Health Effects and Mitigation of Arsenic: Current Research Efforts and Future Directions

Workshop and Webinar Discussion Report

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Part I: On-Site Workshop

Executive Summary

The National Institute of Environmental Health Sciences (NIEHS) Superfund Research Program (SRP) organized a two-day workshop and panel discussion webinar series that gathered leading scientists doing research on arsenic exposure and health effects. Bill Suk, Ph.D., director of the SRP, and Danielle Carlin, Ph.D., health scientist administrator at the NIEHS, spearheaded this effort. The SRP has a long history of funding arsenic research that spans a range of scientific disciplines and expertise. Interest in arsenic is currently on the rise because of recent findings related to arsenic in foods and remediation. Furthermore, the U.S. Environmental Protection Agency (EPA) Integrated Research Information Service (IRIS) is currently performing a risk assessment to inform current policies and regulations, and is seeking data to inform the assessment. Major themes and findings from these discussions are summarized here.

Aggregate Exposures and Exposure Assessments

There is a need for more information about aggregate arsenic exposure in humans, particularly in light of growing evidence for the presence of arsenic in an array of foods. Drinking water can be a primary driver of exposure in areas where water concentrations are very high, but non-drinking water, diet, soil, and dust also need to be assessed. In contrast, diet can be a primary driver of exposure in areas with low drinking water arsenic concentrations. Although expensive to implement, a duplicate diet study paired with biomarkers of exposure in a large human population would provide a wealth of information that is difficult to piece together. The environmental media that contain arsenic (e.g., water, food, and soil) are themselves complex matrices that contain co-contaminants, bacteria, and other microbes that may influence arsenic bioavailability and metabolism.

Total arsenic, inorganic arsenic, and major known arsenic metabolites should also be measured more often in environmental media, and in human blood and tissues as part of exposure and epidemiological studies. Unless arsenic species in foods are identified, it is difficult to make recommendations for food intake. For example, there are a number of arsenoproteins, arsenolipids, and arsenocarbohydrates in foods that have not been characterized for their toxicity and metabolic properties.

Exposure Mitigation

Exposure mitigation requires interventions from the exposure source to the community level, including behavior changes at the individual level. Development of rapid, cost-effective, accurate on-site field measurements of arsenic species in human samples, water, soil, and dust is an essential component for exposure assessment and mitigation. Kits need to be easy to use so that community members can test their own private wells and they need to work under challenging field conditions. Some field testing kits for water are available and have been validated for some uses, and other new kits are in development. One new testing approach measures specific arsenic-induced gene expression in certain microbes, and shows promise for sensitivity and accuracy in field testing for a variety of sample types.

Knowledge of contaminated sources is not sufficient for inspiring changes in behavior to avoid exposure. Effective education and motivation at the community level is a crucial component to convert

information to prevention. Researchers must consider community culture and capacity when designing and implementing remediation options. Any remediation or mitigation strategy will work only if the community uses the system, and uses it correctly.

Alternatively, some approaches to mitigating dietary exposure circumvent a need for motivating community action. New approaches to reduce arsenic content in foods include modification of plant uptake of arsenic, and selection of plant varieties that naturally absorb less arsenic – both topics have been integrated into ongoing studies involving rice. Tracing the genes and biochemical pathways involved in plant uptake of arsenic may also provide insights toward the use of plants to remediate contaminated soils.

Biomarkers, Health Effects, and Susceptibility

There is a great need for data that links aggregate exposure information to health effects. Integrating omics data with epidemiological data is the next step toward linking biomarkers of exposure first with intermediate effects that are precursors for disease, and then with disease outcomes. These biomarkers may be useful for predicting disease risk or susceptibility prior to the onset of disease and for quantitative risk assessment that could inform recommendations for risk mitigation.

Biomarkers of susceptibility provide clues to uncover susceptibility factors to inform prevention and intervention. For example, studies of single nucleotide polymorphisms in arsenic metabolism genes illustrate how a gene polymorphism is linked to altered arsenic metabolism, but also open the possibility for intervention by micronutrient supplementation to reduce susceptibility to arsenic-related health effects. Other susceptibility factors are under investigation, including developmental stage, age, sex, and nutritional status. The role of the microbiome in susceptibility and health effects is largely unknown although accumulating data suggests the microbiome in the environment and in the human body likely plays a substantial role in bioavailability, exposure, and health effects.

Conclusion

Integration of data and information was a theme throughout the workshop and webinar series. The time has come to integrate data by linking DNA methylation to gene expression, and ultimately to health outcomes in large population studies. Increased integration between toxicologists and epidemiologists can inform research in both disciplines. Understanding arsenic speciation is critical for understanding how environmental fate and transport of arsenic, exposure, susceptibility, and health effects are inextricably linked. We also need to develop a more detailed picture of arsenic exposures in the complex matrices of water, food, and soil in order to develop strategies to mitigate exposure and predict risk.

This report takes a step in the direction toward integration. Addressing data gaps calls for deeper collaborations and more integration among molecular biologists, toxicologists, epidemiologists, microbiologists, risk assessors, geochemists, statisticians, and other diverse disciplines. NIEHS staff members extend a warm thank you to all of the participants in the workshop and the webinar panels for their contributions to these rich and productive conversations.

Overview: Arsenic and Health Effects (Linda Birnbaum, *NIEHS*)

Linda Birnbaum, Ph.D., Director of NIEHS and the National Toxicology Program, opened the workshop with an overview of the global issue of arsenic exposures and health effects. Exposure via air, soil, water, and food occurs primarily from natural geological sources, but also from pesticide use, electronics manufacturing processes, metal smelting, wood treatment, and mining processes. Routes of entry include ingestion, inhalation, and, to a lesser extent, dermal exposure. Growing evidence shows that many organs are impacted with growing concerns about developmental effects. The long list of known health effects includes cancer of multiple organs, gastrointestinal effects, cardiovascular effects, diabetes, anemia, impaired immunity, skin lesions (Figure 1), and lung disease. Furthermore, Birnbaum noted that evidence suggests early life exposure can result in health effects into adulthood. Currently, the U.S. EPA has set the Maximum Contaminant Level (MCL) of 10 µg/L in drinking water, based not only on health effects research, but also on the limitations of what can be accomplished in remediation. Therefore, Birnbaum said that research is focusing on reducing arsenic concentrations in exposure media, and mitigating health effects. Current approaches to limit exposure include:

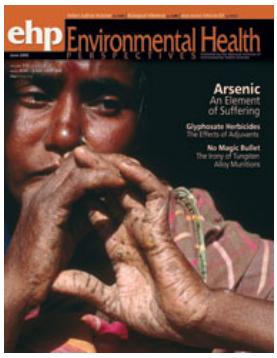

Figure 1: An Environmental Health Perspectives journal special issue (September 2005) featured arsenic research on health effects including skin lesions

- Placement of deeper wells
- Adsorbents
- Phytoremediation
- Bioremediation (e.g., microbial intervention)
- Nanotechnology
- Monitoring for geological environments with high arsenic
- Importance of testing drinking-water sources and marking wells
- Monitoring foods for arsenic (e.g., rice, organic brown rice syrup, apple juice)
- Nutritional intervention

During the question and answer period with workshop participants, researchers agreed that there is still much that we do not know about exposure to arsenic. How much are we getting exposed to? How much is too much? Where are we getting exposed? Food sources have only just begun to be explored. Participants suggested that more exposure data that can be used in pharmacokinetics modeling is needed to calculate protective arsenic standards and inform the U.S. EPA risk assessment. The goals of this workshop and this meeting report are to highlight emerging research on exposure to arsenic, environmental bioaccessibility and endogenous bioavailability of arsenic, susceptibility to arsenic effects, vulnerable populations, advanced techniques for understanding arsenic in health and the environment, and current mitigation and remediation efforts in the U.S. and globally.

Figure 2: Drinking water is a concern for arsenic exposure

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Arsenic and Children's Health: A Multidisciplinary Approach from Superfund to Children's Center (Carol Folt, Chancellor, *University of North Carolina*)

Arsenic presents a multi-faceted problem that requires a broad range of expertise to solve, and with SRP support, the interdisciplinary approach can be very productive, according to Carol Folt, Ph.D., Chancellor at the University of North Carolina. Folt has been involved in interdisciplinary research with the Dartmouth SRP and with the NIEHS Children's Health Center at Dartmouth for more than 20 years. She noted that, 25 years ago, interdisciplinary research programs as well as women leaders in science were uncommon. The SRP was a new kind of program with a mandate for a wide breadth of expertise to tackle complex problems and translate research findings that brought a new kind of research with a new team approach. Folt characterized SRP as being ahead of its time by bringing biomedical researchers together with engineers working on technological development. At that time, she said, those distinct disciplines were not intermingled, and there was much more hierarchy in the workplace. Since the start of SRP, science has moved from single investigator to multiple-investigator teams and 'big' science. Folt noted that complex problems do not respect disciplinary boundaries.

Folt put current interdisciplinary approaches in a historical perspective. When Folt arrived at Dartmouth College, Karen Wetterhahn, Ph.D., was one of the first and few tenured woman scientists. The interdisciplinary Superfund team started when Wetterhahn sent a campus-wide message asking about interest in metals in the environment. A number of researchers responded, and in the end women led more than half of the projects. Folt thinks that initially women in particular were drawn to the interdisciplinary team approach. She applauds diversity in efforts to solve problems, stating that, "You don't get anywhere when people look and sound the same." Interdisciplinary training of young scientists spurned their excitement, she said, and they launched interdisciplinary research careers that continue to be successful today.

Figure 3: Folt says interdisciplinary approaches draw women scientists (Mary Lou Guerinot, Ph.D., and Ilda Bajraktari, a trainee; photo courtesy of C. Folt)

The Dartmouth SRP currently consists of six projects that intertwine different scientific disciplines, an approach that is made possible by SRP's funding and research center structure. The six projects are investigating exposure sources, exposure biomarkers, response biomarkers, health outcomes, and applications in communities, clinical practice, and policy. Overall, Dartmouth SRP's interest has focused on consequences of metal exposures at levels that can be found in the United States. Initially, Folt said, her research focused on mercury variation across lakes and rivers, as well as fish diet effects on mothers and children. Her research then extended to include arsenic, based on epidemiologic findings. The earliest studies looked at arsenic exposure from private wells in the region and led to health endpoint studies including cancers and developmental effects of low-level concentrations, particularly with regard to long-term health effects. She relied heavily on collaborations with the epidemiologists and molecular biologists on the SRP team that developed into new cross-disciplinary areas.

To investigate effects of developmental exposures, Dartmouth SRP established the New Hampshire Birth Cohort, funded by SRP and lead by Margaret Karagas, Ph.D., that has followed 1,000 mother/infant pairs

Figure 4: Diet is an important source of early life arsenic exposure (photo courtesy of C. Folt)

from pregnancy through early childhood. Arsenic researchers were linked by the established cohort research efforts, expanding research data and tools, and providing opportunities to early career researchers. In particular, 2010, Karagas, Folt and a team of scientists established the NIEHS Children's Center that extended their work on arsenic on pregnancy to include a focus on children's health. The Dartmouth researchers uncovered rice, rice-based foods, organic rice syrup, and some infant formulas as important sources of exposure for infants and children.[1] Ongoing studies are revealing immune system effects that include higher infant infection rates, and will be investigating the developing microbiome and vaccine efficacy. In regards to exposure via drinking water, Dartmouth researchers documented that, in New Hampshire, 40% of residents use private water, and >10% of those wells are estimated to have arsenic concentrations greater than the current MCL.

Folt credits the growth of arsenic research at Dartmouth in the SRP and the Children's Center to its interdisciplinary approach that includes engineering, environmental health sciences, statistics, communication, and humanities expertise. She urged scientists to develop multilingual approaches to be able to speak to diverse people about their work. Science is moving quickly toward cross-institutional programs, industry/university partnerships, with industry increasingly turning to universities, and universities being asked to explain the value of their research. Developing a national model for interdisciplinary approaches, Folt said that SRP has led the way in complex problem solving as demonstrated in the approach to solving multi-faceted problems like those related to mercury and arsenic exposures and toxicity. She emphasized that the interdisciplinary approach is suited to problem-driven, solution-oriented research to best address problems associated with these and other contaminants.

[1] Jackson et al. 2012. *Pure Appl Chem,* 84(2):215-223; Jackson et al. 2012. *Environ Health Perspect,* 120(5):623-6

Session 1: Global Environmental Cycling and Bioavailability of Arsenic

(Moderator: Karen Bradham, Ph.D., *U.S. EPA*)

Management of Arsenic-Contaminated Irrigation Water for Rice Production (Matthew L. Polizzotto, *North Carolina State University*)

Throughout Southern and Southeast Asia, groundwater is naturally contaminated with very high levels of arsenic, affecting more than 100 million people. Local residents use well water for drinking but also extensively for irrigation. Rice is a staple crop in Bangladesh, and a staple food for more than half of the world's population. In Bangladesh, rice provides more than half of the calories in their diet[2], so the arsenic content of rice is an important public health issue. Arsenic-contaminated irrigation water contributes to the arsenic content of rice, and arsenic limits rice yields. Matthew Polizzotto, Ph.D., at North Carolina State University is developing strategies to mitigate arsenic contamination in irrigation water for rice production in Bangladesh.

Rice cultivation requires large volumes of water for irrigation. Polizzotto said that an irrigation system design should allow for three potential types of mitigation approaches: 1) to reduce arsenic concentrations in the water source, 2) to reduce arsenic concentrations in the agricultural fields, and 3) to reduce the amount of arsenic taken up by plants. Polizzotto and his team of researchers focus their work on reducing arsenic concentrations in the rice fields using engineered channels, in-channel filtration and trapping systems, and chemical amendments to pull arsenic out of the system. Their goal is to develop low-cost, practical, and sustainable strategies using local materials.

Figure 5: Irrigation channel in Bangladesh (photo courtesy of M. Polizzotto)

Channels that traverse the rice fields (Figure 5) carry groundwater, exposing the water to oxygen and components in soil that together chemically modify the concentrations of arsenic in the water. The channel width and length, water flow rate, soil composition, and other parameters impact arsenic removal from flowing irrigation water, according to Polizzotto. For example, his data showed that channels that are 3-times wider than typical, control channels can remove over 20% of dissolved arsenic and channels that are 4-times longer than typical channels can remove ~45% of dissolved arsenic from the water, as compared to about 8% removal with typical, control channels.[3] There is a complex web of interwoven chemical reactions that can take place in these channels, and they are not yet fully understood. Polizzotto said that researchers will continue to study channel design, water flow conditions, and soil and water chemistry to optimize the system. Ultimately, the researchers plan to

[2] IRRI (International Rice Research Institute) 2014. World Rice Statistics Online Query Facility. http://ricestat.irri.org:8080/wrs2/entrypoint.htm

[3] Lineberger et al., 2014. *J Environ Qual*, 42(6):1733-1742

develop a model that can predict how a new channel system would work in specific rice fields so that systems can be tailored to local conditions.

From the Soil to the Seed: Arsenic in Rice (Mary Lou Guerinot, *Dartmouth College*)

Rice is a staple for more than half of the world's population, and many rice-based foods, including baby foods, are found on the market in many countries. A recent study calculated that consumption of 0.56 cups/day of cooked rice was comparable to drinking 1 liter/day of 10 µg arsenic/liter water, the current U.S. maximum contaminant limit.[4] On average, U.S. rice consumption averages ~0.5 cups/day while Asian Americans consume an average of >2 cups/day.

One strategy to reduce arsenic content in rice is to utilize plant biology and genetics with the goal of growing plants that take up less arsenic. Mary Lou Guerinot, Ph.D., at Dartmouth College is studying ways to reduce arsenic in rice by focusing on two contributing factors: 1) mobilization of As^{III} in flooded rice paddies, making As^{III} available for inadvertent uptake and transport through silicon transporter pathways and 2) inadvertent uptake of As^{V} through phosphate transporters.

Growing rice in flooded conditions results in the release of As^{III} from iron oxyhydroxides in the soil into the water, making it accessible for uptake by the plants, according to Guerinot. Unfortunately, growing rice in unflooded conditions changes the soil chemistry to reduce arsenic uptake, but it also increases cadmium uptake in rice plants. Merely changing growing conditions is not sufficient to address the problem.

Figure 6: Different types of rice plants vary in their uptake of arsenic, and compounds in the soil can affect uptake (courtesy of M. Guerinot)

One of Guerinot's goals is to find and develop rice cultivars (plant varieties) that restrict arsenic accumulation in rice grains. This approach has the potential to be one of the simplest, fastest, and most cost-effective approaches to solving the problem. She says such cultivars could be used immediately in arsenic-rich soils, and could be expanded for use in modern commercial rice production. Guerinot has collaborated with David Salt, Ph.D., at the University of Aberdeen and Shannon Pinson, Ph.D., at the U.S. Department of Agriculture (USDA) on their rice grain ionomics project. Ionomics is the measurement of elements like iron and arsenic within an organism in relation to the biology of that organism.

Guerinot's team and collaborators grew rice at locations around the world and screened more than 1,800 cultivars held in the USDA national rice collection; they found a wide range of arsenic accumulation levels. They are using genome-wide association studies in combination with ionomic phenotypes to identify genes that play a role in arsenic accumulation.[5] Guerinot's team has identified two cultivars with higher arsenic accumulation, and they plan to identify

[4] Gilbert-Diamond et al. 2011. *Proc Natl Acad Sci USA,* 108(51):20656-60
[5] Norton et al. 2012. *New Phytol,* 193(3):650-4; Norton et al. 2014. PLoS One 9(2):e89685.

mechanisms of high arsenic accumulation toward the goal of reversing that process to reduce accumulation.

Another angle of her research aims to characterize the distribution of arsenic in the rice plants and grains. Guerinot and her team are using synchrotron X-ray fluorescence microprobe tomography to identify where specific elements (e.g., arsenic, manganese, and iron) are accumulating in rice grains and tissues at a microscopic level. Guerinot's recent results suggest that the more toxic form of arsenic, As^{III}, accumulates in the outer bran layers whereas less-toxic methylated forms of arsenic accumulate in the inner layers.[6] The bran is present in brown rice, and polished off to make white rice. She said that this finding explains why brown rice, although higher in nutrients, can have higher levels of arsenic than white rice.

