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

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A B S T R A C T

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 factor for inorganic As.

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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 bottled 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 risks presented by inorganic As exposure via apple juice consumption 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 the previously published reports of elevated As and Pb concentrations in certain apple juice brands, the current study expanded 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 bioavailability of the metal in the soils (Intawongse and Dean, 2006; McBride, 2013). The purpose of this study was to present analytical findings of Al, As, Cd, Cr, Cu, Pb, Mn, Hg, and Zn measured in nine apple juice products. The measured metal concentrations were then compared to FDA bottled water regulatory standards, and the potential human health risks (non-carcinogenic and carcinogenic) associated with metal exposure resulting from the consumption of apple juice were investigated.

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 Aliso Viejo, CA. Items were stored in their original containers at room temperature until sampled, then stored at ≤6 °C until analysis. All containers were plastic, except for store brand #3 and Chinese brand #3 which were juice boxes, and organic brand #2 and #3 which were stored 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 (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 aliquoted into a polypropylene vial. All samples were then digested with aliquots of concentrated nitric acid (HNO3), concentrated hydrochloric acid (HCl), and hydrogen peroxide (H2O2) in a heat block reactor. 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, Cu, Cd, Cr, Pb, Mn, and Zn. A known volume of each sample was aliquoted into a polypropylene vial. All samples were then digested with aliquots of concentrated nitric acid (HNO3), concentrated hydrochloric acid (HCl), and hydrogen peroxide (H2O2) in a heat block reactor. The resulting digests were diluted to a known final volume with reagent water prior to analysis via ICP-DRC-MS.

The estimated method detection limits (eMDLs) for As(III), As(V), and DMA were 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 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 based on the analytical technique. 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 or estimated in each brand of juice are reported in Table 1. Significant differences in the 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, DMMAs, 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 nine apple juice combinations. 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, the 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 eMDLs (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 or estimated in the apple juice samples were used to calculate the chronic daily intake (CDI) which was then used to characterize the exposure to metals resulting from apple juice consumption. The following equation was used to determine the CDI of the nine metals analyzed in this study (EPA, 1992b):

\[ \text{CDI} = C \times \text{DI} / \text{BW} \]

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 (mL/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 ten age groups, including infants, children, and adults, was based on the reported consumption 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 each 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:

\[ \text{HQ} = \text{CDI} / \text{RfD or Screening level RfD} \]

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 eight metals considered in this analysis (EPA, 2005). This provides a worst-case scenario assessment of the non-carcinogenic risks that these metals may pose due to apple juice consumers.

\[ \text{HI} = \text{HQ}_{\text{As}} + \text{HQ}_{\text{Cd}} + \text{HQ}_{\text{Cu}} + \text{HQ}_{\text{Cr}} + \text{HQ}_{\text{Mn}} + \text{HQ}_{\text{Al}} + \text{HQ}_{\text{Pb}} + \text{HQ}_{\text{Hg}} \]

Estimation of a HQ for Pb is problematic because of the lack of an oral risk 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 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.
To characterize the risks of Pb exposure via apple juice consumption, changes in blood Pb concentrations in children ages 0.5 to 7 years were estimated using the U.S. EPA’s Integrated Exposure Uptake Biokinetic Model (IEUBK) of Pb in Children (Syracuse Research Corporation, 2007). The age-specific geometric mean blood Pb concentrations were predicted based on default model inputs for environmental exposure duration for the exposure group (years); and AT is a lifetime of 70 years.

The estimated LADD was then used to estimate carcinogenic risk by implementing the following equation:

\[
\text{Risk} = \text{CSF} \times \text{LADD}
\]

where Risk is the estimated cancer risk (ILCR) and CSF is the oral cancer slope factor for inorganic As. Table 2 details the information used to estimate the ILCR. Both the California Office of Environmental Health Hazard Assessment (OEHHA) and the U.S. EPA cancer slope for inorganic As were used to estimate the ILCR. The cancer slope factor for OEHHA and the U.S. EPA were 9.8 (mg/kg-day)⁻¹ and 1.5 (mg/kg-day)⁻¹, respectively.

