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The authors have prepared a brief fact sheet to address consumer questions raised by this report. The fact sheet is available at http://www.dartmouth.edu/~toxmetal/assets/pdf/arsenicinfoodfaq.pdf

24 February 2012: In the manuscript originally published online, the two OBRS formulas were incorrectly identified as infant formula when they are in fact toddler formula. Toddler formula is not intended for infants under 1 year of age unless specified by a healthcare professional. This has been clarified in the text, and Figure 1B has been recalculated to reflect a 9-kg body weight, the median weight for a 12-month-old baby.

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Arsenic, Organic Foods, and Brown Rice Syrup

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Short title: **Arsenic, Organic Foods, and Brown Rice Syrup**

Key words: Arsenic, Brown Rice Syrup, Cereal Bars, Energy Bars, Baby Formula, Organic Foods, Speciation

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Abbreviations

As	arsenic
As_i	inorganic arsenic
As_{total}	total arsenic
DMA	dimethylarsenate
MMA	monomethylarsenate
IC	ion chromatography
ICP-MS	inductively coupled plasma mass spectrometry
OBRS	organic brown rice syrup

Abstract

BACKGROUND: Rice can be a major source of inorganic arsenic (As_i) for many sub-populations. Rice products are also used as ingredients in prepared foods, some of which may not be obviously rice-based. Organic brown rice syrup (OBRS) is used as a sweetener in organic food products as an alternative to high fructose corn syrup. We hypothesized that OBRS introduces arsenic into these products.

OBJECTIVE: We determined the concentration and speciation of arsenic (As) in commercially available brown rice syrups, and in products containing OBRS including toddler formula, cereal/energy bars, and high energy foods used by endurance athletes.

METHODS: We used ICP-MS and IC-ICP-MS to determine total As (As_{total}) concentrations and As speciation in products purchased via the internet or in stores in the Hanover, NH area.

DISCUSSION: We found that OBRS can contain high concentrations of As_i and dimethylarsenate (DMA). An 'organic' toddler milk formula containing OBRS as the primary ingredient had As_{total} concentrations up to six times the EPA safe drinking water limit. Cereal bars and high energy foods containing OBRS also had higher As concentrations than equivalent products that did not contain OBRS. Inorganic As was the main As species in the majority of food products tested in this study.

CONCLUSIONS: There are currently no US regulations applicable to As in food, but our findings suggest that the OBRS products we evaluated may introduce significant concentrations of As_i to an individual's diet. Thus, we conclude that there is an urgent need for regulatory limits on As in food.

Introduction

Arsenic (As) is an established carcinogen based on studies of populations consuming contaminated drinking water (Smith et al. 2002). Recently, attention has focused on As exposure from food, in particular fruit juices (Rock 2012) and rice (Stone 2008). Arsenic in rice reaches total concentrations up to 100-400 ng/g and includes both inorganic arsenic (As_i) and the organic species dimethylarsenate, DMA (Williams et al. 2005), with much lower concentrations (relative to DMA) of monomethylarsenate (MMA) also occasionally detected. Total As (As_{total}) in rice and relative proportions of DMA and As_i differ both geographically (Meharg et al. 2009) and as a function of genetic and environmental controls (Norton et al. 2009).

Inorganic As is more toxic than DMA or MMA (Le et al. 2000) and food regulatory limits, where they exist, are based on As_i. Infants fed rice cereal at least once daily may exceed the daily As exposure limit of 0.17 μg As per kg body weight per day based on drinking water standards (Meharg et al. 2008). Rice products such as cereals and crackers (Sun et al. 2009) and rice drinks (Meharg et al. 2008) have been found to be potentially significant dietary sources of As. Infants and young children are especially vulnerable, since their dietary As exposure per kg body weight is 2-3 times higher than that of adults (EFSA 2009; Meharg et al. 2008).

DMA is a metabolite of As_i. While considered less toxic than As_i, its toxicological potential has not been studied extensively. Although the presence of DMA in rice is likely due to natural soil microbial processes, DMA was used as a pesticide before being banned by the USEPA in 2009. Organic food consumers may therefore object to its presence in organic foods even in the absence of direct evidence of human health effects of DMA.

