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Co-exposure to methylmercury and inorganic arsenic in baby rice cereals and rice-containing teething biscuits

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ABSTRACT

Background: Rice is an important dietary source for methylmercury (MeHg), a potent neurotoxin, and inorganic arsenic (As), a human carcinogen. Rice baby cereals are a dietary source of inorganic As; however, less is known concerning MeHg concentrations in rice baby cereals and rice teething biscuits.

Methods: MeHg concentrations were measured in 36 rice baby cereals, eight rice teething biscuits, and four baby cereals manufactured with oats/wheat (n = 48 total). Arsenic (As) species, including inorganic As, were determined in rice baby cereals and rice teething biscuits (n = 44/48), while total As was determined in all products (n = 48).

Results: Rice baby cereals and rice teething biscuits were on average 61 and 92 times higher in MeHg, respectively, and 9.4 and 4.7 times higher in total As, respectively, compared to wheat/oat baby cereals. For a 15-g serving of rice baby cereal, average MeHg intake was 0.0092 μg day^{-1} (range: 0.0013–0.034 μg day^{-1}), while average inorganic As intake was 1.3 μg day^{-1} (range: 0.37–2.3 μg day^{-1}). Inorganic As concentrations in two brands of rice baby cereal (n = 12/36 boxes of rice cereal) exceeded 100 ng/g, the proposed action level from the U.S. Food and Drug Administration. Log_{10} MeHg and inorganic As concentrations in rice baby cereals were strongly, positively correlated (Pearson's rho = 0.60, $p < 0.001$, n = 36).

Conclusions: Rice-containing baby cereals and teething biscuits were a dietary source of both MeHg and inorganic As. Studies concerning the cumulative impacts of MeHg and inorganic As on offspring development are warranted.

1. Introduction

Methylmercury (MeHg) is a potent neurotoxin; the fetal period is considered the most sensitive exposure window, while young children are a secondary population of concern (U.S. Environmental Protection Agency (U.S. EPA), 1997). Fish ingestion is the primary dietary source of MeHg (National Research Council (NRC), 2000). Fish tissue is also a rich source of beneficial nutrients, including omega-3 fatty acids, which benefit offspring neurodevelopment (Innis, 2007). Rice ingestion is an important exposure pathway for MeHg (Feng et al., 2008; Hong et al., 2016). Most rice is cultivated in flooded rice paddies, where anaerobic microbes convert less toxic inorganic mercury (Hg) to MeHg, which is bioaccumulated in rice grain (Windham-Myers et al., 2014). In a comprehensive review of 51 studies reporting rice Hg concentrations (Rothenberg et al., 2014), the highest quintile of rice MeHg (10.5–57.7 ng/g) was 5.2–29 times lower than the maximum acceptable MeHg level in fish (300 ng/g) (U.S. EPA, 2001a). However, rice does not contain the same beneficial nutrients as fish, and therefore MeHg's toxicity from rice may differ from fish (Rothenberg et al., 2011a, 2014).

Rice is also an important dietary source for inorganic arsenic (As) (Zhao et al., 2010), a non-threshold human carcinogen (International Agency for Research on Cancer (IARC), 2004). In flooded rice paddies, inorganic As(V) is reduced to As(III), while As(V) is more concentrated

Abbreviations: ANOVA, one-way analysis of variance; As, arsenic; AsO, arsenic oxide; AsT, total arsenic; CDC, Center for Disease Control and Prevention; CVAFS, cold vapor atomic fluorescence spectrometry; DDI-H_2O, double-distilled H_2O; FAO, Food and Agricultural Organization of the United Nations; Hg, mercury; DMA, dimethylarsinic acid; IARC, International Agency for Research on Cancer; MDL, method detection level; MeHg, methylmercury; MMA, monomethylarsonic acid; NRC, National Research Council; SD, standard deviation; SF-ICP-MS, sector field inductively coupled plasma-mass spectrometer; THg, total mercury; U.S. EPA, U.S. Environmental Protection Agency; U.S. FDA, U.S. Food and Drug Administration

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near the roots due to oxygen leakage (Zhao et al., 2010). Both As(III) and As(V) are transported into rice plants by different pathways, and accumulated in rice grain (Zhao et al., 2010). Under submerged conditions, inorganic As is converted to dimethylarsinic acid (DMA) or monomethylarsonic acid (MMA) through microbial methylation (Zhao et al., 2010). DMA and MMA are also applied directly to agricultural fields as pesticides or herbicides (Zhao et al., 2010). Of the methylated As species, both MMA(III) and DMA(III) may be more cytotoxic and genotoxic than As(III) (Thomas et al., 2001); however, further studies are needed.