3. Results

3.1. Trace metal concentrations

The metal concentrations in the nine apple juice brands studied in this analysis are reported in Table 1. The concentrations of the majority of the metals detected in the apple juice brands were below their respective established FDA contaminant levels for bottled water. For example, while total As was detected in all nine apple juice samples analyzed (total As ranged from 1.8 to 8.6 µg/L), none of the apple juice brands contained total As concentrations above the current FDA bottled water standard or the proposed FDA action level for inorganic As in apple juice of 0.01 mg/L (10 µg/L). In eight of the nine apple juice brands studied, inorganic As was the
largest contributor to the total As concentration. In two of three organic brands and one of the store brands, As(V) was the only detected As species. In one apple juice brand from China, MMA was the largest contributor to the total As concentration. MMA was detected in three and DMA was detected in six of the nine apple juice brands examined (Table 1). SIMilar to total As, the Cd (range 0.15 to 0.70 μg/L), Cr (range 5.2–18 μg/L), Cu (range 5.1–180 μg/L), Hg (range non-detect to 0.14 μg/L), and Zn (range 76–320 μg/L) concentrations observed in the study were below their respective FDA contaminant levels established for bottled water (Table 3). This analysis revealed that concentrations of Mn, Al, and Pb exceeded FDA bottled water standards in a number of the apple juice brands assessed (Table 1). Mn and Al were found to be the most concentrated metals, with average concentrations of 450 μg/L and 800 μg/L, respectively. Mn concentrations (range 170–1200 μg/L) exceeded the current FDA bottled water standard of 0.05 mg/L (50 μg/L) in all nine apple juice brands tested (Table 1). Al concentrations (range 38–4900 μg/L) exceeded the FDA bottled water standard of 0.2 mg/L (200 μg/L) in six of the nine apple juice brands examined in this study (Table 1). Pb concentrations in one major store brand (7.4 μg/L) and one apple juice brand imported from China (9.1 μg/L) were also found to exceed the current FDA bottled water standard of 0.005 mg/L (5 μg/L) (Table 1). It is worth noting that the FDA bottled water standards for Mn and Al are based on the EPA National Secondary Drinking Water Regulations (NSDWR). The NSDWRs are not health based guidance values but are instead non-mandatory water quality standards that have been established to help manage public drinking water for aesthetic purposes such as taste, color and odor (EPA, 2013b).

With the exception of DMA, Zn, and Cu, the average metal concentrations measured in the organic apple juice brands were less than those found in the store brands and the brands imported from China; however, these differences were not statistically significant. The average concentrations of Zn and Cu were highest in the organic apple juice brands with the mean Cu concentration being significantly higher in the organic brands relative to the Chinese brands (p < 0.05). The average concentrations of As(III), Cd, Mn, Al, Cr, and Hg were highest in the three store brands and the average concentrations of As(V), inorganic As, MMA, total As, and Pb were highest in the three apple juice brands imported from China; however, these differences were not statistically different.

3.2. Trends in apple juice consumption amongst children

Results from the 2009–2010 NHANES were used to identify the age group with the largest apple juice consumption on a per kg basis (Table 2) (CDC, 2012). The largest consumers of apple juice, per body weight, were children between the ages of one and two years (26.5 g apple juice/kg body weight), followed by children two to three years of age (17.6 g apple juice/kg body weight), infants six to 12 months of age (15.0 g apple juice/kg body weight), and infants three to six months of age (13.5 g apple juice/kg body weight). Older children (6 < 16 years), young adults (16 < 21 years), and adults (21 < 70 years) consumed the least amount of apple juice on a per kg basis (Table 2).

Children three to 12 months of age who consume apple juice drink approximately a half cup of apple juice per day, assuming that 250 g equals approximately 1 cup (8 oz) of juice. On average, children between the ages of one and two years drink just under one and a half cups of apple juice per day and children between the ages of two and 15 years drink about one cup of apple juice per day. It is important to note that since the NHANES database provides only a snapshot of food-consumption for a limited period of time, intake estimates based on this information are generally a conservative measure of the average daily chronic intake or average daily intake over a lifetime for individuals within the surveyed population.

