg) were excluded from the analysis.   Data obtained from the FDA (FDA 2011).

	tAs[a] (µg/kg)	iAs[b] (µg/kg)	DMA[c] (µg/kg)	MMA[d] (µg/kg)	iAs per Serving (45g)
Average	217	106	104	6	5
Min	47	20	10	3	1
Median	199	99	87	4	4
Max	854	249	687	25	11
SD	118	44	91	5	2

[a] tAs refers to total arsenic [iAs + DMA(III) + DMA(V) + MMA(III) + MMA(V)]
[b] iAs refers to inorganic arsenic
[c] DMA refers to dimethylarsenic acid and dimethylarsinous acid
[d] MMA refers to monomethylarsonic acid and monomethylarsonous acid

As and metabolite levels in rice by country of origin

Among the rice samples, the mean iAs concentration was higher for products originating in the United States (N=439, mean=109 µg/kg) compared to India (N=34, mean=71 µg/kg) and, within the United States, rice samples from Texas contained the highest levels of iAs (N=41, mean=124µg/kg) (Table 2). Levels of DMA were over four times higher in rice grown in the United States compared to India (108 and 26 µg/kg, respectively), although there was much variability within United States-based rice, as the DMA content of Texas-based rice (266 µg/kg) was six times greater than the DMA content of rice packaged in California (40 µg/kg). However, caution should be taken in interpreting these results, because the country or state of origin for each rice sample is reflective of the origin-of-distribution information provided on each packaging label and may not accurately reflect the country or state where the rice was actually grown.

Table 2. Average tAs and arsenical levels by state or country of origin. Country or state of origin refers to information on the packaging label and may not accurately reflect where the rice was grown. Data obtained from the FDA (FDA 2011).

	Arkansas (μg/kg)	California (μg/kg)	Texas (μg/kg)	Louisiana (μg/kg)	US National Average (μg/kg)	India (μg/kg)
tAs	207	130	428	235	226	102
iAs	101	88	124	105	109	71
DMA	96	40	266	121	108	26
MMA	3.7	1.9	10.3	4.1	4.9	3.0

As and metabolite levels in rice species and products

Brown rice was found to have the highest iAs content, with over twice the iAs content of basmati rice and one and a half times the iAs content of white rice (Figure 1). Samples of both short- and medium-grained rice had similar iAs levels as basmati rice, although long-grained white rice had approximately 20% more iAs than all three. Rice snacks and cereals had similar levels of tAs, iAs, and DMA, and were comparable to white rice in tAs and iAs levels (Appendix Figure 1). Rice cakes had the highest levels of both tAs and DMA of any rice or rice-based product, and had iAs levels comparable to those in brown rice. Rice-based beverages had the lowest levels of any of the arsenicals, although this is likely due to the low amounts of rice within each product. Of particular concern, levels of tAs, iAs, and DMA were high in infant and toddler cereals, consistent with previously published results (Carbonell-Barrachina et al. 2012, Jackson et al. 2012).
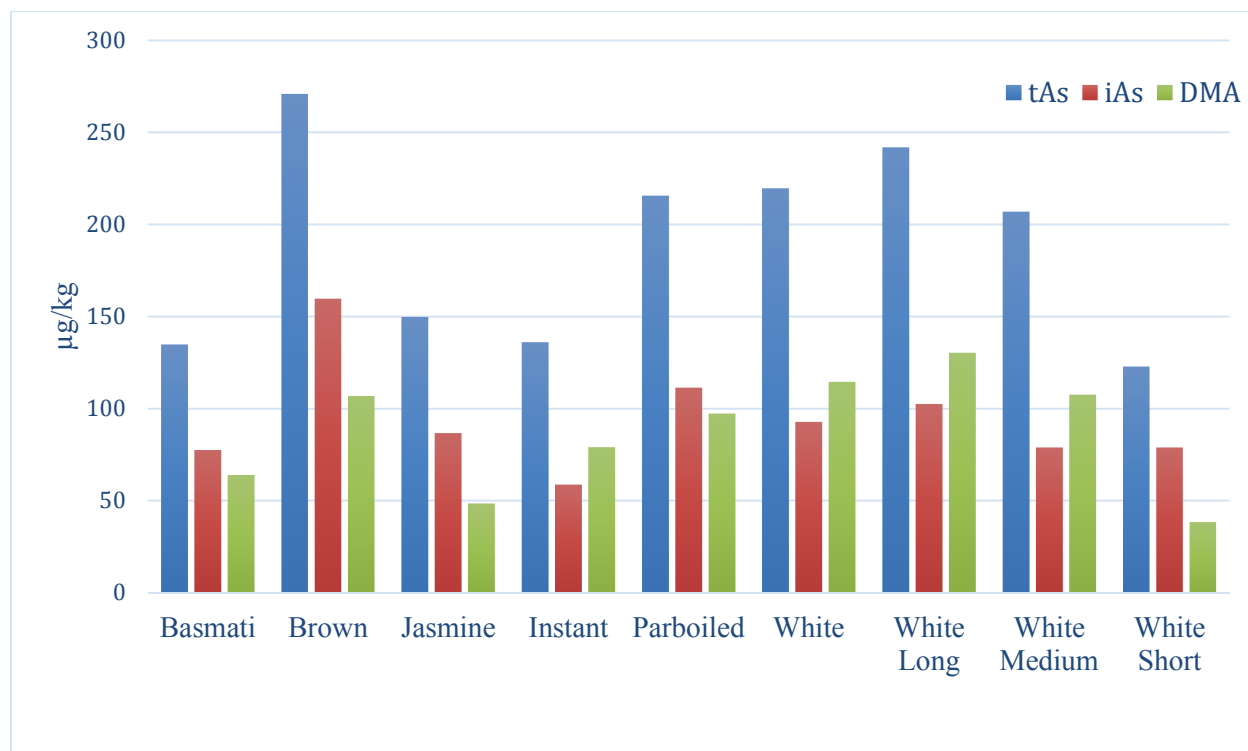
Figure 1. Mean levels of tAs, iAs, and DMA in different rice varieties. Data obtained from the FDA (FDA 2011) for basmati (N=51), brown (N=99), jasmine (N=13), instant (N=14), parboiled (N=34), white (N=251), white long (N=147), white medium (N=81), and white short (N=81).

