United States Environmental Protection Agency **Office of Research and Development**

National Exposure Research Laboratory
Research Abstract

Government Performance Results Act (GPRA) Goal #2
Annual Performance Measure #205

Significant Research Findings:

Preliminary Database on Arsenic Species in Target Foods/Groups to Improve Arsenic Risk Characterization

Scientific Problem and Policy Issues

The maximum contaminant level (MCL) for inorganic arsenic in drinking water will undergo a six year review in 2008. The MCL is influenced by a wide variety of factors including best available treatment technology, analytical monitoring capability and health risk reduction benefit analyses (based on health effects and multiple source exposure estimates). The health risk reduction benefit analysis considers US population exposure to arsenic from all significant sources. The two major arsenic sources and exposure routes are dietary and drinking water ingestion. Also, improved estimates of arsenic dietary exposure in human epidemiology studies will lead to more accurate cancer dose/response estimates. While drinking water sources contain inorganic arsenic, food can contain numerous forms or species of arsenic (arsenicals) which vary significantly in relative toxicity. Generally, inorganic arsenic is considered the most toxic form followed by dimethylarsinic acid (DMA), monomethylarsonic acid (MMA), arsenosugars (associated with seafood), and finally non-toxic arsenobetaine (also associated with seafood). Currently, most of the existing dietary arsenic exposure data reports only a total arsenic value that does not differentiate between inorganic arsenic and arsenobetaine. While total arsenic concentrations can be helpful in identifying target foods which contain a large percentage of the cumulative dietary arsenic exposure, the risk from these exposures cannot be predicted without species specific or chemical form specific information. The development of a preliminary database of the arsenic species in target foods would provide species specific information on a large percentage of the arsenic found in food and would improve exposure source estimates. Thus, the main objective of NERL's dietary arsenic exposure research has focused on developing a preliminary species specific database for target foods including seafood, rice, carrots and apples.

Research Approach

Target foods were chosen for study based on total arsenic analyses obtained from the FDA's market basket survey. Because most of the arsenic in a diet is associated with only a few foods, identifying the arsenicals present

in these foods will provide a fairly robust estimate of arsenic exposure from the dietary route. To accomplish this, extraction procedures were developed and optimized to release as much of each arsenic species present in a food without causing degradation products. The resulting extraction protocols varied considerably based on whether the sample contained predominately protein (e.g., seafood) or starch (e.g., rice). The separation of arsenic species was accomplished via Ion Chromatography (IC) in both the cation and anion modes. The chromatographic detector was an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) which provided low part-per trillion detection for injected arsenicals. In addition, Ion Chromatography was coupled to an Electrospray Ionization Tandem Mass Spectrometer (IC-ESI-MS/MS). This technique allowed the identification of previously unknown arsenic species, and those forms that could not be separated by chromatography.

A mass balance approach was utilized to determine the percentage of the total arsenic (injected on the analytical column) that was separated and identified in each food sample. Without this approach, uncertainty would arise in assessing the risk because the chemical form / toxicity of the arsenicals that were not released by the extraction procedure would be unknown. Initial research indicated that mild extraction conditions often produced a mass balance which was very dependent on the particular food sample. For example, a water/methanol extraction solvent could extract 100% of the arsenicals from a particular rice sample, but only 55% from another rice sample. This variability in method performance ultimately led to the development of a more chemically aggressive extraction procedure to maximize the arsenicals liberated from dietary samples. These extraction conditions minimized the across matrix variability but increased the potential for degradation by-products. Arsenosugars (typically associated with seafood matrices) are the most chemically labile (unstable) of the arsenicals. Their degradation was studied under a wide variety of extraction conditions in order to verify that the final extraction process did not change the native distribution (actual ratios) of the arsenicals.

Results and Impact

Significant findings of this research were as follows:

- Mild extraction procedures (methanol/water) were evaluated using finfish and a Standard Reference Material (SRM). The resulting extraction removed and speciated over 90% of the total arsenic in these samples. While mild extraction solvents were found to produce a good mass balance for samples containing almost exclusively arsenobetaine (a non-toxic arsenical), samples containing high fat (e.g., salmon) were found to produce lower extraction efficiencies and, in turn, increased the uncertainty associated with the chemical form of the unextracted arsenic fraction (*JAAS,* 1999, **14**, 607). Precision and recovery were documented for this extraction technique using laboratory fortified

blanks and the SRM (*JAAS*, 2002, **17**, 581). These data represent preliminary arsenic speciation data for finfish and the accompanying data quality information.