3.3. Evaluation of non-carcinogenic risk

To understand the potential health impact of metals in juice, non-carcinogenic risk levels, represented as HQs, were estimated for all metals except Pb. Since children between the ages of one and two were found to be the largest consumers of apple juice on a per kg basis, the CDI was calculated for this age group and subsequently used to estimate HQs and HI. The CDI was estimated using both the mean contaminant concentration and the 95% UCL of the mean. The parameters used to calculate the CDI and the resulting non-carcinogenic risk (HQ) estimations for metal exposure resulting from apple juice consumption are shown in Tables 2 and 4.

HQs for each metal contaminant were determined using the mean contaminant concentration and the 95% UCL of the mean (Table 1) and then compared to the appropriate guidance value (RFD or EPA screening level RFD) (Table 4). The HQ for As was based on...
on the measured inorganic As concentrations as the RDF is for inorganic As and not for total As or organic As (EPA, 2012b). When estimating the non-carcinogenic risk using the mean contaminant concentrations, inorganic As, Mn, and Cr yielded the highest HQs of 0.19, 0.08 and 0.08, respectively. Similarly, when estimating the non-carcinogenic risk using the 95% UCL of the mean, inorganic As, Mn, and Cr again yielded the highest HQs of 0.27, 0.14, and 0.11, respectively (Table 4).

To estimate the cumulative non-carcinogenic risk, a hazard index (HI) was calculated. The HI (sum of the individual HQs) calculated from the mean contaminant concentration and the 95% UCL of the mean were both less than one (Table 4). To put the risk estimates into context, a HI was also calculated based on each metal’s respective FDA maximum contaminant level for bottled water (Table 3). The HI derived from these concentrations far exceeded one (Table 4).

For Pb, human risk is best characterized in terms of blood Pb concentrations rather than comparing exposure concentrations to an acceptable daily intake value or reference dose. Therefore, the IEUBK model was used to estimate changes in blood Pb concentrations resulting from background Pb exposure plus daily apple juice consumption of juice containing 7.4 μg Pb/dL, the 95% UCL of the mean Pb concentration found in the nine apple juices sampled (Table 1). Using the default IEUBK input parameters and incorporating Pb exposure from apple juice consumption, the geometric mean and the 95th percentile blood Pb concentrations in children between the ages of one and two (who were found to consume the most apple juice on a g/kg/day basis) increased by 0.3 and 0.8 μg/dL respectively (Table 5). It is noteworthy that utilizing the background default values in the IEUBK model, approximately 15% of children between the ages of one and five have blood Pb concentrations greater than 5 μg/dL. Interestingly, recent NHANES data report that approximately 2.5% of children between one and five years old have blood Pb concentrations greater than 5 μg/dL suggesting that the default IEUBK values overestimate current Pb exposures (CDC, not dated). More accurate background default values would likely result in a decreased fraction of children with blood Pb concentrations above 5 or 10 μg/dL.

### 3.4. Evaluation of carcinogenic risk

With respect to carcinogenic risk, the ILCR was estimated to characterize the probability of cancer, beyond one’s existing chance of developing cancer, resulting from apple juice consumption (EPA, 1989a). The California OEHHA has recently performed a risk assessment assessing the health effects of inorganic As in drinking water (OEHHA, 2004). Based on the incidence of lung cancer and bladder cancer in epidemiological studies in Taiwan, Chile, and Argentina and the background mortality rates for these cancers in the U.S., OEHHA derived a public health goal of 4 ng/L for inorganic As in drinking water. This value was based on a unit risk of 2.7 × 10⁻⁴ (μg/L)⁻¹ and an oral cancer potency of 9.5 (mg/kg-d)⁻¹ (OEHHA, 2004).