In the United States, organic brown rice syrup (OBRS) is used as a sweetener as a healthier alternative to high fructose corn syrup in products aimed at the 'organic foods' market. Added sugar is often the main ingredient in infant and toddler formula, and the addition of sucrose to a main brand organic formula was the feature of a popular press article in relation to possible childhood obesity (Moskin 2008). Many products – including some baby milk formulas, cereal bars, and high energy performance products for athletes – list OBRS as the major ingredient. Brown rice is usually higher in both As_{total} and As_i than white rice because As_i is localized to the aleurone layer which is removed when rice is polished, while DMA passes into the grain (Carey et al. 2011; Sun et al. 2008). Ranges of As concentration in rice products, including OBRS, are similar to As concentrations in brown rice (Signes-Pastor et al. 2009).

We posit that consumers of organic food products are generally attempting to make educated eating choices, and that this consumer group would be particularly interested to know if, and to what extent, OBRS introduces As_i, DMA and MMA into these products. As such, we measured As_{total} and As speciation in 3 commercially available brown rice syrups, 15 infant formulas without OBRS, two toddler formulas with OBRS, 29 cereal bars (13 with OBRS), and 3 flavors of a high energy performance product.

Materials and methods

Three commercial organic brown rice syrups were purchased from local or on-line stores. For one syrup, two bottles of the same product (from different lots) were tested. Fifteen infant formulas and two toddler formulas (initially purchased as part of a parallel study on As content of formulas and infant foods), 29 cereal bars and 3 energy 'shots' were all purchased from local stores in the Hanover, NH, area.

Sample preparation

All samples were analyzed for As_{total} and extracted for As species. For formulas As_{total} was determined after closed vessel microwave digestion with Optima HNO_3. Approximately 0.25 g of formula was digested in 2 ml 50% HNO_3. After heating at 180°C for 10 minutes the samples were allowed to cool and diluted to 10 ml with deionized water. Cereal bars and energy blocks were homogenized using a ceramic knife and were not dried prior to digestion. A sub-sample was digested in 2-3 ml Optima HNO_3 and was heated at 95°C for 30 minutes. The digested sample was diluted with deionized water to 25-50 ml. This digested sample was diluted a further 10X prior to analysis to reduce the acid concentration in the sample to <5%. All digestions and dilutions were recorded gravimetrically. Samples were extracted for As speciation using 1% HNO_3 and open vessel heating in a microwave digestion unit following a heating profile of 55°C for 5 min, 75°C for 5 min, and 95°C for 20 min (Foster et al. 2007; Huang et al. 2010). An aliquot of the extracted sample was then centrifuged at 13,300 rpm for 30 min and aliquot of that supernatant was further spin-filtered at 10 KDa.

Total As and As speciation determination

Total As was determined by ICP-MS (7700x, Agilent, Santa Clara, CA) using He as a collision gas at a flow rate of 4.5 ml/min. Samples were analyzed by either external calibration or the method of standard additions. Arsenic speciation of the 1% HNO_3 extracts was determined by ion chromatography (IC) coupled to ICP-MS using a Hamilton PRP X100 anion exchange column and a 20 mM ammonium phosphate eluant at pH 8. Formulas were evaluated in triplicate, 5% duplicate and duplicate spikes were performed for the cereal bars and energy blocks.

NIST 1568a rice flour (Gaithersburg, MD) was used as a quality control material for both As_{total} measurements and As speciation. Although As species are not certified for SRM 1568a, reproducible consensus values have been demonstrated from many studies (Meharg and Raab 2009; Raab et al. 2009; Williams et al. 2005). We determined As_{total} in NIST 1568a to be 279 ± 31 ng/g (mean ± 1 SD, n = 6); the certified value is 290 ± 30 ng/g. For As speciation (n = 5), we determined DMA to be 186 ± 21 ng/g, MMA to be 9.4 ± 3.7 ng/g and inorganic As to be 101 ± 15 ng/g, which are in the range reported by other studies.