Dietary Arsenic - What Do and Don't We Know? (Margaret Kurzius-Spencer, *University of Arizona*)

At the University of Arizona, Margaret Kurzius-Spencer, Ph.D., focuses her environmental research on dietary exposures to arsenic. She said that existing data on dietary arsenic are limited and that the available data show high variability among different samples of the same food items. The forms of arsenic in the diet are generally divided into organic and inorganic forms although most of the existing data measure total arsenic only. The arsenic in most foods is primarily inorganic arsenic. However, total arsenic in seafood is generally 1–2 orders of magnitude greater than in non-seafood items. While most of that is organic and considered non-toxic, seafood may contain up to 30% inorganic arsenic, methylated arsenic species, arsenite sugars, and other species of arsenic. Kurzius-Spencer said that there are two key sources of data:

- The U.S. FDA total diet study[7], a market basket survey conducted annually, looking at the total arsenic levels in about 280 food items
- A report by Schoof et al.[8] of a market basket survey of total and organic arsenic in 40 foods collected in North America

Relative contributions of diet and drinking water as sources of human exposure are important parameters to determine. Many studies have shown correlations between drinking water exposure and urinary arsenic; however, Kurzius-Spencer found that, in U.S. populations, dietary total and inorganic arsenic consumption are better predictors of urinary arsenic than drinking water concentrations.[9] Other

[6] Carey et al. 2010. *Plant Physiol,* 152(1):309-19; Carey et al. 2011. *New Phytol,* 192(1):87-98
[7] http://www.fda.gov/food/foodscienceresearch/totaldietstudy/default.htm
[8] Schoof et al. 1999. *Food Chem Toxicol,* 37(8):839-46
[9] Kurzius-Spencer et al. 2013. *J Expo Sci Environ Epidemiol,* 23(4):442-9

studies show that consumption of certain foods (e.g., rice and wine) is associated with urinary[10] or toenail[11] arsenic concentrations.

What is the contribution of dietary arsenic intake to total arsenic exposure through ingestion? Kurzius-Spencer tackled this question in a recent study of dietary exposure in relation to the current U.S. EPA Maximum Contaminant Level (MCL) of 10 µg/L in drinking water.[12] The researchers modeled exposure to arsenic from foods based on subjects' dietary records (non-seafood eaters) and food residue analysis, and compared those levels to drinking and cooking water contributions to total ingestion exposure. The results show that diet contributes less than half of the aggregate inorganic arsenic exposure for people with tap water >10 µg/L; however, diet is the major source of aggregate inorganic arsenic exposure for people with tap water concentrations <10 µg/L (54-85% of exposure in different populations) (Figure 7).

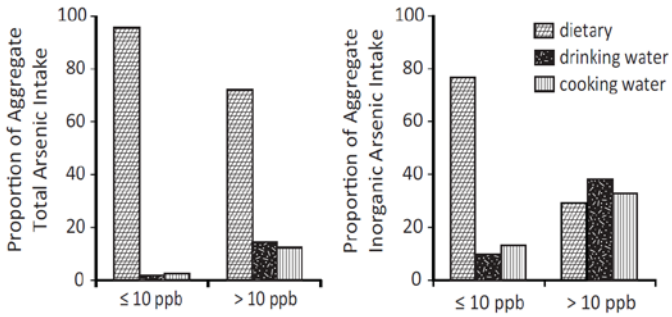

Figure 7: Contribution of dietary arsenic to total arsenic (left panel) and inorganic arsenic (right panel) exposure at different drinking water concentrations (adapted from Kurzius-Spencer et al. 2014)

At the current MCL, dietary arsenic is the dominant exposure, and exposure from food may be 5–20 times higher than from water, according to Kurzius-Spencer. Overall, she said, we currently know that modeled dietary arsenic intake is highly correlated with measured intake but does not accurately estimate exposure. Given that more research is accumulating to show health effects at relatively low-dose arsenic exposures, she thinks that it is very important to consider aggregate exposure, including diet, in relation to health effects and risk assessment.

Kurzius-Spencer also noted that there remain major data gaps regarding exposure from food:

- Data on organic, inorganic, and other arsenic compounds in more foods and more samples of the same foods
- Identification of the other forms of arsenic found in seafood, given that even a small percent of the high levels of total arsenic in seafood may contribute to considerable exposure
- Better understanding of the sources, metabolism, and toxicity of arsenic species found in individual foods and in composite diet

[10] Cascio et al., 2011. *J Environ Monit*, 13:257-265; Gilbert-Diamond et al., 2011. *Proc Natl Acad Sci USA*, 108(51):20656-20660; Davis et al., 2012. *Environ Health Perspect*, 120(10):1418-1424; Banerjee et al., 2013. *Nature Sci Reports*, DOI: 10.1038/srep02195

[11] Cottingham et al., 2013. *Nutr J*, 12:146-154

[12] Kurzius-Spencer et al. 2014. *J Exp Sci Environ Epidemiol,* 24(2):156-62

Incidental Ingestion of Arsenic Contaminated Soil and Dust: Refining Exposure through the Assessment of Relative Bioavailability and Bioaccessibility (Albert Juhasz, *University of South Australia*)

An important parameter when assessing arsenic exposure in various media (e.g., food, soil, and dust) is contaminant bioavailability and / or bioaccessibility. Albert Juhasz, Ph.D., of the University of South Australia, is investigating these issues in the context of arsenic-contaminated soil. Juhasz noted that concentrations of arsenic in soil can range up to tens of thousands of milligrams per kilogram. But arsenic bioavailability can vary considerably, depending on the source of arsenic, its speciation, and soil properties. To refine exposure estimates derived from arsenic concentrations in soil and other media, bioavailability and bioaccessibility need to be considered.

What happens when arsenic in soil is ingested? Juhasz said that there are many processes that occur in the gastrointestinal (GI) tract that affect the extent of arsenic absorption (Figure 8). Contaminant interactions within the GI tract, transport of contaminants to physiological sites, passage across physiological membranes, organ accumulation, and toxicokinetics can all impact the biological effects of arsenic exposure.

Swine, monkeys, and mice have been used as animal models to assess the *in vivo* bioavailability of arsenic. Urine or blood arsenic is measured after dosing animals (single or multiple doses), and arsenic relative bioavailability is calculated by comparing the relationships between arsenic excretion and dose for contaminated soils and sodium arsenate. Juhasz noted that there can be considerable variability in results depending on the physico-chemical properties of the sample, and animal bioassays are ethically challenging, time-consuming, and expensive.

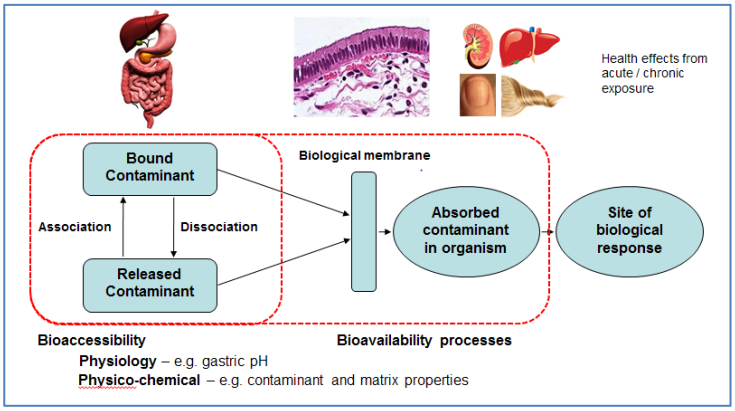

Figure 8: Bioaccessibility and bioavailability processes that play a role in exposure (figure courtesy of A. Juhasz)

To overcome some of these limitations, *in vitro* gastrointestinal extraction methods that mimic key processes in the GI tract have been developed to estimate arsenic relative bioavailability. Approaches range from assays that simulate the gastric phase only to some that include saliva and intestinal flora. In many cases, Juhasz stated, *in vitro* bioaccessibility estimates vary among the different techniques for a given sample. However, comparing *in vitro* to *in vivo* results, a good correlation has been

found between specific tests (e.g., SBRC gastric phase and swine bioassays), but in some cases and for some samples the correlation is poor (e.g., SBRC gastric phase and monkey steady state urinary excretion bioassay).[13]

Further studies are needed to focus on validating the relationships between different *in vitro* bioaccessibility and *in vivo* bioavailability methodologies. Juhasz called for more testing of multiple soil samples, different types of samples, and different methodologies. He placed special emphasis on the need to focus on soils for which *in vitro* data does not match up with *in vivo* data to help understand mechanisms that control bioaccessibility and bioavailability. That understanding might lead to better methods to limit arsenic exposure from soils and other media.

Session 1 Panel Discussants: Brief Research Overviews

Due to inclement weather, the panel discussions were postponed, and later held as a webinar series (see the Arsenic Panel Discussion Webinar Series report). Two panelists from this session gave brief overviews of their research that are summarized here.

Arsenic Entering Groundwater and Rice (Scott Fendorf, *Stanford University*)

Many people are aware of arsenic contamination of well water in Bangladesh, but Scott Fendorf, Ph.D., of Stanford University works on arsenic-related problems throughout South Asia (Figure 9). Arsenic contamination is a problem for areas that drain from the big river systems coming out of the Himalayas, leaving material deposits throughout the deltas. Fendorf said that use of arsenic-contaminated groundwater for drinking and for rice crop irrigation is extensive in the region. Together with colleagues, Fendorf couples bench work with fieldwork in geochemistry and plant biology to investigate exposure reduction strategies related to arsenic in rice crops.

Figure 9: Stanford University arsenic research projects throughout South Asia (graphic courtesy of S. Fendorf)

In their laboratory, Fendorf's group is investigating causal mechanisms for arsenic conversion from the solid phase in soil to the aqueous phase, and for arsenic speciation changes. In the field, the researchers are using

[13] Juhasz et al. 2007. *Chemosphere,* 69(6):961-966; Bradham et al. 2011. *Environ Health Perspect,* 119(11):1629-1634; Brattin and Casteel, 2013. *J Toxicol Environ Health* A, 76(7):449-457; Rodriguez et al. 2003. *J Environ Qual*, 32:876-884; Juhasz et al. 2009. *Environ Sci Technol,* 43(24):9487-94

hydrology and biochemistry to examine soils and subsurface sediments to understand mechanisms and develop effective spatial and temporal models of arsenic partitioning and migration in groundwater systems.

Research is also ongoing with a focus on rice plant root systems. In collaboration with Mary Lou Guerinot, Ph.D., from Dartmouth University, researchers are using optical imaging and x-ray fluorescence mapping of arsenic distribution in rice roots to understand transport of arsenic from soils and groundwater up into the roots. They are doing extensive research into the biology of rice uptake—the role of microbes within the soil, delivery of arsenic to the roots, and uptake into the rice plants. Effects of soil silica and phosphate on this process are also under investigation.

Aggregate Arsenic Exposure in Arizona (Mary Kay O'Rourke, *University of Arizona*)

Arsenic exposure in mining communities in Arizona is the focus of ongoing research by Mary Kay O'Rourke, Ph.D., at the University of Arizona. O'Rourke started her research career as a geologist and paleoecologist looking at relationships between food changes and extinction in 10,000-year-old animals. She then moved to the University of Arizona to investigate relationships between environmental exposures and respiratory health. The first paper that linked her interest in food exposures was a survey to assess aggregate exposure to arsenic in mining communities.[14] They developed an aggregate exposure modeling approach using media measurements, exposure factors, diaries, and questionnaire information, including data from National Human Exposure Assessment Survey (NHEXAS) Arizona, which is a multimedia, multi-pathway exposure assessment survey.

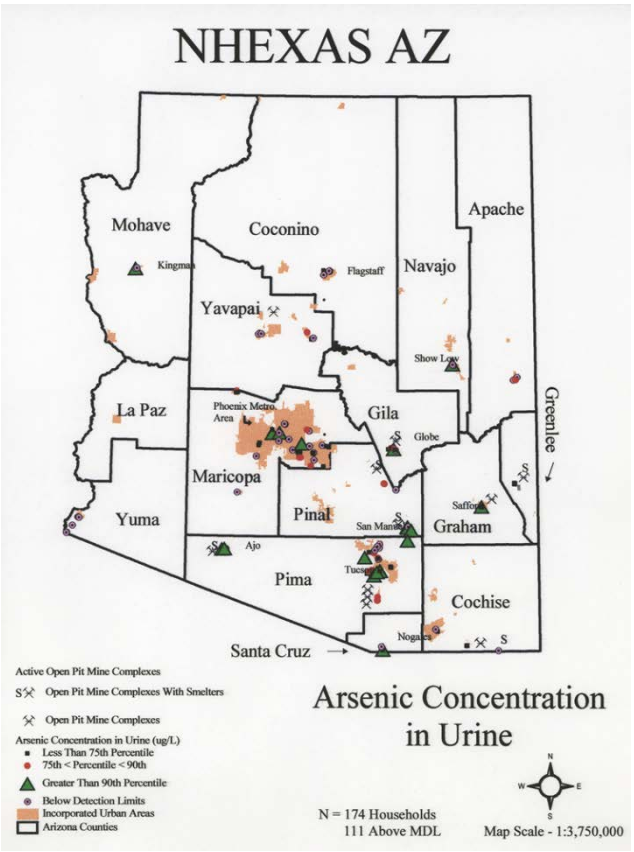

Figure 10: Spatial distribution of arsenic exposure in Arizona based on NHEXAS data (graphic courtesy of M.K. O'Rourke)

Results showed that people living in mining communities had greater arsenic exposure than those living outside these communities (Figure 10).[16] The research team was able to identify exposure sources including house dust collected from floors inside homes in the area. O'Rourke noted that this work has led to other arsenic exposure studies that are continuing at the university.

[14] O'Rourke et al. 1999. *J Exposure Anal Environ Epidemiol,* 9(5):446-55

Session 2: Susceptibility to Arsenic Effects

(Moderator: Janice Lee, *U.S. EPA*)

Developmental Effects of Arsenic (Carmen Marsit, *Dartmouth College*)

In utero arsenic exposures are associated with a number of health effects during early life and into adulthood. Carmen Marsit, Ph.D., of the Children's Environmental Health and Disease Prevention Research Center at Dartmouth College, is focusing on effects of prenatal exposure to arsenic on epigenetic regulation as part of the New Hampshire Birth Cohort Study (NHBCS).[15]

Epigenetics is the study of heritable, stable control of gene expression potential through DNA methylation, histone modification, and other mechanisms. DNA methylation plays a crucial role during development, according to Marsit. He is particularly interested in possible epigenetic mechanisms in immune system development that might explain observed increases in infections in infants.

The NHBCS is recruiting mothers during 22–24 weeks of pregnancy, and collecting biological samples during pregnancy, after birth, during infancy, and during childhood. Using cord blood DNA methylation array analysis, Marsit and his colleagues found that increasing maternal urinary arsenic concentrations were associated with increased proportions of cord blood CD8+ T lymphocytes[16], an immune cell type that is important for immune regulation. Ongoing studies are assessing functional changes in these cells.

Marsit's team is also identifying locus-specific patterns of DNA methylation that are associated with *in utero* arsenic exposure. They found that about 20% of genes were differentially methylated with maternal arsenic exposure, and the preponderance of the loci are in CpG island regions (Figure 11).[18]

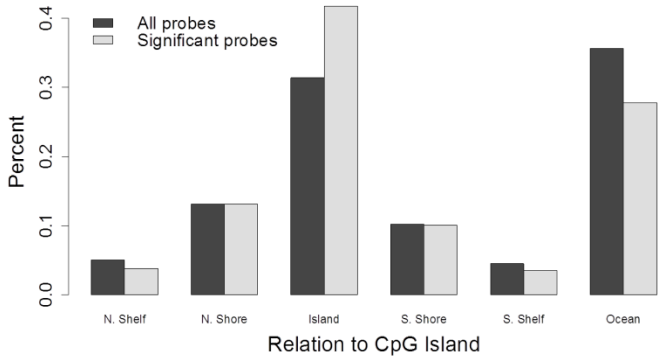

Figure 11: Differentially methylated sites tend to occur in CpG islands (Koestler et al. 2013). Shore, shelf, and ocean refer to location with increasing distance from CpG islands (figure courtesy of C. Marsit)

One of the differentially methylated CpG sites is the leptin receptor, opening the possibility of arsenic-related effects in metabolic outcomes, according to Marsit. He said that leptin is known as the "satiety hormone" that triggers a feeling of being full when eating, but emerging data indicate that leptin might be important for other processes such as immune system development. Infant serum leptin levels were associated with maternal total urinary arsenic levels in preliminary studies from Marsit's collaborators at the Dartmouth Children's Center. This increase in leptin levels and decrease in leptin receptor expression is similar to a phenotype that is seen in obesity, he said. Interestingly, Marsit found that placental leptin methylation decreases in female infants and increases in male infants in relation to increase maternal toenail arsenic

[15] Fei, et al. 2013. *Environ Health* 12:58-62; Koestler et al. 2013. *Environ Health Perspect,* 121(8):971-7

[16] Koestler et al. 2013. *Environ Health Perspect,* 121(8):971-7

concentrations. Marsit's ongoing studies are tracking sex as they continue to research epigenetic changes and their functional implications.

Genetics and Arsenic: Role for Metabolism and Toxicity (Karin Engström, *Lund University, Lund, Sweden*)

At the same arsenic exposure level, some people develop disease and others do not. Karin Engström, Ph.D., a postdoctoral fellow at Lund University in Sweden, is investigating the role of genetics in susceptibility in cohorts in Argentina and Bangladesh. Genes influence how the body handles toxicants, and how the body handles damage. Variations in the genes lead to differences in susceptibility at similar exposure levels. Engström is researching single nucleotide polymorphisms (SNPs) that impact arsenic metabolism, specifically the generation of monomethylarsonous acid (MMA^{III}), the most toxic arsenic metabolite.

It has been known for a long time that the fraction of MMA as a fraction of total metabolites varies among different populations, e.g., 10-15% of metabolites in most populations but only about 5% of the metabolites in an Andean population.[17] The primary methyltransferase in arsenic metabolism is arsenic (+3 oxidation state) methyltransferase (AS3MT), which has conserved regions across several species.[18] Several studies have found associations between specific SNPs in the AS3MT region and the arsenic metabolite patterns,[19] and Engström identified several genetic variants in Argentinian and Bangladeshi cohorts.[20] When she analyzed allele frequencies of the specific SNPs, she found a number of them associated with lower % MMA that occurred at a high frequency in Argentina but a low frequency in Bangladesh and Europe. For example, one such polymorphism, rs7085104, with a guanine residue was found in 73% of people in the Argentinian group as compared to 29% of people in the Bangladesh group (Figure 12). Engström also stated that frequencies of different alleles are often similar between each other because they are often in linkage disequilibrium (inherited together).

