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Arsenic Speciation in Rice Cereals for Infants

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ABSTRACT: The aim of this study was to conduct a survey of arsenic (As) content in rice cereals for infants. The analysis was based on the FDA Elemental Analysis Manual (EAM 4.11). An inductively coupled plasma mass spectrometer (ICP-MS) was used to determine total As. Due to the different toxicities of the chemical forms of arsenic, the ICP-MS coupled to a high-performance liquid chromatograph (HPLC) was used to perform As speciation. The total and speciated arsenic was determined in 31 different infant rice cereals sold in U.S. supermarkets. The mass fraction of total inorganic As (iAs; sum of arsenite As(III) and arsenate As(V)) concentrations ranged between 55.5 ± 1.3 and 158.0 ± 6.0 μg/kg. The average total arsenic and iAs concentrations in infant rice cereal were 174.4 and 101.4 μg/kg, respectively. There was no substantial difference in iAs levels between organic and conventional rice cereals. The mixed-grain rice cereal contained the least total (105 μg/kg) and inorganic arsenic (63 μg/kg). The major detected organoarsenical species was dimethylarsinic acid (DMA). Monomethylarsonic acid (MMA) was not detected, or only trace levels were found. Spiked sample percent recoveries for iAs, DMA, and MMA ranged from a low of 97.3% for iAs to a high of 115.0% for DMA. Results for speciated and total As in the National Institute of Standards and Technology standard reference material rice flour (NIST SRM 1568) were in good agreement with certified values. In the NIST SRM 1568 sample (n = 5) repeatability (%RSD) was 2.8% for iAs, 1.7% for DMA and species sum, and 5.3% for the total arsenic by As total method. The average percent mass balance was 99.9 ± 6.3% for the NIST SRM 1568 sample. This study provides new and much needed information on arsenic levels in rice-based infant cereals.

KEYWORDS: *arsenic, speciation, infant rice cereal, HPLC-ICPMS*

INTRODUCTION

The toxicity of arsenic in foods depends on its chemical form and bioaccessibility. Rice can be one of the main sources of inorganic arsenic (iAs) and organoarsenicals consumed in the current population's diet, especially in areas where arsenic is low or not present in the water.[1] Arsenic occurs naturally in the environment (in soil or from atmospheric inputs due to volcanism).[2] It can also be present as the result of human activity such as introduction into the soil through the use of arsenic-based pesticides prior to the 1970s or the use of seaweed as a fertilizer.[3,4] Transformation processes within soil can result in arsenic forms that are bioavailable for plant uptake, which allow it to enter the food supply. Rice plants are especially efficient at accumulating arsenic from their anaerobic environment because the flooded areas in which they are grown make it easier for them to take up arsenic compounds.[5−7] Inorganic arsenic is a known carcinogen, and chronic exposure to low levels of arsenic has been linked to increased risk of bladder, lung, and skin cancer, type 2 diabetes, and cardiovascular disease.[2,8] Organoarsenicals are less toxic than inorganic arsenic. Due to the different toxicities of the chemical forms of arsenic, there is a need to determine the various arsenic species. Especially important is identification of the more highly toxic inorganic forms of arsenic such as arsenite (AsIII), which is more mobile and toxic than arsenate As(V). Rice samples from the United States have shown higher total arsenic levels when compared to other samples from around the world. Brown rice from all locations was shown to contain more arsenic than white rice.[9,10] The United States and European Union have not set limits for arsenic in food products, including rice. China has a limit of 150 ng/g of inorganic As in rice.[11] It is not clear how harmful arsenic in rice may be to the human population; new data could aid in risk assessment.[12−15] Infants may be at higher risk because they are more susceptible to the harmful effects of arsenic than adults.[16−19]

In 1983 the World Health Organization (WHO) established a provisional maximum tolerable daily intake (PMTDI) guideline for inorganic arsenic of 2.1 μg/kg/day (Food and Agriculture Organization of the United Nations/WHO 1983). The PMTDI was based on a safe drinking water limit for As of 50 μg/L. The U.S. Environmental Protection Agency revised the drinking water limit for arsenic to 10 μg/L in 2001, which is the also new WHO limit.[20] There is a 23 ppb "level of concern" established by the U.S. FDA (2008) for iAs in apple and pear juices. Recent media and consumer reports both advocated a 10 ppb limit to match the EPA drinking water limit.[21,22]

Determination methods of iAs in food samples have required a separation technique, such as HPLC or ion chromatography, with an elemental spectrometer. Early HPLC techniques used C18 ion pairing mode[23,24] but required extensive sample cleanup, especially in higher protein- and lipid-containing material, and required careful attention to pH and buffer concentration to maintain separation but showed separation of iAS species (+3 and +5), organic species monomethylarsonic acid (MMA); dimethylarsinic acid (DMA), arsenobetaine, and arsenocholine.

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Table 1. Rice Flour Standard Reference Materials[a]

SRM	total As (μg/kg)	As(III) (μg/kg)	As(V) (μg/kg)	iAs (μg/kg)	DMA (μg/kg)	MMA (μg/kg)
1568a	290 ± 30*	60 ± 12[b]	39 ± 8[b]	100 ± 20[b]	171 ± 34[b]	11 ± 2[b]
1568	410 ± 50*	85 ± 17[b]	31 ± 6[b]	116 ± 23[b]	285 ± 57[b]	22 ± 4[b]

[a]Certified value with uncertainty expressed as a 95% confidence interval or as a 95% confidence interval plus an allowance for systematic error. [b]Uncertainty expressed as ±20% of the average value from the best available data.