- Arsenosugars (relatively non-toxic arsenicals), commonly associated with clams, oysters and seaweed products, produce erroneous results due to chromatographic co-elution with other toxic arsenic species. Because arsenosugars can be present at parts-per million (ppm) quantities in seafoods, they represent a serious source of chromatographic co-elution and uncertainty. Because arsenosugar standards were not commercially available, they were structurally identified and characterized (*JAAS,* 1999,**14**, 1829). Subsequently, arsenosugars were purified from natural products in microgram amounts to allow quantification of the 4 major arsenosugars found in seafood samples (*Analyst*, 2002, **127**, 781). This combined research minimized the misidentification of the arsenosugars and provided a source of standards essential to the development of a preliminary database for arsenic species in seafoods.

- The analysis of commercially available seaweed and sushi wraps indicated that these samples can contain a mixture of arsenic species in high (ppm) concentrations. Extraction efficiencies for these types of samples only ranged from 26% to 73% meaning that 27% to 74% of the arsenic remained unidentified in the seaweed sample. In addition to the low extraction efficiencies, seaweed contained the highest inorganic arsenic concentration of any seafood analyzed in this research. Ingestion of a gram of Hijiki (seaweed) with a concentration of 104 ppm would represent a 17 microgram exposure to inorganic arsenic, a toxic arsenical. Even though 74% of the arsenic was not extracted from this type of seaweed, this exposure would represent 0.85 times the current drinking water MCL (i.e., a consumption of 2 liters of water at the 10 ppb MCL would equal 20 micrograms of ingested arsenic; *FJAC*, 2001, **369**,71).

- To improve extraction efficiencies, more aggressive acidic (*Analyst*, 2002, **127**, 781) and basic (*Analyst*, 2003, **128**, 1458) extraction procedures were evaluated while monitoring the stability of arsenosugars. These studies indicated that basic extraction conditions minimized the formation of arsenosugar degradation products. This information was critical in assuring that the native arsenosugars are not degraded by the extraction process and provided the scientific foundation for the development of a tetramethyl ammonium hydroxide based extraction procedure (*JAAS*, accepted 08/04). This extraction process was used on shellfish, oysters and clams. Results indicated that while arsenosugars can be the predominant arsenical,

dimethylarsinic acid (DMA), another known toxic arsenical (cancer promoter), are present at significant levels (ranging from 565 - 1581 ppb). In addition, a new sulfur containing arsenosugar was identified which can represent up to 50% of the extractable arsenic.

- Using the same mass balance approach, a trifluoroacetic acid extraction was developed for rice to break down the starch backbone and liberate as much arsenic as possible for speciation analysis. The extraction procedure did not alter the native distribution of arsenicals found in rice. The resulting extraction procedure removed between 84 to 99% of the total arsenic present in rice samples. Both DMA and inorganic arsenic were commonly found, but the ratio between DMA and inorganic arsenic was not uniform across the rice samples analyzed. Therefore, this ratio cannot be used to estimate the amounts of these toxic arsenicals from existing databases which only contain total arsenic concentrations for rice (*JAAS*, 2001, **16**, 299). The inorganic arsenic concentration ranged from 21 - 96 ppb. In this example, the inorganic arsenic exposure from ingesting 2 ounces of uncooked rice ranges from 1 - 5.4 µg, which is equivalent to 5 to 27% of the MCL (i.e., 20 micrograms), assuming a 2 liter/day intake.

- Extraction efficiencies for carrots ranged from 80 to102%, and an accelerated solvent extraction procedure (methanol/water) did not cause degradation of the extractable arsenicals. Both inorganic arsenic and MMA were commonly found. Similar to rice, the ratio between inorganic and MMA was not a constant in the carrots analyzed. The MMA concentration ranged from non-detects to 13 ppm, while the inorganic arsenic concentration ranged from 14 to 325 ppb. In this example, the inorganic arsenic exposure from ingesting 2 ounces of carrots ranges from 0.8 - 18.4 µg, which is equivalent to 5 to 91% of the MCL (Analyst, 2001, **126**, 1011).

- The extraction efficiency for apples ranged from 79 to117% using a 2 stage extraction (an enzymatic extraction using amylase is followed by an acetonitrile-water extraction). The inorganic arsenic concentration ranged from 7.6 to 46.6 ppb. In this example, the inorganic arsenic exposure from a 4 ounce ingestion of apples ranges from 0.9 - 5.3 µg, which is equivalent to 4 to 26% of the MCL (*Analyst*, 2001, **126**, 136).

- Finally, research indicated that seafood is the predominant source of total arsenic for adults while infant exposures predominantly stem from cereals and other pureed foods. Inorganic arsenic was detected in rice, sweet potatoes, carrots, green beans, peach and mixed cereals (*Journal of AOAC International*, 2004, **87**, 244).

