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Arsenic concentration and speciation in infant formulas and first foods

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Abstract

Arsenic exposure to humans is pervasive, and, increasingly, studies are revealing adverse health effects at ever lower doses. Drinking water is the main route of exposure for many individuals; however, food can be a significant source of arsenic to an individual, especially if their diet is rice-based. Infants are particularly susceptible to dietary exposure, since many first foods contain rice and they have a low body mass. Here we report on arsenic concentration and speciation in infant formulas and first foods. Speciation is essential for food analysis because of the much greater toxicity of inorganic arsenic species and the possibility that arsenic in food (unlike water) may be present in either inorganic or organic forms. Infant milk formulas were low in total arsenic (2.2–12.6 ng g^{-1}, n=15). Non-dairy formulas were significantly higher in arsenic than dairy-based formulas. Arsenic in formula was almost exclusively inorganic and predominantly arsenic(V). Arsenic concentration in purees (n=41) and stage 3 foods (n=18) ranged from 0.3–22 ng g^{-1}. Rice-fortified foods had significantly higher total arsenic concentrations than non rice-based foods. Again arsenic speciation was predominantly inorganic; arsenic(III) was the main species with lower concentrations of DMA and arsenic(V) also present. These data confirm that infants are exposed to arsenic via diet, and suggest that careful attention to diet choices may limit this.

Introduction

Arsenic (As) is a ubiquitous metalloid, typically present at low concentrations in rocks, soils and natural waters. The notable toxicity of arsenic has led to its widespread anthropogenic use in insecticides and herbicide, first as an inorganic compound then later as the less toxic organic arsenic compounds. Use of arsenic in agriculture, industry and from arsenic extraction operations has led to localized areas of severe soil contamination which pose immediate human health risks from contaminated water, dust or soil particles. More insidious, though, and affecting many millions more people, is exposure to ‘natural’ geogenic arsenic, resulting in both increased soluble arsenic species in drinking water (1–3) and agricultural soils (4, 5). The detrimental health effects of arsenic exposure via drinking water are well accepted and have led to a lowering of environmental regulatory limits to 0.01 mg/L promoted by both WHO and the US EPA(6). The exposure of millions of people to elevated levels of geogenic arsenic in drinking water is happening now in the Asian sub-continent, particularly in Bangladesh but also in India, Vietnam, and Cambodia (7). Indeed, anywhere where access to drinking water is via groundwater wells there is the potential for elevated arsenic depending on the prevailing geology and sub-surface biogeochemical conditions.

Although arsenic is not generally readily taken up by crops or transported to the edible parts, a notable exception is rice, a staple food that can take up arsenic from soil and transport it to the grain (4, 8–10). The magnitude of this uptake varies widely between cultivars, but the ability to take up elevated concentrations of arsenic (in comparison with other cereal crops)

appears to be a trait found in the entire rice germplasm. Elevated arsenic concentrations in rice (relative to other food sources) were first reported in 1999 (11) However, it is only recently that the potential human health implications for populations consuming rice-based diets has been fully appreciated, largely through the work of Meharg and colleagues in identifying food products containing arsenic, quantifying and speciating arsenic in these products, and showing that arsenic intake through food can be equivalent to or greater than drinking water at the current safe drinking water limit(4, 9, 12–19).

Speciation of arsenic in food products is necessary because of the differential toxicity of different arsenic compounds. Organic arsenic compounds, such as arsenobetaine found in seafood, are non-toxic and can be consumed without health concerns while others, such as inorganic arsenic(III) and arsenic(V) are toxic and pose a potential health risk(20). Depending on growing conditions, rice contains predominantly dimethylarsenic acid (DMA) or inorganic arsenic (14, 21). Inorganic arsenic is more toxic than the methylated arsenic species. While direct speciation of the solid compound would be preferable, current analytical techniques do not possess the necessary detection limits to quantify multiple arsenic species at sub mg/kg levels. Speciation studies of solids generally require extraction of the arsenic from the solid and analysis of the resulting extracted solution. Ideal extraction techniques are those that extract all of the arsenic from a particular sample without changing the speciation from that in the solid. Moreover, the ideal analytical system would identify all the arsenic species in that extracted solution even at very low concentrations; currently HPLC-ICP-MS is closest to the ideal analytical system. Recently, dilute (1–2 %) extractant solutions of HNO_3 have been shown to give excellent recovery of arsenic species from reference materials and from rice and rice products (22), while being simpler than earlier trifluoroacetic acid TFA extraction procedures (23).