Table 2. Concentration of As by Speciation and Total Method in SRM 1568

sample name (separate preperations)	As(III) (μg/kg)	DMA (μg/kg)	MMA (μg/kg)	As(V) (μg/kg)	iAs[a] (μg/kg)	total As (μg/kg) (speciation method)	total As (μg/kg) (totals method)	% mass balance[b]
certified value	85 ± 17	285 ± 57	22 ± 4	31 ± 6	116 ± 23	410 ± 50	410 ± 50	
1568_rep1	65.0	289.9	24.8	40.6	105.6	420.3	398.4	105.5
1568_rep2	68.5	289.0	24.6	42.5	110.9	424.5	445.1	95.4
1568_rep3	66.9	300.6	25.0	44.7	111.6	437.2	405.2	107.9
1568_rep4	70.3	288.7	21.6	37.5	107.8	418.1	442.0	94.6
1568_rep5	69.8	289.4	22.8	43.3	113.1	425.4	442.3	96.2
av	68.1	291.5	23.8	41.7	109.8	425.1	426.6	99.9
SD	2.2	5.1	1.5	2.8	3.1	7.4	22.8	6.3
%RSD	3.2	1.7	6.3	6.7	2.8	1.7	5.3	6.3

[a]iAs equals the sum of As(III) and As(V). [b]Mass balance = total As from speciation (As(III) + DMA + MMA + As(V))/total As from totals method.

Ion exchange chromatography has had more use in the determination of rice samples[1,25] with simpler buffer systems with isocratic separation that separates the arsenicals mentioned above. The connection of these separation systems to ICP is simple, without having any system or chemical modification to change the detection of the arsenicals. Detection limits for arsenicals from rice materials and the FDA EMA 4.11 method were capable of determinations at sub-parts per billion levels. Other detection systems, such as atomic emission spectroscopy (AES), can achieve low-level parts per billion amounts but require hydride generation (HG), which ionizes the arsenicals but loses detection of arsenobetaine. Hydride generation improves signals from interfering elements, such as arsenochloride ($ArCl^+$, m/z 75), but helium collision gas in the mass spectrometer of ICP-MS fragments arsenochloride, reducing any mass elemental interference of the arsenicals measured at the same arsenic mass to charge ratio (As^+, m/z 75).

For rice samples the extraction technique has been a critical aspect and has evolved to increase the recoveries of arsenicals. Other food materials, such as mussels, required organic extraction using solvents such as chloroform and ethanol, but these methods showed a loss of arsenocholine. Because of rice's high carbohydrate content and low protein and fat contents, Narukawa[24] used microwave energy (heat) to extract iAS species and organoarsenicals with excellent recoveries in about 3 h. Liang[26] showed that adding acid from the previous work helped to recover the same arsenicals from 76 to 97% when 0.2 M TFA was used. Nishimura studied several rice varieties and used 0.15 M nitric acid, comparing increased temperature from 80 to 100 °C, and showed the higher temperature has recoveries that were >90% at the higher temperature for spiked varieties and showed a 97% recovery for the total recovery (As^{3+}, As^{5+}, MMA, DMA) of species when compared to the total value of the NIST reference material 1568a.[27] A 1% nitric acid extraction was used with microwave temperature gradient in brown rice syrup that recovered 289 ng/g for the total of iAs (+3 and +5), MMA, and DMA from NIST CRM 1568a with a reference value of 290 ± 30 ng/g.[1] FDA EAM 4.11 uses 0.28 M nitric acid with a quality assurance aspect that uses NIST CRM 1568a to determine the recovery of all arsenic species determined to the total reference value.

The methodology employed for this body of work has the ability to determine an arsenic species based on mass spectrometer (MS) detection of arsenic m/z 75. Speciation is accomplished by mild hydrolysis of samples in dilute acid that enables the extraction of organic and inorganic arsenic species. The aim of this study was to conduct a survey of arsenic content in infant rice cereals (white, brown, and mixed grain, both organic and conventional) purchased at U.S. supermarkets. This work was based on the Elemental Analysis Manual (EAM 4.11) method from the FDA.[23]

■ MATERIALS AND METHODS

Instrumentation. Speciation analysis was done using an Agilent 1200 HPLC system (Agilent, Palo Alto, CA, USA), consisting of a vacuum degassing unit, a binary pump, an autosampler, and a 10-port valve (2 ports were plugged). The HPLC system was connected to the ICPMS (7700x, Agilent Technologies, Inc., Tokyo, Japan) using 1/16 × 0.0025 × 12 in. (o.d. × i.d. × length) peek tubing (Upchurch Scientific, Oak Harbor, WA, USA). The ICPMS was equipped with a micromist nebulizer and a Scott-type double-pass spray chamber. The operating conditions used for this method were as follows: RF power at 1500 W, plasma gas flow rate at 15 L/min, auxiliary gas flow at 0.15 L/min, nebulizer gas flow rate at 0.95 L/min, sampling depth at 8.5 mm, peristaltic pump speed at 0.41 rps (waste line flow rate should be >1 mL/min), spray chamber temperature held at 2 °C, collision cell gas of He at a flow rate at 4.3 mL/min, data acquisition mode set to time-resolved analysis analyzing for m/z 75 for $^{75}As^+$and for m/z 77 for $^{40}Ar^{35}Cl^+$, with dwell times of 0.8 s for m/z 75 and 0.2 s for m/z 77. The HPLC analytical and guard columns used for the separation of the arsenic species were Hamilton (Reno, NV, USA) PRP X 100, 4.1 × 250, stainless steel, 10 μm analytical column and the matching guard column (Hamilton part 79446). Ammonium phosphate dibasic (10 mM) at pH 8.25 (adjusted with ammonium hydroxide) made fresh everyday was used to elute the arsenic species. The external (extra) pump was used (LC-20AD, Shimadzu, Co., Japan) for the internal standard spike introduction.