The current U.S. EPA cancer slope factor is not based on the most relevant endpoints; as such, the risk of cancer from exposure to inorganic As in apple juice was estimated using both the California OEHHA and U.S. EPA cancer slope factors (Table 6). The ILCR was calculated using reported daily apple juice consumption rates for ages 0–6, ages 6–21, ages 21–50, and ages 50–70 (Supplemental Table 2) (CDC, 2012). Both the mean contaminant concentration in apple juice and the 95% UCL of the mean were used to estimate the ILCR. Using the California OEHHA oral cancer slope factor of 9.5 (mg/kg-d)⁻¹, the ILCR for adults (ages 0–70) ranged from 1 × 10⁻⁴ to 2 × 10⁻⁵. Using the U.S. EPA oral cancer slope factor of 1.5 (mg/kg-d)⁻¹, the ILCR for adults (ages 0–70) ranged from 2 × 10⁻⁴ to 3 × 10⁻⁵ (Table 6). For reference, the ILCR was estimated using the current FDA standard for inorganic As in bottled water (0.01 mg/L or 10 μg/L) as the theoretical inorganic As concentration in apple juice. The resulting ILCR was estimated to be 6 × 10⁻⁶ or 8 × 10⁻⁷ for adults, using either the California OEHHA or U.S. EPA oral cancer slope factor, respectively (Table 6).

### 4. Discussion

Recent concerns have been raised regarding the potential health impact associated with the presence of measurable amounts of metals in fruit juices, especially apple juice (Braganca et al., 2012; ConsumerReports, 2011; Williams et al., 2009; Wilson et al., 2012). Metals are ubiquitous in the environment. While most metals are a natural element of the earth’s crust, metal contamination can also occur from a number of anthropogenic sources. Thus, the presence of metals in apple juice can arise from metals that occur naturally in the soil or as a result of anthropogenic activities such as the application of pesticides, herbicides, fungicides, and insecticides.

Unlike drinking water and bottled water, which have enforceable exposure standards for a number of metals, similar guidelines and exposure limits do not currently exist for metals in juice. However, the FDA recently proposed an action level of 0.01 mg/L (10 μg/L) for inorganic As in apple juice which is similar to the EPA drinking water standard and the FDA bottled water standard for inorganic As (Carrington et al., 2013). In general, children are the largest consumers of fruit juices (American Academy of Pediatrics, 2001). Specifically, children between the ages of one

<table>
<thead>
<tr>
<th>Metal</th>
<th>Mean chronic intake (μg/kg-day)</th>
<th>95% UCL chronic daily intake (μg/kg-day)</th>
<th>FDA chronic daily intake at bottled water limits (μg/kg-day)</th>
<th>Guidance value (μg/kg-day)</th>
<th>Mean HQ</th>
<th>95% UCL HQ</th>
<th>FDA HQ</th>
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<tbody>
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<td>Al</td>
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<tr>
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<td>0.11</td>
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</tbody>
</table>

Chronic Daily Intake (CDI) = daily juice intake (L/d) × contaminant concentration (μg/L)/body weight (kg) × μg/kg-day.

CDI based on apple juice consumption for a child between 1 and 2 years of age compared to the oral reference dose (RfD) or screening level (RfD). Appendix C in C.F. CDC, 2012. Based on the average body weight of a child between 1 and 2 years of age (Table 2).

*No RfD is established for this metal; therefore, the EPA screening level (RfD) is listed.

No RfD or screening level RfD was available in the NHANES survey (CDC, 2012). Body weight based on the average body weight of a child between 1 and 2 years of age (Table 2).
Estimated changes in blood Pb concentrations in children resulting from apple juice consumption.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Pb exposure from apple juice (µg/day)</th>
<th>Change in blood Pb concentrations (µg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Geometric mean</td>
<td>95th percentile</td>
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<td>0.2</td>
</tr>
</tbody>
</table>

Table 5

Changes in the geometric mean and 95th percentile blood Pb concentrations for a one year exposure scenario were modeled using the following input parameters: the 95% UCL of the mean Pb concentration in all brands of apple juice sampled (7.4 µg Pb/L), the apple juice consumption rates listed in Table 2, and the normal background exposure as estimated by the U.S. EPA’s IEUBK model for lead in children.

and two were found to consume the most apple juice per kg body weight and, as a result, represent the group with the highest potential exposure to metals from apple juice consumption. Therefore, it is important to understand any potential health risks posed to children as a result of metal exposure from apple juice consumption. 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.

In this study, the organic apple juice brands generally contained lower concentrations of metals when compared to the three store brands and the three brands that contained concentrate from China; however, these differences were not statistically significant. The lack of statistical significance may be due to the small sample size and different results may have been obtained with a larger sample size. The highest average concentration of DMA and Cu was found in the organic apple juice brands sampled in this study. While DMA was detected in only one of the three organic apple juice brands, the measured concentration was higher than that reported in the two store brands with measurable DMA levels and was also higher than the DMA levels found in all three apple juice brands containing concentrate from China.

