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Screening level health risk assessment of selected metals in apple juice sold in the United States

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ABSTRACT

Concerns have recently been raised about the presence of metals in apple juices. As such, the concentration of aluminum (Al), arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), manganese (Mn), mercury (Hg), and zinc (Zn) were measured in six commercially available brands of apple juice and three organic brands. The concentrations of total As, Cd, Cr, Cu, Hg, and Zn in all nine apple juice brands sampled were below each metal's respective U.S. Food and Drug Administration (FDA) maximum contaminant level for bottled water. However, in some apple juices the levels of Al, Pb, and Mn exceeded FDA maximum contaminant levels for bottled water. Therefore, a screening level risk assessment was carried out to assess the potential non-carcinogenic and carcinogenic risks that may result from metal exposure via apple juice consumption. Changes in blood Pb concentrations were also estimated to characterize potential risk from Pb exposure. Our results suggest that the exposure concentrations of the studied metals do not pose an increased non-carcinogenic risk (Hazard Index < 1). Incremental lifetime cancer risk (ILCR) resulting from apple juice consumption was also estimated using both the California Office of Environmental Health Hazard Assessment (OEHHA) and the U.S. EPA cancer slope.

1. Introduction

The largest consumers of juice, per body weight (g juice/kg body weight), are children, and apple juice is one of the favorite juice flavors in the United States (U.S.) with Americans consuming about 2.6 billion liters of apple juice in 2012 (USDA, 2012). The U.S., however, produces only about one sixth of its apple juice supply; two thirds of the apple juice sold in the U.S. comes from China and the remaining apple juice originates from other countries such as Chile, Argentina, Brazil and Canada (USDA, 2011; USGS, 2011).

Recent studies have reported metal concentrations in both apple juices and other fruit juices that exceeded current U.S. Environmental Protection Agency (EPA) drinking water standards and U.S. Food and Drug Administration (FDA) bottled water regulatory standards. For example, in January 2012, Consumer Reports published an article indicating that approximately 10% of the apple and grape juice samples analyzed from five different juice brands had total As levels that exceeded the federal drinking water standard for inorganic As of 0.01 mg/L (10 μg/L) (ConsumerReports, 2011; FDA, 2012). The study also reported that more than one-third of the juices exceeded the FDA's bottled water standard for Pb [0.005 mg/L (5 μg/L)] (ConsumerReports, 2011; FDA, 2012). More recently, researchers from the University of Washington reported that out of 37 juices (apple, grape, citrus, or apple ciders) tested, 27% contained total As concentrations above the FDA bot-

Abbreviations: Al, aluminum; As, arsenic; Cd, cadmium; Cr, chromium; Cu, copper; Pd, lead; Mn, manganese; Hg, mercury; Zn, zinc; HQ, hazard quotient; HI, hazard index; ILCR, incremental lifetime cancer risk; EPA, U.S. Environmental Protection Agency; FDA, U.S. Food and Drug Administration; MMA, monomethylarsonous acid; DMA, dimethylarsinous acid; eMDLs, estimated method detection limits; CDI, chronic daily intake; C, concentration of each metal found in the apple juice; DI, the average daily intake rate of apple juice; BW, body weight; RfD, EPA oral reference dose; PTWI, provisional tolerable weekly intake value; IEUBK, U.S. EPA's Integrated Exposure Uptake Biokinetic Model; LADD, lifetime average daily dose; CSF, cancer slope factor; JECFA, Joint FAO/WHO Expert Committee on Food Additives; EFSA, European Food Safety Authority; ATSDR, Agency for Toxic Substances and Disease Registry; MRL, minimal risk level; PPRTV, EPA provisional peer reviewed toxicity value; CDC, Centers for Disease Control and Prevention; MCL, maximum contaminant level; MCLG, maximum contaminant level goal.

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tled water standard, which is based on inorganic As concentration, not total As concentration, and almost a quarter of the samples tested exceeded FDA's standard for Pb in bottled water (Wilson et al., 2012). In addition, in July 2013, the FDA released the results of a study that provided quantitative estimates of long-term cancer risk presented by inorganic As in apple juice and proposed an As action level for inorganic As in apple juices similar to that of bottled water [0.01 mg/L (10 μg/L)] (Carrington et al., 2013).