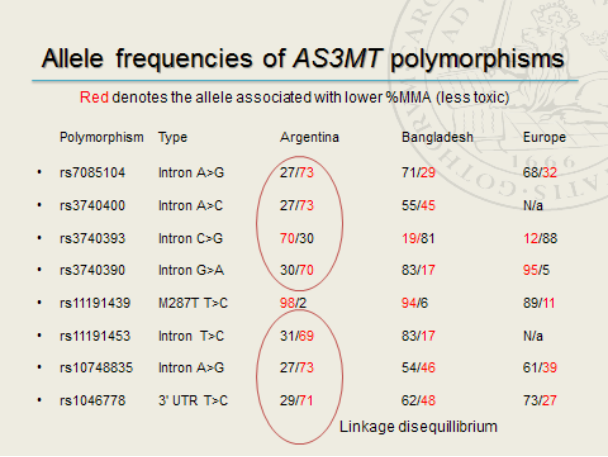

Figure 12: Frequencies as a percentage of the total population that have specific SNPs in the AS3MT gene (figure courtesy of K. Engström)

[17] Vahter et al. 1995. *Eur J Pharmacol,* 293(4):455-62
[18] Li et al. 2005. *Toxicol Appl Pharmacol,* 204(2):164-9
[19] Pierce et al. 2012. *PLoS Genet,* 8(2):e1002522
[20] Engström et al. 2007. *Environ Health Perspect,* 115(4):599-605; Engström et al. 2011. *Environ Health Perspect,* 119(2):182-8

In the context of risk assessment, Engström said, it is import to understand the large variations in AS3MT allele frequencies between populations. This wide range in frequencies of susceptibility alleles may have a stronger impact on the reference values for arsenic in water than what the uncertainty factors account for. More genotyping of different populations is needed in order to have the ability to estimate a percentage of a population that might be more susceptible to arsenic for risk assessment purposes.

Engström is also investigating SNPs in other genes that are relevant to the one-carbon metabolism pathway, a pathway centered around folate and important in DNA methylation and purine synthesis. The pathway includes glutathione transferases and DNA methyltransferases. Engström is investigating polymorphisms in a metal transporter gene; the transporter is involved in hormone metabolism and may play a role in sex differences in susceptibility. More mechanistic studies are needed to confirm causal relationships between polymorphisms and health effects. During the question answer period, Barry Rosen, Ph.D., suggested that this information might be valuable for a therapeutic approach. The more active the enzyme, the less MMA^{III} and more DMA^{IV} that is produced, and such an enzyme might improve elimination of arsenic from the body.

Effects of Prenatal Arsenic Exposure on DNA Methylation (Molly Kile, *Oregon State University*)

The Harvard University School of Public Health has been working on a number of projects related to arsenic exposures and effects in Bangladesh for approximately 15 years. Molly Kile, Ph.D., trained at Harvard University and is now an Assistant Professor at Oregon State University. She spoke about the history of Harvard's involvement in Bangladesh, the range of health effects studies that Harvard has conducted, and her research on DNA methylation.

According to Kile, in the late 1990s, the director of the Dhaka Community Hospital was trying to gain international attention on the arsenic crisis in Bangladesh. This was a challenge because there was resistance to believing that the groundwater was the source of the arsenic. Then he met David Christiani, MD, at an international meeting, and Christiani agreed to collaborate. Since that time, Kile said that Harvard researchers in collaboration with Dhaka Community Hospital have published 36 peer-reviewed articles, trained eight U.S. and five Bangladeshi students. This collaboration between Harvard School of Public Health and Dhaka Community Hospital has also strengthened local community capacity to provide health care and reduce exposures. Dhaka Community Hospital has become a leader in arsenic research and remediation. Health effects studied include skin lesions, skin cancer, reproductive health, type 2 diabetes, neurodevelopment, and immune function.

Kile became interested in arsenic-induced DNA methylation changes when animal studies showed that the rate of hepatocellular carcinoma as well as the severity and multiplicity of the lesions were associated with changes in DNA methylation in specific tissues. She wondered whether epigenetic mechanisms might be at play in arsenic health effects in humans, particularly at lower doses of exposure. Kile worked with a Bangladesh birth cohort recruited in 2008 exposed to low-to-moderate doses of arsenic in drinking water, and reported that prenatal exposure (drinking water concentration

and total maternal urinary arsenic) was associated with increased DNA methylation, particularly in cord blood leukocytes.[21] Similar results with increased global methylation were reported by the Columbia University group, also working in Bangladesh.[22] By analyzing affected genes using the Kyoto Encyclopedia of Genes and Genomes (KEGG), Kile found that some specific biological pathways were affected (e.g., onset of diabetes of the young at maturity, hematopoietic cell lineage, renin-angiotensin system, and TGR-beta signaling pathway). Kile urged caution in interpreting KEGG analyses, and a workshop audience member pointed out that KEGG as a pathway-based software program is designed to analyze gene expression and not DNA methylation.

As Kile probed further, she found that prenatal arsenic exposure was associated with changes in the fraction of different types of leukocytes in cord blood; granulocytes and CD8+ cells were significantly increased, whereas natural killer and CD4+ cells were decreased.[23]

Recently, Kile also measured methylation in other fetal tissues: placenta, umbilical cord artery, and the endothelial cell lining of the umbilical cord vein. Using a new statistical method developed by Andy Houseman, Ph.D., Kile determined that there are more statistically significant methylation differences in placenta than the other two tissues with arsenic exposure, and no significant changes in the endothelial cells. Kile said these results, particularly for endothelial cells, were surprising. Perhaps the methylation changes in these early-life, low-level exposures to arsenic is perturbing the immune system and might be altering the cellular composition of tissues, although the biological effects of these changes are currently unknown..

Arsenic and Susceptibility to Cardiometabolic and Liver Disease (Eric Ditzel, *University of Arizona*)

Metabolic disease is a spectrum of clinical conditions contributing to the risk of increased cardiovascular disease. Conditions include high fasting glucose, abdominal obesity, high serum triglycerides, low HDL cholesterol, and hypertension. At the University of Arizona, Eric Ditzel, graduate research assistant with Todd Camenisch , Ph.D., is focusing on questions related to arsenic exposure impacts on energy metabolism and fatty liver disease in the context of a high fat diet.

Several studies have shown that arsenic exposure can impact the cardiovascular system (e.g., hypertension and increased acute myocardial infarction mortality).[24] Furthermore, evidence of steatosis and inflammation in the liver as well as high cholesterol following arsenic exposure have been reported.[25] On the foundation of these and other data, Ditzel designed an experiment in which mice were exposed to arsenic (100 ppb $NaAsO_2$) during different life stages, and fed a high fat Western diet

[21] Kile et al. 2012. *Environ Health Perspect,* 120(7):1061-6
[22] Pilsner et al. 2012. *PLoS ONE,* 7(5):e37147
[23] Kile et al. 2014. *Epigenetics,* 9(5):774-82
[24] Yuan et al. 2007. *Am J Epidemiol,* 166(12):1381-91; Sanchez-Soria et al. 2012. *Toxicol Pathol,* 40(3):504-12; Rhaman et al. 1999. *Hypertension,* 33(1):74-8
[25] Mazumder et al. 2001. *J Toxicol Clin Toxicol*, 39(7):665-74

after weaning. Mice were exposed *in utero* from day 5 until birth (*in utero,* or IU, only), or through 13 weeks of age (IU+); a third group was exposed postnatally (PN) from weaning through 13 weeks of age.

The IU and the IU+ groups showed significantly increased liver to body weigh ratios. Liver histology showed steatosis among all groups which was an expected consequence of the high fat diet. However, the ballooning degeneration found in the arsenic-treated groups was more widespread and more severe in the liver than it was in the control group. Lipid accumulation and limited fibrosis in the liver was also higher in the treated groups, and highest in the IU and IU+ groups.

Ditzel then analyzed energy metabolism pathways in the mice. He found statistically significant changes in levels of multiple metabolites suggesting that there is a disruption in the TCA cycle, concomitant with increased ketogenesis in the IU and IU+ groups. Ditzel postulates that arsenic disrupts the TCA cycle, forcing acetyl Co-A in the liver to use ketogenesis for energy production (Figure 13). Increased triglycerides in plasma further suggests that the liver is overwhelmed with lipid and exporting excess as triglycerides. Together the data provide evidence for arsenic-mediated cardiometabolic disease in the context of a high-fat Western diet.

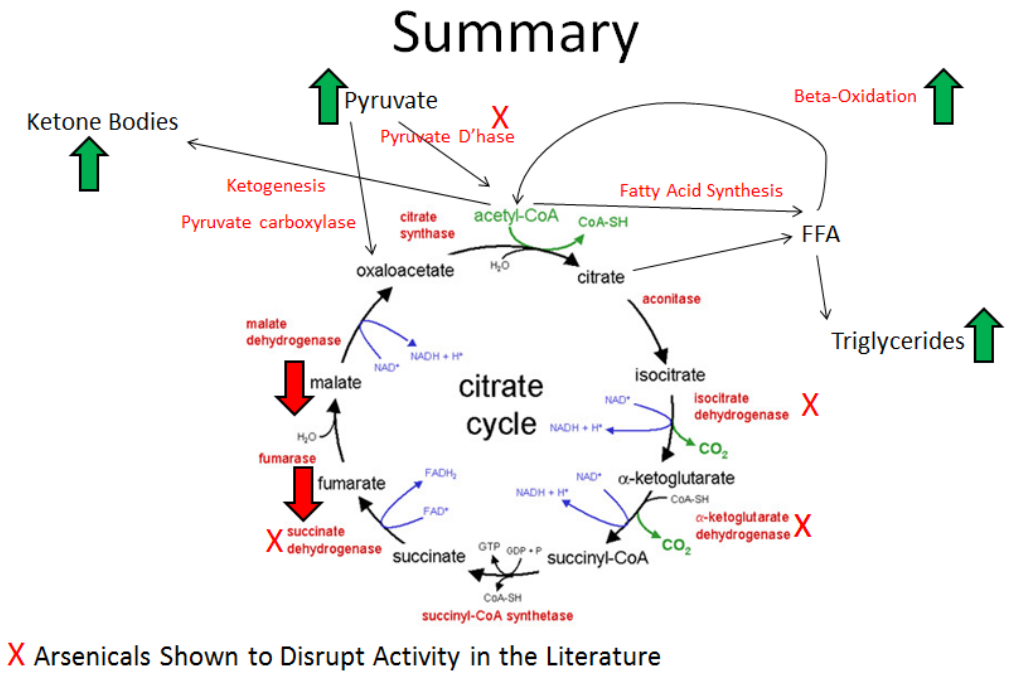

Figure 13: Metabolic intermediates and pathways (large arrows) shown to be affected by arsenic in this study and in the literature (graphic courtesy of E. Ditzel)

Session 2 Panel Discussants: Brief Research Overviews

Moderator: John Cowden, U.S. EPA

Due to inclement weather, the panel discussions were postponed, and later held as a webinar series (see the Arsenic Panel Discussion Webinar Series report). Two panelists from this session gave brief overviews of their research that are summarized here.

An Experience with Exposure Intervention (Mary Gamble, *Columbia University*)

Several projects at Columbia University are focusing on arsenic-exposed populations in Bangladesh, where millions of people are being exposed to arsenic in drinking water. Mary Gamble, Ph.D., of Columbia University focuses primarily on nutritional biochemistry toward understanding the role of nutrition in the metabolism of arsenic and risk for arsenic-related health outcomes; details about this research can be found in the Panel Discussion Webinar Series section of this report. For this brief talk, she shared an experience that she had working with study participants in Bangladesh. As part of the study, participants were given water filters to remove arsenic from their drinking water for a six-month period. When they revisited participants about a year later, 95% of the participants had stopped using the filters. The participants said that the filters became clogged over time and that the filtering time became an inconvenience. Gamble indicated that this remediation strategy does not seem to be a good long-term option. Other workshop participants shared similar experiences with filter use, and noted that filter maintenance and replacement can be a stumbling block for people who use filters.

Experiences with the Chilean Cohort (Craig Steinmaus, *University of California at Berkeley*)

Researchers at the University of California at Berkeley have been studying long-term health effects from exposures to arsenic in drinking water for a population in Antofagasta, Chile that was exposed to high concentrations of arsenic in the city water supply from 1958-1970, when a water treatment plant was installed. Craig Steinmaus, Ph.D., shared his experience with filter systems in a conversation with Gamble. He said the primary problem with filter use is that people do not maintain them. Maintenance costs as well as developing maintenance strategies should be part of the equation when developing home filtering systems.

Gamble asked Steinmaus about nutrition in the Chilean cohort. Specifically, she asked whether he has looked at health effects after folate supplementation was started in that cohort. He said they are now looking at lung cancer cases from 2007-2010, and that includes a period of supplementation though not when the subjects were children. They collected dietary data for all research subjects, so that information can be analyzed. An audience member also mentioned that this population consumes pinto beans, which is a typical food source in this region, and intake of pinto beans, in particular, might be noteworthy due to their high folate content.

Session 3: Contributions of Advanced Techniques to Understanding Arsenic in Health and the Environment

(Moderator: Claudia Thompson, *NIEHS*)

Pathways of Exposure to Arsenic (Miranda Loh, *University of Arizona*)

Potential sources of arsenic in the environment include metal smelters, mining activities, power plants, pressure-treated wood, and cigarette smoke. Exposure to arsenic occurs via food, water, soil, and house dust. Miranda Loh, Ph.D., of the University of Arizona, studies arsenic pathways of exposure for individuals and populations. Loh's work includes a project called "Metals Exposure Study in Homes" (MESH) that measures multi-media exposures in rural Arizona homes. Specifically, she focuses on measuring and modeling arsenic exposure via multiple pathways of exposure.

Pathways of exposure describe how a substance travels from its source to the receptor (e.g., a person), and routes of exposure describe how a substance gets into the body. For exposure assessment, biomarkers of exposure, such as arsenic concentrations in blood, hair, and nails, can show the sum total of exposure via different exposure pathways. However, arsenic biomarkers may vary due to differences in how individuals metabolize arsenic and which species of arsenic they are exposed to. Measurements of arsenic in hand wipes and nasal samples could also inform exposure assessments, as they provide an intermediate measure that might help better quantify the amount arsenic in the air and soil or dust that may be inhaled or ingested. According to Loh, there are limitations to measurement approaches for estimating exposure, such as potential uncertainty in sampling methodology and limitations in the number of samples that can be measured over time.

Fate and transport models, as well as simulation models, can complement measurement techniques by providing a means to estimate exposures in situations where samples are limited. For example, an indoor transport model coupled with an exposure simulation model can be used to estimate the non-dietary arsenic intake of a population near a contaminated site, where only outdoor soil measurements are available. Another potential use for modeling to fill exposure gaps is in simulating exposures over a longer period of time, when it may not be possible to take many samples, for example, in estimating dietary exposures over a year. However, probabilistic simulation models tend to be better at predicting population-level exposures rather than individual-level exposures, Loh said. She described different modeling approaches used by the EPA and others to estimate population exposures under different scenarios. For example, in one study, researchers were able to model dietary exposure and urinary excretion estimates that were similar to results from duplicate diet measurements of arsenic in food.

Loh also indicated that inhalation and ingestion of air particles are important exposure routes to consider, particularly with contaminated soil and dust. One study in Tacoma, Washington explored children's exposure near a copper smelter with contaminated dust and soil.[26] Within the population, children 0-6 years old had higher urine and hair concentrations, compared to older individuals, and for the population nearest the smelter, coarse indoor particles and hand wipe concentrations were associated with their urine arsenic levels. For risk assessment, simulations of surface contact,

[26] Polissar et al. 1990. *Environ Res,* 53(1): 29-47

adherence, and hand-to-mouth exposures for metals have been performed and could provide estimates of non-dietary intake exposures for children in areas where arsenic may be high in soil or dust (e.g., SHEDS-Wood model; Cumulative and Aggregate Simulation of Exposure (CASE)). Linking these intake models to PBPK models allows for prediction of urine concentrations. These predicted concentrations can then be compared to measured concentrations for model validation.

These types of analyses are valuable for risk assessment at Superfund sites, Loh said. From soil sample concentrations, for example, researchers can link different models together to estimate indoor dust and air concentrations as well as urinary concentrations. In addition to soil, dust, and water as potential pathways of exposure to arsenic, food is another important means by which people may be exposed to arsenic. Loh emphasized that more information about arsenic species in food and how they are transformed in the body is needed to gain a better understanding of links between exposures and health effects. Understanding how all of these different exposure pathways contribute to people's exposure will help improve our ability to assess risks to both the general population and to vulnerable populations near contaminated areas.

Combined Arsenic and Fluoride Exposure (Luz Maria Del Razo Jiménez, *Cinvestav, Mexico*)

High inorganic arsenic concentrations in groundwater often naturally co-occur with high inorganic fluoride levels in many geographic regions of the world, mainly in China, India, America, Argentina, and Mexico.[27] Luz Maria Del Razo Jiménez, Ph.D., at Cinvestav in Mexico City, is investigating the implications of co-occurrence for human environmental health.

Fluoride has been implicated in osteosarcoma as well as several non-cancerous diseases that include cardiovascular diseases, neurodevelopmental disorders, decreased renal function, bone disorders, and disrupted glucose homeostasis,[28] according to Del Razo. The most recognized mode of action for fluoride toxicity is via oxidative stress pathways. Fluoride exposure leads to increases in reactive oxidation species (ROS), and inhibition of several enzymes such as superoxide dismutase and glutathione oxidase. These changes link to inhibition of several metabolic

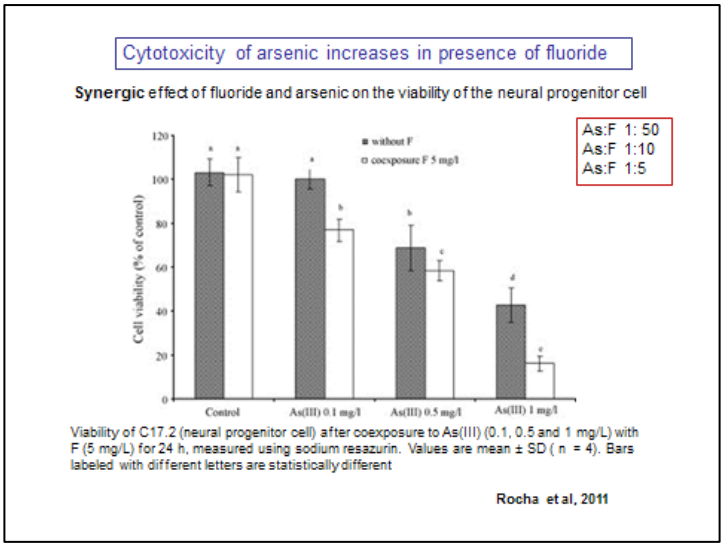

Figure 14: Fluoride and arsenic co-exposure can have synergistic effects (figure courtesy of L. Del Razo)

[27] Amini et al. 2008. *Environ Sci Technol,* 42(10):3669-75
[28] Barbier et al. 2010. *Chem Biol Interact,* 188(2):319-33

processes.

Although it is commonly known that fluoride accumulates in bones, fluoride also accumulates in soft tissues such as kidney, brain, testicles, liver, muscle, spleen, and, at very high levels, in the pineal gland.[29]

Does co-exposure to arsenic with fluoride differ from responses to either agent alone? Del Razo said that studies have shown that fluoride can be synergistic or antagonistic depending on the effect. Fluoride increases arsenic-induced cytotoxicity in neural progenitor cells *in vitro* (Figure 14).[30] In contrast, mice exposed to arsenic and fluoride together showed significantly less of an increase in ROS production in liver than mice exposed to either agent alone.[31] Co-exposure is associated with increased risk of reduced IQ in Mexican children.[32] New unpublished data from Del Razo suggests that arsenic metabolism is altered by co-exposure to fluoride in mice, resulting in a different distribution of metabolites including less DMA production.