In summary, the arsenic speciation analyses of specific target foods indicates that the inorganic arsenic concentration can produce exposures which range from 4 to 91% of the exposure estimated from consuming 2 liters of drinking water at the MCL. Therefore, the contribution of inorganic arsenic from dietary sources can range from insignificant to approximately equal to that from drinking water. Since these estimates are based on a very limited sample set, a broader sampling of these target foods is warranted. In addition, drinking water regulations attempt to address sensitive sub-populations (e.g., infants and/or the elderly) and provide a additional level of safety for these sub-populations, and the data on infant foods indicates that certain exposures may be significant relative to the adult exposure calculated from the drinking water MCL.

Research Collaboration and Research Products

This research has been a collaborative effort between the U.S. EPA's National Exposure Research Laboratory and the U.S. Food and Drug Administration (FDA).

U.S. EPA Publications

Fricke, M.W., Creed, P.A., Parks, A.N., Shoemaker, J.A., Schwegel, C.A., Creed, J.T. "Extraction and Detection of a New Arsine Sulfide containing Arsenosugar in Mollusks by IC-ICP-MS and IC-ESI-MS/MS." JAAS, accepted Aug. 2004.

Gamble, B.M., Gallagher, P.A., Shoemaker, J.A., Parks, A.N., Freeman, D.M., Schwegel, C.A., Creed, J.T. "An investigation of the chemical stability of arsenosugars in basic environments using IC-ICP-MS and IC-ESI-MS/MS." Analyst, **128**: 1458-1461, 2003.

Gamble, B.M., Gallagher, P.A., Shoemaker, J.A., Wei, X., Schwegel, C.A., Creed, J.T. "An investigation of the chemical stability of arsenosugars in simulated gastric juice and acidic environments using IC-ICP-MS and IC-ESI-MS/MS." Analyst, **127**: 781-785, 2002.

Gallagher, P.A., Creed, J.T. Wei, X., Murray S., Brockhoff, C.A. " An Evaluation of Sample Dispersion Medias with ASE for the Extraction and Recovery of Arsenicals in LFB and DORM-2 with ICP-MS Detection." JAAS, **17**: 581-586, 2002.

Gallagher, P., Creed, J.T. Wei, X., Shoemaker, J., Brockhoff, C.A. "Extraction and Detection of Arsenicals in Seaweed via Accelerated Solvent Extraction with Ion Chromatographic Separation and ICP-MS Detection." Fresenius Journal of Anal. Chem., **369**: 71-80, 2000.

Gallagher, P., Creed, J.T. Wei, X., Shoemaker, J., Brockhoff, C.A.

"Detection of Arsenosugars from Kelp Extracts via IC-ESI-MS/MS and IC Membrane Hydride Generation ICP-MS." JAAS, **14**: 1829-1834, 1999.

McKeirnan, J., Brockhoff, C.A., Creed, J.T., Caruso, J. "A Comparison of Automated and Traditional Methods for the Extraction of Arsenicals from Fish." JAAS, **14**: 607-613, 1999.

U.S. FDA Publications

Vela, N.P., Heitkemper, D.T. "Total Arsenic Determination and Speciation in Infant Food Products by IC-ICP-MS." *Journal of AOAC International* Special Section, **87**: 244-252, 2004.

Vela, N.P., Heitkemper, D.T. "Arsenic Extraction and Speciation in Carrots using Accelerated Solvent Extraction, Ion Chromatography and Plasma Mass Spectrometry." Analyst, **126**: 1011-1017, 2001.

Heitkemper, D.T., Vela, N.P., Stewart, K.R., Westphal, C. "Determination of Total and Speciated Arsenic in Rice by Ion Chromatography and Inductively Coupled Plasma Mass Spectrometry." JAAS, **16**: 299-306, 2001.

Heitkemper, D.T., B'hymer, C. B., Caruso, J.A. "Evaluation of Extraction Techniques for Arsenic Species from Freeze Dried Apple Samples." Analyst, **126**: 136-140, 2001.

Future Research

The data presented in each of these manuscripts represents documentation regarding method performance and preliminary findings in each of the target dietary matrices. To obtain a more robust estimate of arsenic present in rice, data has been collected (but not yet published) on 25 different rice samples. A new interagency agreement with the FDA has been initiated which will expand infant food data in hopes of improving this exposure estimate. The FDA has also begun to look at poultry (another target food), but is having problems extracting and positively identifying all arsenicals present in the extracts.

Currently, the seafood methodology has only been applied to 15 different shellfish samples with the goal of expanding this to include a wider variety of finfish. The ultimate goal is to expand the sampling to a minimum of 25 in each of the target food groups.

Finally, while chemical extractions have been developed to maximize the amount of arsenic extracted from target foods, it is not known how well these procedures mimic physiological based processes occurring in the human body. Future research will emphasize estimating what portion of

the arsenic present in seafoods is bioaccessible.

Contacts for Additional Information

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Federal funding for this research was administered through an interagency agreement between the U.S. EPA and the U.S. FDA (#DW75939361).