Chemicals and Reagents. All solutions were prepared using deionized water with resistance >18 MΩ·cm with a Purelab Ultra

Table 3. Summary of the Infant Rice Cereals Used (Product Name, Location of Purchase, and Other Ingredients)

brand	sample ID	product name	other ingredients	location of purchase
A	Baby_1	mixed-grain cereal	whole wheat flour, rice whole grain oat flour, soybean oil	Illinois
A	Baby_2	organic brown rice	organic whole grain brown rice flour, soy lecithin	Illinois
B	Baby_3	organic, whole grain rice cereal	organic whole grain brown rice, tocopherols (vitamin E), electrolytic iron	Illinois
C	Baby_4	organic, rice cereal,	organic whole grain brown rice flour, sunflower lecithin, electrolytic iron	Illinois
A	Baby_5	rice single-grain cereal	organic whole grain brown rice flour, sunflower lecithin, electrolytic iron	Illinois
C	Baby_6	organic, rice cereal,	organic whole grain brown rice flour, sunflower lecithin, electrolytic iron	Texas
D	Baby_7	rice cereal, single grain	rice flour, soybean oil, soy lecithin	California
E	Baby_8	rice cereal	rice flour, soybean oil, soy lecithin	North Dakota
A	Baby_9	organic brown rice cereal	organic whole grain brown rice, tocopherols (vitamin E), electrolytic iron	North Dakota
B	Baby_10	organic brown rice cereal	organic whole grain brown rice, tocopherols (vitamin E), electrolytic iron	North Dakota
B	Baby_11	organic brown rice cereal	organic whole grain brown rice, tocopherols (vitamin E), electrolytic iron	California
A	Baby_12	organic brown rice cereal	organic brown rice cereal	Texas
F	Baby_13	rice cereal	rice flour, sunflower lecithin	North Dakota
F	Baby_14	rice cereal	rice flour, sunflower lecithin	California
F	Baby_15	rice cereal	rice flour, sunflower lecithin	California
A	Baby_16	rice cereal	rice flour	Texas
F	Baby_17	rice cereal	rice flour, sunflower lecithin	Texas
A	Baby_18	mixed-grain cereal	whole wheat flour, rice whole grain, oat flour, soybean oil	California
A	Baby_19	mixed-grain cereal	whole wheat flour, rice whole grain, oat flour, soybean oil	Texas
A	Baby_20	mixed-grain cereal	whole wheat flour, rice whole grain, oat flour, soybean oil	North Dakota
A	Baby_21	mixed-grain cereal	whole wheat flour, rice whole grain, oat flour, soybean oil	California
A	Baby_22	mixed-grain cereal	whole wheat flour, rice whole grain, oat flour, soybean oil	North Dakota
A	Baby_23	mixed-grain cereal	whole wheat flour, rice whole grain, oat flour, soybean oil	California
G	Baby_24	organic brown rice cereal	organic whole grain brown rice flour, sunflower lecithin	California
G	Baby_25	organic brown rice cereal	organic whole grain brown rice flour, sunflower lecithin	California
A	Baby_26	rice single-grain cereal	rice flour, tri- and dicalcium phosphate, soybean oil, soy lecitin, tocopherols, vitamins A and B, B group	California
A	Baby_27	mixed-grain cereal	whole wheat flour, whole grain oat flour, tri- and dicalcium phosphate, soybean oil, soy lecitin, tocopherols, vitamins A and B, B group	California
B	Baby_28	organic whole grain rice cereal with apples	organic whole grain brown rice flour, organice apple puree, vitamin E, iron	California
B	Baby_29	organic whole grain rice cereal	organic whole grain brown rice, vitamin E, iron	California
B	Baby_30	organic whole grain rice cereal	organic whole grain brown rice, vitamin E, iron	California
A	Baby_31	DHA and probiotic single-grain rice cereal	rice flour, vitamins, minerals, soybean oil, soy lecitin, tocopherols, tuna oil	California
A	Baby_31	DHA and probiotic single-grain rice cereal	rice flour, vitamins, minerals, soybean oil, soy lecitin, tocopherols, tuna oil	California

(Siemens, Broadview, IL, USA). Nitric acid and hydrogen peroxide were both OPTIMA ultrapure grade and from Fisher Scientific (Pittsburgh, PA, USA). Ammonium phosphate was purchased from Acros (Morris Plains, NJ, USA). Ultrapure reagent ammonium hydroxide, HPLC grade isopropanol, and optima grade nitric acid were from Thermo Fisher Scientific (Pittsburgh, PA, USA). Arsenite stock standard (As(III)) and arsenate stock standard (As(V)) were purchased as 1000 mg L^{-1} solutions from Spex Certiprep (Metuchen, NJ, USA). Monomethylarsonic acid (98.5% purity) and dimethylarsinic acid (98.9% purity) were from Chem Service (West Chester, PA, USA). Arsenobetaine (AsB) was purchased from Fluka.