In summary, Del Razo said that both synergistic and antagonistic interactions can occur depending on exposure levels and specific physiological endpoints or metabolic processes. More studies are needed to characterize possible health risks associated with co-exposure to fluoride and arsenic. Lastly, she said that because fluoride often naturally occurs together with arsenic in groundwater, the potential role of fluoride in health effects previously attributed to arsenic alone should be systematically studied.

Functional Interactions between the Gut Microbiome and Arsenic Exposure (Kun Lu, *University of Georgia*)

The human microbiome is the full complement of microbes in and on the human body. According to Kun Lu, Ph.D., at the University of Georgia, there are 10-times more microbial cells than human cells in and on the human body. Lu is studying the role of the human microbiome in arsenic metabolism and subsequent health effects.

Microbiome by the numbers:

- ~1,000 species in the human microbiome (~150 per person)
- ~3 million microbial genes versus 23,000 human genes
- ~3 lbs. of microbes in the human gut
- ~60% of stool dry matter is microbial mass

The composition of the microbiome is highly dependent on the location in or on the human body. The microbiome in the ear, oral cavity, skin, and gut can differ tremendously, as can their impact on human health. In fact, the gut microbiome functions like a virtual organ, says Lu. The gut microbiome impacts processes like food digestion, energy metabolism, and pathogen resistance and induce systemic effects. For example, fecal transplantation from obese mice into germ-free mice resulted in increased percentage body fat as compared to those with transplants from lean mice.[33] Microflora can

[29] Inkielelewicz and Krechniak, 2003. *Fluoride* 36(4):263-266; Luke, 2001. *Caries Res,* 35(2):125-8
[30] Rocha-Amador et al. 2011. *Environ Toxicol and Pharmacol,* 32(3):399-405
[31] Mittal et al. 2007. *Drug Chem Toxicol,* 30(3):263-81
[32] Rocha-Amador et al. 2009. *NeuroToxicol* 30(6):1149-1154
[33] Turnbaugh et al. 2006. *Nature,* 444(7122):1027-31

also regulate levels of trimethylamine, a compound highly associated with heart disease.[34] The gut microbiome can also be influenced by outside factors such as diet, antibiotics, other drugs, and bacterial infections.[35]

How does arsenic affect the gut microbiome? To analyze effects on the microbiome, Lu performed metabolomic global profiling and analysis as well as 16S rRNA sequencing to look for shifts in the populations or in the metabolic pathways. Arsenic exposure changed the types of microbes present in the gut. Arsenic also changed the metabolomics profile, showing about 400 metabolic changes in feces of exposed mice (Figure 15).[36]

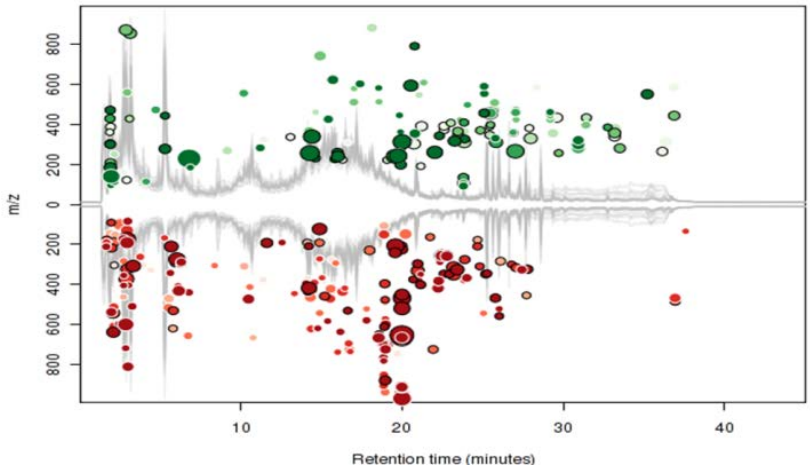

Figure 15: Arsenic perturbed the metabolic profiles of the gut microbiome in fecal samples of mice (370 molecular features were significantly changed compared with controls with a fold change >1.5 and p<0.05), here showing increases (green) and decreases (red) in molecular features (Lu et al. 2014. *Environ Health Perspect* 122(3):284-91)

How does the microbiome affect arsenic metabolism and toxicity in the host? It is known that there is individual variability in the susceptibility to arsenic-related effects and in the individual microbiomes, and this work set out to see if the two might be linked. Lu studied arsenic metabolite profiles for mice exposed to arsenic, comparing mice with altered or unaltered microbiomes. Lu altered the microbiome by two methods: bacterial infection and IL-10 gene knockout. In both cases, the arsenic metabolic profiles changed when the microflora composition changed.[37] With infection, the relative abundance of DMA(V) decreased and inorganic As(V) increased. Similar results were obtained in arsenic-exposed IL-10 knockout mice.[38] Together these data demonstrate impacts of arsenic on the microbiome, and impacts of the microbiome on arsenic metabolism. Lu suggested that the gut microbiome structure might be a new risk factor for individual susceptibility to arsenic.

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[34] Wang et al. 2011. *Nature,* 472(7341):57-63
[35] Cotillard et al. 2013. *Nature,* 500(7464):585-8; David et al. 2014. *Nature,* 505(7484):559-63
[36] Lu et al. 2014. *Environ Health Perspect,* 122(3):284-91
[37] Lu et al. 2013. *Chem Res Toxicol,* 26(12):1893-903
[38] Lu et al. 2014. *Chem Res Toxicol,* in press

Field-Deployable Arsenic Sensor to Assess Personal Exposure (Badawi Dweik, *Giner, Inc.*)

Monitoring arsenic concentrations in samples collected from the field has been expensive, requiring transport of samples from the field to laboratories where specially trained people run tests on expensive mass spectrometry equipment. The sample shipping and processing can itself introduce variables in terms of sample stability and storage temperatures during transport and handling. Badawi Dwiek, Ph.D., of Giner, Inc., has been developing a new portable benchtop arsenic sensor system that overcomes many of the limitations of current arsenic detection methods.

Figure 16: Field-deployable urine arsenic test kit using PalmSens (photo courtesy of B. Dweik)

Other commercially available field test options have been available, including Hach tests, Wagtech's Arsenator, and Metalyser. According to Dweik, none of these systems can detect organic arsenic or different species of arsenic; there are major safety issues with each of them; and they were developed primarily to test for arsenic in water and not urine.

Dweik and his team set out to develop a portable monitor for on-site measurement of arsenic from urine samples in near-real time (Figure 16). Such a system would provide major benefits to epidemiological studies to determine human exposure. Dweik described the system as one that uses an electrochemical approach with a specially engineered electrode, a microarray microelectrode configuration to improve detection limits, and unique electrochemical sample processing. He said that the system allows for rapid analysis (30 minutes) with sensitivity down to 1-5 parts-per-billion of As(III) and of As(III)+As(V) (Figure 17). The concentration of As(V) is calculated from these two measurements. It is also possible to use this system to measure monomethylarsonic acid (MMA) by measuring conversion to As(V).

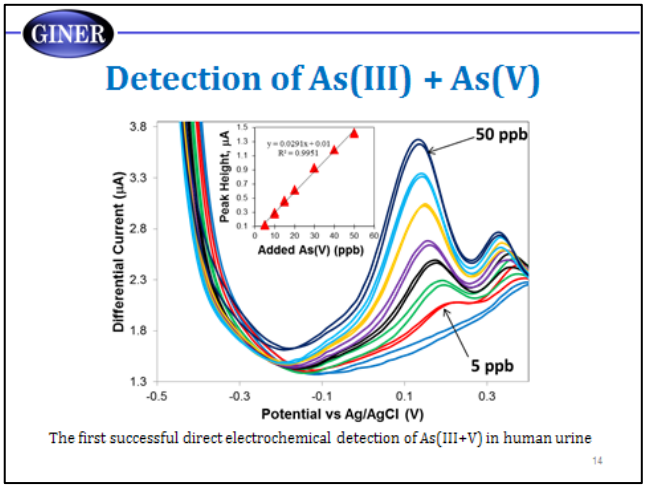

Figure 17: Urine arsenic detection using the Giner test kit (figure courtesy of B. Dweik)

Dweik tested his system using urine samples with known arsenic concentrations from Bangladesh residents provided by David Christiani, M.D., of Harvard University. Giner measured As(III) and As(V) in fifteen field samples with known elevated total arsenic concentrations (100-200 ppb) and five with low levels, and the results were comparable to results measured using HPLC-ICP-MS.

Giner is also developing the technology to detect other toxic metals in urine including cadmium, mercury, lead, and manganese.[39] This work was funded by several grants from the NIEHS.

Session 3 Panel Discussants: Brief Research Overviews

Moderator: Erik Tokar, *NIEHS National Toxicology Program*

Due to inclement weather, the panel discussions were postponed, and later held as a webinar series (see the Arsenic Panel Discussion Webinar Series report). Two panelists from this session gave brief overviews of their research that are summarized here.

Methyltransferase Reactions and Biotransformation of Arsenic (Barry Rosen, *Florida International University*)

Arsenic metabolism utilizes a methyltransferase called AS3MT. Barry Rosen, Ph.D., is investigating the biochemical reaction scheme for this enzyme during arsenic metabolism in a number of different organisms, including humans. Rosen and his team developed a new reaction scheme for AS3MT methyltransferases (Figure 18).

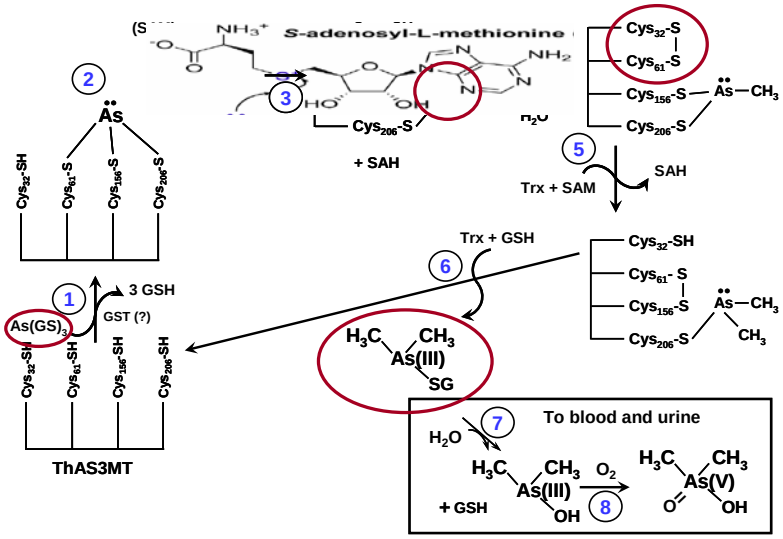

Figure 18: New biochemical reaction scheme forAS3MT (diagram courtesy of B. Rosen)

Key features to note, according to Rosen, are: 1) the substrate is triglutathione conjugate of arsenic, $As(GS)_3$, and it binds to AS3MT with a higher affinity than inorganic arsenic; 2) the products are trivalent (pentavalent forms are generated by oxidation in air); and 3) there are disulfide bonds in the methyltransferase protein that Rosen proposed are important for recognizing trivalent arsenic products.

[39] Argun et al. 2013. *Anal Chim Acta,* 773:45-51

Rosen's team is also investigating microbial biotransformation of arsenic, and recently identified new pathways that take place in human and plant microbiomes. They isolated two novel genes from soil and rhyzosphere organisms that confer resistance to toxic organoarsenicals, herbicides, and anti-microbial agens like salt. One enzyme, ArsH, oxidizes pentavalent arsenic species to the less-toxic trivalent species. A second new enzyme, ArsI (with a capital "I"), degrades organoarsenical herbicides.

Why would bacteria use arsenic in these ways? Rosen thinks that bacteria have found these ways to use arsenic to their own advantage as part of their ongoing battle with other bacteria for resources, using arsenic as an antibiotic to kill their neighbors, but also to develop mechanisms of resistance to arsenic to defend themselves.

Health Effects of Arsenic Longitudinal Study (Maria Argos, *University of Illinois at Chicago*)

Bangladesh is a country that is impacted by high levels of arsenic in their groundwater. Maria Argos, Ph.D., of the University of Illinois at Chicago, provided an overview of two ongoing cohort studies in the region.

The Health Effects of Arsenic Longitudinal Study (HEALS) was established in 2000 through the Columbia University SRP. She is collaborating with the HEALS principle investigator, Habib Ahsan, M.D., M.Med.Sc., at the University of Chicago. The prospective cohort has approximately 30,000 adults and has been followed longitudinally to study disease outcomes from chronic arsenic exposure. Outcomes investigated include skin diseases, cancers, cardiovascular disease, and non-malignant respiratory diseases. Several dose response associations with arsenic have been established in this cohort:

- Incident skin lesions[40]
- All-cause mortality[41]
- Respiratory disease mortality[42]

Figure 19: Villagers in Bangladesh draw water from tube wells (photo courtesy of G.Sarwar and M. Argos)

Another study in Bangladesh is a non-melanoma skin cancer prevention trial.[43] The cohort includes adults (ages 25–65 years old) in rural areas who manifest arsenical skin lesion. The trial is 2x2 factorial, double-blind, placebo-controlled, randomized trial, testing selenium (selenomethionine at 200 μg daily) and vitamin E (α-tocopherol, 100 mg daily) supplementation for cancer prevention, and is expected to be completed in 2015.

[40] Argos et al. 2011. *Am J Epidemiol*, 174(2):185-94
[41] Argos et al. 2010. *Lancet,* 376(9737):252-8
[42] Argos et al. 2014. *Epidemiol,* in press
[43] Argos et al. 2013. *Eur J Clin Invest,* 43(6):579-88

These two population-based studies have also served molecular epidemiologic investigations looking at molecular-level effects of arsenic, including a genome-wide gene expression study.[44] Additional ongoing studies are looking at epigenome-wide DNA methylation patterns in relation to arsenic exposure, and genome-wide associations with arsenic skin lesions.

Session 4: Prevention and Remediation Strategies for Arsenic Exposure

(Moderator: Michelle Heacock, *NIEHS*)

The Influence of Nutrition on Arsenic Metabolism (Megan Nina Hall, Sc.D., *Columbia University*)

At the intersection of nutritional and environmental epidemiology, Megan Hall, Sc.D., at Columbia University is investigating how nutritional factors modify the health effects of environmental exposures. Currently, she is focusing on nutritional influences on arsenic detoxification in Bangladesh in collaboration with several investigators at the Columbia University SRP.

The arsenic in Bangladesh drinking water is largely arsenite. According to the Challenger pathway, arsenite can undergo methylation *in vivo*, catalyzed by arsenic methyltransferase (AS3MT), to the pentavalent monomethyl form (MMA^{V}), which can be converted to the more toxic trivalent form (MMA^{III}). MMA^{III} can undergo a second methylation to the pentavalent dimethyl form (DMA^{V}), the least toxic and most rapidly excreted arsenic metabolite. S-adenosyl methionine (SAM) serves as the methyl donor in these reactions.

Why be concerned about arsenic methylation? Individuals vary in their ability to methylate arsenic, and high % MMA in urine is associated with increased risk for several health outcomes.[45] Part of the reason for differences in methylation is nutritional status. Therefore, Hall and colleagues are interested in nutritional interventions that might alter arsenic metabolism in favor of reducing levels of the more toxic forms of arsenic – inorganic arsenic (arsenite and arsenate) and MMA.

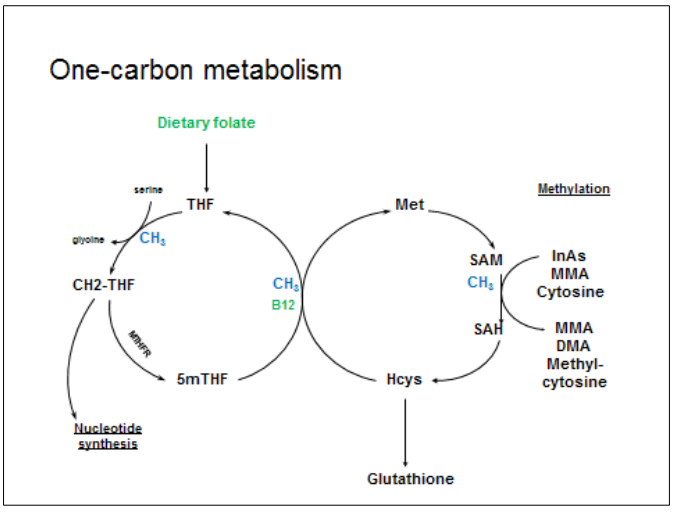

Figure 20: Schematic of core components of one-carbon metabolism (figure courtesy of M. Hall)

One key to this process is SAM. SAM is synthesized via one-carbon metabolism, a

[44] Argos et al. 2006. *Cancer Epidemiol Bormarkers Prev,* 15(7):1367-75
[45] Steinmaus et al. 2010. *Toxicol Appl Pharmacol,* 247(2):138-45

biochemical pathway that plays a role in methylation of many substrates, including arsenic and DNA (Figure 20). Folate, vitamin B12, choline, betaine, and homocysteine are involved in this pathway and can affect arsenic methylation. For example, Mary Gamble, Ph.D., showed that folate supplementation in a folate-deficient population resulted in decreased inorganic arsenic and MMA in blood and urine, and increased DMA in urine.[46]

Vitamin B12 is critically important in this pathway. It is a co-factor for methionine synthase, the enzyme that catalyzes remethylation of homocysteine to methionine, which can be activated to SAM. Hall reported decreased % inorganic arsenic and increased % MMA in urine with increasing plasma concentrations of vitamin B12 in folate-sufficient individuals.[47] Surprisingly, there was no association between vitamin B12 and urinary % DMA. Hall postulates that people who were deficient in folate were lacking in methyl groups to donate. She also noted that there may be differences in one-carbon metabolism in children as compared to adults.

In other studies, Gamble, Hall, and other investigators from Columbia University showed that low vitamin B-12, low folate, and hyperhomocysteinemia were associated with increased odds of arsenic-induced skin lesions. Hall is also exploring whether choline and betaine supplementation might shift the metabolism to lower % inorganic arsenic and %MMA and to increase %DMA. Betaine is found in beets, whole grains, and spinach; choline is found in meat and eggs. Folate and betaine are interdependent and serve as complementary methyl donors that can impact methylation of arsenic.