The difference in Cu concentration between the organic brands and the brands containing concentrate from China was statistically significant. This difference may be due to a number of factors including the maturity of the apple, differences in agricultural practices, location of production as well as a number of environmental factors including the chemical composition of the soil (Perez-Lopez et al., 2007). Metal content in soil can vary greatly depending on geographical location, thus variation in average metal concentrations among the three different apple juice types as well as variation seen between all nine brands of apple juice sampled may be highly dependent upon the origin of the apple juice. Further, it has been reported that organic fruits and vegetables tend to be higher in essential nutrients such as Cu, Zn and Mn. This may be the result of differences in the materials and approaches used in organic farming versus conventional farming (Perez-Lopez et al., 2007). However, in this study, only the average concentration of Cu was higher in the organic apple juice brands when compared to the store brands and the brands that contained concentrate from China.

The measured metal concentrations in apple juice from this study were compared to the FDA bottled water standards since similar standards or guidance values do not currently exist for juice. The FDA often adopts drinking water standards set by the U.S. EPA (i.e., Al, inorganic As, Cd, Cr, Mn, Hg and Zn) and it is important to note that most drinking water standards are set as a fraction of the tolerable or acceptable daily intake value for a given contaminant divided by the daily drinking water consumption rate. The MCL also takes into account “analytical methodology, treatment technology and costs, economic impact, and regulatory impact” (EPA, 1992a, p. 52). As such, exceeding this value does not necessarily imply an increased health risk. Further, the amount of juice consumed per day is generally considerably less than the amount of water consumed per day. Specifically, the average water consumption for a child between the ages of one and two years is 1.2 L/day (based on the average body weight of 11.4 kg) and the average apple juice consumption is estimated to be 0.3 L/day (CDC, 2012; EPA, 2009). Thus, comparing our results to bottled water standards is a conservative approach as far more water is generally consumed on a daily basis when compared to juice.

While the metal concentrations in the nine apple juice brands sampled in this study were generally below the FDA bottled water standards, some juice brands contained Mn, Al, and Pb at concentrations that exceeded the FDA bottled water standards. While Mn is considered an essential element, exposures to high concentrations of Mn have been associated with certain neurodegenerative diseases (ATSDR, 2012). Although all nine apple juice brands assessed in this study were found to exceed the FDA bottled water standard of 0.05 mg/L (50 µg/L) for Mn, the HQs calculated for Mn did not suggest a significant non-carcinogenic risk as a result of apple juice consumption. This finding is not surprising as the FDA bottled water standard for Mn is based on the EPA recommendation of 0.05 mg/L (50 µg/L), which is not a health based guidance value, but rather a value established to help manage public health risks.
drinking water “for aesthetic considerations, such as taste, color and odor” (EPA, 2013b). The EPA does not enforce “secondary maximum contaminant levels,” such as the one for Mn, because these contaminants are not considered to present a risk to human health at or below this exposure level. Therefore, the results from this risk assessment would suggest that there is not a risk for Mn toxicity as a result of apple juice consumption.

Chronic exposure of Al is known to be toxic to humans under certain exposure conditions and, as a result, various guidance values have been recommended (ATSDR, 2008). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a provisional tolerable weekly intake (PTWI) for Al of 2 mg/kg-week (0.29 mg Al/kg-day), the European Food Safety Authority (EFSA) has suggested a lower guidance value of 1 mg Al/kg-week (0.14 mg/kg-day), and the Agency for Toxic Substances and Disease Registry (ATSDR) recommends a considerably higher “minimal risk level” (MRL) for chronic duration oral exposure of 1 mg Al/kg-day (7 mg Al/kg-week) (Aguilar et al., 2008; ATSDR, 2008; WHO, 2011). The EPA screening level RDF is also 1 mg Al/kg-day and is based on the EPA’s provisional peer reviewed toxicity value (PPRTV) (EPA, 2013a). The guidance value of 1 mg Al/kg-day was used for the risk analysis in this study because the JECFA and EFSA guidance values were likely to be exceeded following normal dietary intake of Al (Aguilar et al., 2008). Despite exceeding the FDA bottled water standards, the risk estimates from this analysis indicated no significant non-carcinogenic risk of Al exposure resulting from apple juice consumption alone, as the maximum HQ value for the highest exposed age group (children between one and two years) was less than one (Table 4). This is not surprising, because similar to Mn, the FDA bottled water standard for Al is not a health based guidance value but is instead based on a non-mandatory EPA water quality standard that has been established to help manage public drinking water for aesthetic purposes such as taste, color and odor.

