

Health Effects and Mitigation of Arsenic Current Research Efforts
and Future Directions workshop panel discussion webinar series

Part 3: Global Environmental Cycling and Bioavailability of Arsenic

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Danielle Carlin: Well, welcome, everybody, to the third of our fourth -- four webinars in the Health Effects and Mitigation of Arsenic: Current Research Efforts and Future Directions workshop panel discussion series. My name is Dr. Danielle Carlin, and I am a Program Administrator with the Superfund Research Program here at the National Institute of Environmental Health Sciences, better known as NIEHS.

So the NIEHS Superfund Research Program is hosting this series of four expert panel discussions that will focus on the current state of knowledge and data gaps in the field of arsenic environmental health research. Topics include exposure sources and mitigation, remediation, bioavailability, contributions of advanced techniques and susceptibility.

These panel discussions stem from the workshop that was held in Research Triangle Park, North Carolina, on March 3 and 4, 2014. Unfortunately, the panel discussions, they were originally scheduled to be held at that workshop but they had to be postponed due to inclement weather. So this webinar is the third in the series, and it's titled Global Environmental Cycling and Bioavailability of Arsenic.

We invited leading researchers to serve as panelists to support discussions on specific questions, and you will hear more about the webinar format from our session moderator shortly. We are grateful to the session moderator and the panelists for their time and effort in making up this webinar -- or, excuse me, making up this panel discussion.

Our next and last webinar in the series will be held June 3 from 3:00 to 4:30 Eastern Time, so I encourage you to please mark your calendars. And you can register for that webinar at this link, which is listed here at the bottom in green. And it's bit.ly/ArsenicSeries.

So, the goal of these panel discussions is to highlight new techniques and identify data gaps to help guide future research directions. The panelists each propose answers to specific questions posed to them, and panelists -- and the panelists will discuss their answers towards the goal of a consensus answer.

We encourage you to submit your written questions and comments during the webinar, as well. That's instructions for our audience. The session moderator will tell you more about the format

of the webinar discussion. Information from the workshop and from this webinar series will be captured in a written report that is currently being written by Dr. Marisa Naujokas from MDB, Inc.

For the next slide, the session moderator for today's webinar is Dr. David Thomas. He is a Research Toxicologist in the Pharmacokinetics Branch, Integrated Systems Toxicology Division at the National Health and Environmental Effects Research Laboratory of the US EPA. David, I just want to say thank you on behalf of the entire SRP staff here today, and thank you so much for moderating this session. So now I'm going to turn it over to you, David.

David Thomas: Thank you, Danielle. I appreciate the opportunity to do this, and I want to thank your staff for organizing these webinars to make up for the last -- the problems at the meeting back in March.

Today's session is Global Environmental Cycling and Bioavailability of Arsenic. At the end of this discussion over the next hour or so we'd like to be able to have some consensus answers to the questions that the panelists discussed. These answers can take many different forms. They could be descriptions of the major points, evaluation of or identification of techniques, concepts or concerns that are important for this -- for the further discussion. Although it's difficult sometimes to integrate and consolidate information like this on a complex topic, it does challenge us to discern what are the most important points that are relevant to the questions and to frame the future discussion of this topic.

And I guess we're ready for Slide No. 5. Yes, so this is the schedule for today's discussion. We're on a fairly tight time schedule. We're at the beginning now. I'm doing the -- describing the format and introducing the panelists, and then we have 12 minutes to discuss each question. Danielle will be marking the time for each discussion of each question, and she'll let us know when 10 minutes are up, and also when 12 minutes are up the session will be -- discussion of that question will be over. If there's time at the end of today's discussion we can revisit some of the questions that might benefit from additional discussion and have an opportunity to maybe cover some issues that are unresolved in the original presentation.

Okay, I guess we're now ready for the next slide, Discussion Format. The panelists were tasked with a specific question. The lead panelist for each question will propose an answer as a starting point for the discussion. And the participants can add or change the proposed answer. And from this discussion we hope to capture the -- identify and capture the data gap (inaudible) future research. NIEHS will collect all written questions and comments for consideration in the final report.