Infants and young children may be particularly vulnerable to dietary sources of arsenic. The 2009 EFSA report found that dietary exposure to inorganic arsenic for children under age three is ~2 to 3 times higher that of adults (24). Many families introduce rice cereal as the first solid food for 4–6 month old infants. These rice-based cereals are particularly high in arsenic relative to other foodstuffs and Meharg et al (15) estimated a median intake of arsenic of 0.21 $\mu g\ kg^{-1}\ d^{-1}$ for an average 20 lb, 1 year old baby, by consumption of a single 20g serving, exposure which exceeds that of an adult drinking water containing 10 ppb arsenic (0.17 $\mu g\ kg^{-1}\ d^{-1}$). This latter adult exposure value is based on daily consumption of 1 liter of tap water as estimated by the US EPA(25). Exposure to arsenic from consumption of rice cereal is high both because of the high concentration of arsenic in rice cereal and the low body mass of a child.

Rice and derived products like starch, flour and syrup are used to fortify a number of processed baby foods, including formula (powdered baby milk), jarred purees and strained foods, and snack items. In this study, we have determined total arsenic and arsenic speciation in a number of infant formulas and first foods. For formulas, we compared dairy- and soy-based products. For first foods, we tested fruit and vegetable purees, plus more complex stage 3 purees that contain meat and grains, and determined whether rice-containing and non-rice containing and meat and vegetarian products differed in their arsenic concentrations. The work was conducted in support of an ongoing birth cohort study focusing on environmental health effects of early life exposure to arsenic.

Materials and methods

Infant formulas and first foods were purchased from supermarkets in the Hanover, New Hampshire area and were chosen from popular brands and to reflect the diversity of ingredients in these foods. Foods were analyzed directly from the sample container and were

not further dried or homogenized. For total As analysis of infant formulas, approximately 250 mg samples were acid-digested using 2 ml 50:50 optima HNO_3:H_2O by microwave digestion (MARS XPRESS, CEM, Mathews, NC) with a 10 minute ramp and 10 minute hold at 180°C. The digested sample was then diluted with deionized water to a final volume of 10 ml. The diluted sample was analyzed for As by ICP-MS (7700x, Agilent, Santa Clara, CA) using He as a collision gas.

Purees and stage 3 foods were 'open vessel'-digested in concentrated HNO_3. Between 1–2 g of each product was weighed into a 50 ml polypropylene tube and 2 ml of acid was added. The vials were lightly capped and heated in a microwave at 95°C for 30 minutes. The samples were allowed to cool, 250 μl of H_2O_2 were added, and the samples were taken through a second heating step. The samples were diluted to 10 ml and the weight was recorded. An aliquot of digested sample was then centrifuged at 13300 rpm for 30 minutes and a 1 ml aliquot for the supernatant was filtered (0.45 μm) and diluted to 4 ml with DI water.

For speciation analysis formula and food samples were extracted with 1% HNO_3. Approximately 2 g of sample was weighed into a 50 ml polypropylene tube and 20–40 ml of 1% HNO_3 was added. The tubes were lightly shaken then taken through a progressive microwave heating program of 10 minutes at 55°C, 10 minutes at 75°C and 30 minutes at 95°C. An aliquot was then centrifuged and/or filtered (0.45 μm) prior to speciation analysis. The samples were further spin-filtered through 10 KDa spin filters (VWR, Radnor, PA) to remove larger molecular weight constituents that could foul the ion exchange columns.