<u>Data analyses</u>

Given our calculated values for As speciation in the formulas, we estimated As concentrations (µg/L) of reconstituted formulas assuming that 1 scoop of powdered formula weighs 8.75 g and that 1 scoop of formula is added to 60 ml of As-free water to make 2 fluid ounces of formula. We then estimated daily intake of As species for a 6 and 9 kg baby assuming consumption of six, four-ounce bottles of milk formula each day, and compared this to 'safe' levels estimated for consumption of drinking water containing As_i at the EPA and WHO maximum contaminant limit of 10 µg/L (Meharg et al. 2008).

Results and Discussion

Rice syrups

Total As concentrations in 3 rice syrups (and from two lots of one of the syrups) ranged from 80 -400 ng/g (Table 1). Inorganic As was 80-90% of As_{total} for two of the three syrups; for the third syrup, only 50% of As_{total} was inorganic. However, because this syrup was much higher in As_{total} it also had the highest As_i concentration of the syrups. All syrups had detectable MMA, ranging from 3 – 4% of As_{total}, but the major organic As species for each syrup was DMA. Our results are similar to a previous study that reported dry weight As_{total} concentrations of 80, 100, 120, and 330 ng/g in four rice syrups, with 71% As_i and 85% extraction efficiency in the highest As syrup (Signes-Pastor et al. 2009). Moreover, given their estimate of 15% moisture content for the syrups (Signes-Pastor et al. 2009), we estimate that the actual As concentration in food products that include OBRS as the dried product – like toddler formulas – would be approximately 1.15 times the concentration listed in Table 1.

Baby formulas

We have analyzed 17 different formulas. Average As_{total} concentrations in the 15 infant formulas that did not contain OBRS were relatively low, in the range 2 – 12 ng/g (Jackson et al. 2012). Those results were consistent with two other studies of As in infant formula (Ljung et al. 2011; Vela and Heitkemper 2004). However, the As concentrations in the two toddler formulas that listed OBRS as the primary ingredient (one dairy-based and one soy-based) were >20 times the As concentrations in infant formulas that did not contain OBRS (Figure 1A). The proportion of As_i varied between products and between lots of the soy-based formula, but the concentration of As_i in both reconstituted formulas with OBRS was at or above the current U.S. drinking water standard (10 µg/L). In addition, the OBRS formulas contained 19-40 µg/L DMA and trace levels of MMA. Expressed as daily As intake per kg body weight, the exposure of infants and toddlers drinking OBRS milk products is even more apparent (Figure 1B). Using web-based search engines, we found only these two toddler formula which used OBRS, so the number of infants using this formula is presumably a very low percentage of US formula-fed infants.

Infants, in a phase of rapid development, are especially vulnerable to contaminants and emerging data suggest that As exposure early in life may pose risks not only during childhood but in adult life (Vahter 2009). This suggests that we need to pay particular attention to the potential for As exposure during infancy. The standards and guidelines for daily intake of As are currently a matter of debate (Meharg and Raab 2009; Meharg et al. 2008). The World Health Organization (WHO) established a provisional maximum tolerable daily intake (PMTDI) guideline of 2.1 µg/kg/d in 1983(FAO/WHO 1983). For either a 6 or 9 kg infant, both of the OBRS formulas would be above this value based on As_{total}; for a 6 kg infant, the soy formulas would be above the guideline based only on As_i. It should also be noted that the WHO 1983 PMTDI is based on a safe drinking water limit of 50 µg/L rather than the current limit of 10 µg/L (EFSA 2009; Meharg and Raab 2009). Currently, only China has a limit for As in food, specifically, a limit of 150 ng As_i per g of rice (Zhu et al. 2008). Although the OBRS toddler formulas would not exceed this limit on average, As_{total} and As_i concentrations of these OBRS formulas are cause for concern.

Cereal and energy bars

Organic brown rice syrup is also a popular sweetener for many cereal/energy bars and high energy athletic performance products. Our web- and store-based market survey of 100 bars indicated that about 50% contain either OBRS (31%), other rice products (5%) or both (14%). We tested 29 bars and three types (flavors) of an energy product obtained from a local Hanover supermarket. The results for the cereal/energy bars are shown in Table 2. All of the bars had detectable As_{total} with a range of 8 – 128 ng/g. The seven bars that did not list any rice product among the top 5 ingredients were among the eight lowest As-containing bars we tested. The remaining bars listed at least one of four rice products – organic brown rice syrup, rice flour, rice grain and rice flakes – in the first five ingredients and had As_{total} concentrations ranging from 23 – 128 ng/g.