Next slide. Okay, I'm going to briefly cover the -- review the questions and identify the panelists for today's session.

So, Question No. 1, are data sufficient to allocate exposures to different sources in US populations or in other populations? The panelist for this is Margaret Kurzius-Spencer, who's an Assistant Professor of Pediatrics in the Section of Medical and Molecular Genetics at the University of Arizona College of Medicine, with a joint appointment in the College of Public

Health.

Question No. 2, how do we assess bioavailability/bioaccessibility of arsenic from different sources? The two panelists, Albert Juhasz, who's an Associate Research Professor at the Center for Environmental Risk Assessment and Remediation at the University of South Australia, and Mary Lou Guerinot, who is the Ronald and Deborah Harris Professor of Sciences at Dartmouth College.

Question 3, do we have satisfactory biomarkers to assess arsenic exposure in humans? And the panelist is Mary Kay O'Rourke, who is an Associate Professor of Public Health and a Research Associate Professor of Medicine at the University of Arizona.

Question 4, is understanding arsenic speciation in the environment more relevant for risk -- exposure/risk assessment or for determining fate and transport? The panelist is Matthew Polizzotto, Assistant Professor in the Department of Soil Science at North Carolina State University.

And Question 5 is do available models adequately represent aggregate exposure to arsenic? What is limiting -- the model or the data? And we do not have a -- the lead panelist was not able to attend today's session, so this question's going to (inaudible) by all.

Okay, Margaret, we should now proceed to the first question.

Margaret Kurzius-Spencer: Okay. So the question I was asked to address is are data sufficient to allocate arsenic exposures to different sources in US populations or in other populations? And the answer to this question is no, existing data are inadequate for allocating exposures to different sources.

Aggregate arsenic exposure occurs primarily through ingestion of food and water. Exposure via water may be either direct, through drinking, or indirect, through use of water for food preparation, such as in cooking water, powdered drinks, condensed soups, etc. There may be other routes of exposure besides ingestion, such as inhalation or through the skin, but these are thought to be relatively minor for arsenic in most nonindustrial settings.

Arsenic in drinking water has been a recognized public health problem for over half a century, probably much longer, and as a result groundwater arsenic has been fairly well studied. The World Health Organization, EPA, US Geological Survey and others have been involved in assessing arsenic in well water and drinking water samples over the last 40 years or more, and the World Health Organization and EPA have also been involved in efforts to determine a maximum contaminant level, an MCL, based on existing data on toxicity and health effects, particularly cancer.

Generally speaking, indirect water use has not been measured or differentiated as a source of aggregate exposure. In the US people often use different sources of water for drinking, such as bottled or filtered water, and for food preparation most often they use tap water.

What I will mostly be focusing on today in this webinar is exposure to arsenic from food. Existing data on arsenic exposure from food is limited mostly to total arsenic, and little is known about the various arsenic compounds or species that comprise total arsenic.

The US FDA conducts the Total Diet Study, TDS, which is a market basket survey of 280 table-ready foods collected in different geographic areas of the US, and this is conducted annually. The TDS analyzes 30 or more other analytes besides arsenic, including pesticides, various elements, radionuclides, etc., but only analyzes total arsenic, not any arsenic species in their food samples.

In 1999, Rosalind Schoof and coauthors published a market basket survey of inorganic arsenic in 40 food commodities thought to comprise over 90% of the total dietary intake of inorganic arsenic. They included such foods as rice, wheat flour, corn meal, peanut butter, beef, chicken, pork, various types of fish, eggs, milk, sugar, a few fruits and vegetables, juices and beer, and analyzed four samples of each for total and inorganic arsenic content. The 1999 Schoof paper is the only source of information on the inorganic arsenic content of a variety of foods.