Modeled daily As maximum allowable dose

Three models were constructed to estimate age-specific As daily thresholds. The first model extrapolated a daily threshold based on the EPA's MCL for As in drinking water and the proportion of As exposure from drinking water. The second and third models were constructed based on food-associated As values selected by the EPA for sensitivity analysis. Safe consumption thresholds of 0.68, 0.71, and 1.0 µg As/kg/day were determined. Until age 16, female daily As thresholds differed from male thresholds by no more than 3%, so all subsequent analysis were performed using female daily As thresholds (Appendix Figure 2).

As expected, results between modeled thresholds of 0.68 and 0.71 µg As/kg/day were comparable, and a similar trend was noted for a threshold of 1.0 µg As/kg/day. Using the modeled

thresholds of 0.68 and 0.71 µg As/kg/day, consumption of a single serving of mean-tAs rice will contribute at least 50% to this modeled threshold for children under eight years of age (or under four years of age using a modeled threshold of 1.0 µg As/kg/day). Based on modeled thresholds of 0.68 and 0.71 µg As/kg/day, consumption of a single serving of rice with high tAs content (27.6 µg As) by children under 14 years old will likely exceed the modeled daily thresholds, and consumption of this rice will exceed 50% of the daily thresholds at any age (Figure 2).

Figure 2. Age-specific As exposure from a serving of rice or a rice product with the mean tAs content or high tAs content (27.6 µg As) for (A) ages 1-10 and (B) ages 12-20+. Percent of modeled daily As threshold based on a threshold of 0.68 µg As/kg/day.
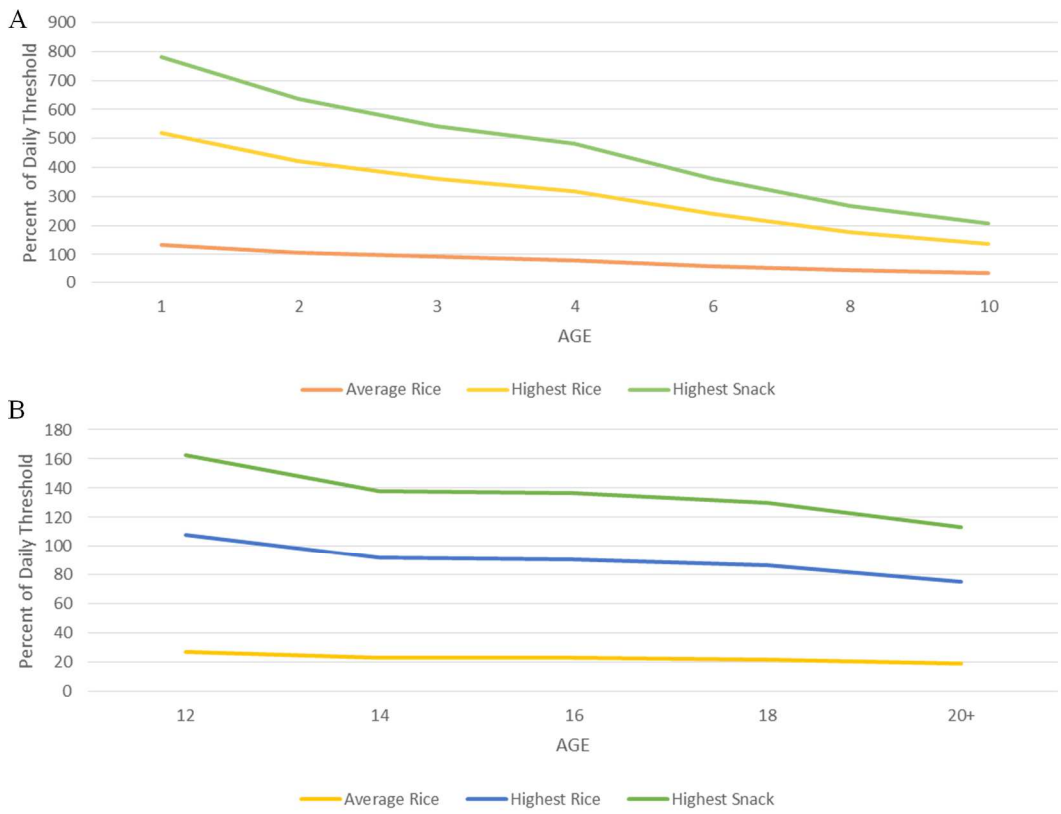
Estimation of excess cancer incidence and mortality in relationship to rice consumption