One of the most consistent findings in terms of predictors of arsenic methylation, or the types of arsenic metabolites in urine, is urinary creatinine (uCRN). uCRN is positively associated with % DMA in urine, and negatively associated with % inorganic arsenic and % MMA in urine consistently across several studies (e.g., Gamble et al., 2005[48]). Higher uCRN levels are also associated with decreased risk of arsenic-induced skin lesions.[49] The reasons for the strong associations are unclear, and are a focus for future investigations.

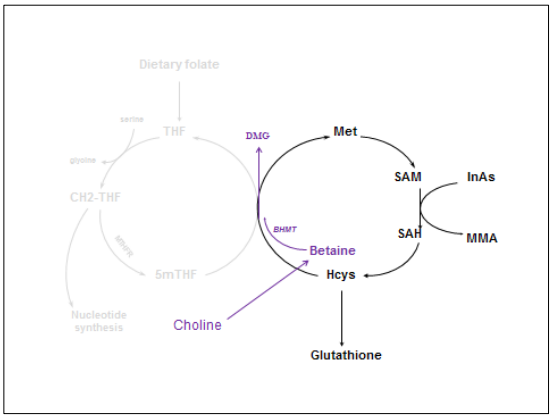

Figure 21: Choline and betaine in one-carbon metabolism (figure courtesy of M. Hall)

The biochemical interactions described thus far are just a small part of the one-carbon metabolism pathway. The pathway is a set of interlocked biochemical cycles that can buffer against fluctuations in nutrient intake to ensure that critical reactions continue to proceed. To complement the epidemiological approach, Hall and Gamble, in collaboration with researchers at Duke University, have developed a mathematical model of arsenic methylation that takes into account kinetics of known reactions and interactions based on the

[46] Gamble et al. 2006. *Am J Clin Nutr,* 84(5):1093-101; Gamble et al. 2007. *Am J Clin Nutr,* 86(4):1202-9
[47] Hall et al. 2009. *Environ Health Perspect,* 117(11):1724-9
[48] Gamble et al. 2005. *Environ Health Perspect*, 113(12):1638-8
[49] Hall et al. 2009. *Environ Health Perspect*, 117:254-60

biochemistry of the system. Using the modeling approach, this research group can conduct multiple *in silico* experiments. The model performed well in tests that compared modeled results with actual data. Hall and colleagues used the model to calculate changes in body burden of arsenic following folate supplementation, and the model predicted that liver burden would be reduced by 19%, and body burden by 26%.

Gamble and Hall have trials in progress that are testing the effects of supplementation with other nutrients involved in one-carbon metabolism on arsenic methylation (Figure 21). They are testing folic acid and/or creatine supplementation in one trial, and phosphotidylcholine and/or betaine supplementation in another trial.

In summary, information gained about one-carbon metabolism biochemistry is opening windows to the possibility of reducing arsenic-related health effects through nutritional supplementation. The biochemical pathways are complex and intertwined, but studies suggest that several nutrients may be effective in preventing or ameliorating the adverse health effects of chronic arsenic exposure. By continuing epidemiological studies that evaluate nutrient supplementation combined with mathematical modeling, Hall hopes to better understand how nutrients might help prevent or mitigate arsenic-related health effects.

Phytostabilization of Arsenic in Mining Wastes (Raina Maier, *University of Arizona*)

Legacy metal mining and smelting sites often have mine tailings piles that are laden with metals, including arsenic. In Dewey-Humboldt, Arizona, the Iron King Mine and Humboldt Smelter Superfund site contains tailings with high levels of arsenic and lead (~3,000 mg/kg each).[50] Given the semi-arid climate and the absence of natural plant cover, the tailings become airborne and present exposure risks for the local population. Raina Maier, Ph.D., at the University of Arizona is leading a team of scientists that is developing strategies to use plants to stabilize the tailings as a cost-effective approach to reducing exposures.[51] Maier's team is in the 5th year of a phytostabilization field study to identify effective plants and optimize growing conditions.

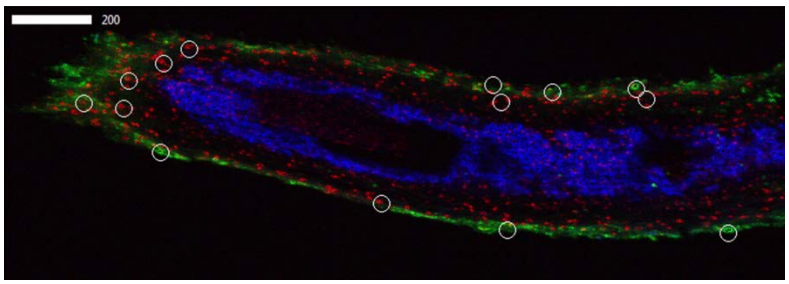

Figure 22: Cross-section of mesquite root where µ-XRF and µ-XANES were combined to spatially resolve metals and their oxidation state, showing arsenic thiols (red), As^{V} (green), and potassium (blue) (photo courtesy of C. Hammond).

Initially Maier's team hypothesized that plants grown at the site would mobilize arsenic in the tailings and run the risk of migration to groundwater, but this was not observed to be the case. Working with Jon Chorover, Ph.D., Rob Root, Ph.D., and Corin Hammond, Ph.D. candidate,

[50] Hayes et al. 2014. *Geochim Cosmochim Acta* 141:240-257
[51] Mendez and Maier 2008. *Environ Health Perspect* 116:278-283

Maier's team used X-ray absorption spectroscopy and found that oxidized arsenic in the soils was associated with minerals like iron hydrides.

As researchers delved deeper to identify what is preventing mobilization, they turned to cutting-edge technology to localize metals on biological and mineral surfaces in the root zone, and within plant tissues. First, using micro X-ray fluorescence (μ-XRF) and micro X-ray absorption spectroscopy (μ-XANES), Maier's team spatially identified metals and their oxidation states at the microscopic level in mesquite roots (Figure 22). Oxidized arsenic was found at the root surface, and arsenic thiols on the interior of the root near the surface, but not in the channel that moves compounds from the root to the shoot. Further analysis showed that oxidized arsenic (As^{V}) was co-localized with oxidized iron (Fe^{III}), and that the plant was concentrating arsenic at the root surface in the rhizosphere.

How does the plant do this? Maier said that, based on other data, bacteria can reduce the bioavailability of metals by oxidizing arsenic and by adsorbing metals, so Maier and her team then turned their focus to bacteria. Using a microprobe that can resolve down to the 2 μm scale, they observed different metals (arsenic, iron, and zinc) were localized in the root tissues. Researchers used fluorescent *in situ* hybridization (FISH) that uses DNA probes to identify bacterial groups that were co-localized with the metals. They found that 40–60% of metabolically active *Actinobacteria* were associated with iron or arsenic as compared to 20–30% of *alpha-Proteobacteria*. Maier said that *Actinobacteria* are known to live in harsh conditions and are often metal resistant, and some are known to oxidize arsenic, so these bacteria have potential to be a valuable arsenic remediation tool.

According to Maier, the mining industry is very interested in sustainable mining approaches given that social protests can shut down mines, leading to losses of tens of millions of dollars per day.[52] Some contaminated sites are more than 2,000 acres, and it is not feasible to remove soil for remediation. The long-term goal of this project, Maier said, is to establish a permanent vegetative cover that over time will stabilize arsenic in a mineral form that, even if ingested or inhaled, exhibit low bioavailability.[53]

Reducing Arsenic Exposure from Drinking Well Water in South and Southeast Asia: Obstacles and Opportunities (Alexander van Geen, *Columbia University*)

Efforts at reducing arsenic exposure in Bangladesh and other countries have resulted in a cycle of optimism, excitement, and disappointment, according to Alexander van Geen, Ph.D., of Columbia University. van Geen and colleagues are working on a number of different strategies to reduce exposures that have had varying levels of success.

In three locations (Punjab province, India; Araihazar upazilla, Bangladesh; and the Irrawaddy delta, Myanmar), Columbia University researchers have been measuring arsenic concentrations in tube wells used for drinking water since 2000. In their main study area in Bangladesh, they found that about 50% of

[52] Maier et al. 2014. *Rev Environ Health* 29:83-89
[53] Menka et al. 2014. *Rev Environ Health* 29:23-27

wells were unsafe but about 90% of households lived within 100 meters of a safe well.[54] In 2006, they tested what impacts their efforts had on safe well use, and found that 57% of the population continued to be exposed to high levels of arsenic in drinking water. The team has since continued to put concerted efforts into reducing use of unsafe wells.

One important step in reducing exposure is to inform the community of well testing results. Researchers worked with local governments to devise a system using signs with visuals of two glasses of water on a blue sign as better wells, and a glass of water on a red sign with a "X" through it for unsafe wells (Figure 24). On another project, van Geen aggregated a large set of geochemical data to the village level that informed what a safe depth would be for drilling new wells. Unfortunately, the local government blocked dissemination of the information for reasons that are unclear.

Figure 23: Signs indicating safe and unsafe wells (photo courtesy of A. van Geen)

Can the community be persuaded to test their own wells? In Bangladesh, van Geen and colleagues implemented a field trial of an inexpensive quick-test kit with GPS location identifiers. Researchers trained local women health workers, who, over the course of 1½ years, tested 50,000 wells. More than 50% of the wells had high arsenic concentrations. Although the test kits are inexpensive and feasible, surveys showed that the cost is still more than most households would pay, so some type of subsidy is needed.

Can the community be persuaded to switch to healthy wells? Again in Bangladesh, van Geen reported that only 27% of people who were told of high arsenic in wells actually switched. Reasons for not switching were that the safe well was too far; they were not aware of health risks; or they said they did not care. Researchers have been looking into parameters that affect decision-making regarding switching (e.g., household wealth and walking distance to well), but more research is needed.

One approach to persuade people is to educate schoolchildren about the issue (Figure 24). Of families whose children learned about arsenic, 81% reported switching to lower-arsenic wells, as compared to 43% of control families. Unfortunately, urinary arsenic levels before and after the intervention suggested the percentage who switched might be lower than they reported. Just the same, van Geen said that these results are encouraging as the researchers continue to search for effective, persuasive approaches.

Figure 24: Educating Bangladeshi children about arsenic exposure and health effects in an effort to persuade families to switch to safe wells (photo courtesy of K. Khan)

[54] van Geen et al. 2002. *WHO Bull* 80:732-737; van Geen et al., 2013. *Sci Tot Environ,* 488–489:484–492

Closing Remarks for the On-Site Workshop (Bill Suk, *NIEHS*)

Arsenic exposure is a significant global health problem. Bill Suk, Ph.D., Director of the NIEHS SRP, stated that this problem can only be addressed through inter-, multi-, and, hopefully at some point, transdisciplinary research and training. Reducing the burden of exposure and disease will improve the quality of life for millions, if not billions, of people.

Suk remarked on what he called the "incredible work over the years" contributed by a member of the audience, Vas Aposhian, Ph.D., relating to arsenic research. Suk said that Aposhian performed the initial landmark studies that triggered Suk's interest in supporting arsenic research at the SRP. At Suk's request, Aposhian stood up to allow the audience to thank Aposhian for his efforts through their applause.

Suk asked all participants to contemplate the questions posed for discussion by the workshop committee as everyone considers future research directions. Suk closed the meeting by expressing appreciation for the organizers, particularly Danielle Carlin, Ph.D., and for the contributions of all participants for an outstanding meeting.

Last Thoughts

- *What are the biggest challenges and opportunities for preventing arsenic exposures?*
- *What are the major exposure routes and environmental media that need to be considered for prevention?*
- *How can communities be made aware of potential exposure to arsenic and opportunities for prevention?*
- *What types of prevention/remediation options are needed considering each exposure route? And each media?*
- *Arsenic is an interesting toxicant because much of the exposure occurs from natural sources; do you see some exposures occurring because of anthropogenic processing (e.g., mining)?*

--Bill Suk, Ph.D.

Part II: Panel Discussion Webinar Series

As part of the workshop, "Health Effects and Mitigation of Arsenic: Current Research Efforts and Future Directions," SRP originally planned to include panel discussions for leading research to highlight new findings and identify data gaps to help guide future research. Due to inclement weather, the on-site panel discussions were postponed and later held in a series of four webinars titled:

- Contributions of Advanced Techniques to Understanding Arsenic in Health and the Environment
- Susceptibility to Arsenic Effects
- Global Environmental Cycling and Bioavailability of Arsenic
- Prevention and Remediation Strategies for Arsenic Exposure

Panelists proposed answers to specific questions posed to them prior to the webinars, and participants discussed the answers with the goal of a forming a consensus statement. Webinar audience participants submitted written comments and questions that were captured during the webinars and incorporated into this report. This section of the report contains the summary of the discussions, highlights, and data gaps in response to the questions posed, and together represent the consensus of the expert panels.

Session 1: Contributions of Advanced Techniques to Understanding Arsenic in Health and the Environment

Moderator: Mike Waalkes, *NIEHS*
May 6, 2014, 1:30–3:00 pm ET

The following sections of this report summarize consensus answers to the specific questions posed to panel members. Each section lists detailed highlights of current understanding and data gaps.

1. What are the most appropriate assessment methods for acute and chronic arsenic exposure in humans? (Miranda Loh, *University of Arizona;* Badawi Dweik, *Giner, Inc.*)

Summary:

Although urinary arsenic is a generally accepted technique for measuring acute and recent exposures, further studies are needed to refine chronic exposure assessments. For chronic exposure, a combination of toenail concentrations, urine concentrations (several measures over time), external exposure measurements (e.g., water concentrations), and probabilistic modeling based on intake source concentrations (e.g., diet) may be the best approach.

We need to gain a better understanding of intake sources of arsenic and develop guideline levels for chronic exposure based on toenail concentrations. We also need to develop on-site biomonitoring systems to measure arsenic species in urine to overcome challenges in sample handling and increase the use of speciation analysis to assess exposure.

Highlights:

- The minimum lethal arsenic dose is defined as 1–3 mg/kg. The current EPA non-cancer risk level is 0.3 µg/kg-day.
- Currently, there is no exposure level range defined for acute exposure, but generally total arsenic concentrations >100–200 µg/L in urine over a 24-hour time period could be considered an indicator for further examination for possible sources, arsenic species, and treatment.
- Urinary arsenic is reflective of ~2-4 days exposure, and collecting repeat samples is recommended to validate the measurements. For epidemiological studies, repeat samples for a subset of the study population may be sufficient.
- Urinary arsenic is a generally accepted method for assessing recent exposure (24-hour samples are recommended but spot samples can be adequate) with the recommendation of no seafood ingestion for 2–3 days prior to testing. Measurements can also inform chronic exposure assessment if the population or individual has had steady state exposure over time.
- Assessing chronic exposure is challenging, and the best approach is likely to use a combination of biomonitoring, biomarkers, and modeling intake (e.g., probabilistic modeling).
- Toenail arsenic concentrations reflect several months of past exposure and are easy to collect, but there currently are no guideline levels.
- Hair can be contaminated by external dilution and, thus, might not reflect internal exposures.
- It is very important to measure what species of arsenic are present in samples when assessing exposure.
- Regarding correcting for dilution of urine, researchers are moving away from creatinine to specific gravity or no adjustment because creatinine levels can vary for other reasons.

Next Steps to Address Data Gaps:

- Increasing the use of speciation analysis in assessing exposures; the current recommended analysis profile is total inorganic arsenic, monomethylarsonic acid (MMA), and dimethylarsinic acid (DMA)
- Overcoming difficulties in measuring speciation due to changes in samples during shipping and processing (e.g., some arsenic species are sensitive to the environment such as oxygenation of arsenite [As^{III}] to arsenate [As^{V}])
- Developing guidelines for interpreting toenail arsenic concentrations (some ongoing epidemiological studies are generating informative data)
- Measuring arsenic levels in a greater variety of body fluids (e.g., mucous, sputum), and in tissues
- Collecting more information about intake sources and external exposures

- Developing biomonitoring systems or field test kits that will rapidly determine urinary or blood arsenic levels on-site in all of its toxic forms at a lower cost, and reduce variability associate with sample handling and transport from the field to the lab
- Investigating the partitioning sources of DMA in studies because food sources can contribute DMA to human exposure that may differ in toxicity from metabolically generated DMA

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2. What biomarkers are best to predict human arsenic-induced diseases? Are there disease-specific biomarkers? (Maria Argos, *University of Illinois at Chicago;* Barry Rosen, *Florida International University*)

Summary:

In the coming years, we will be identifying new biomarkers using emerging –omics technologies including transcriptome, epigenome, metabolome, proteome, and microbiome technology. We need to identify biomarkers that reflect intermediate changes that may relate arsenic exposure to disease onset. One biomarker (MMA species in urine) has been broadly effective but there have been limitations.

Molecular epidemiology studies are needed to evaluate whether exposure-related effects predict long-term disease. We also need to evaluate –omic data sources in an integrative manner, particularly within the same study sample, to identify new biomarkers of effect and susceptibility.

New techniques need to be developed to measure exposure. One promising new technique detects arsenic-inducible gene expression in the human microbiome triggered by environmental exposures to arsenic as a measure of internal exposure via different routes (e.g., inhalation, ingestion, dermal).

Highlights:

- The use of urinary arsenic species as biomarkers has been broadly effective (e.g., associations between increased urinary MMA and health effects).
- In regards to susceptibility, polymorphisms in the AS3MT gene affect arsenic methylation capacity, and are associated with susceptibility to health effects. Other potential biomarkers of susceptibility are being studied.
- In coming years, researchers will be identifying –omics markers of intermediate arsenic-related changes that could be precursors to later health effects and diseases. Panels of biomarkers may provide enhanced specificity. The long-term goal is to link arsenic-related effects to long-term disease.
- There are new assays in development that utilize quantitative PCR to detect arsenic-induced gene expression from bacteria (e.g., arsenic-resistant system [Ars] genes) and fungi (e.g., ACR gene family) that exist in the human microbiome (Barry Rosen, Ph.D.).
    - The microbial gene expression serves as a measure of human exposure from environmental sources.

- Assays using quantitative PCR can be performed on fecal specimens for oral exposure, dermal swabs for skin exposure, and nasal swabs for inhalation exposure. The tests are sensitive (<1 ppb), noninvasive, rapid, inexpensive, and can be incorporated into a single chip for –omics analysis.