So determination of aggregate exposure in human populations and subgroups is technically difficult and cost-prohibitive. However, direct measurement is essential, given that diet is the primary source of exposure in much of the world, existing data on arsenic residue in foods is limited, and even low-dose exposure to arsenic appears to have deleterious health effects.

We've compared estimates of dietary total arsenic exposure based on TDS mean values to measure total arsenic from duplicate diet samples and found that the TDS mean values grossly underestimated intake and were poorer at predicting urinary total arsenic concentrations than measured dietary exposure. On the other hand, the estimates of dietary total arsenic exposure based on Schoof mean values overestimated intake in the same populations.

Characterizing dietary arsenic presents a distinct challenge in that the arsenic concentration and the species composition in specific food types are highly variable, and the analysis of arsenic compounds in food is complex. Fish and other seafood are the primary source of dietary exposure to total arsenic, but most of the arsenic in seafood is organic in the form of arsenobetaine, which is generally considered nontoxic. But the various species of seafood also contain differing concentrations of additional arsenic compounds, including inorganic arsenic, arsenal lipids, arsenal proteins, arsenal sugars and methylated arsenicals. The toxicity of many of these compounds is undetermined.

Arsenic in agricultural crops varies by geographic region, growing conditions, cooking methods and other factors. While most of the arsenic in grains, fruits and vegetables are a result of naturally appearing arsenic in the soil and irrigation water, some agricultural soils have been treated historically or more recently with arsenical pesticides and/or herbicides.

A report on arsenic in rice published by Consumer Reports in 2012 found high variability within and among types of rice and different rice products in total inorganic and organic arsenic, and in the amount of total arsenic unaccounted for. In this study, total arsenic ranged from about 55 to nearly 600 parts per billion, with inorganic arsenic comprising anywhere between 11 and 80

percent of the total. Organic arsenic, defined in this report as the sum of DMA and MMA, the mono and dimethylated arsenicals, made up as much as 70 percent of the total arsenic in some rice products. Studies have also found differences in arsenic contamination and arsenic speciation in rice grown in different regions.

A survey of arsenic in commercial beverages -- apple juice, grape juice, milk and broths -- was published by Roberge, et al., in 2009. They found extensive variability within and between brands and among lots of the same brand, reporting up to a sevenfold difference among samples. The FDA and Consumer Reports also assessed arsenic in apple juice. They found 5 to 10 percent of samples exceed the MCL for arsenic in water of 10 parts per billion, and they recently proposed an action level of 10 parts per billion for arsenic in apple juice.

FDA also recently published analytical results for total arsenic, inorganic arsenic and methylated arsenic species in pear juice. Their samples showed tremendous variation, a huge range of concentrations, from 0 to 700 parts per billion, and some samples had very high levels of inorganic arsenic and MMA.

Some seafoods, rice products, apple and pear juice are among the very few foods that have been analyzed to any extent for arsenic species. The variability in the analytical results from sample to sample underscores the uncertainties inherent in modeling and exposure and a lack of understanding of the factors involved in arsenic uptake by plants and animals.

Other commonly consumed foods, including many fruits and vegetables, other fruit drinks, wheat and other grain products, also may have relatively high concentrations of arsenic compounds but have not been thoroughly studied. Although costly and time-consuming, use of duplicate diet methods in which exposure to arsenic compounds in foods as it's prepared and eaten is measured will provide the best estimates of human exposure.

Based on existing data on dietary total and inorganic arsenic content in foods, inorganic arsenic intake accounts for less than 50 percent of the dietary total arsenic intake among non-seafood eaters in population studies. So which arsenic compounds comprise the rest of the total arsenic consumed? How are the different arsenicals metabolized? What is their toxicity? To move forward on arsenic exposure research, before we can allocate exposure to different sources, we need more complete data on arsenic species composition in a much wider variety of foods and human dietary exposures and on the toxicity of the assortment of arsenical compounds found in food.

Thank you for your time. I'll be happy to take any questions.

Danielle Carlin: David, we have about two or three minutes for discussion.