Speciation analysis was by anion exchange chromatography coupled to ICP-MS. An Agilent LC1120 was used as the liquid chromatography system. Two different exchange columns were used, a Hamilton PRP X100 and a Dionex AS16 column. For the Hamilton column the eluant was 20 mM $NH_4H_2PO_4$ at pH 6 and a flow rate of 1 ml min^{-1}. For the Dionex AS 16 column the gradient elution method reported in Jackson and Bertsch(26) was used with tetramethyl ammonium hydroxide as the mobile phase and a flow rate of 1 ml min^{-1}. In both methods, the effluent was introduced directly to the ICP-MS equipped with a seaspray nebulizer (Glass Expansion, Pocasset, MA). The ICP-MS was operated as described above.

Inorganic As standards were obtained from Inorganic Ventures (Christiansburg, VA), while MMA and DMA were prepared from salts of monosodium methane arsonate (Chem Services, West Chester, PA, USA) and cacodylic acid (Sigma Aldrich, St Louis, MO), respectively. Calibration standards were prepared daily from serial dilution of stock species standards. Quality control for total digestion and analysis included triplicate analysis for all formula samples and duplicate and spike analysis at a frequency of one each per batch of 20 samples for the purees and stage 3 foods. Rice flour National Institute of Standards and Testing Standard Reference Material 1568a (Gaithersburg, MD) was used as a quality control material for both total As measurements and As speciation. Although As species are not certified for this SRM, reproducible consensus values have been demonstrated from many studies. We determined Total As in NIST 1568a to be 318 ± 26 ng g^{-1} (n = 8), the certified value being 290 ± 30 ng g^{-1}. For As speciation we determined DMA to be 200 ± 17 ng g^{-1} and inorganic As to be 105 ± 17 ng g^{-1} which are in the range reported by other studies(9).

We used mixed model, nested ANOVA (JMP version 8.0.2) to test whether mean total As concentrations differed with product formulation. For example, we compared formulas (1) with and without dairy and (2) with and without rice. For the purees, we compared fruit versus vegetable-based products, and (after noticing that pears were particularly high in As), pears vs. other kinds of fruits. In each model, we treated the factor of interest (dairy/soy,

rice/not-rice, fruit/vegetable) as a fixed main effect, with a random effect of the product name (e.g., Brand A sweet potatoes) nested within that main effect to account for replicate measurements for most of the products tested. Finally, for the stage 2/3 foods, we compared products with and without rice and with and without meat, in a two-factor ANOVA, with product name nested within each rice x meat treatment. For the formulas, As concentrations met the assumptions for analysis without transformation, but for the purees and stage 2/3 foods, $\log_{10}$-transformation was required to homogenize variance.

We also make some estimates of average As exposure for infants through to 1 year old babies based on personal experience of infant diets and feeding frequency and average of the 50th percentile weights for 3,6 and 12 month old children taken from the WHO child growth standards (http://www.who.int/childgrowth/standards/Chap_4.pdf). For comparison, we use an upper exposure metric suggested by Meharg (15) that a 60 kg adult consuming 1 L of drinking water at the EPA/WHO limit would consume 0.17 μg arsenic per kg body weight per day.

Results and Discussion

Infant Formulas

We analyzed 15 infant formulas comprising of 5 main brands. The formulas were further classified as to whether they were dairy- vs. non-dairy based, and whether they contained rice starch. Arsenic totals were then statistically evaluated between these classes for significant differences. Arsenic was detectable in all infant formulas, with values ranging from 2.2–12.6 ng g^{-1} (Table 1). The mean arsenic concentration was significantly lower in dairy-based formulas than those without dairy (nested ANOVA $F_{1,13}$=13.3, P=0.003). Most (92.9%) of the variability not explained by the fixed effect of dairy was explained by formula type; triplicate samples had coefficients of variation <16.9%.