We analyzed As speciation in 12 of the rice-containing bars. Eleven of the 12 bars contained As_i concentrations >50% , with an average of 70% As_i. All organic As was DMA. The percent recovery (sum of As species as a percent of As_{total}) ranged from 67% - 124%; however, some of this variability is due to the fact that the bars were not dried prior to analysis and were analyzed 'as is', with limited homogenization using a ceramic-bladed knife . The amount of As_i ingested when eating one of these bars is a function of the As concentration of the bar and the size (weight) of the bar. The bars we analyzed ranged in weight from 28 – 68 g; at the upper limit of bar weight and As_i content, an individual bar contained up to 4 µg As_i. For example, bar ID 27 weighs 45 g and contains 101 ng/g As_{total} and 79% As_i equating to an As_i content of 3.64 µg.

Energy Shots

We also analyzed As concentration and speciation in three high energy products for endurance athletes known as 'energy shots', each of which contained OBRS. Although an educated consumer might be aware of the potential for rice to contain As (and therefore know that products containing rice ingredients might also contain As), the energy shots are gel-like blocks, so that it would not be immediately apparent to the consumer that these too are rice-based products.

The As concentration in one of the energy shot blocks containing OBRS was 84 ± 3 ng/g As_{total} (n=3), which was 100% As_i. The other two shot blocks were very similar to one another in As_{total} concentrations (171 ± 3.6 ng/g, n=6) and speciation (53% As_i). No MMA was detected in the shot blocks. All three flavors contained 2.5 – 2.7 µg As_i per 30 g serving. The manufacturer recommends consuming up to two servings (60 g) per hour during exercise, so an endurance athlete consuming four servings during a two-hour workout would consume more than 10 µg As_i per day, equal to the As_i intake resulting from consumption of 1 L of water at the current EPA and WHO limit of 10 µg/L. Athletes consuming the two flavors with 171 ng/g As_{total} would also consume 2.5 µg DMA per 30 g serving.

Conclusions

Food is a major pathway of exposure to As for most individuals (EFSA 2009). Rice and rice products can contribute to an individual's As_i exposure (Meharg et al. 2008; Williams et al. 2005). There is a growing body of information about As concentration and speciation in rice in the peer reviewed literature and thus in the public domain, but much less information on rice-based food products. Rice products are used in a variety of foods, including gluten- free products and, as we show here, in products where OBRS is used as an alternative to high fructose corn syrup. The formulas with OBRS – which could be the sole sustenance for an individual over a critical period of development – can result in consumption of milk with As concentrations much higher than the drinking water standard, yet there are no US regulations to deal with this particular scenario. Similarly, endurance athletes who consume 12 OBRS-containing energy shots (manufacturer-recommended maximum for two hours of physical activity) may be exposed to as much as 10 µg As_i and 20 µg As_{total} in a single day. Moreover, the major As species is the more toxic As_i in the overwhelming majority of food products we have tested, a finding which, although noted in other studies (Sun et al. 2009), is particularly troubling given the non-threshold relationships between cancer risk and exposure to As_i (NRC 2001).

There are currently no US regulations applicable to As in food, but our findings suggest that the OBRS products we evaluated may introduce significant concentrations of As_i to an individual's diet. Thus, we conclude that there is an urgent need for regulatory limits on As in food.

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Table 1. Arsenic concentrations and As speciation for three organic brown rice syrups (OBRS). Analyses were done in triplicate.

