Presence of Arsenic in Commercial Beverages

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Abstract: Problem statement: This study’s goal was to assess the arsenic concentration of various beverages and broths purchased from a local chain supermarket. A source of chronic arsenic exposure occurs via food and beverage consumption. Groundwater levels of total arsenic are regulated (<10 µg L\(^{-1}\)) by the Environmental Protection Agency (EPA) but few studies have examined arsenic concentrations in common beverages. Approach: In the initial analysis of 19 items, total arsenic concentration was assessed from a variety of fruit juices, sports drinks, sodas and broths. Items found to contain levels of total arsenic \(\geq\) 5.0 µg L\(^{-1}\) were further evaluated. Additional analysis included purchasing multiple brands of items \(\geq\) 5.0 µg L\(^{-1}\) and analyzing them for total arsenic and chemical species of arsenic. Results: Among the beverages in the initial analysis, apple juice (10.79 µg L\(^{-1}\)) and grape juice (49.87 µg L\(^{-1}\)) contained the highest levels of total arsenic. Upon examination of items with As concentrations above 5.0 µg L\(^{-1}\), varying concentrations of total arsenic were found in apple cider (range: 5.41-15.27 µg L\(^{-1}\)), apple juice (range: 10.67-22.35 µg L\(^{-1}\)), baby fruit juice (range: 13.91-16.51 µg L\(^{-1}\)) and grape juice (range: 17.69-47.59 µg L\(^{-1}\)). Conclusion: Many commercially available juices contained concentrations of arsenic that were higher than the standard for total arsenic allowed in groundwater as set forth by the EPA. The concentration of As in these juices varied between and within brands. In general, those consuming apple and grape juices are the young and elderly and it is these populations that may be more vulnerable to over exposure of heavy metals.

Key words: Arsenic, species, apple, grape, beverage, juice

INTRODUCTION

Arsenic (As) is a widely distributed element found naturally in the earth’s soil\(^1\). Arsenic is classified as a known human carcinogen\(^2\) and has been placed on a list of top 20 hazardous materials\(^3\). Chronic arsenic exposure is known to be associated with various cancers, including lung, bladder and skin cancer\(^4-6\). Arsenic exposure has also been linked to diabetes\(^7,8\) and cardiovascular disease\(^9\).

The most common source of human exposure to arsenic occurs through natural contamination of drinking water\(^10\). Inorganic arsenic is consumed as either arsenite \([\text{As(III)}]\) or as arsenate \([\text{As(V)}]\). These forms of arsenic may be present in ground water supplies depending on the geology of the region. The current standard for total arsenic in groundwater, as set forth by the Environmental Protection Agency (EPA) is 10 µg L\(^{-1}\), which was lowered from 50 µg L\(^{-1}\) due to the association of chronic arsenic exposure as a risk factor for various adverse health outcomes\(^11\).

The metabolism of inorganic arsenic involves reduction followed by methylation. Arsenate is first reduced to As(III) prior to methylation. This is considered the first step in detoxifying inorganic arsenic\(^12\). Arsenite is then methylated via hepatocytes to form Monomethylarsonic Acid (MMA) and Dimethylarsinic Acid (DMA). MMA(V) is methylated from inorganic As and then reduced to MMA(III). This is then methylated to DMA(V) and then reduced to DMA (III). The process is not complete and some inorganic arsenic and MMA remain\(^13\). The trivalent
forms of MMA and DMA are more toxic than their inorganic compounds or their pentavalent forms. The trivalent forms have been shown to inhibit enzymatic reactions and should be considered as a potential factor for the development of various cancers.

Data are readily available for groundwater concentrations of arsenic, however there are few studies that have examined arsenic concentrations (total or chemical species) in commercially available and commonly consumed beverages. The primary oversight of food contaminants in the US is through the Food and Drug Administration (FDA). The Center for Food Safety and Applied Nutrition (CFSAN), a division of the FDA, conducts the Total Diet Study (TDS) annually. The goal of the TDS is to measure levels of various contaminants in approximately 280 foods and beverages and to estimate the dietary intake of those contaminants. The TDS includes the assessment of total arsenic concentration.