In the U.S., rice baby cereal is a common first food, which is introduced between four and six months (Karagas et al., 2016). Rice baby cereals and rice-containing infant snacks are dietary sources of inorganic As (Carbonell-Barrachina et al., 2012; Jackson et al., 2012a; Karagas et al., 2016; Meharg et al., 2008; Signes-Pastor et al., 2016; U.S. Food and Drug Administration (U.S. FDA), 2013). In 2015, the European Commission stipulated that rice used for the production of food for infants and young children must have inorganic As concentrations below 100 ng/g (Commission Regulation (EU), 2015). In 2016, the U.S. FDA issued a proposed action level of 100 ng/g of inorganic As for rice products marketed to infants (U.S. FDA, 2016a).

Rice baby cereals are also likely a source of MeHg. To the best of our knowledge, just one study reported total Hg (THg) and MeHg concentrations in infant rice cereals (n = 7, Brombach et al., 2017), while another study reported THg and total As (AsT) concentrations in rice baby cereals (n = 91, Hernández-Martínez and Navarro-Blasco, 2013). There are no studies reporting both MeHg and inorganic As concentrations in rice baby cereals; however, adverse health impacts due to co-exposure may be synergistic (Cardenas et al., 2015). It is important to evaluate whether rice baby cereals and teething biscuits, which meet the FDA proposed action level (i.e., < 100 ng/g inorganic As) (U.S. FDA, 2016a), are dietary sources of MeHg.

To address this knowledge gap, we quantified concentrations of THg, MeHg, AsT, inorganic As [= As(III) + As(V)], DMA and MMA, in rice baby cereals and rice teething biscuits purchased in the U.S. In addition, we also compared Hg and As concentrations between rice baby cereals, rice teething biscuits, and baby cereals manufactured with wheat or oats. Wheat/oat cereals served as a control; in previous studies, wheat/oat baby cereals had lower As concentrations compared to rice baby cereals (Carbonell-Barrachina et al., 2012; Hernández-Martínez and Navarro-Blasco, 2013), due to cultivation of wheat/oats in an upland environment.

2. Methods

2.1. Market basket survey

Between September 2016 and June 2017, 48 boxes of baby foods were purchased, including 36 boxes of rice baby cereals, eight boxes of rice-containing teething biscuits marketed to infants, and four boxes of baby cereals manufactured with wheat or oats. Rice baby cereals represented five brands (n = 3–12 boxes per brand), rice-containing teething biscuits represented two brands (n = 1 or 7 per brand), and baby cereals containing wheat or oats represented two brands (n = 2 per brand) (Table 1). Box labels indicated whether rice cereals were manufactured with brown rice, and whether rice grain was organic (Table 1). All products were purchased in the U.S., including South Carolina (n = 28), California (n = 9), Ohio (n = 6), New York (n = 3), and Florida (n = 2). Both brands of teething biscuits were manufactured in China, while the sources of rice used for rice baby cereals and teething biscuits were uncertain. One brand of rice baby cereal (Brand 4) was discontinued in December 2016, while the other four brands of rice baby cereals were still available in June 2017.