Arsenic speciation was evaluated on 1% HNO_3 extracts for the 9 infant formulas with total arsenic >6 ng g^{-1}. The concentration of arsenic species in the extracted formula samples were near the limit of detection of the IC-ICP-MS technique for either ion exchange column. Nevertheless, the results from both ion exchange columns were in general agreement: arsenic speciation in the formulas was almost exclusively inorganic and the major arsenic species was arsenic(V); both ion chromatography columns showed that infant formula (IF) 11 had a higher proportion of arsenic(III) than the other formulas. Low concentrations of DMA (~0.5 μg l^{-1}) were quantifiable only on the AS16 column. Recoveries for the Hamilton column (sum of species/total digested arsenic concentration) ranged from 54–102% with an average of 76%, while for the As16 column they ranged from 72–145% with an average of 80%. Both columns had an arsenic species eluting in the void volume for many of the infant formulas, which may be either arsenobetaine or other arsenic species not retained by anion exchange chromatography.

Few other studies have reported arsenic levels in infant formulas. One study, which used a similar approach to that described here, reported that infant formulas ranged from 12–17 ng g^{-1} and that the major species were inorganic arsenic and DMA(27). A more recent study reported arsenic concentrations for formulas reconstituted per the manufacturer's instructions with deionized water and these ranged from < 1–1.6 μg l^{-1}(28). Applying a similar calculation to our data, 1 scoop formula per 60 ml water and an average scoop weight of 9 g, yields formula arsenic concentrations of 0.3–1.8 μg l^{-1}. Hence there is general agreement between studies on the concentration levels of arsenic in main brand formulas. Using the arsenic concentration range from Table 1, we calculate that the arsenic exposure of a three month old 6.2 kg infant consuming 6, 60 ml bottles of formula daily, would be between 0.036–0.21 μg arsenic per kg body mass per day solely from formula.

This higher range value exceeds the 0.17 μg kg^{-1} d^{-1} limit referred to earlier, of an adult drinking 1 L of water at the WHO/EPA limit and suggests the potential vulnerability of infants, because of their low body weight, to even ostensibly low concentrations of arsenic in food.

Purees

Arsenic concentration was also analyzed in three different brands of fruit and vegetable purees (n = 40) targeted at 6–12 month old infants (Table 2). For two brands (D, G) the total arsenic concentration ranged from 0.32–7.8 ng g^{-1}. For brand E, most purees were low in total arsenic (1–4 ng g^{-1} with a MDL = 0.15 ng g^{-1}), except for the pear-containing products, which had an average arsenic concentration of 16.6 ng g^{-1}. Given the brand-specific nature of these high arsenic pear products, these high concentrations are likely source related rather than a property of pears in general. Overall, fruit purees had marginally higher arsenic concentrations than vegetable purees ($F_{1,40.7}$=3.86, P=0.06), but this result was driven entirely by pear products from one brand; when pear-containing products were excluded from the analysis, fruit and vegetable purees were not different ($F_{1,34.01}$=0.12, P=0.73). Within the fruit purees, pear-containing products had significantly more arsenic ($F_{1,18.17}$=32.3, P<0.0001). Our results are similar to those of Vela and Heitkemper (27) who report an arsenic range of < 1–24 ng g^{-1} for infant puree food products and the prevalence of inorganic arsenic in the speciated samples.

For the high arsenic, pear-containing products, we measured arsenic speciation using the Hamilton PRP X100. Arsenic speciation was very similar for each pear-containing product, with inorganic arsenic > DMA. Inorganic arsenic (as the sum of arsenic(III) and arsenic(V)) ranged from 76–83%, and the overall recovery of arsenic species (sum of species as a percent of the total arsenic determined separately) ranged from 80–96%.