Other smaller studies have been conducted to assess arsenic levels in common beverages, but have been limited to specific beverages. These beverages include Chinese tea, mineral water, breast milk and alcoholic beverages, specifically wine and beer.

Few beverages have been assessed for total arsenic and chemical species of arsenic. Thus arsenic concentration and species distribution were characterized among some commonly consumed beverages and broths that were purchased in Tucson, Arizona and compared to data obtained from the total diet study.

MATERIALS AND METHODS

Item collection: Commercial beverages and broths were assessed for total and speciated arsenic concentration. All beverages and broths were purchased from one chain supermarket. For each beverage and broth, the lot number, container description (plastic, glass), volume and expiration date were recorded for tracking purposes. Items were stored in their original containers under the same conditions as they were obtained from the supermarket (room temperature or refrigerated) until aliquotted for analysis. Aliquotted samples were stored at -20°C until time of arsenic analysis.

Study design: Nineteen brand name items were selected. Two aliquots of 10 mL were obtained from each beverage and broth item and analyzed for total arsenic. These items included apple juice, beef broth, bottled water, chicken broth, cola, fruit punch, grape juice, grapefruit juice, iced tea, lemonade, sports drink and whole milk.

Items found to contain As levels ≥5.0 µg L⁻¹ were further evaluated. Twenty-four additional items were purchased and tested for total arsenic and chemical species of arsenic. These items included apple juice, beef broth, chicken broth and grape juice. Items used in this evaluation were purchased approximately one month after sampling the 19 brand name items and included generic name items along with brand name items. These items were of a different lot number from the initial samples. Four aliquots of 10 mL were obtained from each newly purchased beverage and broth sample and analyzed for total and speciated arsenic.

Sample preparation for analysis: All samples were prepared by microwave digestion. A one mL aliquot of each sample was placed in acid-washed 7 mL Teflon bombs and 0.5 mL of concentrated nitric acid was added. Samples were sealed and heated in a microwave for 5 minutes on the medium-high setting. Samples were cooled and vented and heated for an additional 5 minutes on the high setting. Additional steps of heating on the high setting were performed as necessary to complete sample digestion.

For total arsenic analysis, samples were brought up to volume with Milli-Q water for Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) analysis after digestion. Two samples of NIST SRM 1640, Trace Elements in Natural Water, were processed with the beverage and broth samples as a control.

For arsenic speciation, the samples were filtered through 0.45 µm nylon centrifuge filters after digestion. The samples were then analyzed by High Performance Liquid Chromatography (HPLC).

Arsenic analysis: Total arsenic was analyzed using an Agilent 7500ce ICP-MS (Agilent Technologies, Inc., Santa Clara, CA) with a MicroMist nebulizer (Glass Expansion). An ASX500 autosampler (CETAC Technologies, Omaha, NE) was used to introduce the samples into the Agilent 7500ce. The operating parameters were as follows: RF power, 1500 watts; plasma gas flow, 15 L min⁻¹; carrier flow, 0.85 L min⁻¹; makeup gas, 0.15 L min⁻¹. Acquisition parameters were as follows: Arsenic measured at m/z 75, terbium (internal standard) measured at m/z 159, points per peak 3, dwell time for arsenic was 1.5 sec and dwell time for terbium was 1.5 sec. There were seven repetitions.

Two NIST SRM 1640 control samples were used in the analysis of the 19 items. The control sample has a certified value of 26.71+/−0.41 µg L⁻¹. The two
control samples returned an arsenic concentration of 26.12 and 25.86 µg L⁻¹. In the analysis of the 24 additional items, two NIST SRM 1640 control samples were used with the same certified value. The two control samples returned an arsenic concentration of 26.68 and 27.04 µg L⁻¹.