Given previously published reports of elevated As and Pb concentrations in certain apple juice brands, the current study expands the analysis to include these and other metals that have been used historically in pesticides, herbicides, fungicides, and insecticides. It is worth noting that all metals exist naturally in soils and can be absorbed by fruits and vegetables; the soil-to-plant transfer factor is dependent upon the plant species and the bio-availability of the metal in the soils (Intawongse and Dean, 2006; McBride, 2013). The purpose of this study is to present analytical findings of Al, As, Cd, Cr, Cu, Pb, Mn, Hg, and Zn measured in nine apple juice products, compare them to FDA bottled water regulatory standards, and investigate human health risks (non-carcinogenic and carcinogenic) associated with metal exposure resulting from the consumption of apple juice.

2. Materials and methods

2.1. Item collection

A total of nine apple juice brands were collected for this analysis. Apple juice brands referred to as "store brands" contained apple concentrate produced in a number of countries including the U.S., Brazil, Argentina, Chile, China, Germany, and Turkey. Two of the three apple juice brands referred to as "Chinese brands" were made from apple concentrate originating solely from China and one contained apple concentrate originating from China or Argentina. All store brand and Chinese brand apple juices used in this analysis were purchased from chain supermarkets in the U.S. The three local organic apple juices were purchased from farmers' markets in Boulder, Colorado (CO); San Francisco, California (CA); and New Cuyama, CA. Items were stored in their original containers at room temperature until sampled, then stored at $\leqslant 6\,^{\circ}C$ until analysis. All containers were plastic, except for store brand #3 and Chinese brand #3 which were juice boxes, and organic brand #2 which was in a glass container.

2.2. Sample preparation for analysis

Apple juice samples (10 mL) were analyzed in triplicate by Applied Speciation and Consulting, LLC (Bothell, WA) utilizing inductively coupled plasma dynamic reaction cell mass spectrometry (ICP-DRC-MS), cold vapor inductively coupled plasma mass spectrometry (CV-ICP-MS), or ion chromatography inductively coupled plasma collision reaction cell mass spectrometry (IC-ICP-CRC-MS). All sample preparations were performed in laminar flow clean hoods known to be free from trace metal contamination. ICP-DRC-MS was used to assess total metal concentration of Al, As, Cd, Cr, Cu, Pb, Mn, and Zn. A known volume of each sample was aliquotted into a polypropylene vial. All samples were then digested with aliquots of concentrated nitric acid (HNO_3), concentrated hydrochloric acid (HCl), and hydrogen peroxide (H_2O_2) in a heat block apparatus. The resulting digests were diluted to a known final volume with reagent water prior to analysis via ICP-DRC-MS. The reporting limit for each metal in this analysis was as follows: 2 μg/L for Cd and Pb; 5 μg/L for total As, Cr, Cu, and Mn; and 50 μg/L for Al and Zn. CV-ICP-MS was used to determine total Hg content in the nine apple juice brands sampled in this study. The reporting limit for total Hg was 0.2 μg/L. IC-ICP-CRC-MS was used to directly analyze each apple juice sample for As species. The reporting limit for As(III), As(V), monomethylarsonous acid (MMAs), and dimethylarsinous acid (DMAs) was 1 μg/L.

The estimated method detection limits (eMDLs) for As(III), As(V), and DMA were calculated using the standard deviation of replicate analyses of the lowest standard in the calibration curve.

The eMDL for MMA was calculated from the average eMDL of the three As species contained in the calibration (*i.e.*, As(III), As(V), and DMA). The eMDLs for all total metals and for total Hg were calculated as three times the standard deviation of the method blanks prepared and analyzed concurrently with the submitted samples. Concentrations at or above the eMDL indicate that there are detectable levels of the analyte in the sample; however, concentrations between the eMDL and the reporting limit are estimates. The eMDL for each analyte was as follows: As(V) 0.08 μg/L; total Hg 0.10 μg/L; total Mn 0.11 μg/L; total Pb 0.12 μg/L; DMA 0.14 μg/L; MMA, total As and total Cd 0.15 μg/L; As(III) 0.23 μg/L; total Cr 0.40 μg/L; total Cu 0.49 μg/L; total Mn 0.11 μg/L; total Al 5 μg/L.