Standard Reference Materials (SRMs). NIST rice flour SRMs, namely 1568a and 1568 (National Institute of Standards and Technology, Gaithersburg, MD, USA), were used as quality control materials for both measurements for As speciation and total As (Table 1). The values for total As have been extracted from the Certificate of Analysis and those for individual As species, which are expressed as ±20% of the average value, from the best available literature and shall be considered as the true values for comparison purposes.[23] Table 2 shows results for the NIST 1568 SRMs using the method described in this paper.

Standard Solutions. All standard solutions were prepared daily from serial dilution of stock species standards according to the literature (EAM 4.11). All arsenic stock standard solutions (As(III), DMA, MMA, As(V)) were prepared in water at 1000 μg mL^{-1}. The arsenic concentration of the DMA and MMA standards were verified, using ICPMS analysis. The total arsenic concentrations of 1 μg/g MMA and DMA standards were determined using a calibration curve of ICPMS arsenic standard (1000 μg mL^{-1}, Plasma, Canada). These concentrations were used to recalculate the stock standard concentrations and then applied as new values to future calculations.

Working standards were prepared by diluting the 200 μg mL^{-1} mixed standard stock solution with mobile phase to desired concentrations. All standard solutions were stored in the dark at 4 °C. On the morning of the experiment, a fresh mixed standard solution was made. The internal standard for the speciation method was 2 ng/g of As(V) in mobile phase, and it was injected postcolumn to monitor and to correct for signal drift. The internal standard for the totals method was rhodium/germanium with 18% isopropanol (ICPMS Internal Standard Mix 100 mg L^{-1} (part 5188-6525, Agilent Technologies, Inc.)). Standards for LOD and LOQ determination were prepared at 0.2 ng/g containing each As(III), DMA, MMA, and As(V) in mobile phase. Additional standard was prepared for the arsenic speciation method, which is a resolution check standard at 5 ng/g As(III) and AsB in mobile phase. A new resolution check standard was prepared when ~50% of As(III) has been converted to As(V).

Samples. Thirty-one infant rice cereals were purchased at different locations and from various grocery stores in the following U.S. locations: Chicago, IL; San Francisco Bay area, CA; Texas; and North Dakota. All 31 infant rice cereals were from 7 different manufacturers. Table 3 lists all of the information (product, purchase location, and other major ingredients) that could be obtained from the infant rice cereal containers.

Sample Preparation. All 31 infant rice cereals were analyzed for total extractable arsenic, and all samples were subjected to arsenic speciation analysis. Infant cereals were analyzed directly from the container and were not further dried or homogenized. For the total arsenic analysis, the same hot block digestion method was used, but the infant rice cereals were not run through the HPLC, just straight into the ICPMS for total arsenic concentration. The hot block digestion method[28] used for both the total and speciation analysis was as follows. A gravimetric method was applied to all preparations in this study. One gram of infant rice cereal sample was weighed precisely into a preweighed 50 mL polypropylene centrifuge tube (with lid), and 10 g of 0.28 M HNO_3 was added. The sample was vortexed for 10−30 s. The tightly capped tube was placed in a preheated block digestion system at 95 °C for 90 min. After the sample was cooled, 6.7 g of deionized water was added. Weights were recorded through every step of the sample preparation. The rice cereal suspension was centrifuged at 3500 rpm for 10 min. The supernatant was passed through a 0.45 um nylon syringe filter attached to a 3 mL disposable syringe. The first ∼1 mL was discarded through the filter to waste, and then 1 g of the filtrate was transferred to a new 15 mL tared centrifuge tube. Two grams of pH adjustment solution were added to the extract. The pH adjustment solution was mobile phase with a pH of 9.95 ± 0.05 (it was prepared by adding ammonium hydroxide to the mobile phase). The final analytical solutions (method blank and samples) had a pH between 6 and 8.5. If the pH was not within this range, the pH was adjusted for the pH adjustment solution until the proper pH range was achieved in method blanks and sample extracts. A portion of the resulting solution was placed into a polypropylene autosampler vial for analysis by HPLC-ICP-MS.

In the original method, which was validated by Huang et al.,[29] the extracts were less diluted than samples prepared using EAM 4.11. This method calls for diluted samples, adjusted pH (prolongs column use), and analyte retention times more similar to those of the standards. The negative aspect to this pH adjustment leads to interconversion of As(III) and As(V).

Blank tests were performed to determine the absence of possible arsenic contamination before the analysis was continued.