Arsenic species were analyzed using an Agilent 1100 HPLC (Agilent Technologies, Inc., Santa Clara, CA) with a reverse-phase C18 column (Gemini 5u C18 110A, 150×4.60 mm, Phenomenex, Torrance, CA) and guard cartridge. The mobile phase (ph 5.85) contained 4.7 mM tetrabutylammonium hydroxide, 2 mM malonic acid and 4% (v/v) methanol at a flow rate of 1.2 mL min⁻¹. Column temperature was maintained at 50°C and samples were kept at 4°C in a thermally controlled autosampler. An Agilent 7500ce ICP-MS with a Conikal nebulizer (Glass Expansion) was used as the detector. The operating and acquisition parameters were the same as for total arsenic analysis.

**Arsenic analytes:** Results included concentrations of total arsenic, the inorganic species: As(III) and As(V) and the organic species: Monomethylarsonic acid, MMA(V) and Dimethylarsinic Acid, DMA(V). Analysis of the arsenic species MMA(III) and DMA(III) was not possible due to their unstable nature. The limit of detection for arsenic are as follows: Total arsenic 0.1 µg L⁻¹, As(III) 0.05 µg L⁻¹, As(V) 0.06 µg L⁻¹, MMA(V) 0.04 µg L⁻¹ and DMA (V) 0.04 µg L⁻¹.

**RESULTS**

**Total arsenic concentration for 19 items (lot 1):** The average total arsenic concentration from the two aliquots for each item is shown in Table 1. No two aliquots from the same item differed by more than 1.83 µg L⁻¹. The items with arsenic concentrations greater than 10.0 µg L⁻¹ include: Apple juice (10.79 µg L⁻¹), chicken broth (22.81 µg L⁻¹), beef broth (42.63 µg L⁻¹) and grape juice (49.87 µg L⁻¹). Whole milk had an As concentration of (7.92 µg L⁻¹), the bottled waters, colas, fruit punch, grapefruit juice, iced tea, lemonade, orange juices and sports drinks were all below 5.0 µg L⁻¹.

**Total and speciated arsenic concentrations for 24 additional items (lot 2):** Apple juice 1, grape juice 1, whole milk, beef broth 1 and chicken broth 1 were of the same brand as used in the initial analysis of the 19 items (Table 2). A wide variation of arsenic concentration can be seen among the newly purchased items. The concentration of As in apple juice sample 1 increased from 10.79-22.35 µg L⁻¹, in the beef broth sample the concentration of As decreased from 42.63-19.11 µg L⁻¹, in the chicken broth sample the concentration of As decreased from 22.81-11.13 µg L⁻¹, in the grape juice sample the concentration of As decreased from 49.87-17.69 µg L⁻¹ and in the whole milk sample the concentration of As decreased from 7.92-2.78 µg L⁻¹.

The average total arsenic concentration from four aliquots and the As species for each of the 24 additional items is shown in Table 3. The items with total arsenic concentrations ≥5.0 µg L⁻¹ included one apple cider sample (15.27 µg L⁻¹), five apple juice samples (range: 10.67-29.52 µg L⁻¹), five grape juice samples (range: 6.96-47.59 µg L⁻¹), 1 chicken broth sample (11.13 µg L⁻¹) and 1 beef broth sample (19.11 µg L⁻¹). All of the milk samples contained less than 3.0 µg L⁻¹ of arsenic.

In all of the samples, the measured species that had the largest contribution to the total arsenic concentration were As(III) and As(V). In the apple ciders, nearly three times the amount of As(V) was detected compared to As(III). In the apple juice samples, there was 1.5-5.5 times as much As(V) as there was As(III); however, in the grape juice samples the opposite was observed where there was 1.5-2.5 times as much As(III) as there was As(V).

| Item description | Mean total As (µg L⁻¹)ᵃ |
|------------------|-------------------------|
| Apple juice 1    | 10.79                   |
| Beef broth 1      | 42.63                   |
| Bottled water 1   | 2.11                    |
| Bottled water 2   | <0.10ᵇ                   |
| Bottled water 3   | <0.10ᵇ                   |
| Bottled water 4   | 22.81                   |
| Chicken broth 1   | 2.09                    |
|-Cola 1            | 1.52                    |
| Fruit punch       | 3.66                    |
| Grape juice 1     | 49.87                   |
| Grapefruit juice  | 2.14                    |
| Iced tea          | 2.35                    |
| Lemonade          | 3.00                    |
| Orange juice 1    | 1.96                    |
| Orange juice 2    | 2.53                    |
| Sports drink 1    | 2.09                    |
| Sports drink 2    | 1.91                    |
| Whole milk        | 7.92                    |