An estimation of excess cancer incidence associated with lifetime rice consumption was achieved by multiplying an individual's average daily rice consumption by levels of tAs found in

rice. Rice with mean tAs content (217 µg As/kg) *vs.* high tAs content (854 µg As/kg) yields a daily exposure of 7.0 *vs.* 27.6 µg As, respectively. These values were then multiplied by a unit risk value of 4.8×10^{-4} for males and $7.3 \times 10^{-4}/$ µg/kg for females, an estimation of excess cancer incidence attributed to each µg/kg As in consumed drinking water (NRC 1999). As a result, it is estimated that individuals consuming an average quantity of mean-tAs rice (217 µg As/kg) over a lifetime will experience an excess cancer incidence of 1.7×10^{-3} for males and 2.6×10^{-3} for females, or 1.7 and 2.6 in 1,000 individuals, respectively. These numbers equate to a lifetime cancer risk associated with exposure to 3.5 µg As/L in water. For those individuals consuming an average quantity of rice with high-tAs (854 µg As/kg), an excess cancer incidence of 6.6×10^{-3} for males and 1.0×10^{-2} for females, or 6.6 and 10 in 1000 individuals, respectively. These numbers equate to a lifetime cancer risk associated with exposure to 13.8 µg As/L in water. Among both males and females, average lifetime consumption of rice with mean (217 µg As /kg) and high tAs (854 µg As /kg) levels yields an excess cancer incidence of 4.2×10^{-3} and 1.7×10^{-2}, or 4.2 out of 1,000 and 1.7 out of 100, respectively (Figure 3).
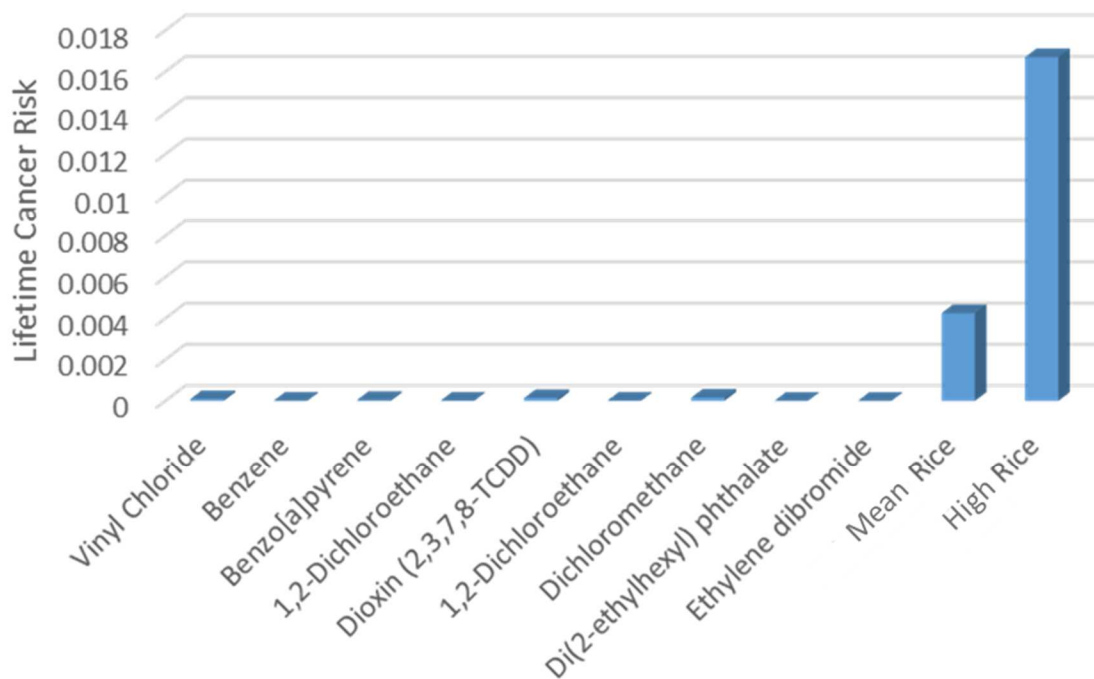
Figure 3. Lifetime risk of cancer for regulated drinking water contaminants and consumption of rice. For regulated chemicals, lifetime consumption of 2 L of water with levels of the contaminant equal to the EPA's MCL was assumed.

To estimate excess cancer mortality associated with the consumption of rice, daily As exposure values corresponding to mean- and high-As rice (7.0 and 27.6 µg As, respectively) were multiplied by a NAS estimation of the lifetime risk of dying from cancer associated with As level in drinking water. Lifetime consumption of drinking water containing 1 µg As/kg was estimated to contribute to a 1 in 5,000 risk of dying from cancer (NRC 1999). As the NAS model assumes linearity without a biological threshold, daily exposure values were multiplied by the 1 in 5,000 risk and divided by two to account for the drinking water assumption of 2 liters per day. As a result, average consumption of rice with mean (217 µg As/kg) and high tAs (854 µg As/kg) content yields a 1 in 1,426 and 1 in 362 risk of dying from cancer, respectively (Figure 4).
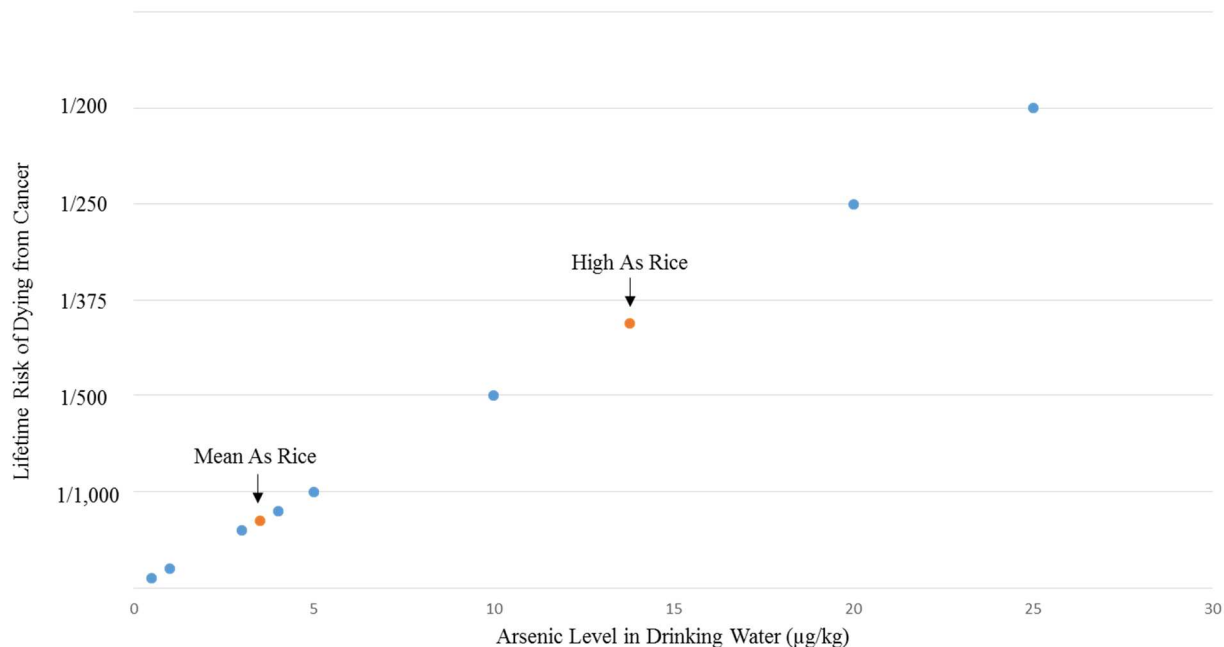
Figure 4. Estimated lifetime risk of dying from cancer associated with a lifetime daily average consumption of rice with mean and high tAs content (7.0 and 27.6 µg As, respectively) plotted against estimated lifetime risk of dying from cancer associated with drinking water with variable As content. Estimations of lifetime risk of dying from cancer for different As water standards were obtained from the NAS (NRC 1999).