Total arsenic concentrations per serving for these fruit and vegetable purees ranged from 0.03–2.3 μg. A typical 7 month old infant (≈8 kg) consuming these products might eat 1.5 full jars daily. At the median exposure (0.25 μg/serving), this infant would be exposed to ≈0.05 μg arsenic per kg body mass per day, or <⅓ of the ‘safe’ adult level derived from drinking water. However, if this infant were eating the median jar of pears daily (1.6 μg arsenic/serving), the exposure would be 0.2 μg per kg per d, above the safe adult level. Also, an infant of this age would still be formula fed, contributing more arsenic to the daily exposure total.

Stage 2/3 Foods—We also determined arsenic in more complex infant foods containing multiple ingredients including meat, vegetable and grain products; these foods are typically marketed at infants aged 9–15 months. N.B. no ‘rice only’ foods, such as cereals, were considered in this study; previous studies have shown these to have high inorganic As concentrations with median values of 110 ng g^{-1}(15). We sub-classified these foods into meat (M) vs. vegetarian (V), and rice or non-rice based. Total arsenic ranged from below detection (ca. 1–3 ng g^{-1} depending on the extent of dilution of the digested solid) to 22 ng g^{-1} (Table 3). Statistically, there was an interaction between meat and rice content ($F_{1,37.25}$=14.42, P=0.0005): foods with both meat and rice had the highest arsenic concentration. Foods without rice had the lowest mean total arsenic concentrations (least-squares mean of 3.75 ng g^{-1} for neither meat nor rice and 2.34 ng g^{-1} for meat but not rice). Of the foods with rice, those without meat had lower arsenic concentrations (LS mean 11.90 ng g^{-1}) than those with meat (LS mean 18.45 ng g^{-1})..

Arsenic speciation was determined in foods were arsenic > 5 ng $g^{-1}$and was again found to be predominantly (> 70%) inorganic arsenic. This was somewhat unexpected given that for these foods the concentration of arsenic is related to the presence of rice and that DMA is

the major species in US rice, although rice from other countries such as Bangladesh and India have higher (≥80%) proportion of inorganic rice(9), Alternatively the rice in these foods may have higher levels of rice bran which is known to be higher in inorganic arsenic than bulk grain(18) or there may be additional (non-rice) sources of inorganic arsenic from other ingredients in these foods. We note that inorganic arsenic is the major speices (75–90%) in rice products such as crackers, noodles and puffed rice(19) and that our results for baby foods show similar levels of inorganic arsenic.

Total arsenic content per 170 g (brand G) or 113 g (brands D and E) serving of these foods ranged from 0.17–3.7 μg, with a median of 1.3 μg. If a 10 kg infant (~1 year old) consumed 3 full jars at the median arsenic concentration each day, s/he would be exposed to 0.39 μg arsenic per kg body mass per day, more than twice the 0.17 $\mu g\ kg^{-1}\ d^{-1}$ safe adult arsenic exposure level. Even at the lowest concentrations, the daily exposure would be 0.05 $\mu g\ kg^{-1}\ d^{-1}$ solely from these jarred foods, before any consideration of other potential arsenic sources such as cereals or water.

Conclusions

Although we report relatively low concentrations of arsenic (< 1–23 ng g^{-1}) in formulas, purees and multiple ingredient infant foods, these levels are potentially of concern because arsenic is present mainly in the more toxic inorganic form. In addition, the low body weight of infants means that when expressed on a $\mu g\ kg^{-1}\ d^{-1}$ basis, even these low concentrations result in exposures that are greater than for an adult drinking water at the WHO/EPA safe drinking water level. Additionally, our results and theoretical dietary intakes do not take into account additional arsenic present in water used to reconstitute the infant formulas, which can be significant in our geographical region (New England. USA)(29) and elsewhere. We also do not consider rice-based cereals, which can be an order of magnitude higher in arsenic than the foods reported here (15).

It is clear that food is a significant route of arsenic exposure for infants that must be considered in any epidemiological study. Although the US has no regulations governing the arsenic concentration of foodstuffs, China has set a level of 150 ng g^{-1} inorganic arsenic for rice(30). However, in the case of infants, where the per kg exposure rate is so much higher, then even this limit would appear too high.