2.2. Lab analyses

For THg, samples (~ 0.6 g) were weighed into 40 mL borosilicate glass vials with Teflon-lined lids. Samples were leached in 5 mL of freshly prepared 3:7 sulfuric acid: nitric acid (v/v) for four hours at room temperature. Vials were transferred to a 60–70 °C water bath and gently heated for two more hours. After samples cooled, 0.35 mL of 0.2 N bromine monochloride was added, and the volume was raised to 35 mL using double-distilled H_2O (DDI-H_2O). Samples were held overnight. Just before analysis, hydroxylamine hydrochloride (0.050 mL) was added, and samples were further reduced with tin chloride, converting all Hg to elemental Hg. THg was analyzed following U.S. EPA Method 1631 (U.S. EPA, 2002), including quantification using cold vapor atomic fluorescence spectrometry (CVAFS) (Merx-T and Model III Detector, Brooks Rand Instruments, Seattle, WA).

Rice MeHg was analyzed using methods from Liang et al. (1996). Briefly, samples (~ 0.6 g) were weighed into a 50 mL polypropylene vial, and digested in 2 mL of 25% (w/v) potassium hydroxide-methanol in a 75 °C oven for three hours. Then 6 mL of dichloromethane and 1.5 mL of hydrochloric acid were added, samples were shaken, centrifuged (4000 rpm = 3000 × *g*, 30 min), and phases were separated. Then 30 mL of DDI-H_2O was added, and vials were heated for 1.5 h at 60–70 °C to remove dichloromethane. MeHg extracts were analyzed following U.S. EPA Method 1630 (U.S. EPA, 2001b), including ethylation with sodium tetraethylborate, and quantification by gas chromatography-CVAFS (Model-III Detector, Brooks Rand Instruments, Seattle, WA).

AsT concentrations were analyzed following U.S. EPA 3050b (U.S. EPA, 1996). Briefly, ~ 1.0 g of sample was weighed into a 70 mL vial, 5 mL of nitric acid was added (Baker), and samples were gently refluxed in a 80 °C water bath for 2 h. Then 5 mL of hydrogen peroxide was added, and vials were heated for one more hour. After vials cooled, the volume was raised to 60 mL using DDI-H_2O. AsT concentrations were analyzed using a Finnigan ELEMENT XR double focusing magnetic sector field inductively coupled plasma-mass spectrometer (SF-ICP-MS) with internal standard Rh or Ir. For sample introduction, a Micromist U-series nebulizer (0.2 mL/min) (GE, Australia), quartz torch, and injector (Thermo Fisher Scientific, USA) were used.

As species, including inorganic As, DMA and MMA, were determined for rice baby cereals and teething biscuits (n = 44/48). We did not speciate As in wheat/oat baby cereals (n = 4) because the AsT concentrations were far below 100 ng/g (range: 15–24 ng/g) (Table 1). All wheat/oat cereals met the FDA proposed action level (i.e., < 100 ng/g inorganic As) (U.S. FDA, 2016a) (Table 1). This differed from a majority (n = 34/44 boxes) of rice-containing cereals and teething biscuits, which had AsT concentrations > 100 ng/g. For rice-containing products, it was important to speciate As to determine whether samples exceeded the FDA proposed action level. A similar approach was used by Jackson et al. (2012b) in a study comparing products with and without organic brown rice syrup.

Methods for As speciation were previously described by Jackson (2015). Briefly, samples were weighed into 15 mL polypropylene vials, and three mL of 2% nitric acid was added. Vials were lightly capped and heated at 95 °C for 45 min. After vials cooled, 0.5 mL of 30% hydrogen peroxide was added [to oxidize As(III) to As(V)], and samples were heated for another 45 min at 95 °C. Samples were cooled, and an aliquot was analyzed. As speciation analysis was performed on an Agilent LC1260 liquid chromatograph coupled to a triple quadrupole ICP-MS (Agilent 8800), using an anion exchange column (Hamilton PRP-X100 10 mm 4.6 × 50 mm; Reno, NV) with a mobile phase of 40 mM ammonium carbonate at pH 9, a column temperature of 30 °C and a flow rate of 1.5 mL min^{-1}. The ICP-MS was operated with the collision cell with oxygen as a reactive gas, and As was detected at *m/z* 91 as arsenic oxide (AsO).

Quality assurance/quality control data are summarized in Table 2. The limits of detection were 0.008 ng/g for THg, 0.002 ng/g for MeHg