Estimation of in U-tAs and in utero exposure

Values for rice with mean and high tAs content (7.0 and 27.6 µg As, respectively), were multiplied by the conversion factors 0.4, 0.5, 1, 2, and 3, representing the conversion of oral As exposure into estimated levels of U-tAs. U-tAs values ranging from 2.8-21 µg/L were determined for rice samples with an As content of 7.0 µg As, and values between 11 and 82.8 µg/L were determined for rice samples with an As content of 27.6 µg As.

These values were then multiplied by the conversion factors of 3 and 9%, reflecting an estimated conversion of U-tAs levels to maternal blood As levels. The conversion factors of 3 and 9% were obtained from studies in which populations were chronically exposed to low levels of As and U-tAs and blood As levels were measured simultaneously (Concha et al. 1998, Hall et al.

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2007). The estimated range of maternal blood As levels associated with consumption of rice with

mean tAs content ranged from 0.084-2.5 µg/L and the range associated with consumption of high-

tAs rice was 0.25-7.5 µg As/L. Because As levels in maternal blood are linearly associated with

levels in fetal cord blood (Concha et al. 1998, Rudge et al. 2009), cord blood levels of As were

assumed to be consistent with the measured maternal blood levels.

Comparison to BEI, MRL, and RfD

The range of predicted U-tAs associated with a single serving of rice with mean and high-

tAs content was 2.8-21 and 11-82.8 µg As/L, respectively. As a result, a urine sample taken after

consumption of a single serving of rice with high-tAs content may surpass the BEI level of 35

µg/L when a coefficient greater than one is utilized. While such a conversion factor may not be

applicable to the entire population, inter-individual differences in As metabolism suggest that in a

segment of the population urine As level could exceed the BEI value following consumption of a

single serving of high-tAs rice. In addition, it is likely that exposure to As from other sources

including other foods, beverages, and water may increase the likelihood of exceeding the BEI

value.

As expected, both the MRL and RfD values of 0.3 µg As/kg/day are lower than any of the

three daily exposure thresholds calculated in the present work (0.68, 0.71, 1.0 µg As/kg/day).

Unlike the MRL and RfD, the current MCL for As in drinking water does not include any safety

factors and the risk assessment process included considerations of the cost that community water

suppliers might incur in reducing the MCL below 10 µg/L (EPA 2000). As a result, both the MRL

and RfD are more protective, and as a result, a single serving of rice will contribute significantly

more to an individual's modeled daily threshold. Children under the age of ten consuming a single

serving of rice with the mean tAs content will likely exceed the MRL and RfD while U-tAs levels of individuals at any age consuming a single serving of rice with high tAs content are likely to exceed the MRL and RfD level (Figure 5).