ᵃ: Derived from two aliquots from each item; b: Limit of detection for total arsenic = 0.1 µg L⁻¹; As: Arsenic

| Item description | Lot 1: Mean total As (µg L⁻¹)ᵇ  | Lot 2: Mean total As (µg L⁻¹)ᵇ |
|------------------|--------------------------------|--------------------------------|
| Apple juice 1    | 10.79 (0.11)                   | 22.35 (0.72)                    |
| Grape juice 1    | 49.87 (0.09)                   | 17.69 (0.73)                    |
| Whole milk       | 7.92 (0.17)                    | 2.78 (0.27)                     |
| Beef broth 1      | 42.63 (0.93)                   | 19.11 (0.91)                    |
| Chicken broth 1   | 22.81 (1.29)                   | 11.13 (0.40)                    |

ᵇ: Derived from two aliquots from each item; As: Arsenic; SD: Standard Deviation
Table 3: Total and speciated arsenic concentrations (µg L\(^{-1}\)) from beverages and broths

| Item description                        | Mean total As (SD)a | As(III)b | As(V)b | MMA(V)b | DMA(V)b |
|-----------------------------------------|---------------------|----------|--------|---------|---------|
| Apple cider 1                           | 5.41 (0.40)         | 0.98     | 2.90   | 0.80    | 0.30    |
| Apple cider 2                           | 15.27 (0.75)        | 4.29     | 11.20  | 0.81    | 0.92    |
| Apple juice 1                           | 22.35 (0.72)        | 4.48     | 13.60  | 0.39    | 0.68    |
| Apple juice 2                           | 10.67 (0.62)        | 2.73     | 4.20   | 2.43    | 0.27    |
| Apple juice 3                           | 12.15 (0.58)        | 1.75     | 9.13   | 0.33    | 0.27    |
| Apple juice 4                           | 29.52 (0.51)        | 3.74     | 21.30  | 0.53    | 0.60    |
| Apple juice (infant juice)              | 16.51 (0.81)        | 3.91     | 9.93   | 0.20    | 0.79    |
| Grape juice 1                           | 17.69 (0.73)        | 2.06     | 7.60   | <0.04   | 0.27    |
| Grape juice 2                           | 6.96 (0.65)         | 3.10     | 2.06   | 0.25    | 0.60    |
| Grape juice 3                           | 16.40 (0.73)        | 7.37     | 5.29   | <0.04   | 2.07    |
| Grape juice 4                           | 47.59 (2.60)        | 35.65    | 15.30  | <0.04   | 0.97    |
| Grape-apple juice blend (infant juice)  | 13.91 (0.27)        | 6.83     | 4.28   | 0.19    | 0.80    |
| Whole milk                              | 2.78 (0.27)         | 0.29     | 0.56   | <0.04   | <0.04   |
| 2% milk                                 | 2.64 (0.19)         | 0.17     | 0.28   | <0.04   | <0.04   |
| 1% milk                                 | 2.76 (0.11)         | <0.05    | 0.56   | <0.04   | <0.04   |
| Fat free milk                           | 2.67 (0.08)         | 0.94     | 1.05   | <0.04   | <0.04   |
| Beef broth 1                            | 19.11 (0.91)        | 5.94     | 6.56   | <0.04   | 0.17    |
| Beef broth 2                            | 7.46 (0.12)         | 1.37     | 0.37   | <0.04   | <0.04   |
| Beef broth 3                            | 7.87 (0.12)         | 0.33     | 0.92   | <0.04   | <0.04   |
| Beef broth 4                            | 9.09 (0.14)         | 1.14     | 1.45   | <0.04   | <0.04   |
| Chicken broth 1                         | 11.13 (0.40)        | 1.38     | <0.06  | <0.04   | <0.04   |
| Chicken broth 2                         | 6.16 (0.11)         | 1.17     | 0.66   | <0.04   | <0.04   |
| Chicken broth 3                         | 6.76 (0.22)         | 0.17     | 0.78   | <0.04   | <0.04   |
| Chicken broth 4                         | 7.54 (0.19)         | 0.27     | <0.06  | <0.04   | 0.29    |