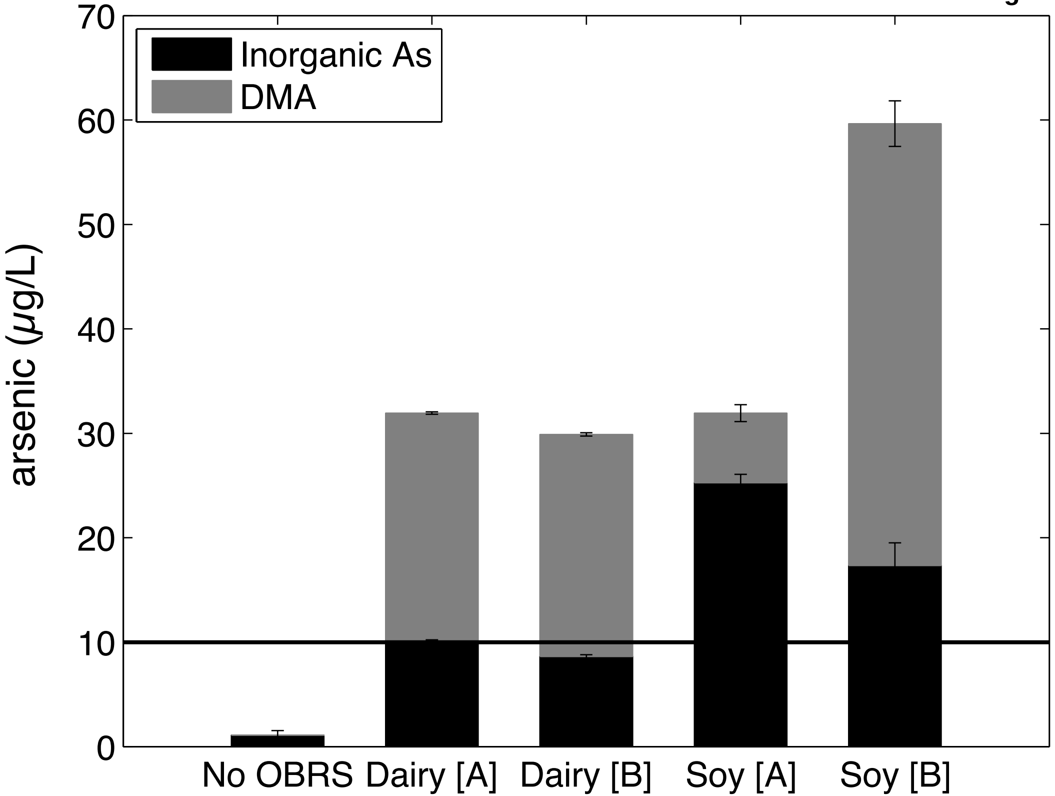
Sample ID	As_{total} ng/g	Speciation analysis % As_i	%DMA	%MMA	$\Sigma As_{species}$ ng/g
A, Lot 1	78 ± 6	89	7	4	81
A, Lot 2	94 ± 8	84	12	4	94
B	136 ± 3	91	6	3	118
C	406 ± 6	51	46	3	294