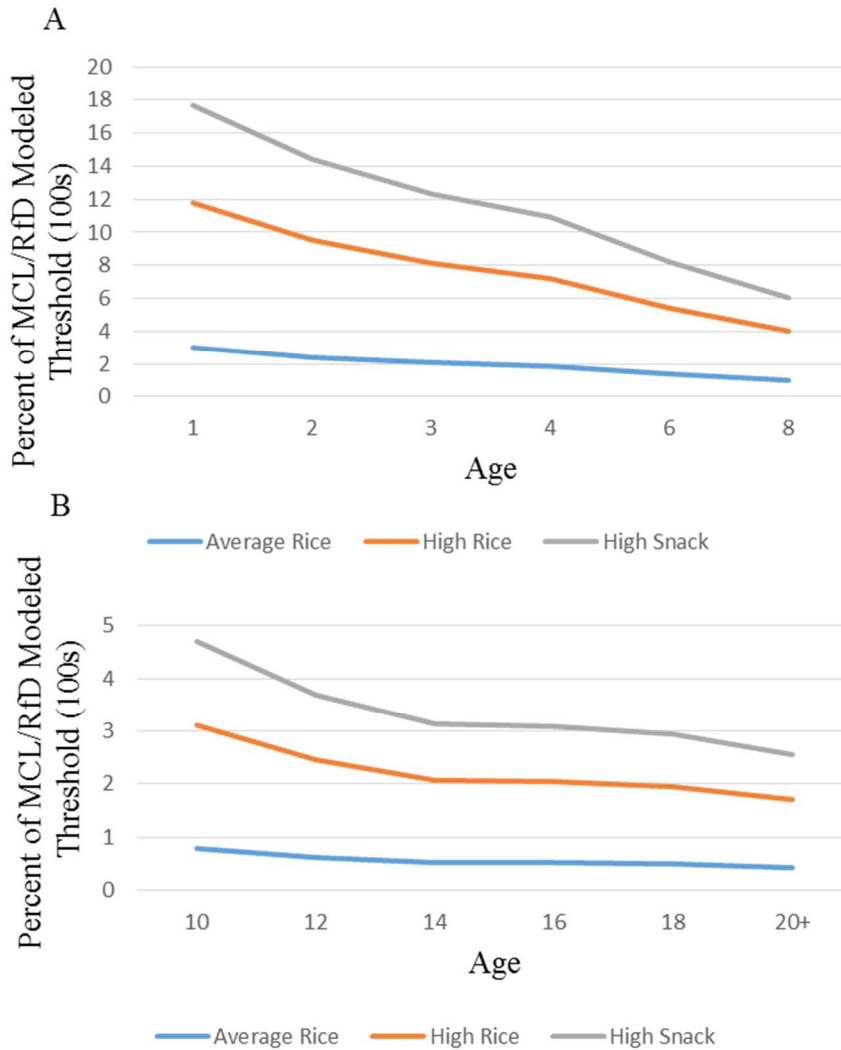**Figure 5.** Age-specific As exposure for (A) ages 1-8 and (B) ages 10-20+ from either a single serving of rice with mean (217 µg As/kg) or high tAs (854 µg As/kg) or a rice snack with high tAs (1931 µg As/kg) content based on the MRL and RfD for As.

CHAPTER 4: DISCUSSION

Because iAs is an IARC Group 1 carcinogen and developmental toxicant, exposure to iAs from any source, including food, presents a serious health concern. Children are especially vulnerable to the health effects caused by exposure to iAs, as they consume more food proportional to their body weight than adults, are more sensitive to the adverse developmental effects of iAs (Davis et al. 2012), and can be at risk for long-term health effects (EPA 2013). While much research and legislation has gone into limiting As exposure from water, and now juices (FDA 2013), no such standardized action levels currently exist for solid foods. In the present work, speciated As testing performed by the FDA was analyzed to quantify levels of iAs and the metabolites DMA and MMA in rice and rice-products. The results were then compared against modeled age-specific daily thresholds to determine whether consumption of a single serving of rice or rice-based products could significantly contribute to an individual's tAs exposure. In addition, the tAs content of the rice-based samples was analyzed to determine if exposure to such products during childhood could significantly contribute to As exposure at levels associated with the development of disease.

Within the FDA's records, some of the rice cereals containing high tAs and iAs levels were infant cereals. Although there was not enough data about specific rice products to accurately quantify possible tAs and iAs exposure directly for infants, evidence suggests the potential for high levels of exposure through formula or baby food (Xu et al. 2008, Carbonell-Barrachina et al.

2012, Jackson et al. 2012). In addition, research has pointed to an increase in U-tAs among pregnant women consuming rice compared to pregnant women who did not report rice consumption (Gilbert-Diamond et al. 2011b). Based on the adverse health effects observed in infants before and after birth (Rahman et al. 2011, Kippler et al. 2012), levels of As in an infants or pregnant women's diet may play a critical role in the future development of disease. This is particularly worrisome, as As readily crosses the placenta, and cord blood As levels closely mirror levels found in the mother's whole blood (Concha et al. 1998, Rudge et al. 2009). While additional data would be needed to accurately estimate if the rice consumption of women differs from the general population during pregnancy, analysis of the As content of rice in the present work suggests that *in utero* exposure to As may be high, with estimated cord blood As levels of 0.084-2.5 µg/L and 0.25-7.5 µg/L associated with mean (7.0 µg As) and high tAs (27.6 µg As) consumption, respectively. However, additional studies are needed, as As in the blood is cleared quickly and, therefore, peripheral or cord blood As level may not be the best measure to estimate chronic *in utero* As exposure due to large fluctuations in measured As levels (Mann et al. 1996).

In addition to elevated exposures during the prenatal period, based on the results from the present study, it is likely that children who consume rice or rice products may be unwittingly exposed to high levels of tAs, specifically high levels of iAs and DMA. This is consistent with previous studies that have shown an increase in children's U-tAs content following consumption of rice (Davis et al. 2012). While the extent of exposure to iAs is not consistent between individuals due to differences in iAs metabolism (reviewed in (Bailey et al. 2013), younger individuals may be at increased risk of cancer as well as non-cancer health effects (Smith et al. 2012), as a child consuming a serving of tAs-rich rice will have a higher iAs exposure proportional to their body weight (reviewed in (Yost et al. 2004). This potential for elevated childhood exposure

is particularly worrisome, because the brain and nervous system of children continue to develop and are, therefore, particularly vulnerable to the effects of toxicants (reviewed in (Vahter 2008, IARC 2012).

When the FDA records were analyzed with regard to country of origin, trends were apparent. Rice originating in the US had higher tAs and iAs content, a finding both consistent with previous geographic distribution studies (Zavala et al. 2008, Meharg et al. 2009), and at odds with other studies that have shown that rice grown in Asia has higher iAs levels than rice grown in the US (Williams et al. 2005, Meharg et al. 2008). In addition, the results from the present study suggest that in US-grown rice contains equal amounts of iAs and DMA, a finding at odds with published research that suggests that As in US-grown rice tends to be primarily DMA, with a smaller percentage of iAs (Zhao 2013). In addition to country-specific trends, rice originating in Texas had higher levels of iAs, MMA, and DMA than rice in California, Louisiana, or Arkansas. However, caution should be taken when trying to draw any inferences from these seemingly contradictory results, as the state of origin may not correspond to the state where the rice was actually grown, and may help to explain why the results from the current work are not always consistent with other published studies.