a: Derived from four aliquots from each item; b: Limit of detection (µg L\(^{-1}\)) for As(III) = 0.05, As(V) = 0.06, MMA(V) = 0.04 and DMA(V) = 0.04; As: Arsenic; SD: Standard Deviation; MMA: Monomethylarsonic Acid; DMA: Dimethylarsinic Acid

Table 4: Subset of arsenic results from the total diet study\(^{(a)}\) and this study

| Item description                          | Total diet study | Present study |
|-------------------------------------------|------------------|---------------|
|                                           | Number of items | Mean total As | Maximum As |
|                                           | analyzed        | (SD) (µg L\(^{-1}\)) | (µg L\(^{-1}\)) |
| Apple sauce, bottled                      | 51              | ND            | ND          |
| Apple juice, bottled                      | 51              | 4 (8)         | 40          |
| Apple juice, strained                     | 51              | 3 (6)         | 24          |
| Grape juice from frozen concentrate       | 51              | 4 (6)         | 23          |
| Whole milk, fluid                         | 51              | ND            | ND          |
| 2% milk, fluid                            | 51              | ND            | ND          |
| Skim milk, fluid (fat free)               | 51              | 1 (3)         | 20          |

a: Total diet study values for µg L\(^{-1}\) were calculated using 1 kg = 1 L; b: Apple cider 100% juice; c: Grape juice was bottled; SD: Standard Deviation; ND: Non-Detectable

For the broths and milk (excluding fat free), the inorganic metabolites composed a small fraction of the total arsenic concentration. In fat free milk, As(III) and As(V) explained the largest proportion of the total arsenic concentration.

DISCUSSION

Out of the initial 19 items five were above 5 µg L\(^{-1}\). Inorganic arsenic was found to be a major contributing component of total arsenic in apple and grape juices. The mean arsenic concentration from the beverages sampled was higher than those found in the Total Diet Study for similar items (Table 4). In the TDS, samples were taken from various apple juices, grape juice and milks. All sample results for arsenic concentrations in the TDS were reported in mg kg\(^{-1}\). Microgram per liter was calculated for comparison of results using a rough estimate of 1 kg = 1 L. Bottled apple juice samples and grape juice samples from frozen concentrate had a
mean arsenic concentration of 4 µg L\(^{-1}\). At least one apple juice sample contained 40 µg L\(^{-1}\) and at least one grape juice sample contained 23 µg L\(^{-1}\).

The average total arsenic values described in the TDS are lower than the average values reported in this study. In our study, the mean total arsenic concentration for the five apple juices sampled was 18 µg L\(^{-1}\) while in the TDS it was 4 µg L\(^{-1}\). In the total diet study, the standard deviation for bottled apple juice was reported as being 8 µg L\(^{-1}\) which puts many of the samples above 10 µg L\(^{-1}\) of arsenic. These levels in apple and grape juices may be of concern as those consuming these beverages are typically the young\(^{[24]}\) or the elderly.

Arsenic concentrations among different lots of the same brand of broths and beverages varied in this study. The high concentration of arsenic found in the brand of broth in the initial sampling was not present in the same brand of broth upon retesting another sample a month later. Variation in arsenic concentrations among the beverages and broths may be due to the origin of the animal product or fruit being used to create the items. Since the lot numbers were different between the two time periods, the beverages and broths of a particular brand may have originated from different production facilities.