In addition to Al and Mn, Pb concentrations in two of the juices sampled were found to exceed FDA bottled water standards in the present analysis. However, the data indicated that the possible incremental increase in children's blood Pb concentrations as a result of apple consumption is expected to be minimal. It is also worth noting that the default background values used in the IEUBK model indicate that approximately 15% of children between the ages of one and five are expected to have blood Pb concentrations greater than 5 μg/dL. However, this is not consistent with recent NHANES data that report that only 2.5% of children between one and five years of age have blood Pb levels greater than 5 μg/dL. These results suggest that the default background assumptions in the model are dated and could be revised. Possible revisions could include the incorporation of blood Pb concentrations in the U.S. and better characterization of background Pb exposure associated with dietary intake. Updating these parameters may influence the total impact of apple juice consumption on children's blood Pb concentrations.

The California Office of Environment Health Hazard Assessment (OEHHA) has proposed a child specific health guidance value for Pb to be used in health risk assessments. The proposed benchmark for risk assessment purposes is an incremental change in blood Pb concentration of 1 μg/dL. The basis of this value reportedly represents the estimated incremental increase in a child's blood Pb concentration that would reduce a child’s IQ by up to one point (Carlisle and Dowling, 2007). The results from this analysis indicate that the maximum change in blood Pb concentration in a child as a result of Pb exposure via apple juice consumption is expected to be less than 1 μg/dL. Therefore, the rise in a child's blood Pb concentration as a result of apple juice ingestion would not be viewed as “significant for purposes of risk assessment” according to OEHHA (Carlisle and Dowling, 2007, p. 1). However, according to OEHHA, an increase in blood Pb concentrations ≤1 μg/dL does not necessarily imply a safe level as no safe blood Pb concentration has been definitively established (Carlisle and Dowling, 2007).

The potential health impacts of metals in juice can be assessed by considering the metal exposure resulting from juice consumption relative to the appropriate regulatory guideline such as the chronic oral RfD. The RfD represents an acceptable total daily exposure considered “safe” for all age groups. The potential non-carcinogenic health impact posed by metal exposure as a result of apple juice consumption was assessed by estimating a HQ for each metal (except Pb) and the total non-carcinogenic risk was then calculated (HI). None of the HQs nor the HI in the current study exceeded one, suggesting that there was not an increased risk for non-carcinogenic health effects. Inorganic As (44%), Mn (19%) and Cr (19%) were found to be the largest contributors to the overall non-carcinogenic risk based on the mean contaminant level. Similarly, these three metals were also found to be the largest contributors to the overall non-carcinogenic risk when the risk estimates were based on the 95% UCL of the mean. Using the 95% UCL of the mean for risk characterization is a conservative approach, and even so, no increase in non-carcinogenic risk was found. Interestingly, if non-carcinogenic risk was estimated using the FDA maximum contaminant level for bottled water instead of the metal concentrations found in the current study, the HI far exceeded one (2.7) with inorganic As and Cr as the largest contributors to the overall non-carcinogenic risk (Table 4). This comparison further demonstrates the low non-carcinogenic risk associated with apple juice consumption as the HI estimated from the FDA maximum contaminant level for bottled water was appreciably higher than the HI estimated from the actual measured metal concentrations in the apple juice brands assessed in this study.