2.3. Statistical analysis of juice samples

Metal concentrations measured in each brand of juice are reported in Table 1. Significant differences in average metal concentrations amongst the three juice types (e.g., store brands, Chinese brands, local organic brands) were assessed using one-way ANOVA followed by the Tukey post hoc multiple comparison test for data with a defined distribution and without non-detect values (Al, total As, As(V), inorganic As, Cd, Cr, Cu, and Mn). For metals with non-detect values or without a defined distribution, (As(III), MMAs, DMAs, Hg, Pb, and Zn) the Kruskal–Wallis non-parametric test was used to test for significant differences amongst the metal concentrations in the three juice types. PROUCL 4.0 was then used to estimate the 95% upper confidence limit of the mean (95% UCL) for each metal tested in all nine apple juices combined. For normally distributed data without non-detects (As, As(V), Cd, Cr, Cu, and Mn), the 95% UCL was calculated using Student's-t UCL. For normally distributed data with non-detects, (As(III) and DMA), the KM (t) UCL was used to determine the 95% UCL. The data set for Al was found to be gamma distributed so the adjusted gamma UCL was used to determine the 95% UCL. For Pb, the data set was non-parametric so the Chebyshev UCL was used. For Zn, one data point (3200 μg/L) was determined to be a statistical outlier using Z-score testing and was therefore removed when determining the mean and 95% UCL for Zn. When the outlier was removed from the Zn data set, the data was normally distributed and the Student's-t-UCL was used to estimate the 95% UCL. Values below the limit of detection (reported as ND in Table 1) were not used in the determination of the mean or the 95% UCL.

2.4. Exposure and risk estimation

2.4.1. Exposure estimation

The metal concentrations measured in the apple juice samples were used to calculate the chronic daily intake (CDI) which was used to characterize the exposure to metals resulting from juice consumption. The following equation was used to determine the CDI of the nine metals analyzed in this study resulting from apple juice consumption (EPA, 1992b):

$$\mathrm{CDI} = C \times \mathrm{DI}/\mathrm{BW}$$

where CDI is the chronic daily intake (mg/kg-day), C is the concentration of each metal found in the apple juice (mg/L), DI is the average daily intake rate of apple juice (L/day), and BW is the body weight (kg) of an individual. The product of C and DI represents daily metal intake (mg/day).

Results from the 2009–2010 National Health and Nutrition Examination Survey (NHANES) were used to estimate apple juice consumption (DI) for the various age groups (CDC, 2012). The average apple juice consumption for 15 age groups, including infants, children, and adults, was based on the reported values (grams per day) for three NHANES food categories: 'apple juice', 'apple cider', and 'apple juice, baby food'. Juice consumption was averaged across apple juice drinkers only. To calculate the CDI using NHANES data, the assumption that 1 kg is equal to 1 L was used. Additional assumptions and estimations used to determine the CDI are summarized in Tables 1 and 2.

2.4.2. Non-carcinogenic risk

To estimate the non-carcinogenic risk of metal ingestion resulting from apple juice consumption, a hazard quotient (HQ) for eight of the nine metals (Al, As, Cd, Cr, Cu, Hg, Mn, and Zn) was determined using standard U.S. EPA methodology (EPA, 2005). The EPA reference doses (RfD) for inorganic As, Cd, Cr, Mn, and Zn were used to calculate the HQs for these five metals. Since no RfD has been established for Al, Cu, and Hg, the EPA screening level RfDs were used to calculate the HQs for these three metals (EPA, 2013a). The RfD is an estimate of a daily oral exposure to the human population (including sensitive subgroups) that is likely to produce no appreciable risk of deleterious effects during a lifetime (EPA, 2012a). The following equation was used to calculate the HQ:

$$\mathrm{HQ} = \mathrm{CDI}/(\mathrm{RfD\ or\ Screening\ level\ RfD})$$

Relevant RfDs and additional guidance values for the metals examined in this analysis are summarized in Table 3.

The cumulative non-carcinogenic risks were expressed as a hazard index (HI) which is the sum of the HQs from all of the metals considered (EPA, 2005). This provides a worst-case scenario assessment of the non-carcinogenic risks these metals may pose to apple juice consumers.

$$\mathrm{HI} = \mathrm{HQ}_{Al} + \mathrm{HQ}_{As} + \mathrm{HQ}_{Cd} + \mathrm{HQ}_{Cr} + \mathrm{HQ}_{Cu} + \mathrm{HQ}_{Hg} + \mathrm{HQ}_{Mn} + \mathrm{HQ}_{Zn}$$

Estimation of a HQ for Pb is problematic because of the lack of an oral RfD or guidance value. At one point, the WHO had recommended a provisional tolerable weekly intake (PTWI) value of 0.025 mg/kg-day for Pb (JECFA, 2010), but upon recent review of the toxicity data for Pb, the Committee concluded that the PTWI was not health protective and was withdrawn; a new PTWI has not been established (WHO, 2011). Therefore, Pb was not included in the HQ or HI analysis. For all other metals, both the mean contaminant concentration and the 95% UCL were used for this risk assessment.