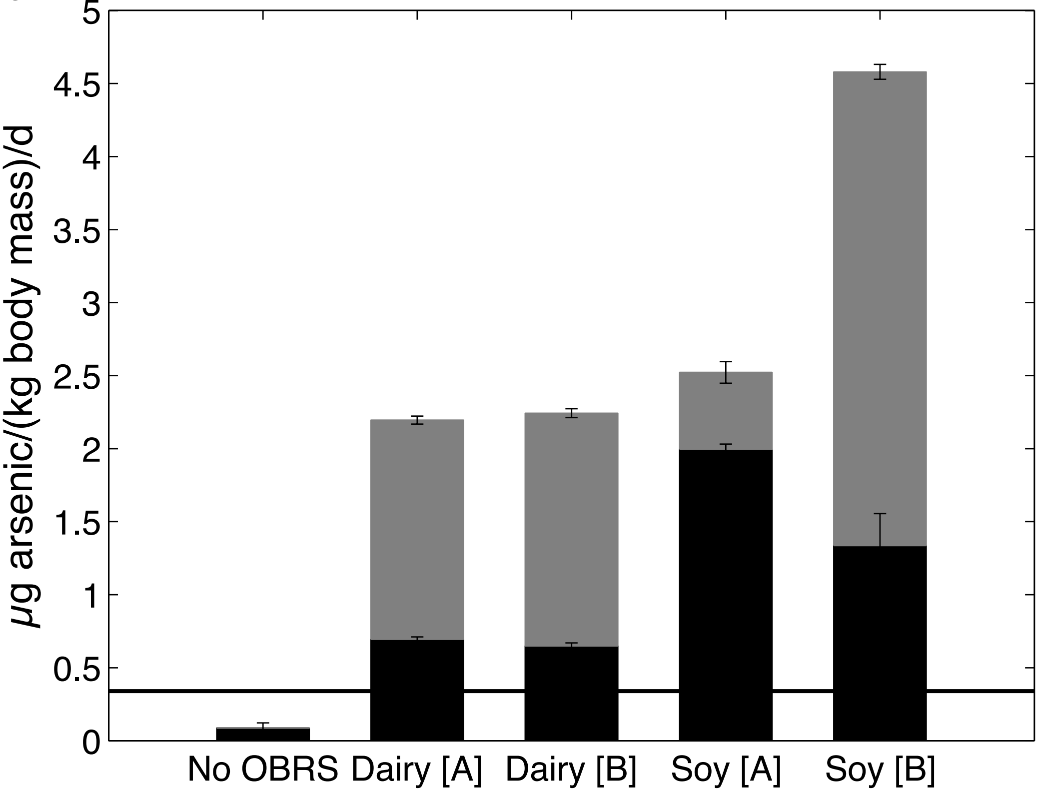
Table 2: Arsenic concentrations and speciation in 29 cereal bars, together with information about their rice-based ingredients. Speciation data and % recovery are presented for a selection of rice-containing bars. ✓ indicates the presence of a rice-based ingredient (flakes, grain, flour, or brown rice syrup) and the number in parentheses is the numerical listing of that ingredient. Only the first five listed ingredients were considered.

	Arsenic Content			Rice Ingredients			
Lab ID	As_{total}, ng/g	% As_i	% recovery	flakes	grain	flour	syrup
29	8						
21	11						
14	12						
28	12						
22	22						
8	23					✓ (4)	
4	27						
17	27						
2	28					✓ (4)	✓ (1)
20	30						✓ (3)
1	33					✓ (3)	
5	34						✓ (5)
9	35					✓ (4)	✓ (2)
101	41					✓ (2)	✓ (1)
7	45	92%	77%				✓ (2)
18	51	53%	119%		✓ (4)		
19	55	38%	120%			✓ (1)	✓ (2)
26	56	73%	101%			✓ (4)	✓ (2)
103	57					✓ (2)	✓ (1)
13	61	76%	77%				✓ (2)
12	64	73%	76%				✓ (3)
16	66	81%	97%		✓ (1)		✓ (2)
11	76	75%	67%		✓ (4)	✓ (5)	
10	83	81%	71%			✓ (2)	✓ (1)
102	86					✓ (2)	✓ (1)
15	90	85%	124%		✓ (1)		✓ (2)
27	101	79%	97%			✓ (3)	✓ (1)
3	119	57%	85%			✓ (1)	✓ (2)
6	128	62%	113%	✓ (1)		✓ (4)	✓ (2)

Figure Legend

Figure 1. A. Comparison of inorganic As (black) and DMA (gray) concentrations in reconstituted milk formulas with and without organic brown rice syrup (OBRS) (vertical bars) to the current WHO and USEPA drinking water standard of 10 µg/L (solid horizontal line). B. Daily As intake for a 9 kg baby drinking six, four ounce bottles of milk formula constituted with As-free water (vertical bars) compared with a 60 kg adult drinking 2 L of tap water at the safe drinking water limit (solid horizontal line). The height of each bar indicates a mean and error bars are standard deviations. The no OBRS bar is calculated from 15 different main brand milk formulas (Jackson et al. 2012). The OBRS bars are based on triplicate analysis from one lot (A or B) of each type.


70
60
50
40
30
20
10
0
arsenic (μg/L)
Inorganic As
DMA
No OBRS
Dairy [A]
Dairy [B]
Soy [A]
Soy [B]


μg arsenic/(kg body mass)/d
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
No OBRS
Dairy [A]
Dairy [B]
Soy [A]
Soy [B]