It is important to recognize that there are several limitations in using the RDF and HI approach to estimate risk. One limitation is that exposures resulting in the same RDF value (i.e., inorganic As and Hg have the same RDF value of 0.0003 mg-kg/day) do not necessarily imply the same level of risk. For example, the RDF for inorganic As is based on a NOAEL of 0.0008 mg As/kg/day for dermal effects in humans and the application of an uncertainty factor of 3 to account for the lack of reproductive data and uncertainty in whether the NOAEL accounts for all sensitive individuals (ATSDR, 2007). On the other hand, the RDF for Hg is derived from three studies using Brown-Norway rats that reported LOAELs of 0.226, 0.317, and 0.633 mg Hg/kg/day for the development of autoimmune effects and the application of a total uncertainty factor of 1000 to account for extrapolation from a LOAEL to NOAEL (10), from a sub chronic exposure to a chronic exposure (10), and from an animal study to humans (10) (ATSDR, 1999; EPA, 2002). While the application of uncertainty factors can be used to account for uncertainty in the data, it does not take into consideration the experimental confidence of individual studies; thus, there can be varying degrees of confidence in the estimated RFDs. Further, the application of uncertainty factors is subjective and may not always capture the true risk. Most importantly, the RDF approach does not take into account dose–response information which is an important consideration when estimating risk. For instance, a chemical above the RDF with a very steep dose–response curve may be associated with a greater likelihood of effect than a chemical above the RDF with a more gradual dose–response curve.

In regards to limitations associated with the HI approach in estimating non-carcinogenic risk, one limitation is that RFDs of varying levels of confidence, with different uncertainty adjustments, are given equal weight and combined to estimate non-carcinogenic risk. Thus, the level of concern does not increase linearly as the HI approaches or exceeds one because the RFDs do not have equal accuracy or precision and are not based on the same severity of effect. Further, the HI approach assumes that dose additivity is the most appropriate way to describe and assess the potential...
interactions between the chemicals of concern. Thus, application of the HI approach to a number of compounds that are not expected to induce the same type of effects or that do not act by the same mechanism could overestimate the potential for non-carcinogenic effects. Despite these limitations, the EPA has concluded that the HI approach is appropriate for a screening level risk assessment, as was performed in this study (EPA, 1989b).

The health hazards associated with inorganic As exposure and the continued potential for exposure from historical and current use of arsenic pesticides in other countries as well as recent reports demonstrating high levels of As in juice, have fueled media attention (ConsumerReports, 2011; USGS, 2002; Wilson et al., 2012). In contradiction to two recent studies (ConsumerReports, 2011; Wilson et al., 2012), total As levels in all juices sampled in this study were below the FDA bottled water standard and the FDA proposed action level for inorganic As in apple juice. The average total As concentration (5.3 μg/L) measured in this study was similar to the average total As concentration (5.2 μg/L) reported in the recent FDA report assessing inorganic As in apple juice (Carrington et al., 2013) suggesting that while the sample size is limited in this study, it does offer a representative picture of potential metal exposure from apple juice consumption.

It is important to note, however, that while these two earlier studies (ConsumerReports, 2011; Wilson et al., 2012) compared total As levels to FDA bottled water standards, total As should not be used to determine whether a food or drink is safe because the FDA and EPA guidance values are based on inorganic As concentration, not total As concentration. Therefore, the speciation of As should be taken into consideration when assessing human health risk. As such, an EPA RfD has been established for inorganic As exposure, not total As exposure, and the total inorganic As concentration [As(III) + As(V)] was used for risk characterization in this study.

The ILCR resulting from inorganic As exposure via apple juice consumption was determined and compared to the target cancer risk range established by the EPA. In general, the EPA has considered cancer risks ranging from 10^{-4} to 10^{-6} as protective of public health (EPA, 2000). Using the California OEHHA oral cancer slope factor, the estimated ILCR using both the mean contaminant concentration in apple juice and the 95% UCL of the mean was 1 \times 10^{-4} and 2 \times 10^{-4}, respectively, for adults. As a point of comparison, when using both the California OEHHA and the U.S. EPA oral cancer slope factors, a considerably higher ILCR for adults was calculated when the current FDA maximum contaminant level for inorganic As in bottled water [0.01 mg/L (10 μg/L)] was used as the contaminant concentration compared to the estimated ILCR when using the mean and 95% UCL of the mean concentrations in the current study. As noted above, using regulatory limits for metals in bottled water to estimate a theoretical cancer risk and comparing this risk estimate to those based on the metal concentrations measured in the juices analyzed in the current study helps to put the ILCR estimate for apple juice consumption into perspective.