Next Steps to Address Data Gaps:

- Integrating -omics analysis, including within the same sample
- Developing panels of biomarkers that, used together, might be most informative
- Identifying biomarkers that link arsenic-related intermediate effects to arsenic-related diseases (using stored samples in longitudinal studies collected prior to the onset of disease could be helpful)
- Identifying new biomarkers of susceptibility in addition to AS3MT/methylation capacity biomarkers by using genome-wide scans
- Identifying new biomarkers of disease for disease-specific processes and more broadly for arsenic-induced diseases
- Expanding biomarker studies in blood to also look at other tissues
- Developing and validating new biomarkers of exposure and new techniques that are faster, cheaper, and better for assessing exposure, and evaluating applicability in population studies with adequate sample sizes to detect moderate effect sizes
- Partitioning food-source DMA from human metabolically produced DMA in studies, because of suggested differences in toxicity for humans
- Prioritizing biomarker research based on the research needs (see Figure 25)

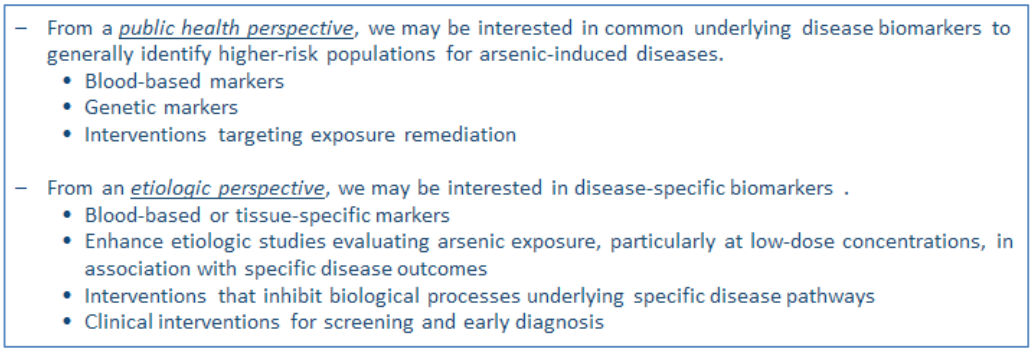

Figure 25: Prioritizing biomarker research (courtesy of M. Argos)

- Refining the use of assays for arsenic-inducible gene expression in fungi and bacteria in human microflora as an indicator of human exposure for different pathways of exposure, particularly because they are faster and less expensive than existing methods

3. What is the impact of the microbiome on arsenic? Does the microbiome alter arsenic metabolism? (Kun Lu, *University of Georgia*)

Summary:

It is well known that the microbiome affects the biotransformation of arsenic, and numerous metabolic reactions can be changed by bacteria and fungi. Gut bacteria are directly involved in arsenic metabolism including methylation and thiolation reactions, particularly because of the high acid and sulfur concentrations in the gut. Questions remain about how the microbiome affects bioavailability and individual susceptibility, and how arsenic affects the microbiome.

Highlights:

- The microbiome can affect the biotransformation of arsenic and is directly involved in arsenic metabolism (e.g., reduction, methylation, and thiolation reactions).
- Thiolated arsenic metabolites are generated in the highly reducing, high-sulfur conditions in the gut by microbes. Some thiolated metabolites have been shown to be highly toxic and efficiently taken up by cells in the gut, raising concerns for toxicity.
- The microbiome may affect the bioavailability of arsenic and, consequently, the toxicity of arsenic and individual susceptibility.
- The gut microbiome can induce systemic responses distant from the location of the microbes (e.g., gene expression in the brain), and change host metabolic activity.

Next Steps to Address Data Gaps:

- Further defining microbiome effects on the bioavailability and metabolism of arsenic, and effects of arsenic on the microbiome
- Characterizing different arsenic sources (soil, diet, food, and drinking water) as complex matrices that also contain microbes, which can alter arsenic biotransformation
- Identifying how the microbiome may consequently impact toxicity of arsenic and individual susceptibility
- Studying microbiomes at different body sites (e.g., nasal cavity) because most studies have focused on the gut microbiomes
- Characterizing microbiomes in different populations (e.g., chronically exposed versus control human populations)
- Exploring the possibility of modulating the gut microbiome to change the arsenic metabolism and understanding the mechanisms of such modulation
- Further defining the generation and toxicity of thiolated metabolites of arsenic (there is controversy about whether thiolated metabolites are generated only by microbes or also by humans)

4. What are other complex exposures that have been associated with arsenic? What data are needed to determine the effects of arsenic and other exposures (e.g., metals, PAHs, etc.)? (Luz Maria Del Razo Jiménez, *Cinvestav, Mexico*)

Summary:

Complex exposures associated with arsenic include many elements, such as magnesium, calcium, and strontium, as well as other metals, pesticides, and polycyclic aromatic hydrocarbons. Concurrent exposure to arsenic with other elements or agents can alter the toxicokinetics and toxicity of arsenic. Exposure levels, specific physiological and metabolic processes involved, and the health endpoints affected are determinants in whether interactions are synergistic, antagonistic, or unaffected.

Highlights:

- In well drinking water, barium, vanadium, and fluoride are commonly associated with arsenic, but magnesium, calcium, strontium, iron, manganese uranium, and chromium are also found (particularly in Argentina and Mexico).
- Other co-contaminants from anthropogenic sources include metals (e.g., lead and cadmium), pesticides, and polyaromatic hydrocarbons.
- Several co-contaminants are known to affect the biotransformation of arsenic and have been shown to work synergistically with arsenic in increasing oxidative stress, interfering with arsenic methylation, and altering immune responses, among other effects.
- Co-contaminant exposures can result in health effects often attributed to arsenic alone (e.g., fluoride is associated with cardiovascular effects similar to those seen in arsenic-exposed populations).

Next Steps to Address Data Gaps:

- Characterizing effects of co-contaminants on toxicokinetics of arsenic (absorption, metabolism, and tissue distribution)
- Teasing out the health effects attributed to exposure to arsenic alone versus co-contaminants
- Measuring co-contaminants in studies of arsenic exposure and effects to account for possible co-contaminant contributions

Responses to write-in questions and comments for Part 1 that were not addressed above:

Audience comments:

- None of the thio-arsenicals tested in laboratory studies has been as toxic as MMA(III) or DMAs(III).

- *In vitro* thiolation has been observed in a non-enzymatic reaction of arsenic with hydrogen sulfide, and arsenic thiolation has been shown to occur naturally and abiotically.

Audience questions not answered during the webinar:

- Question: What are the health implications, if any, of arsenate reduction to arsenite (by respiratory or resistance enzymes) occurring in the colon? Is arsenite more likely to get absorbed into the bloodstream than arsenate?
    - Thomas' response: In direct measurement of oral bioavailability, arsenate and arsenite are quite similar.

Session 2: Susceptibility to Arsenic Effects

Moderator: John Cowden, Ph.D. *USEPA*
May 7, 2014, 1:30–3:00 pm ET

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1) What types of mechanistic data are needed to identify novel susceptibility pathways for inorganic arsenic exposure? (Andrea Allan, *University of New Mexico;* Eric Ditzel, *University of Arizona*)

Summary:

To identify novel susceptibility pathways, more data from multiple –omics approaches are needed. Epigenomic, transcriptomic, proteomic, and metabolomic changes with arsenic exposure at different life stages and across a range of doses and organ systems may reveal new arsenic targets and toxicity pathways. Influences of diet, stress, sex, and co-exposure to other metals in the context of pathways of susceptibility are additional data gaps that need to be investigated.

Types of mechanistic data needed:

- In-depth research into changes in mRNA (RNAseq, miR array) and protein expression (e.g., global proteomics using protein-seq) to identify novel arsenic targets and pathways
- Transcriptome and epigenome analysis to identify arsenic-induced changes and possible biomarkers
- Various life-stage and organ-level metabolomics analyses to identify affected pathways
- Transgenerational epigenetic assessments

- Mechanistic studies that elucidate and validate mechanisms by interfering with and reversing arsenic-induced effects
- Epidemiological studies based on more input from toxicologists and animal studies
- Studies of intersections between arsenic with other metals (e.g., competition with zinc or collaboration with lead)

Next Steps to Address Data Gaps:

- Using a whole transcriptome shotgun approach to identify pathways related to intermediate arsenic effects that may lead to identification of other outcomes
- Comparing response pathways between low- and high-dose exposures and across developmental stages with careful consideration of which target tissues to analyze
- Assessing multiple arsenic concentrations at different developmental stages and with more complete coverage of various organ systems
- Consideration of species choices including heterogeneous or outbred stocks
- Influence of diet/stress/gene interactions in the context of arsenic exposure
- Following through on one-carbon metabolism impacts by testing arsenic demethylation of the promoter to reverse the effect as proof of mechanism
- Identifying alterations in genetic imprinting during gestation
- Performing detailed epidemiological studies that include:
    - Duration and level of exposure, and generational exposure
    - Nutritional factors and other metal co-exposures particularly with affected and unaffected cohorts
- Focusing on interplay between arsenic and other metals (e.g., competition with zinc or collaboration with lead)
- Further characterizing arsenic disruption of transcription factor function (e.g., disrupted localization, steric inhibition, and persistent activation)
- Studying effects of early life exposure and early life epigenetic modifications on later life health effects like carcinogenesis and, importantly, other health outcomes
- Finding better biomarkers for early life exposure and risk
- Investigating sex differences in outcomes, metabolic profiles, gene expression, and additional outcomes

2) What types of data on susceptibility are needed to inform the dose-response relationship for human health effects related to inorganic arsenic exposure (e.g., variability in response to a particular dose)? What types of susceptibility information are needed to inform cumulative risk for individuals/populations? (Craig Steinmaus, *University of California Berkeley*)

Summary:

The U.S. EPA is currently re-evaluating the arsenic risk assessment, and an important question is what types of data can inform that assessment. Steinmaus listed his thoughts on the five most important factors in determining susceptibility and risk based on reports in the literature and consistency of evidence in human studies: metabolism, genetics, diet, age of exposure, and co-exposures to other agents (Figure 26). A large amount of data exists, but relevant data needs to be summarized and assessed in the context of risk quantification and risk assessment. Additional studies of potential markers of quantifiable risk (e.g., single nucleotide polymorphisms, MMA/DMA ratios, and studies that measure lifetime exposures) are needed.

Types of data needed:

- More frequent reporting MMA/DMA ratios in research studies (higher MMA/DMA ratios have been shown to be associated with health effects)
- More studies of humans with exposures over time (including lifespan exposures), but especially childhood and *in utero* exposures
- Studying co-exposures to other metals, smoking, pesticides, and occupationally associated agents (e.g., asbestos, silica, and wood dust), and other agents for possible synergistic effects

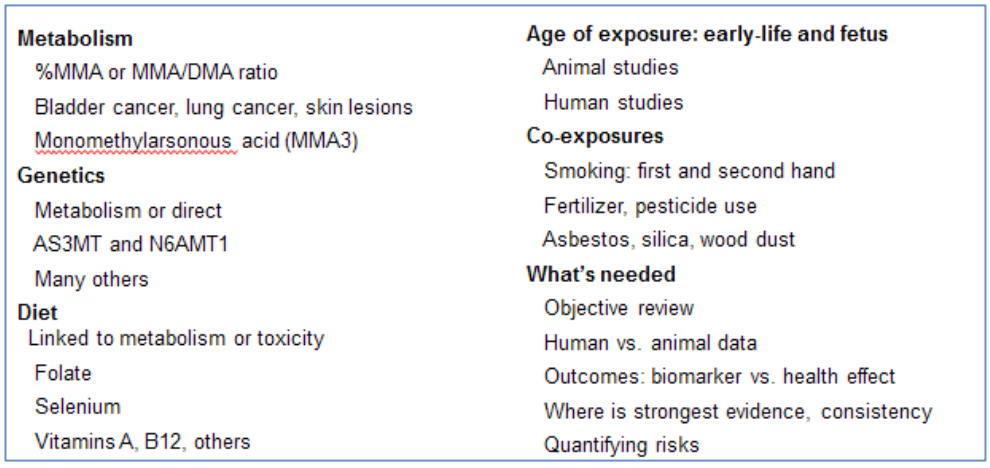

Figure 26: Examples of types of data that may inform risk assessment (courtesy of C. Steinmaus)

Next Steps to Address Data Gaps:

- Understanding implications of poor nutrition with relevance to subsets of the U.S. population that may have nutritional deficiencies
- Synthesizing existing data on genetic associations with susceptibility and risk — e.g., which SNPs markers have the strongest evidence for association with susceptibility and risk?
- Exploring the use of existing data on susceptibility in quantitative risk assessment by combining data (how much does risk increase for a given parameter, e.g., dose or genetic susceptibility)
- Studying possible links between specific genetic polymorphisms and % MMA, and links between % MMA and risk toward a goal of linking genetic polymorphisms to risk quantification
- Continuing to perform high and medium-dose studies because there are benefits (e.g., increased power of the study, less likelihood of important bias and confounding (e.g., the Bradford Hill criteria) and most environmental regulatory standards are based on high-dose data
- Critical review of low dose studies for all major elements of causal inference: confounding, bias, dose-response, consistency with other studies, biologic plausibility, and chance

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3) What methods/data are needed to identify susceptible individuals/populations? Alternatively, what types of data are needed to consider a mechanistic event a "biomarker" of susceptibility? (Karin Engström, *Lund University, Lund, Sweden*)

Summary:

Susceptibility to arsenic is being studied *in vitro* and in epidemiological studies, and research needs to shift to more strongly link the two approaches. Putative biomarkers of susceptibility need to be closely examined for relevance to human populations. DNA polymorphisms, for example, may be useful for estimating population risk if the markers are linked to risk of adverse outcomes.

Types of data needed:

- Translational research that links *in vitro* studies with epidemiological studies of large, well-defined populations
- Epigenetic studies using primary cells and not cancer cells
    - Cancer cells are substantially different from normal cells after malignant transformation.
    - Cancer cell lines have aberrant epigenetic patterns.

Next Steps to Address Data Gaps:

- Large-scale genotyping of the AS3MT gene to determine the frequency/prevalence of the polymorphisms in populations (valuable information for quantitating risk in a population)
- Linking polymorphisms to effects on methylation, and then in turn linking that to epidemiological studies

4) What mechanistic data are needed to inform susceptible lifestage exposures, particularly the late onset of health effects following early life exposure? (Carmen Marsit, *Dartmouth College*)

Summary:

Layers of data from multiple approaches, including –omics approaches, need to be integrated to identify new biomarkers of susceptibility, and to measure exposures over time. Arsenic-associated epigenetic marks may serve as long-term indicators of exposure, possibly providing an estimate of developmental exposures in adult tissues. Integrating molecular information across the genome could provide a more comprehensive understanding of risk and susceptibility.

Next Steps to Address Data Gaps:

- Identifying integrated biomarkers of exposure and effect that can be used to comprehensively understand windows of susceptibility
    - Carefully collecting and analyzing repeated measures that can inform the utility of more integrated and accessible markers (e.g., toenail arsenic as marker of exposure over time, imprinted genes)
    - Using integrated markers to better assess risks
    - Testing whether DNA methylation remains stable, which would offer an advantage as a biomarker of exposure over time (e.g., testing samples stored from years ago with current fresh samples)
    - Exploring the use of epigenetic marks to estimate the life stage the exposure occurred—if exposure occurred early in life during development or at stem cell stages, then a greater proportion of adult cells and cell types might carry the epigenetic mark than if exposure occurred later in life
- Integrating –omics data across the genome related to DNA methylation, miRNA or non-coding RNA expression, chromatin modifications, genetic features, and gene expression to inform mechanisms of toxicity and identify biomarkers
    - Understanding differences in information gleaned from these data: DNA methylation may be a stable indicator of exposure over time whereas gene and protein expression may be more easily perturbed by arsenic in the short-term, and each approach can serve different goals; RNA studies can help reveal perturbations in pathways and can likely better link effects to mechanisms.
    - Linking chromatin changes to gene expression
- Better characterizing where model systems can inform human biology and where they cannot
    - Examinations of parallel tissues (blood, placenta, etc., that are accessible to human studies) but cautiously comparing species depending on the tissue (e.g., the placenta varies significantly across species)

- Identifying differences and similarities in mechanisms involved, particularly epigenetic mechanisms
- DNA methylation may not be affecting the adjacent gene, but could be affecting other genes downstream, given that DNA is a three-dimensional structure.
- Increasing cross-disciplinary collaborations, especially in moving information between animal and human studies

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5) What is the impact of the different susceptibility factors on epigenetic regulation? Which factor or factors have the biggest impact on arsenic susceptibility? (Molly Kile, *Oregon State University*)

Summary:

To expand from existing knowledge of arsenic susceptibility factors and impacts on epigenetic regulation, toxicologists, and epidemiologists could consider increasing integration to better address data gaps. Early life stages and nutritional status are known susceptibility factors, but more details need to be unraveled. Characterizing the role of specific nutrients, including folate and antioxidants, is crucial before using nutritional supplements as interventions. Are there other susceptible life stages, especially periods of rapid growth and cell turnover? In addition, applying epigenetic regulation and susceptibility information to risk assessment is new territory, but very important to pursue.

Highlights:

- *In utero* arsenic exposure influences susceptibility to epigenetic alterations in developing tissues. For example in one study, cord blood showed more methylation changes than paired mother's blood with arsenic exposure.
- One-carbon metabolism is known to impact epigenetic regulation, and arsenic metabolism and DNA methylation both require one-carbon nutrients.
- Folate status has been shown to modify arsenic-induced alterations in DNA methylation in leukocytes.
- Arsenic induces oxidative stress and inflammatory responses that have been shown to alter epigenetic mechanisms, including DNA methyltransferase activity.
- Sex differences in epigenetic changes with arsenic exposure have not been noted (Kile), though sex differences in the relationship between epigenetic marks and gene expression have been observed (Marsit).

Next Steps to Address Data Gaps:

- Gathering more human data, with epidemiologists and toxicologists working together to better address specific data gaps

- Collecting co-exposure data in epidemiological studies (e.g., lead, diet)
- Identifying life stages other than the prenatal period that confer susceptibility — consider menopause and periods of rapid growth such as childhood and adolescence as well as specific tissues that have a rapid turnover
- Identifying other one-carbon nutrients that could influence susceptibility to arsenic-induced changes in DNA methylation
- Further unraveling the relationships between epigenetics and folate status (deficient and replete) before using supplements
- Exploring whether other nutrients (e.g., zinc) might play a role toward the goal of dietary interventions, and better understanding nutritional status as a factor in epidemiological studies
- Testing whether antioxidants and/or reactive oxygen species influence epigenetic susceptibility (some evidence suggests this is the case)
- Applying epigenetic information to risk assessment—important to consider and new territory

Responses to write-in questions and comments for Part 2 that were not addressed above:

Audience questions not answered during the webinar:

- Question: I am from India and working with an arsenic-exposed population. Recently we have found that in our Genetics Clinic we had 30 Down syndrome cases whose parents have a body burden level [sic] of arsenic but their children have a toxic level. Is there any relationship?
  - Kile's response: This is an interesting observation. A literature review conducted on the Web of Science and PubMed did not find any existing literature related to arsenic and Down syndrome. This appears to be an unstudied relationship.
  - Allan's response: There are reported cases in Hungary of a similar relationship between arsenic exposure and the frequency of Down's syndrome. The mechanism for the relationship has not yet been fully explored. However, it is known that arsenic reduces the activity of PARP-1 which in turn leads to a reduction in DNA repair (Sun et al., 2014; Toxicol.Appl. Pharmacol.)