In addition to trends in the country or state of origin, large differences in As content were observed between different varieties of rice. Brown rice contained higher levels of tAs and iAs than any other type of rice, while white-long grained rice had the highest level of DMA. White short rice had the lowest concentrations of every arsenical, although level of the arsenicals in basmati and jasmine rice were approximately half that of brown rice. These findings are consistent with previous published reports and are expected because rice predominately accumulates iAs and DMA in the hull of rice, which is removed in the dehulling process which produces varieties such

as polished white rice (Rahman et al. 2007). In addition, differences in arsenical concentrations may be due to inter-species and inter-genotype differences in which different rice varieties accumulate or metabolize As at different rates (Abedin et al. 2002a, Abedin et al. 2002b, Williams et al. 2005, Liu et al. 2006, Ma et al. 2008). In addition, the present of environmental factors such as concentrations of silicon or phosphate in the soil can affect the accumulation of arsenicals (Williams et al. 2005).

The three daily exposure thresholds calculated in the present work (0.68, 0.71, and 1.0 µg As/kg/day) were two to three times higher than the MRL and RfD value of 0.3 µg As/kg/day from the ATSDR and EPA. Although values utilized in the present work are based on extrapolations of the EPA's As drinking water standard and assumed food-based As values, it must be noted that the current EPA limit for As in drinking water includes results from feasibility studies assessing the economic cost to community water suppliers of reducing the level below 10 µg/L (EPA 2000). As a result, comparing the levels of As found in rice to the current MCL may not truly reflect a benchmark of levels of "safe" As exposure, but rather, simply a comparison to the current legal standard.

With regards to cancer endpoints, consumption of contaminated rice may be associated with high levels of cancer incidence and mortality. Based on an individual's average annual rice intake, consumption of rice over a lifetime containing the mean tAs content from the present analysis (7.0 µg As) is associated with an increased incidence of cancer (based on lung and bladder cancer estimates) of 420 out of 100,000 with a 1 in 1,426 lifetime cancer mortality risk. Individuals consuming this same quantity of rice over a lifetime with the high tAs content (27.6 µg As) would have a lifetime cancer incidence of 1,700 out of 100,000 and a cancer mortality risk of 1 in 362. Although an individual in the US in unlikely to chronically consume rice with the high tAs content

(27.6 µg As), even rice with the mean tAs content (7.0 µg As) is predicted to be associated with cancer risks at levels much higher than any substances currently regulated by the EPA. In addition, these values are based on average national consumption of rice and do not accurately reflect the range of cancer risk for populations consuming larger quantities of rice. In addition to populations consuming larger quantities of rice for dietary reasons, such as individuals with celiac disease or individuals on a gluten-free diet, there is also a high variability in rice consumption between different ethnicities. For example, populations such as Asian Americans and American Indians reported consuming up to six times the national average (Batres-Marquez et al. 2009, Cleland et al. 2009).

Based on the estimates of U-tAs associated with rice consumption, certain populations consuming high-tAs rice (27.6 µg As) may frequently exceed the BEI level of 35 µg As/L in urine. In addition, it should be noted that these U-tAs estimates do not account for As exposure from other sources such as food, water, or occupational exposures. While the BEI is a useful benchmark for industrial hygienists to estimate non-cancer health effects, recent studies have shown non-cancer health effects such as increased rates of lower and upper respiratory infections in populations exposed to As levels similar to those observed in the present study (Farzan et al. 2013). As a result, it is likely that individuals in general population may experience non-cancer health effects due to As exposure associated with their rice consumption.

CHAPTER 5: LIMITATIONS, FUTURE DIRECTIONS AND CONCLUSIONS

Further studies

Given the levels of As present in rice, which is available for purchase in the United States, and the later-life health effects associate with these levels, it is clear that further studies are warranted. First, additional studies are needed to accurately estimate both childhood and adult rice consumption from all sources, including rice-based products. While large national surveys such as NHANES routinely ask participants about their rice consumption, limited data exists concerning quantity, and the results do not accurately reflect consumption of rice-based products. In addition to the NHANES data, studies that routinely estimate childhood consumption of particular foods often neglect to ascertain rice consumption, and those that do are particularly scarce. Given the elevated levels of As present in rice-based products such as cereals, snacks, and energy bars, additional studies are needed to quantify the As exposure from these seldom-studied products in an individual's diet. As an example of the need for further research, an often-cited study estimating childhood consumption-based As exposure utilizes a value of 3.2 µg iAs per day (Yost et al. 2004), although as the present study demonstrates, a single serving of rice or rice-based products such as cereal can, and often will, exceed this daily estimate.

In response to additional research into the iAs content of apple juice, the FDA proposed an action level of 10 µg/L in July of 2013 (FDA 2013b). This recommendation was supported by a quantitative risk assessment in which the lung and urinary cancer rate of approximately 1 in

100,000 was estimated due to United States apple juice consumption with As levels below 10 µg/L (FDA 2013a). Based on the data in the present work, the average iAs content of rice is twenty-four times higher than the average iAs content found in apple juice (FDA 2013a). Given this proportion, as well as the lifetime frequency and ubiquity of rice consumption, it stands to reason that either an action level for rice or dietary recommendations concerning the frequency of rice consumption are warranted.