Table 4. ASDL, ASQL, LOD, and LOQ for Arsenic Speciation Analysis by HPLC-ICPMS

	As(III)	DMA	MMA	As(V)
ASDL (ng/g)	0.034	0.017	0.024	0.035
LOD (μg/kg)	1.68	0.87	1.18	1.74
ASQL (ng/g)	0.262	0.135	0.184	0.273
LOQ (μg/kg)	13.1	6.8	9.2	13.6

RESULTS AND DISCUSSION

Method Validation. To validate the method for arsenic speciation and total methods, the NIST SRM 1568 rice flour was analyzed. Results for speciated and total As in the standard reference material were in good agreement with certified values (Table 2). Repeatability (%RSD, $n = 5$) was 2.8% for iAs, 1.7% for DMA and species sum, and 5.3% for the total arsenic by As total method. A mass balance was calculated between the sum of all arsenic species detected and the total As determined in each NIST SRM 1568 sample. The average mass balance was 99.9 ± 6.3% for the NIST SRM samples, which ensured that the majority of the total arsenic in the samples was accounted for in the speciation analysis. The results are shown in Table 2.

The analytical solution detection limit (ASDL), analytical solution quantitation limit (ASQL), method LOD, and method LOQ were determined for the arsenic speciation analysis in rice using the HPLC-ICPMS method. The ASDL and ASQL were based on standard deviation of replicate ($n = 10$) analyses of low-level mixed standard (each species = 0.2 ng/g). The LOD and LOQ are calculated using the ASLD and ASQL,

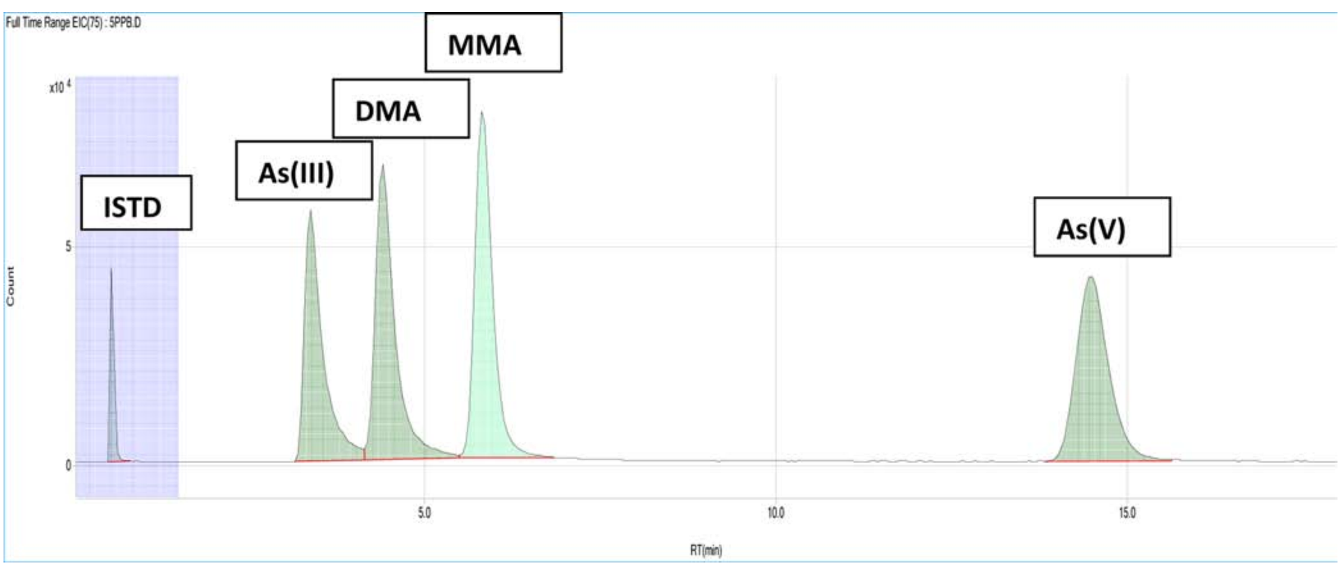

Figure 1. Chromatographic separation of arsenic standards at 5 ng/g.

Table 5. Recovery of Arsenic ($n = 2$)

	% recovery				
sample name	75 As(III)	75 DMA	75 MMA	75 As(V)	iAs
Spk LL-50 ppb	104.1 ± 38.2	115.0 ± 1.2	104.8 ± 1.1	109.1 ± 38.3	106.5 ± 0.6
Spk LH-75 ppb	85.1 ± 1.3	108.6 ± 2.9	106.8 ± 0.8	116.4 ± 0.9	100.5 ± 1.1
Spk LL-100 ppb	67.8 ± 32.8	112.4 ± 2.2	105.0 ± 0.8	143.7 ± 28.5	105.2 ± 2.6
Spk LL-150 ppb	74.7 ± 10.1	104.0 ± 9.4	106.0 ± 6.1	120.7 ± 7.7	97.3 ± 8.9
Spk LH-150 ppb	67.9 ± 15.6	112.9 ± 2.8	106.6 ± 0.5	142.8 ± 12.4	104.8 ± 1.8
Spk LH-225 ppb	88.1 ± 1.5	109.3 ± 1.2	105.2 ± 1.0	118.2 ± 3.0	102.9 ± 2.2