David Thomas: So I guess we'll ask the panelists if someone has a comment or a question. Danielle, you have the written comments. So can I just throw out one general question, Margaret? So if you have this issue about identifying the -- looking for arsenic species in food, how are you going to -- among all the food types, what's going to be the strategy that would be best to choose the foods that are going to be looked at? Is it based on food consumption patterns?

And do we have a sense of how far down the ladder we have to go in terms of identifying these different species in food?

Margaret Kurzius-Spencer: Those are really good questions, Dave. I think that we at least need to evaluate foods by consumption patterns, those foods that are consumed by a large number of people in the US, for example, for at least inorganic arsenic and total arsenic and to do a study that includes many samples just to -- again, it's very expensive to do this, but it's a very good question.

I mean, we could focus on what we expect to have the highest total arsenic level after rice, basically, and then try to evaluate, well, maybe, wheat or corn flour to see what arsenic species they contain and to do a large enough sample size and from plants growing in different parts of the country to figure out what their arsenic composition is. But another approach would be to look at specific arsenic compounds in those foods that contain relatively high concentrations of arsenic compounds that we are concerned about. But I think we also do not know that much about toxicity, as yet, and need to evaluate that further.

Danielle Carlin: So, David, we have some questions, and Michelle Heacock, from our SRP staff, she's going to be fielding those questions.

David Thomas: Good.

Michelle Heacock: Okay. So the first question is, at this point can we assume that the arsenobetaines in seafood are not toxic?

Margaret Kurzius-Spencer: So, from what I have read, and I am not a toxicologist, I'm an epidemiologist, but from what I have read I don't think that we can necessarily assume that 100 percent. I think that's been the common thinking, but there is some indication that I think, from what I've read, that arsenobetaine sometimes is metabolized into the methylated arsenicals, and I don't know how well they're captured, that has been captured. But I think we -- there's a lot to learn.

Michelle Heacock: We could also open this question up to the rest of the panel if any of the other panelists can provide a little bit more clarity on the answer. The question again is at this point can we assume that the arsenobetaines in seafood are not toxic? If anyone else on the panel has an answer that they'd like to chime in, please do so.

Okay, we'll move on to the next question. The next question has to do with dealing with the high variability and samples from different regions, such as rice, such as those in -- such as rice samples.

Margaret Kurzius-Spencer: Right. So I'm guessing that Mary Lou might be able to answer this better, but I would think it would be important to evaluate the soils in which the rice is being grown in the Southeastern US as well as in California. From what I have read California has high naturally occurring arsenic in the soils, at least in certain rice-growing areas, and in the Southeastern US I have -- am under the impression that the use of arsenical pesticides, I guess

specifically on cotton previously, may be increasing the methylated arsenical species found in rice to a greater extent grown in the Southeast. So please correct me if I'm not right or I misunderstand.

Danielle Carlin: David, we need to move on to the next question.

David Thomas: Well, thank you, all of you. So I guess we will go -- we're going to have some time at the end to come back to some of these questions. Now I'm going to be considering Question No. 2. How do we assess the bioavailability/bioaccessibility of arsenic from different sources? Our panelists are Albert Juhasz, again, University of South Australia, and Mary Lou Guerinot, from Dartmouth College. And I guess we're starting with Albert. Is that correct?

Albert Juhasz: Thanks, mate. I can start off if you want. I'll split the answer into two, because the question addresses two different points, bioavailability and bioaccessibility.

So in terms of bioavailability assessment of arsenic, and in this particular example I'll be talking about contaminated soils and dust in relation to human health exposure assessment, relative bioavailability can be assessed using a number of different animal models. And over the last 10 to 15 years the most predominant animal models that have been utilized include swine, mice and also monkey models.

But within those methodologies or within those animal models there are different methodologies that may be utilized. So you may assess arsenic relative bioavailability by looking at urinary excretion (inaudible) urinary excretion. You can also assess bioavailability by looking at urinary excretion following a single administration of the contaminated soil. An alternative approach is also looking at area under the arsenic (blood) time curve following a single administration of arsenic.