Limitations

While the data presented suggest that As exposure via rice consumption is of concern, there are several critiques and limitations of the present work. First, the model that derived a daily threshold of 0.68 µg/kg assumes that the EPA guidelines regarding As were created to limit exposure to As in water proportional to all other sources of exposure. While the use of a historical proportional constant for As exposure associated with water may be incorrect in light of the current work, the value is very close to one of the daily thresholds (0.71 µg/kg) based on values agreed to by the EPA's limit derived from their risk assessment. Within the United States, drinking water was considered one of the primary sources of exposure to As and, therefore, the regulation of As levels in drinking water was likely viewed as an effective means of regulating total As exposure (NRC 1999). However, if rice consumption is associated with higher As exposure than previously thought, it seems prudent that additional research is conducted to determine new values that are more protective for children and adults.

A second limitation of the current work is the estimation of U-tAs and maternal and cord blood As levels. Because As metabolism differs between individuals (Vahter 2000, Loffredo et al. 2003), a single coefficient value was not appropriate in the conversion between levels of U-tAs

and maternal blood As levels. With regard to estimating U-tAs from As exposure, several values were selected in an attempt to mitigate these inter-individual differences (Biggs et al. 1997, Kurttio et al. 1998). However, these values are based on small cohort studies in which individuals were routinely exposed to much higher levels of As than what would be commonly seen within the United States. As a result, extrapolation to lower As levels may not be accurate. In addition, the estimations of maternal blood levels from U-tAs levels are likely to be limited. As is readily cleared from the blood into urine, because As in the blood has a half-life of approximately one hour (Mann et al. 1996, NRC 1999). Consequently, spot measures of blood As levels are much more sensitive to the timing of the sample collection than measures of U-tAs levels (Mann et al. 1996). Therefore, it is probable that the conversion factors chosen are inaccurate due to highly variable blood As levels.

Conclusion

Based on the current work, average consumption of rice available for purchase in the United States may significantly contribute to an individual's As exposure at levels associated with increased risk for cancer and non-cancer health effects. Children consuming rice are likely exposed to As at levels equivalent or exceeding age-adjusted daily As thresholds based on the MCL set for drinking water. Given the association between *in utero* or childhood As exposure and later-life adverse health effects, new regulations or recommendations are warranted to limit the national burden of disease associated with rice consumption. At present, it seems prudent that vulnerable populations such as pregnant women, infants, and children eat a varied diet and reduce their rice consumption in order to mitigate their As exposure.

Appendix Table 1. Sources used to estimate coefficients factors in the conversion of As consumption to U-tAs. Because excretion of As in urine is dependent on inter-individual differences in As metabolism, five values were utilized for sensitivity analysis purposes.

Coefficient	Source	As dose (µg)	U-tAs (µg/L)	U-tAs/As
0.4	(Meza et al. 2004)	665 (405-1192)	240 (51-500)	0.36
	(Kurttio et al. 1998)	1489.9 (16.2-4905.4)	582.4 (61.2-1892.6)	0.39
0.5	(Kurttio et al. 1998)	20 (13-27)	9 (3-34)	0.48
1	(Meza et al. 2004)	65.5 (18–108)	64.5 (46-90.6)	0.98
2	(Biggs et al. 1997)	29.8 (11.5-78.3)	58.8 (4.4-266.5)	1.97
3	(Meza et al. 2004)	11.9 (3.1–24.5)	38.4 (26-56.7)	3.23
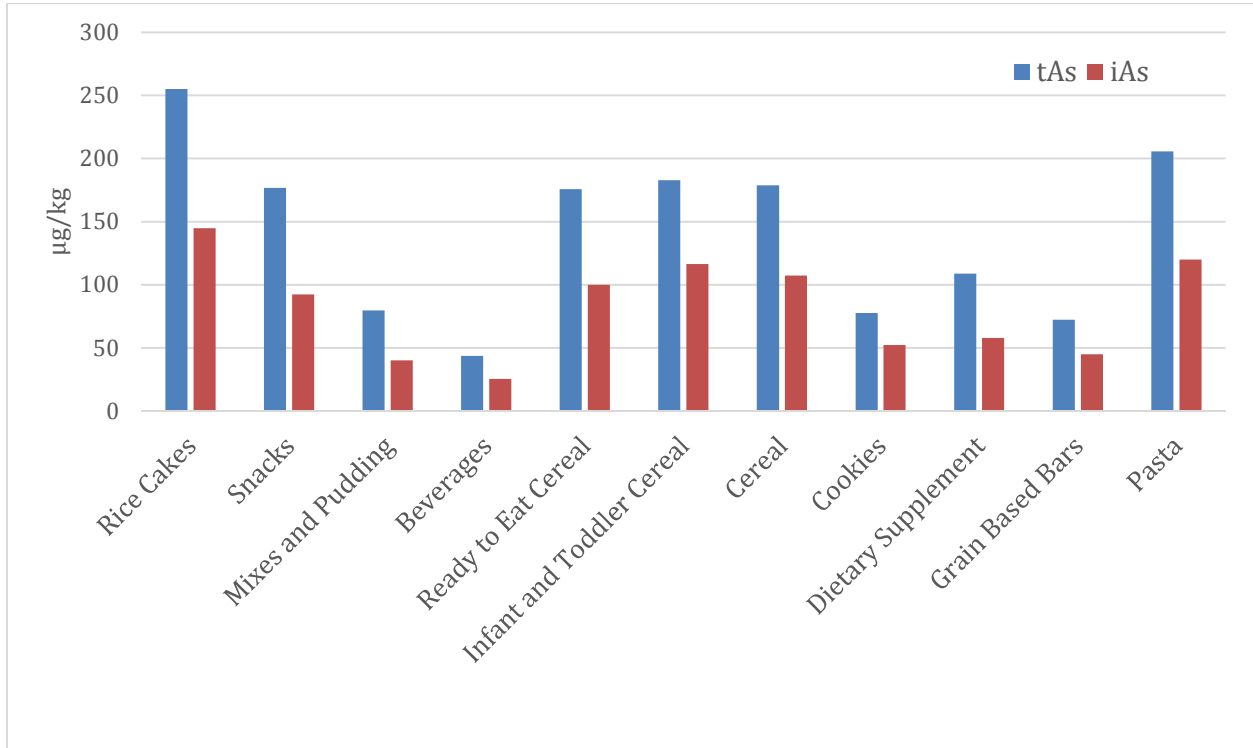
Appendix Figure 1. Average levels of tAs and iAs in various rice-based products. Data obtained from the FDA (FDA 2011) for rice cakes (N=59), snacks (N=190), mixes and pudding (N=36), beverages (N=222), ready to eat cereal (N=110), infant and toddler cereal (N=85), cereal (N=195), cookies (N=43), dietary supplements (N=12), grain based bars (N=115), and pasta (N=23).