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Table 6. Arsenic Concentration by Speciation Method and Totals Method of Infant Rice Cereals[a]

sample ID	As(III) (μg/kg)	DMA (μg/kg)	MMA (μg/kg)	As(V) (μg/kg)	iAs[b] (μg/kg)	total As (speciation)[c] (μg/kg)	total As (totals)[d] (μg/kg)	% mass balance	inorganic As per serving (μg/serving)
mixed-grain cereal_1	20.6 ± 7.7	27.5 ± 0.2	TR (1.4)	36.6 ± 8.4	57.2 ± 0.7	86.2 ± 0.5	88.3 ± 1.3	97.6	0.9
mixed-grain cereal_18	18.1 ± 3.3	35.2 ± 1.4	TR (1.9)	44.9 ± 4.1	63.0 ± 0.7	100. ± 2.0	112. ± 5.7	89.1	0.9
mixed-grain cereal_19	12.1 ± 9.2	23.0 ± 0.7	TR (1.5)	43.3 ± 7.9	55.5 ± 1.3	79.9 ± 1.4	88.0 ± 1.4	90.9	0.8
mixed-grain cereal_20	4.84 ± 1.3	50.5 ± 0.6	TR (2.3)	58.9 ± 1.1	63.7 ± 0.1	116. ± 0.6	124. ± 0.5	93.8	1
mixed-grain cereal_21	13.0 ± 2.1	48.2 ± 0.4	TR (2.4)	58.5 ± 2.0	71.6 ± 0.1	122. ± 0.5	126. ± 0.4	96.4	1.1
mixed-grain cereal_22	4.83 ± 0.5	50.0 ± 1.7	TR (2.5)	64.0 ± 1.1	68.8 ± 0.6	121. ± 2.7	131. ± 9.1	92.2	1
mixed-grain cereal _23	9.89 ± 9.6	49.1 ± 0.6	TR (3.0)	55.4 ± 9.1	65.3 ± 0.4	117. ± 0.7	128. ± 4.6	91.5	1
mixed-grain cereal _27	19.3 ± 0.2	38.1 ± 0.5	TR (1.3)	37.7 ± 5.3	57.1 ± 5.0	96.5 ± 3.9	109. ± 0.4	88.1	0.9
organic, rice cereal_4	37.8 ± 8.6	153. ± 0.6	TR (8.9)	67.3 ± 4.9	105. ± 3.7	267. ± 3.8	272. ± 7.9	98.2	1.6
organic, rice cereal_6	26.0 ± 0.4	51.8 ± 1.5	TR (2.8)	64.7 ± 1.9	90.7 ± 1.4	145. ± 3.1	146. ± 7.3	99.3	1.4
rice single-grain cereal_5	65.7 ± 5.7	100. ± 0.1	TR (4.9)	72.0 ± 3.7	137. ± 1.9	243. ± 2.5	230. ± 10.	106	2.1
rice single-grain cereal_7	32.9 ± 0.8	89.2 ± 0.9	TR (5.1)	85.9 ± 0.3	118. ± 1.1	213. ± 0.7	212. ± 8.5	100	1.8
rice single-grain cereal_8	56.6 ± 1.8	126. ± 1.0	TR (6.6)	76.1 ± 0.5	132. ± 1.3	265. ± 0.8	256. ± 0.4	104	2
rice single-grain cereal_13	27.6 ± 0.1	35.3 ± 0.0	TR (1.5)	45.2 ± 1.7	72.8 ± 1.6	109. ± 1.2	108. ± 1.7	101	1.1
rice single-grain cereal_14	24.7 ± 1.0	32.2 ± 0.9	TR (3.0)	43.5 ± 0.0	68.2 ± 0.9	103. ± 1.8	101. ± 1.7	102	1
rice single-grain cereal_15	24.1 ± 1.6	30.0 ± 0.3	TR (1.6)	33.8 ± 0.0	58.0 ± 1.7	89.7 ± 3.1	92.5 ± 3.2	97.1	0.9
rice single-grain cereal_16	59.9 ± 22.	107. ± 1.5	TR (3.4)	65.6 ± 14.	125. ± 8.1	236. ± 9.6	227. ± 1.6	104	1.9
rice single-grain cereal_17	35.2 ± 10.	34.3 ± 0.9	TR (1.8)	54.7 ± 7.0	90.0 ± 3.1	126. ± 3.1	127. ± 1.4	99.2	1.4
rice single-grain cereal_26	60.1 ± 8.3	97.1 ± 1.8	TR (2.7)	47.4 ± 8.2	107. ± 0.1	207. ± 2.1	241. ± 2.4	85.8	1.6
rice single-grain cereal_31	58.6 ± 3.7	104. ± 1.6	TR (2.8)	39.1 ± 2.7	97.8 ± 0.9	205. ± 3.5	237. ± 0.9	86.4	1.5
organic, whole grain rice cereal_3	16.2 ± 7.3	15.9 ± 0.5	0	51.7 ± 5.4	68.0 ± 1.9	84.4 ± 3.0	85.1 ± 1.7	99.1	1
organic brown rice cereal_2	21.7 ± 0.2	42.9 ± 0.1	TR (4.2)	111. ± 1.2	133. ± 1.0	180. ± 0.2	178. ± 0.1	101	2
organic brown rice cereal_9	21.7 ± 10.	27.7 ± 1.0	0	34.3 ± 9.1	56.0 ± 1.7	84.7 ± 2.6	83.9 ± 2.6	101	0.8
organic brown rice cereal_10	24.2 ± 1.3	206. ± 4.2	TR (6.5)	39.5 ± 2.6	63.8 ± 4.0	277. ± 8.0	261. ± 7.0	106	1
organic brown rice cereal_11	21.3 ± 3.1	38.5 ± 0.3	TR (2.8)	107. ± 0.3	128. ± 2.7	169. ± 2.0	165. ± 7.8	103	1.9
organic brown rice cereal_12	23.0 ± 3.5	107. ± 1.0	TR (3.4)	65.7 ± 1.3	88.8 ± 2.2	200. ± 2.9	206. ± 13.	97.2	1.3
organic brown rice cereal_24	18.0 ± 0.9	25.8 ± 0.0	TR (1.7)	69.4 ± 0.7	87.4 ± 1.6	114. ± 2.0	127. ± 1.9	90.2	1.3
organic brown rice cereal_25	23.1 ± 1.8	27.6 ± 0.0	TR (2.0)	73.0 ± 0.6	96.2 ± 2.4	125. ± 1.9	133. ± 0.0	94.2	1.4
organic, whole grain rice cereal with apples_28	41.8 ± 1.2	32.2 ± 0.0	TR (1.5)	63.3 ± 0.4	105. ± 0.7	139. ± 0.9	160. ± 1.0	86.6	1.6
organic, whole grain rice cereal_29	51.5 ± 2.2	65.0 ± 0.3	TR (2.6)	89.6 ± 1.2	141. ± 0.9	208. ± 0.1	242. ± 4.4	86	2.1
organic, whole grain rice cereal_30	81.7 ± 7.8	51.8 ± 0.6	TR (1.2)	76.7 ± 13.	158. ± 6.0	211. ± 5.4	244. ± 0.0	86.4	2.4