Because arsenic does not degrade, it can continue to be problematic wherever it resides. Many orchards throughout the world are contaminated with past use of arsenical pesticides. In the US, lead arsenate pesticides were widely used in apple orchards from 1925-1955 until DDT became available. Residual soil pesticide concentrations are dependent upon the length of time a property was an orchard and to this day higher amounts of lead and arsenic can be found in the soil of former orchards\(^{[25]}\). One study out of Washington indicated that As concentrations in lead arsenate contaminated soils could be of concern\(^{[26]}\) since pesticide residuals in orchards have the potential of being absorbed by the fruit tree.

The data in this study are limited but we show that arsenic concentrations vary among and within brands and are well above 10.0 µg L\(^{-1}\) in some beverages and broths. It is unknown why the apple juice samples had more As(III) than As(V) while some grape juice samples had more As(V) than As(III). It may be a result of the stability of the arsenic species\(^{[27]}\) or that the water used for irrigation in the orchards may contain a larger proportion of one species of arsenic over another. In Table 3, there were a few items in which the sum of the arsenic species was greater than the total As concentration. This was due to the total arsenic concentration being an average of four samples while the arsenic species concentration was from a single sample.

Information regarding the source of arsenic species in food is limited. The uptake of arsenic in plants and animals is likely from water containing inorganic arsenic. Residual arsenical pesticides may also contribute to the arsenic found in fruits. When inorganic arsenic is consumed by plants or animals the inorganic arsenic is metabolized resulting in a combination of inorganic and methylated arsenic. It may be through this biological process that we observe varying ratios of arsenic species between fruits.

With the global market in the US, we should be examining arsenic in beverages more routinely as it may be present at levels above those for drinking water in the US. Random spot checking of a few samples per year may not be enough to identify beverages with levels of arsenic greater than 10 µg L\(^{-1}\). In the US, the exposure to arsenic from drinking water is decreasing due to stricter regulations causing foods and beverages to play a larger role as the primary source of arsenic exposure.

The Agency for Toxic Substances and Disease Registry (ATSDR) lists the Maximum Risk Limit (MRL) of chronic arsenic exposure as 0.0003 mg kg\(^{-1}\) day\(^{-1}\)\(^{[28]}\). For a 190 pound male, the maximum limit of arsenic consumption would be approximately 26 µg L\(^{-1}\) day\(^{-1}\). For a 163 pound female, the MRL would be approximately 22 µg L\(^{-1}\) per day. Based on As concentrations we found, those consuming large amounts of apple juice or grape juice may be consuming more arsenic than the MRL for their weight. Typically, individuals consuming these beverages are children and the elderly. Children may be the most susceptible to the toxic effects of arsenic due to this time period of growth and development\(^{[29]}\).

The number of samples taken and the fact that these samples were taken from one supermarket is a limiting factor. We have learned that arsenic concentration among beverages and broths vary among and between brands. Levels of inorganic arsenic in some commercially available apple juices and grape juices are higher than the standard for total arsenic allowed in groundwater in the US.

Foods and beverages consumed in the US originate from around the world. Residents in the US purchased nearly $2 trillion of imported goods in 2007 based on FDA estimates. These goods entered into the US from more than 300 ports of entry by 825,000 importers\(^{[30]}\). This is of concern since the production of foods and beverages use standards from the country of origin, rather than US standards.
CONCLUSION

Commonly consumed juices bought at a local supermarket contained arsenic levels that were higher than the allowable limit of arsenic set forth for drinking water by the EPA. The levels among one brand of apple juice, grape juice, whole milk, beef broth and chicken broth varied across lot numbers. This demonstrates the variability of arsenic in consumed products which is likely due to the source of ingredients coming from different locations. The beverages with the highest levels of arsenic (apple and grape juice) tend to be consumed by the young and the elderly, individuals that may be more vulnerable to over exposure of heavy metals.

Further investigation is needed to acquire a better measure of exposure to arsenic from commonly consumed beverages. Data from the total diet study are based on 12 samples of any particular food or beverage per year. This may not be enough samples given the variability of arsenic levels across lot numbers as demonstrated by our study. Future research into arsenic levels in food and beverages should include examination of the underlying arsenic content of soils and irrigation water sources.

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