APPENDIX 3: FIGURE 2
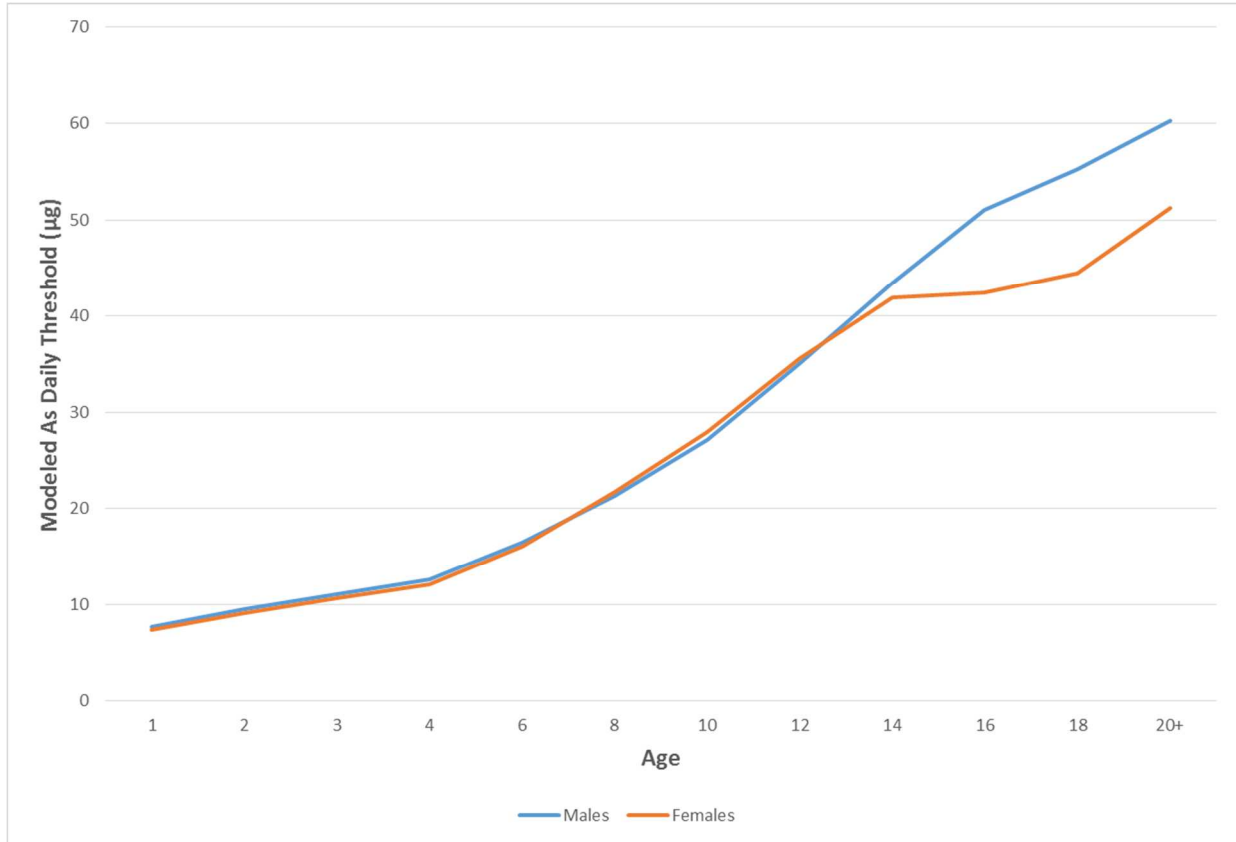
Appendix Figure 2. Modeled daily thresholds of As for males and females based on an extrapolated daily threshold of 0.68 µg/kg. Until age 16, modeled daily thresholds differed by less than 3%. Age-specific body weights for males and females was determined by childhood anthropometric reference data collected from the National Health and Nutrition Examination Survey (NHANES) (CDC 2012).

Appendix Table 2. Derivation of the lifetime cancer risk associated with exposure to chemicals in drinking water at MCL levels. To compare against lifetime cancer risk associated with consumption of mean (7.0 µg As) and high tAs rice (27.6 µg As), MCLs were multiplied by drinking water unit risk to determine the associated lifetime cancer risk.

	MCL (mg/L)	MCL (µg/L)	Drinking Water Unit Risk (per µg/L)	Cancer Incidence
Vinyl Chloride	0.002	2	4.2E-05	8.4E-05
Benzene	0.005	5	1.6E-06	8.0E-06
Benzo[a]pyrene	2.0E-04	0.2	2.1E-04	4.2E-05
1,2-Dichloroethane	0.005	5	2.6E-06	1.3E-05
Dioxin (2,3,7,8-TCDD)	3.0E-08	3.0E-05	4.5	1.4E-04
1,2-Dichloroethane	0.005	5	2.6E-06	1.3E-05
Dichloromethane	0.005	5	3.0E-05	1.5E-04
Di(2-ethylhexyl) phthalate	0.006	6	4.0E-07	2.4E-06
Ethylene dibromide	5.0E-05	0.05	3.0E-05	1.5E-06

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