- Question: Is diet-gene-exposure or diet-exposure interaction an important area to consider in terms of susceptibility to health effects such as cardiovascular effects?

  - Kile's response: Yes, there is considerable evidence that genetic polymorphisms influence arsenic metabolism and arsenic-related toxicity. With regards to studies that are specific to cardiovascular outcomes, previous research by Liao et al. (Toxicol Appl Pharmacol 2009) observed that genetic polymorphisms in the human paraoxonase gene was associated with a higher incidence of electrocardiogram anomalies. Another study

by Wu et al. (Atherosclerosis 2011) reported that genetic polymorphisms in the heme oxygenase-1 gene was associated with a differential risk of being hypertensive.

- Allan's response: Yes, given the basic work on epigenetic modifications and the interaction of arsenic with critical metalloproteins, a careful evaluation of diet and other metals within the system would be an important area of research both in increasing susceptibilities and in mitigating arsenic health effects. For example, it appears that the levels of available glutathione can impact the ability of arsenic to interact with certain proteins.

- Question: Can you comment on doses/concentrations of inorganic arsenic and MMA^{III} needed to replace Zn in Zn-finger protein?
  - Thomas' response: The literature indicates that exchanges can be driven at low levels of arsenic. Work from the Arizona group (Gandolf and colleagues) with PARP is a nice example of this metal replacement model.
  - Allan's response: The concentrations of inorganic arsenic and MMA^{III} needed to replace Zn in zinc-finger protein depend on multiple factors, including cell type, incubation time, the specific zinc-finger protein, etc. However, the concentration is typically much lower than cytotoxic concentration of arsenic. There are no studies that I know of exploring this *in vivo*; however, Jim (KJ) Liu, Ph.D., has done *in vitro* research in this area using HaCaT cells and has several papers on the topic. The paper published in Chemical Research in Toxicology (Zhou et al., 2014) is the most recent. It was reported that 2 µM arsenic or MMA incubated with the cells displaced zinc from the zinc finger domains of the proteins tested.

Session 3: Global Environmental Cycling and Bioavailability of Arsenic

Moderator: David Thomas, Ph.D. *USEPA*
May 22, 2014, 11:30 am – 1:00 pm ET

1) Are data sufficient to allocate exposures to different sources in U.S. populations or in other populations? (Margaret Kurzius-Spencer, *University of Arizona*)

Summary:

Data are currently inadequate for allocating exposures to different sources given substantial data gaps in our understanding of arsenic in food sources. The identification of species of arsenic, and the amounts and toxicities of those species need to be defined more thoroughly. Direct measurement and

identification of arsenic species in a wide variety of foods is essential for understanding aggregate human exposure to arsenic.

Highlights:

- The primary route of aggregate exposure is thought to be ingestion of water and food, with generally minor contributions from inhalation and dermal routes.
- Groundwater and drinking water have been a primary focus over the last 50 years, although some indirect water sources (e.g., water used for food preparation) have been overlooked.
- Available information on arsenic content in foods is very limited (currently reports include the FDA nationwide Total Diet Study of total arsenic, and the Schoof et al. 1999 market based survey of total and inorganic arsenic).
- Determination of aggregate arsenic exposure in humans is technically difficult and cost-prohibitive, but direct measurement is essential because diet is a major source of arsenic exposure for many people worldwide.
- In a comparison of estimates of food arsenic content based on TDS mean values to actual measured values, the Total Diet Study grossly underestimated intake, and was poorer at predicting urinary total arsenic concentrations than measured dietary exposure. In contrast, estimates based on the Schoof study overestimated intake as compared to measured values.
- Few foods have been tested, and high variability among samples of the same foods were seen in a 2012 Consumer Reports study (e.g., rice and apple juice, even between samples from the same company). This study highlights uncertainties in our knowledge and the need to measure multiple samples to quantify arsenic quantities in diet.
- Arsenic content in agricultural foods varies widely depending on soil arsenic concentrations and other growing conditions (e.g., rice grown in the southeastern U.S. grown on fields previously treated with arsenical pesticides).
- Arsenic species in fish and seafood includes arsenobetaine, arsenoproteins, arsenosugars, and arsenolipids, many of which have limited toxicity data.

Next Steps to Address Data Gaps:

- Identifying and quantifying arsenic species in food samples via direct measurement, starting with select foods based on consumption patterns
- Greatly expanding the types and numbers of foods tested for total and inorganic arsenic
- Performing duplicate sample diet studies to test food that is actually prepared and eaten to best estimate human diet exposure (expensive studies, but essential)
- Identifying currently unknown arsenic species in foods
- Characterizing the toxicity of different arsenical compounds (e.g., arsenobetaine and newly characterized species)

2a) How do we assess the bioavailability/bioaccessibility of arsenic from different sources? (Mary Lou Guerinot, *Dartmouth College*)

Summary:

To reduce the bioavailability of arsenic, concerted efforts should focus on rice as a food source because rice is a staple food eaten by about 50% of the world's population every day. One promising approach is to restrict arsenic accumulation in rice grains, and both agricultural practices and plant uptake mechanisms are the focus of current research. The use of cultivars that restrict arsenic accumulation in the grain is one of the simplest, fastest, and most cost-effective approaches to solving the problem of arsenic contamination of rice and rice products. More data are needed on the genetics of arsenic uptake, and the identification, bioavailability, and toxicity of arsenic species found in rice. The call for a regulatory standard in rice will be difficult to address without knowing more about the identification and toxicities of different arsenic species in rice.

Highlights:

- Identifying plant genes responsible for arsenic uptake with a long-term goal of altering the tissue distribution of arsenic in rice and other edible plants
- Surveying many rice cultivars for arsenic uptake to identify currently available cultivars that farmers can grow to reduce arsenic content in rice
- Testing agronomic practices that influence the bioavailability of arsenic (e.g., flooded conditions can increase bioavailable arsenic)
- Testing influence of soil and water conditions on arsenic uptake

Next Steps to Address Data Gaps:

- Identifying arsenic species and their distribution in rice plant tissues
- Understanding toxicities of different arsenic species in rice in order to develop a regulatory standard for levels of arsenic in rice
- Investigating differences between water and food exposures (current estimate is that 0.5 cups of cooked rice is roughly equivalent to drinking 1 L of drinking water containing 10 parts per billion of arsenic, the current U.S. Maximum Contaminant Level, or MCL)
- Investigating metabolism pathways of different arsenic species found in rice (e.g., can the metabolism of arsenobetaine lead to generation of toxic arsine gas under anaerobic conditions in a biologically relevant manner?) — the resemblance of the arsenobetaine/trimethylarsine metabolism pathway to the arsenocholine/trimethylamine pathway raises concerns
- Characterizing the role of the microbiome in the soil and in humans in regards to metabolism of different arsenic species
- Managing agricultural conditions and other parameters to minimize uptake of arsenic as well as cadmium and other metals because of shared transport mechanisms (e.g., arsenic is mobile in anaerobic conditions, but cadmium is mobile in aerobic conditions)

- Eliminating use of arsenical fertilizer and medications in livestock as immediate remedies to reduce human exposures

2b) How do we assess the bioavailability/bioaccessibility of arsenic from different sources? (Albert Juhasz, *University of South Australia*)

Summary:

There are numerous methods available for measuring the bioavailability and bioaccessibility of arsenic. Tests for bioavailability utilize *in vivo* methods in animal models, and tests for bioaccessibility utilize *in vitro* methods under conditions that mimic gastrointestinal environments. *In vitro* assays have the potential to be used as a surrogate method for predicting bioavailable arsenic concentrations, but the correlation between these methods needs to be better established and validated.

Highlights:

- Arsenic relative bioavailability (RBA) is quantified by measuring arsenic urinary excretion factors (UEF) following single or multiple doses, or area under the arsenic blood time curve (AUC) following a single dose in swine and monkeys.
- Arsenic *in vitro* bioaccessibility (IVBA) is quantified using numerous assays (e.g., gastric ["G"] and intestinal ["I"] phase extraction using SBRC, IVG, PBET, DIN, and UBM assays).
- Correlations between arsenic RBA and IVBA have been determined for SBRC-G (correlation with swine UEF and AUC, and mouse and monkey arsenic RBA), IVG-G (correlation with swine arsenic RBA), and UBM-G (correlation with swine arsenic RBA).
- Arsenic IVBA-RBA correlations vary depending on the RBA and IVBA methodologies utilized.

Next Steps to Address Data Gaps:

- Comparing different methods of *in vivo* bioavailability testing methods to determine the relationship between methodologies
- Investigating correlations between different methods of *in vitro* testing of arsenic bioaccessibility because different tests can yield different estimates (currently about 5–6 methods have been demonstrated to have good correlation)
- Expanding testing to increase the number and types of samples tested
- Validating linear regression methods developed for predictive capability for risk assessment purposes using independent data sets

3) Do we have satisfactory biomarkers to assess arsenic exposure in humans? (Mary Kay O'Rourke, *University of Arizona*)

Summary:

Existing biomarkers of exposure can be adequate depending on the research question asked, but it is unclear whether existing biomarkers are the gold standard that we might think they are. Researchers need to consider the complexity of what they are measuring to gauge whether the biomarker is valid for answering a specific research question. In addition, field conditions, stability of samples, and participant compliance and reliability are important parameters to consider. Notably, current biomarkers are not sufficient for assessing exposure from food because many questions remain unanswered about the complexities of dietary arsenic exposure.

Highlights:

- Common biomarkers include:
  - As^{III} and As^{V} and methylated metabolites $MMA^{III,V}$ and $DMA^{III,V}$
  - Total arsenic or sum of species
  - Arsenobetaine and arsenocholine
- Arsenic concentrations are measured in different media, including urine, blood, hair, toenail, and bladder epithelium.
- Different biomarkers are appropriate for acute versus chronic exposure (e.g., blood shows recent exposure, hair indicates about one month's exposure, and toenails show longer term exposure).
- Multiple parameters should be weighed in deciding on the use of specific biomarkers in epidemiological studies (e.g., legitimacy of the biomarker to the research question, field conditions for sample stability, practical implications of sample collection, and study participant willingness and compliance).

Next Steps to Address Data Gaps:

- Comparing biomarkers of exposure for different media and routes of exposure
- Investigating how intake of different foods impact arsenic biomarkers, including effects of micronutrients (e.g., folate) on arsenic absorption and metabolism — Is the biomarker stable relative to the foods consumed? Does uptake of other nutrients alter the yield of the biomarkers?
- Improving the feasibility of using biomarkers in the field and testing stability of arsenic species in samples after collection
- Further characterizing hair and toenail arsenic measurements (e.g., relationship to blood arsenic levels, and stability of different arsenic species in hair and toenails)

- Investigating methods for standardizing urine arsenic concentrations to account for dilution — the creatinine correction is under debate
- Identifying speciation and distribution of arsenic in different tissues, particularly in humans
- Defining half-life of different arsenic species (e.g., one study in humans showed that methylation of arsenic increases clearance, but few other studies are available)

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4) Is understanding arsenic speciation in the environment more relevant for exposure/risk assessment or determining fate and transport? (Matthew Polizzotto, *North Carolina State University*)

Summary:

Understanding arsenic speciation is critical to both evaluating the potential for exposure and determining fate and transport. Exposure as well as fate and transport are intimately linked. More data are needed on speciation in different exposure media, and the impacts of speciation on remediation, bioavailability, and bioaccessibility.

Highlights:

- Speciation controls the mobility of arsenic in the environment because speciation defines pathways for arsenic to bind to soils and sediments, accumulate in water, and be taken up by food sources.
- Efforts to mitigate arsenic exposure often rely on controlling arsenic speciation and the geochemical environment in order to limit arsenic mobility and transport.
- Understanding speciation of arsenic is extremely important in plants, foods, soils, and dust, and provides more information to couple with bioavailability and bioaccessibility data.
- Soil conditions affect plant uptake of different arsenic species (e.g., As^{III} is mobile under anaerobic conditions).

Next Steps to Address Data Gaps:

- Investigating remediation approaches, such as adding amorphous ions or phosphate to decrease bioavailability, although such conditions may increase bioaccessibility
- Identifying how physicochemical properties and microbial composition of soil affects arsenic speciation in soil
- Identifying speciation in multiple media (e.g., soil, dust)
- Exploring the use of different fertilizers that have agents (e.g., silicate and phosphate) that compete with arsenic for plant uptake or bioavailability
- Gaining a better understanding of bioavailability from inhalation of dust, including consideration of the importance of particle size on exposure
- Identifying the role of the microbial population in soils that can affect speciation and uptake

5) Do available models adequately represent aggregate exposure to arsenic? What is limiting - the model or the data? (Open panel discussion)

Summary:

Based on discussion of the first four questions of this session, panelists agreed that we are sorely lacking data for the modelers to use. There is a paucity of data on food exposures in particular — what foods contain arsenic, and how much and which species of arsenic are present? In order to do more pharmacokinetic modeling, more human studies of tissue uptake are needed but are difficult to design and implement. Furthermore, exposure models have not yet reached a high level of validation without sufficient human data to test the models.

Highlights:

- Panelists agreed that the paucity of data related to dietary exposure must be addressed.
- High variability in arsenic content of foods, even within food samples from the same food brand, introduces uncertainty in exposure assessments.
- There is variability in the metabolism of arsenic within plants as evidenced by variability in the ratio of inorganic-to-organic arsenic among rice cultivars.
- Interactions with other micronutrients in food likely affect arsenic metabolism and absorption.
- Models for aggregate exposure have not been fully developed or validated yet, in part because of the paucity of human aggregate exposure data to test the models.

Next Steps to Address Data Gaps:

- Measuring organic and inorganic arsenic as well as arsenic species in a wider range of foods and multiple samples of the same food including some that are not known to contain arsenic
- Analyzing total arsenic and inorganic arsenic in biological samples and foods on a regular basis (e.g., NHANES and the FDA)
- Performing duplicate diet exposure assessments in conjunction with biomarkers of exposure
- Analyzing arsenic metabolism and uptake in human tissues combined with assessment of aggregate exposure — one approach could be cadaver tissue, combined with hair and toenail measurements as an estimate of exposure, with the caveat that arsenic in tissues might change post-mortem

Responses to write-in questions and comments for Part 3 that were not addressed above:

Additional Audience comments (includes panelists' discussion during the webinar):

- Hair might provide a temporal record of arsenic exposure given that hair grows out from the root over time (about 0.5 inches per month). Temporal analysis of mercury in hair has been well characterized, but it has not yet been well characterized for arsenic. Arsenic bands in hair have been observed so temporal analysis is possible.
- Comparing several different types of biomarkers with total duplicate diet information might together provide adequate information for modeling purposes, but the complexity of other nutrients in the food must be considered. Just as arsenic and cadmium uptake in plants can be affected by soil conditions, it is possible that the same interactions occur in the human body.
- Some examples of *in silico* models for arsenic accumulation exist in the literature, but they lack sufficient data to validate the models.
- There are numerous biomarkers of effect for arsenic that have been published in the literature. Correlations between exposure and DNA methylation changes and oxidative stress markers are some examples. The important question is whether they are sensitive and specific.
- There is little data on the intracellular localization of arsenic in cells, as well as molecular targets of arsenic, and more data are needed.
- There are few studies on exposure via inhalation of arsenic from traffic particulate or dust. The challenge in assessing particulate matter and dust exposures is distinguishing the pathway of exposure, which could be inhalation or ingestion.
- Panelists were unaware of any arsenic data on meconium samples. Data on arsenic in fecal matter is available in mice for bioavailability studies, but not in humans.
- Arsenic in dust exposure would be highly relevant to large-scale operation chicken farmers that use roxarsone and then spread the feces as fertilizer on soils. Roxarsone survives the gastrointestinal tract, but the bacteria in feces will break down the carbon-arsenic bond, making subsequent exposure to inorganic arsenic in dust possible.
- More research is needed to characterize microbial reactions, especially anaerobic reactions, that change the speciation of ingested inorganic arsenic and organic arsenic compounds. This would be relevant for arsenic transformations in the colon where anaerobes proliferate.
- Little is known about the arsenobetaine degradation pathways in anoxic environments. Arsenobetaine degradation might follow that of its analog, glycine betaine, that degrades to acetate and trimethylamine (TMA), followed by TMA degradation to methane, CO_2, and ammonia. For trimethylarsine (TMAs), it is possible that arsine gas (H_3As) could be produced in lieu of ammonia, but this has not been tested. If so, it could be toxic if absorbed from human GI tracts. More work on the metabolism and toxicity of arsenobetaine is needed.
- Rice doesn't methylate arsenic. Rhizosphere bacteria do that.

Audience questions not answered during the webinar:

- Question: Is there any sense that consumption of seafood (i.e., seaweeds, shellfish, and fish) could be a significant portion of the aggregate exposure to harmful forms of arsenic?
    - Thomas' response: In the case of seaweed (nori), it appears that there can be a significant exposure to dimetylated arsenic. This may reflect not only the presence of dimethylated arsenic in the seaweed, but also catabolism of arsenosugars to liberate a dimethylated arsenic. There is a lack of quantitative data on the significance of degradative metabolism of complex organic arsenical.
    - Kurzius-Spencer's response: It is likely that some seafood (depending on the type of seafood ingested) contributes to exposure to arsenic compounds other than arsenobetaine, the primary organic arsenic compound. Some seafood also contains inorganic arsenic, arsenosugars, arsenolipids, and/or methylated arsenic compounds.
- Question: Does arsenic exposure alter creatinine excretion?
    - Thomas' response: In most epidemiological studies, urinary creatinine is identified as a covariate with the levels of inorganic and methylated arsenical in urine.
    - Kurzius-Spencer's response: In our analyses, we found that urine creatinine was associated with urinary arsenic excretion but showed no relation to dietary arsenic intake. In other studies, urine creatinine was associated with race/ethnicity, sex, age, BMI, and smoking.
- Question: Is arsenic in "organic" rice and if so, why?
    - Polizotto's response: "Organic" rice can have just as much arsenic as conventionally grown rice, depending on the amount of arsenic in the soil and the watering regime. If arsenic is in the soil or applied to it and rice is grown under flooded conditions, arsenic may be mobilized and taken up by the plant. Both organic and conventionally grown rice may be grown under such conditions.
    - Kurzius-Spencer's response: Arsenic is found in organic and inorganically grown rice. Rice plants accumulate inorganic arsenic, a mostly naturally-occurring contaminant of water and soils. Organically-grown food means that the food is produced without the use of synthetic pesticides and chemical fertilizers. Other restrictions may apply. In chemistry, an organic compound contains carbon. Hence, organic arsenic compounds are those that contain carbon as well as arsenic, and inorganic arsenic compounds do not contain carbon.
- Question: Has anybody looked at meconium arsenic and cord blood relationships, along with measures of mother's exposures?
    - See these references:
        - Yang et al., 2013. A preliminary study on the use of meconium for the assessment of prenatal exposure to heavy metals in Japan. J UOEH 35(2):129-35
        - Vall et al., 2012. Assessment of prenatal exposure to arsenic in Tenerife Island. PLoS One 7(11):e50643

Session 4: Prevention and Remediation Strategies for Arsenic Exposure

Moderator: Heather Henry, Ph.D. *NIEHS*
June 3, 2014, 3:00–4:30 pm ET

1) Nutrition is a preventative strategy that can reduce the adverse health effects of arsenic exposure. What are the considerations, limitations, and challenges to using this approach? What are some of the other more recent nutritional interventions that we should be aware of? (Mary Gamble, *Columbia University;* Megan Hall, *Columbia University*)

Summary:

Nutrition has the potential to be a low-cost, low-risk preventative strategy for minimizing health effects from contaminant exposures, and has broad implications beyond arsenic. Nutritional status and interventions affect arsenic methylation and metabolism, but outcomes can be difficult to predict because of the complexity of metabolic pathways like the one-carbon pathway. Mechanistic studies in humans are critically essential. Nutritional supplements under investigation include folate, creatine, choline, betaine, vitamin E, and selenium.