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Table 1

Total arsenic concentrations, speciation and formula type for 15 main brand formulas

total As (ng g^{-1})	dairy	rice	species recovery	% inorganic As	Brand
5.36 ± 0.21	YES	NO	n.s.		A
11.27 ± 0.35	NO	NO	88.20%	100%	A
9.29 ± 0.43	NO	NO	88.61%	100%	A
11.89 ± 0.64	NO	YES	66.76%	100%	A
5.76 ± 0.4	YES	NO	n.s.		B
6.95 ± 0.43	NO	NO	102.84%	100%	B
11.43 ± 1.09	NO	NO	84.25%	100%	B
6.02 ± 0.26	YES	YES	n.s.		B
8.19 ± 0.63	YES	YES	54.48%	100%	B
8.14 ± 0.77	YES	NO	55.31%	100%	C
9.38 ± 0.31	YES	NO	62.75%	100%	D
2.92 ± 0.33	YES	NO	n.s.		D
9.62 ± 1.35	NO	NO	77.58%	100%	E
3.42± 0.2	YES	NO	n.s.		F
2.6 ± 0.44	YES	NO	n.s.		F

n.s. = not speciated

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TABLE 2

Total arsenic concentration in 41 1st food purees.

ingredients	As (ng g^{-1})	brand
pears & mango	15.01	E
sweet potatoes	1.45	E
apples & apricots	1.48	E
winter squash	0.68	E
sweet potatoes	2.78	E
apples & blueberries	0.93	E
apples	0.69	E
prunes & oatmeal	1.74	E
first prunes	1.25	E
apples & plums	0.97	E
pears	13.55	E
pears & raspberries	20.20	E
carrots	1.68	E
peas	3.14	E
squash	1.90	E
corn & butternut squash	0.48	E
pears	17.52	E
apples	2.51	E
banana	3.99	D
pear and wild blueberry	1.00	D
sweet potatoes	5.03	D
apples	2.47	D
green beans	3.21	D
pears	4.64	D
carrots	1.75	D
squash	1.14	D
apple strawberry	1.2	D
apples	6.74	D
peaches	3.34	D

ingredients	As (ng g^{-1})	brand
prunes	2.01	D
sweet peas	1.06	D
Select prunes	1.63	D
Select sweet potatoes	7.81	D
green beans	0.9	G
squash	0.48	G
pears	3.17	G
sweet peas	0.74	G
sweet potatoes	4.28	G
sweet carrots	2.07	G
bananas	0.32	G
applesauce	0.65	G

Table 3

Arsenic concentration and selected speciation in 2nd/3rd stage foods.

food type	total As (ng g^{-1})	rice	species recovery	% inorganic As	Brand
meat and veg	<3.4	NO	n.s.		D
meat and fruit	4.41	NO	n.s.		D
meat, rice, and veg	22.34	YES	74.28%	72.1%	D
meat and veg	12.52	YES	n.s.		D
meat and pasta	18.32	YES	79.19%	77.9%	D
veg and rice	10.33	YES	91.18%	87.4%	E
2 veg	6.29	YES	133.04%	90.8%	E
rice and pulses	9.61	YES	92.67%	83.7%	E
veg medley	11.28	YES	82.12%	85.4%	E
meat and fruit	18.84	YES	88.56%	89.8%	E
meat and fruit	13.42	YES	67.30%	87.5%	E
meat and broth	13.81	YES	75.33%	86.2%	E
meat and rice	14.15	YES	110.29%	75.2%	E
fruit and oatmeal	1.74	NO	n.s.		E
fruit and rice	17.8	YES	98.35%	77.2%	E
meat and broth	<3.4	NO	n.s.		G
meat and broth	5.43	NO	n.s.		G
meat and broth	<3.4	NO	n.s.		G

n.s. = not speciated