[a]All samples were analyzed in duplicate (n = 2, mean ± SD). [b]iAs equals the sum of As(III) and As(V). [c]% recovery = total As from speciation (As(III) + DMA + MMA + As(V)). [d]Total As from totals method.

respectively, multiplied by nominal dilution factor (DF = 50). The LOD values for As species ranged from 0.9 to 1.8 ng/g and the LOQ from 7 to 14 μg/kg (Table 4).

The calibration curves for arsenic species were determined using As(III), DMA, MMA, and As(V) standards with concentrations of 0.25, 0.4, 1.0, and 5.0−20 ng/g (Figure 1). The standard curves were linear for the range of 0.25−20 ng/g, with R^2 of 1.00). Calibration check standard (10 ng/g of each: iAs(III), DMA, MMA, and As(V)) percent recovery was 99 −113% with %RSD < 4%.

The samples were spiked before extraction, and generally the recoveries for iAs, DMA, and MMA were excellent. Spiked sample recoveries for iAs, DMA, and MMA ranged from a low of 97.3% for iAs to a high of 115.0% for DMA. Spiked sample recoveries for iAs at lower concentration levels (50, 100, and 150 ng/g) were 106.5 ± 0.6, 105.2 ± 2.6, and 97.3 ± 8.9%, respectively (number of replicates, n = 2). Spiked sample recoveries for iAs at the higher concentration levels (75, 150, and 225 ng/g) were 100.5 ± 1.1, 104.8 ± 1.8, and 102.9 ± 2.2%, respectively (Table 5).

Total and Speciation Arsenic Concentrations. Table 6 shows the total and speciation results for arsenic in infant rice cereals. Of the 31 infant rice cereals tested, all 31 contained detectable arsenic as well as iAs. The average total arsenic and iAs concentrations in rice cereals were 174.4 and 101.4 μg/kg, respectively. This gives an average iAs concentration of 58.2% in the infant rice cereals tested. The mass balance between the As speciation and total methods (sum of all As species by the speciation method divided by the As concentration from the total method) ranged from 85.8 to 106.0%, with an average mass balance of 96.7%. All mass balances are shown in Table 6, and no bias was seen between replicates 1 and 2. The concentrations of the arsenic in various types of infant rice cereals were compared. Results show that the average total arsenic and iAs concentrations were lower in mixed-grain cereals (105.1 and 62.8 μg/kg, respectively) than in plain rice. The mean iAs concentration for the baby mixed-grain cereals was 59.8%. The types of rice grain used to make the infant rice cereals were analyzed as listed in Table 3, with 31 infant rice cereals made from either organic rice whole grain, mixed-grain flour (whole wheat flour, rice whole grain, and oat flour), organic rice, or rice flour. Results show that levels of inorganic arsenic (iAs) greatly varied among all rice cereals (Table 6). There was no