Highlights:

Considerations:

- The panelists urged that remediation should be the first priority for reducing arsenic exposure and related health effects.
- Nutritional interventions have the potential to be low-cost, low-risk tools with broad implications beyond arsenic, particularly for low- and middle-income countries (LMICs).
- The 10 countries with the most significant known problems of environmental arsenic exposure represent roughly 45% of the world's population. Most of them are low- and middle-income countries in which nutritional deficiencies are common.
- Interventions that are most likely to be successful consider our understanding of the underlying physiology of arsenic metabolism and mechanisms of arsenic toxicity; the dimethyl form of arsenic has a much shorter circulating half-life and is rapidly excreted in urine.
- S-adenosylmethionine (SAM) serves as the methyl donor for arsenic methylation.
    - Folate is a precursor for SAM and is a likely candidate for intervention for arsenic-related health effects. A study in Bangladesh has shown that folate supplementation can decrease %MMA in blood and in urine and increase %DMA in urine, thereby lowering total blood arsenic concentrations.
    - Other methyl donors (e.g., choline and betaine) may also be important.
- Additional methyltransferases may be impacted by nutritional status.

- There are >100 known methyltransferase enzymes and substrates that undergo methylation.
- Enzyme kinetics measures (Km and Vmax) for SAM vary widely; published values for AS3MT also vary by experimental conditions.
- DNA methyltransferases have very low Km's for SAM, and folate is weakly correlated with global DNA methylation.
- The ability of arsenic to modify DNA methylation might be dependent on folate status.

- Prevention of health effects should not be confused with treatment. For example, folate may be protective of arsenic-related health effects. But this does not suggest that folate supplementation should be used as a treatment for people with arsenic-induced bladder or lung cancer because rapidly dividing cells, such as cancer cells, need folate for survival.
- Proving effective prevention approaches require very large studies that are costly and difficult. Studies of prevention in historically exposed populations might be feasible, but difficult.
- Dose is an important parameter given that nutritional supplements (e.g., niacin) can have pharmacological properties at high doses.
- Human studies of arsenic health effects should consider nutritional status, age (changes in age can influence metabolism and nutritional status), and pregnancy state (e.g., taking vitamin supplements and naturally occuring wide variations in plasma folate levels due to pregnancy).

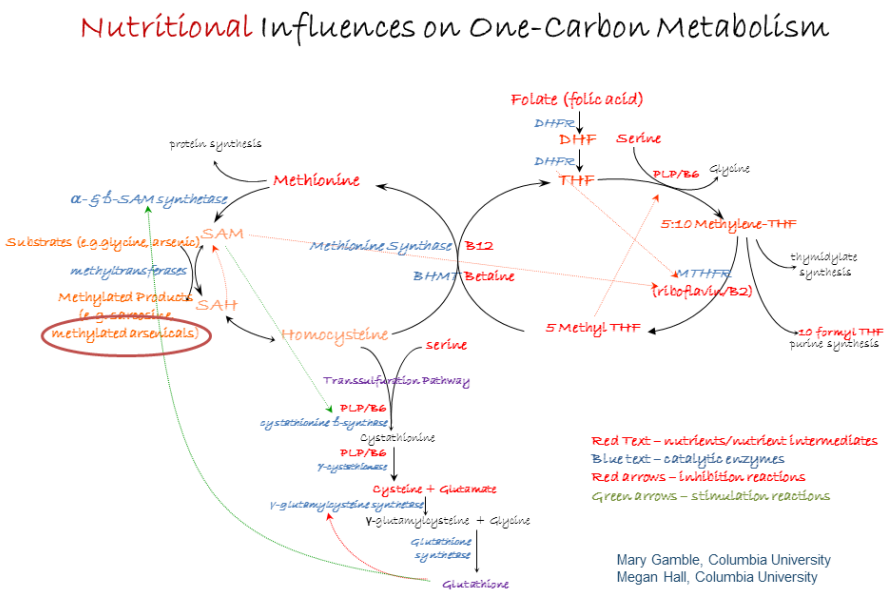

Figure 27: One-carbon metabolism pathway intermediates as potential nutritional intervention targets (figure courtesy of Drs. Hall and Gamble)

Challenges and Limitations:

- Because of the complexities of biochemical metabolic pathways *in vivo*, not all nutrient supplements behave in the predicted manner. For example, cysteine (a precursor for glutathione) could increase arsenic methylation by increasing arsenic methylation, but may also increase arsenic toxicity, if it reduces pentavalent arsenicals to trivalent forms.
- Mathematical modeling of arsenic methylation is a tool for exploring outcomes of nutritional supplementation prior to testing in randomized control trials. The combination of epidemiological, *in vivo*, and modeling data can inform clinical intervention study design.
- Diet studies based on food recall data (e.g., Food Frequency Questionnaires) can provide insights, but need to be designed carefully to minimize measurement error. For instance, it is essential for researchers to:
    - Validate the questionnaire for the specific population being studied.
    - Account for different food preparation habits.
    - Adjust for caloric intake.
- Measurement error can lead to attenuation of findings. For example, while a smaller cross-sectional study showed weak but significant associations between folate levels and arsenic methylation, a subsequent larger intervention trial showed a substantial effect.
- The utility of food recall data is nutrient- and population-specific (e.g., food recall questionnaires are not accurate for folate studies in Bangladesh, if they do not take into account cooking habits that can affect folate content in foods).
- Mechanistic studies in humans are limited to accessible cells and tissues, primarily blood cells. In the case of arsenic exposure, there is good evidence that bone marrow progenitor cells are a target tissue of arsenic. Thus, while the use of PBMCs may not be appropriate for all environmental exposures, PBMCs are actually a very good cellular target for study in the case of arsenic exposure.

Next Steps to Address Data Gaps:

- Proving that nutritional interventions prevent disease outcomes (Data from most human studies infer prevention of adverse health effects, but proving prevention requires large-scale, long-term, costly clinical trials. Nested-case control study design can improve efficiency as compared to other study designs. Prevention studies of cardiovascular disease (CVD) may be more feasible than cancer because CVD is more common and therefore would require fewer study participants and/or a shorter follow up period.)
- Identifying nutritional interventions that can reduce risk even after exposure is reduced, because increased risk of arsenic-related diseases persists long after exposure reduction
- Understanding population-specific parameters that could impact micronutrient interventions prior to scaling up the interventions
- Ongoing nutritional intervention studies include:
    - Folic Acid and Creatine Trial (FACT; Mary Gamble, lead researcher)
    - Bangladesh Vitamin E and Selenium Trial (BEST; Habibul Ahsan, lead researcher)

- Choline and Betaine Pilot Study (CABS; Megan Hall, lead researcher)

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2) How can communities be made aware of potential exposure to arsenic and opportunities for prevention? Should blanket testing of private wells for arsenic throughout the U.S. be offered or imposed? (Alexander Van Geen, *Columbia University*)

Summary:

About 5 million U.S. residents are exposed to water containing arsenic at >10 µg/L (the current U.S. Maximum Contaminant Level) via private wells and municipal water sources. It is important to educate communities about testing private wells for arsenic, and their options for mitigating exposure to arsenic. Educational media through local governments, such as county health departments, can be effective, and test kits for personal use are commercially available. Requiring water testing through regulations can be helpful, but a substantial proportion of people may not change their behaviors even when informed of the risks. More effective education and intervention approaches are needed to reduce arsenic exposure risks.

Highlights:

- Fifteen percent of the U.S. population (43 million) relies on private wells as a source of water for drinking and cooking.
- Seven percent (~3 million) of the 2,100 wells tested nationally by USGS contain >10 µg/L arsenic.
- Another ~2 million people rely on 500–800 public water systems that contained >10 µg/L arsenic in 2011.
- County health departments are often most closely involved with private well issues and can educate communities about options for reducing exposures. Potential strategies to promote awareness include:
  - Use of flyers and videos from county health departments to educate residents
  - Providing information about reputable laboratories and commercial providers of home water-treatment systems (e.g., reverse osmosis, Fe-based adsorption media).
  - Monitoring treatment effectiveness.
- Encourage personal water testing, and consider endorsing validated field kits for home screening of wells (although existing kits might present safety issues, because of the generation of arsine and the mercury content of the test strip). There are test kits available on Amazon.com that have been used in research studies in the field (e.g., test kits by ITS). See the inset box below. The following factors are also important to take into consideration:
  - Home testing can be less costly than lab tests.
  - Test results might be more impactful in changing community behaviors if community members test their own water.

- Some test kits have been validated for field use in Bangladesh studies, although those well water concentrations are much higher than those generally found in the United States. Data suggests that the test kits are linear at lower concentrations.

More on test kits:

Write-in comment from the audience: An EPA researcher with the Small Public Water System Technology Center at Penn State University - Harrisburg has explored the question of arsenic field test kits and concluded that "water professionals should be cautious is choosing field test kits." Spear, J M. et al. "Evaluation of Arsenic field test kits for drinking water analysis," Journal AWWA Dec 2006 pages 97- 105. Selecting a test kit from Amazon is not being cautious.

van Geen's response: The suggestion of the ITS kit, which can indeed be purchased from Amazon in various formats (including the Quick II included in the 2006 J AWWA paper), was based on two more recent studies carried out testing a large number of groundwater samples in Bangladesh (van Geen et al., 2014; George et al., 2012). In other words, this was an informed suggestion. As also pointed out during the webinar, the greater concern may be the risk of arsine generation in an enclosed space and inadvertently touching mercuric bromide strip without washing hands afterwards.

The Hach EZ Arsenic kit was also shown to be effective provided the reaction time is increased from the manufacturer's recommendation of 20 minutes to 40 minutes (van Geen et al., 2005). The 2006 J AWWA study was a useful early contribution – although deionized water rather than actual groundwater was used in the trial. In our experience, this reduces the Hack kit recovery beyond what it would be in a groundwater matrix – which is more representative of what people actually drink.

van Geen A, EBA Sumon, L Pitcher, JL Mey, H Ahsan, JH Graziano, KM Ahmed. Comparison of two blanket surveys of arsenic in tubewells conducted 12 years apart in a 25 km2 area of Bangladesh. Science of the Total Environment S 488–489, 484–492, 2014.

George, CM, Y Zheng, JH Graziano, SB Rasul, JL Mey, A van Geen, Evaluation of an arsenic test kit for rapid well screening in Bangladesh, Environmental Science and Technology 46, 11213–11219, 2012.

van Geen, A., Z.Cheng, A. A. Seddique, M. A. Hoque, A. Gelman, J. H. Graziano, H. Ahsan, F. Parvez, and K.M. Ahmed, Reliability of a commercial kit to test groundwater for arsenic in Bangladesh, Environmental Science and Technology 39(1); 299-303, 2005.

- The 1974 Safe Drinking Water Act does not apply to domestic wells.
- There are some existing testing requirements by government entities, for example:
  - Many counties are responsible for "potability" tests but they are typically limited to nitrate and bacteria.
  - The 2001 Private Well Testing Act in New Jersey only mandates water testing for arsenic when a house is sold.

    - Private well regulations were proposed in North Carolina, New York, and Maine, but have failed.
- At least five states, including Maryland, regulate dealers and operators of homeowner treatment systems.
- Changing behaviors may be challenging. For example, 27% of well owners who volunteered for testing in Maine continued to drink from wells, knowing it contained >10 µg/L arsenic.
- Outside funding sources, or governmental support for water testing in low-income areas, could be considered.
- Testing crop irrigation water is important, but the very large volume of water used makes it difficult to remediate. Surface water is an alternative in some cases.
- References:
    - Flanagan et al. Science of the Total Environment 2014a http://dx.doi.org/10.1016/j.scitotenv.2014.03.079
    - Flanagan et al. Science of the Total Environment 2014b http://dx.doi.org/10.1016/j.scitotenv.2014.05.017

Next Steps to Address Data Gaps:

- Developing new and validating existing field test kits for arsenic that are safe, reliable, and accurate within the concentration ranges being tested
- Developing methods for measuring bioaccessible and bioavailable arsenic in water
- Considering regulations for private well testing
- Developing effective education strategies for reducing exposures from drinking water

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3) What are the biggest challenges and opportunities for preventing arsenic exposures? What types of prevention/remediation options are needed considering each exposure route and each media? (Julie Zimmerman, *Yale University*)

Summary:

Preventing arsenic exposure via drinking water requires a multi-pronged approach. Consideration of intended use is needed to make remediation decisions, to match remediation technology to community capacity, and to implement community education and outreach.

Highlights:

- Provision of a safe water supply for:
    - Drinking (requires the lowest levels of contamination)
    - Food preparation (also requires low levels of contamination)
    - Irrigation of crops, which is important for plants that absorb arsenic (e.g., rice)
- Awareness, education, and outreach:

    - There is a need to develop and validate low-cost, accurate, and easy-to-use testing methods for field and community uses.
        - There have been observed differences in results between test kits and lab results in some cases, so validation of testing kits requires more investigation.
        - Educating and training consumers about the use of the test kits is important once they are validated.
- Match water quality to intended use (i.e., drinking, food preparation, and crop irrigation).
    - It will be a challenge to move away from one standard for all water sources and develop several standards that correspond with different uses.
- Develop sustainable, resilient arsenic removal systems and ensure appropriate disposal of removed arsenic. When addressing this, it is important to consider the following:
    - Systems at the household and community scale present different challenges.
    - Community culture and capacity are factors in planning and implementation.
    - Community differences make it difficult to create generalizable and transferable strategies for other communities.

Next Steps to Address Data Gaps:

- Examining the possibility of setting different standards depending on intended use
- Investigating community parameters to best match the technology to the community
    - Identifying parameters that allow grouping of communities that mitigate costs of planning by reducing the number of communities that require individual analysis
- Developing reliable, real-time, sensitive field detection methods
- Developing effective education and outreach activities to maximize community understanding and motivation to reduce exposures

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4) Arsenic is an interesting toxicant because much of the exposure occurs from natural sources. What exposures occur due to anthropogenic processing (e.g., mining)? How can these exposures be evaluated? What types of prevention strategies are there to minimize arsenic exposures from anthropogenic sources (e.g., the Garden Roots Project)? (Raina Maier, *University of Arizona*)

Summary:

Arsenic exposure risks increase whenever the earth's crust is disturbed (e.g., mining-related activities). Historic and present use of arsenical pesticides is also a source of exposure. Prevention strategies include remediation as well as community education for informed decisions to reduce exposures from water, food, soil, and dust.

Highlights:

Anthropogenic sources:

- Arsenic occurs in association with >200 different metals, including arsenopyrite, in the minerals making up the earth's crust. Activities that disturb the earth's crust can release arsenic into the environment and leach into groundwater.
    - Mining and mining-related activities release arsenic (usually from metal mine when arsenopyrite oxidizes to release arsenic into soil and water).
    - When burning coal that contains arsenopyrite, or other arsenic bearing minerals, arsenic is released into the air. Relevant issues of concern include:
        - As the supply of high-quality coal diminishes, arsenic emissions will become a bigger challenge.
        - Clean coal technology is designed to clean about 85% of arsenic from emissions.
    - Increased use of geothermal power is a concern, because geothermal fluids can contain arsenic.
    - Dust is a concern in arid climates, like the US Southwest. Exposures through inhalation of arsenic associated with dust particulates and ingestion (especially for children) of arsenic associated with dust or soil could become more widespread with climate change.
    - Fracking flowback water is of concern as a possible source of high arsenic water concentrations.
- Activities that use arsenic to control pests can release arsenic into the environment. Some examples include the following:
    - Wood treated with chromated copper arsenate.
    - Feed additive for poultry and swine production (roxarsone) – a voluntary suspension of roxarsone use occurred in 2011.
    - Agricultural insecticides – organic arsenicals were used on a large number of crops (e.g., apples); use was largely phased out from 2009 to 2013 except for cotton but legacy sites remain.

Evaluating these exposures:

- Food, soil, and dust become more important sources of exposure in areas with relatively low water arsenic concentrations, as compared to areas with high arsenic concentrations (e.g., Bangladesh).
- Studies are needed that measure total arsenic exposure combined with biomonitoring, so partnered studies should measure:
    - Source concentrations and speciation of arsenic in water, soil, dust, and food
    - Speciated arsenic exposure in human populations

Prevention strategies:

- Remediation is a top priority, but there are situations where the contamination is so substantial or widespread that remediation is not feasible. In these cases, community-level prevention and education is essential.
    - Education on the need for well-water testing (both public and private) to ensure drinking water arsenic levels are below the MCL.
    - Provide information on home behaviors (e.g., removing outdoor shoes/clothing and hand-washing) that can minimize tracking of soil/dust into homes.
    - Expand our knowledge of arsenic food sources, and provide information on foods that have high levels of arsenic to allow informed dietary decisions. Food sources that may include high levels of arsenic include:
        - Rice and rice products
        - Apple juice
        - Dark meat fish
    - Provide information to communities with high soil arsenic concentrations (either endemic or anthropogenic) on levels of vegetable uptake and on vegetable washing procedures.

Next Steps to Address Data Gaps:

- Developing tests for bioaccessibility and bioavailability for soil testing as well as water
- Expanding knowledge of food sources of arsenic to make informed dietary decisions
- Measuring speciated arsenic in humans in more studies, and pairing the data with environmental exposure data
- Providing information to more communities on actions they can take to reduce exposures

Write-in questions and comments for Part 4 that were not addressed above:

Audience comments:

- Several counties in the U.S. have their own legislation requiring testing of private wells before selling a property, even though there is no statewide requirement.

Audience questions not covered above:

- Question**:** With increasing burning of coal in countries like China, is it anticipated that their populations are more susceptible to arsenic toxicity in the future?
    - Panel answer: Arsenic is only one of many components in the air pollution to which the Chinese are exposed, and arsenic is likely to be a contributor to adverse health effects. In addition, the increasing use of lower quality coal worldwide is likely to increase arsenic exposures.