The FDA has recently released a report estimating the cancer risk resulting from exposure to inorganic As in apple juice (Carrington et al., 2013). Carrington et al. (2013) estimated a per capita urinary tract and lung cancer rate from exposure to inorganic As in apple juice of approximately one in one hundred thousand based on the modeled disease rate of 8 cases per million people at average levels of consumption. While the estimated ILCR in the current study is higher than that estimated by Carrington et al. (2013), there are a few key differences between the two analyses. First, in the report issued by Carrington et al. (2013) it was assumed that apple juice consumption was constant over a life time; this assumption is not consistent with NHANES data which indicate that the rate of apple juice consumption changes with age. As such, in this analysis, apple juice consumption was adjusted based on age when calculating the LADD used to determine the ILCR. Second, while the assessment by Carrington et al. (2013) included several additional NHANES categories (e.g., fruit juice blends and various apple containing baby products) that were not used in this current analysis to determine apple juice consumption rates, our assessment determined apple juice consumption based on individuals who actually reported drinking apple juice, whereas consumption in Carrington et al. (2013) was determined on a per capita basis. Due to these differences, the apple juice consumption rates used in our analysis were higher on a kg/day basis than those used in the FDA assessment, subsequently increasing the average metal exposures used to estimate risk. As a result, this risk analysis may have been more conservative than the recent FDA assessment. Another difference between the current study and the FDA assessment was the method used to estimate the ILCR resulting from exposure to inorganic As in apple juice. In the current study, the EPA oral CSF, which was derived from the prevalence of skin cancers associated with the ingestion of inorganic As, was used to estimate the ILCR. Carrington et al. (2013), on the other hand, used the prevalence of urinary tract and lung cancer in different cohorts to estimate the ILCR resulting from inorganic As exposure from apple juice consumption.

There are limitations to the current analysis. First, risk from metal exposure was estimated based on one source of exposure, apple juice consumption. It is possible that an increased risk would be observed when taking into account other potential routes of exposure (e.g., food, water, soil, etc.). Second, although the juice samples analyzed in this study were collected from several different supermarkets and three different organic farms (two in CA and one in CO), the sample number is small (n = 9). This study comprises a limited sampling of apple juice brands currently available for sale in the U.S. and, as such, past and future measurements of metal concentrations in apple juices may reflect varying metal content. In addition, the authors acknowledge that statistical comparisons between the three types of juice (store brands, Chinese brands, and organic) is not optimal due to the small sample size; however, the main purpose of the study was to analyze metal content in select samples of apple juice, compare these results to current regulatory standards, and investigate human health risks associated with metal exposures resulting from apple juice consumption. The comparison of metal content between store brands, Chinese brands, and organic apple juices was an initial attempt to understand if notable differences in metal content exist among these three groups which may drive future studies in this area. Although it would have been ideal to collect multiple samples from different apple juice lots over multiple years to account for natural variability in metal concentrations by apple juice brand and type (store brand, Chinese brand, organic brand) over time, this was not possible in the current study. It is acknowledged by the authors that similar comparisons and analysis using a larger sample size could result in different data outcomes; however, the approach used in the current analysis to assess carcinogenic and non-carcinogenic health risks from apple juice consumption are applicable with data sets of various sizes. Additionally, the results of our study are intended to be used to direct future research in this area.

5. Conclusion

This study presents a screening level risk assessment investigating whether apple juice consumption is likely a significant source of metal exposure and whether this exposure poses an increased risk to human health. The data in the current study suggest that metal exposure via apple juice consumption does not pose an increased non-carcinogenic risk to human health. The HQs for each individual metal as well as the aggregate HI were both below one,
indicating that the daily metal intake from apple juice consumption estimated in this analysis is not likely to cause any deleterious effects during a lifetime of exposure in humans. In addition, both the aggregate HI and the ILCR for children and adults, when calculated using the data from this study, were far less than those estimated based on the current FDA bottled water standards.

Conflict of Interest

The authors declare that there are no conflicts of interest and that no external funding was provided for writing this manuscript.

Transparency Document

The Transparency document associated with this article can be found in the online version.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.05.015.

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