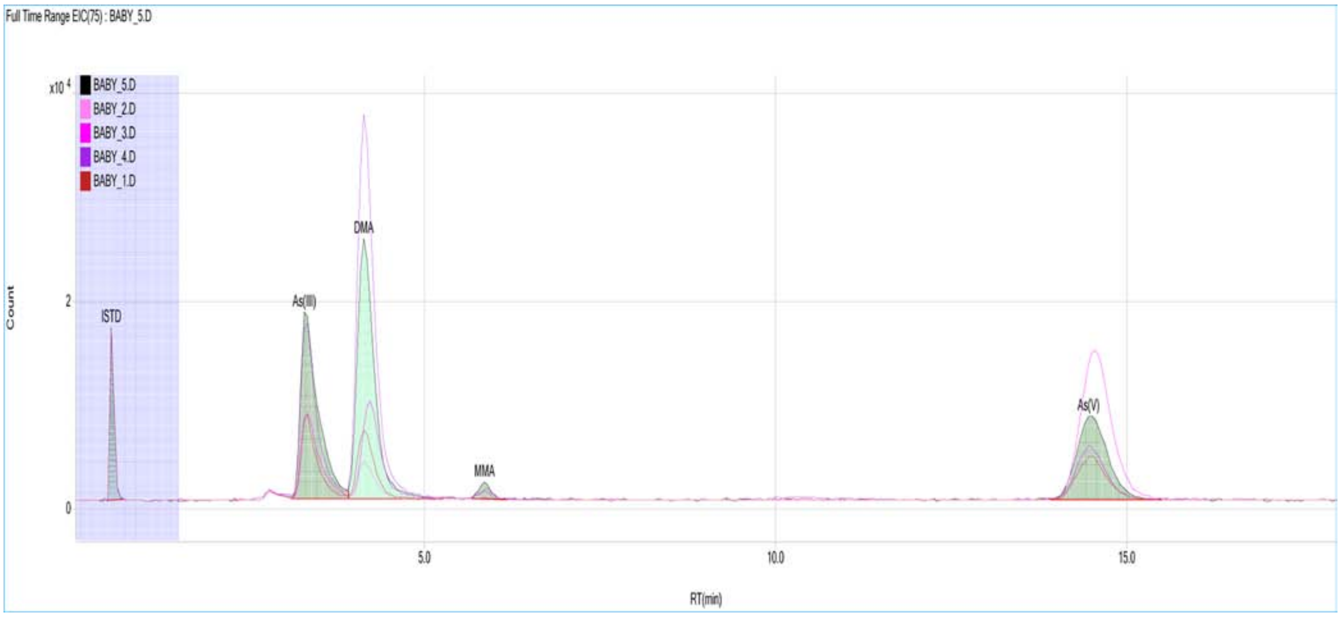

Figure 2. Representative chromatogram of arsenic speciation in five infant rice cereals.

substantial difference in iAs levels between organic and conventional rice cereals (an average of 98 ng/g in organic white rice cereal versus 101 ng/g in conventional white rice cereal). In 31 infant rice cereals, the total arsenic concentration ranged from 84.5 to 267.4 ng/g. The iAs as a percent of total arsenic ranged from 23 to 81%. The major detected organoarsenical species was DMA (Figure 2). MMA was not detected, or only trace levels were found (Table 6).

On September 19, 2012, the FDA released the first analytical results of nearly 200 samples of rice and rice products collected in the U.S. marketplace. Among these samples 32 were rice cereal samples, but only 3 were infant rice cereal samples (Table 7). The FDA reported values much higher than our results on iAs amount per serving. Average iAs was 3.5 μg/serving in FDA-tested rice cereals (range of 1.5–9.7 μg/serving) with an average of 2.7 μg/serving in infant rice cereals. The 23 infant rice cereal samples that were analyzed at IFSH had on average 1.5 μg/serving, and 8 mixed-grain cereals had 0.9 μg/serving on average. The average iAs range was from 0.8 to 2.4 μg/serving in infant rice cereals (Table 6).

Table 7. FDA Summary Analytical Results from Rice/Rice Product Sampling, September 2012

sample ID	product category	sample description	country of origin	inorganic As per serving (μg/serving[a])
70145	rice cereal	organic whole grain rice cereal (infant)	NR	3.2
720341	rice cereal	organic whole grain rice cereal (infant)	NR	2.9
719983	rice cereal	rice single grain (infant)	NR	2

[a]Serving size based on Reference Amount Customarily Consumed (RACC) per 21CFR 101.12 for infant rice cereals, which used a value of 15 g to calculate the mcg inorganic arsenic per serving.

The results of the infant rice cereals were compared with other similar sample types, infant and toddler formulas, run by Heitkemper[30] and Jackson.[6] Concentrations of total arsenic and inorganic arsenic were not as high as some of the infant and toddler formulas already in the literature. This could be due to the fact that none of the infant rice cereals in this study contained organic brown rice syrup (OBRS). It has been published by Jackson[6] that toddler formulas that have OBRS as the primary ingredient can give results >20 times higher than non-OBRS formulas. (Most times the peaks were properly and consistently captured using Autointegration parameters (software determined) in the EAM 4.11 method (Table 8), which frees analyst time and reduces the cost of sample analysis.)

Table 8. EAM 4.11 Method Parameters for MassHunter Peak Integration

general (tab)	
detector	
data point sampling: 1	start threshold: 0.3
smoothing: (checked)	stop threshold: 0.5
detection filtering: 5 point	peak location: top
baseline allocation	
baseline reset (# points) > 10	
if leading or trailing edge <50	
baseline preference drop else tangent skim	
peak filter (tab)	
peak area [counts] 2000 (select this bullet only)	
leave all other input fields unchanged	

This study determined levels of different arsenic species in infant cereals that present a potential health risk to the infant population. Our results for iAs in 30 infant rice samples indicated that American rice cereal did not exceed the Chinese maximum allowed concentration of 150 ng/g iAs in rice. One sample (organic whole grain rice cereal) did exceed the maximum allowed concentration of 158 ng/g. The mixed-grain rice cereal contained the least total (105 ng/g) and inorganic arsenic (63 ng/g). Speciation of arsenic in rice products is necessary because of the growing consumer and regulatory concern and the lack of information or regulation on arsenic levels in foods. This study provides new and much needed information on arsenic levels in rice and rice-based infant cereals.

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Notes

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