The methodologies that are probably most prevalent are the steady-state urinary excretion methodologies. However, there's been a small attempt to date to see what is the relationship between arsenic relative bioavailability that has been derived using those different animal models. So that's (inaudible). But really I think there needs to be some more concerted effort to see the relationship between these different animal models and the different approaches for assessing arsenic relative bioavailability.

In terms of assessing arsenic bioaccessibility using in vitro gastrointestinal extraction methods, again, there's a wide variety of different methodologies that have been utilized. So these methodologies might include a single [gastric phase], so extraction at low pH, somewhere between pH 1.5 and 2, to look at arsenic dissolution and dissolution of other phases that arsenic may be associated to. Or they may then transit the [assay] into an intestinal (phase) where you increase the pH up to (inaudible) and you may include other constituents in there that they may be representative of intestinal [phase] extraction. But, again, depending on which methodology you utilize and depending on which phase of the assay you utilize, then you can get significantly different results.

So there has been an effort within the last 10 or so years, and more so pushing in the last five

years, to correlate the in vitro methodologies to in vivo arsenic relative bioavailability. And there have been about five or six studies that have shown that, depending on which in vitro methodology you utilize, there is a pretty good relationship or strong correlation between arsenic relative bioavailability determined using the respective animal models and arsenic bioaccessibility. And of the studies that have demonstrated this correlation, three of them have shown for the (inaudible) gastric phase, which is sometimes called (inaudible) or (inaudible) analysis.

But one of the limitations of the work thus far is the number of samples that have been utilized to derive these correlations, the type of samples that have been utilized. Predominantly they have been non-impacted materials. There's only been a few studies that have incorporated other arsenic sources such as herbicide, pesticide impacted materials.

And I suppose the other main issue in terms of bioavailability and bioaccessibility assessment is that although correlations have been demonstrated between in vivo and in vitro approaches, there hasn't been a validation of these methodologies to date, so utilizing independent data sets to validate the linear regression models that have developed that express the predictive capabilities of in vitro methodologies for predicting arsenic relative bioavailability.

So there's still a little bit of way to go. There are a number of approaches that can be utilized, but we need to have confidence in the methods that have been utilized so that we can be confident in the way that exposure can be refined for human health risk assessment.

With that I might leave it and then hand over to Mary Lou for her section.

Mary Lou Guerinot: Okay, thank you. So I took a slightly different approach on this question, because my own area is not really bioavailability and bioaccessibility. Those are really important pieces of data that we need, but since one of the major sources of arsenic exposure is rice, I decided to just take a few minutes to say we really need to focus on rice.

And rice is a very efficient accumulator of arsenic, especially when it's grown under flooded conditions, and it's a staple food, so it's eaten by 50 percent of the world every day. So there are some really sort of long-term studies that need to be conducted to like look at the distribution and speciation of arsenic in rice. And I'm a molecular geneticist, so I want to understand ultimately what genes are responsible so that we could maybe alter the distribution and/or speciation of arsenic in rice. But we don't really know that much yet about what forms are there, and we've already heard we don't know much about which forms are more toxic than others.

And so some of the variability that's already been pointed out when you sample rice is that different cultivars accumulate different amounts of arsenic, and that's going to depend on the genetics of the cultivar, but also on the soil and the water, the amount of arsenic that's in the soil and the water, and then the agronomic practices can also influence how much arsenic's available to be taken up, for example, flooded or not flooded and whether you're putting on fertilizer that has arsenic in it.

So what we are hoping to accomplish really is doing a lot of surveys of cultivars and trying to get

an idea about what the best cultivars are and then getting that information out there so that farmers can grow rice that is currently available that has the lowest levels of arsenic. And then with the combination of agronomic practices, like maybe not having the plants flooded for the entire time, managing the water, because that will really help limit the amount of arsenic that's available -- it becomes very bioavailable to the rice plants under flooded anaerobic conditions.

So there are some short-term things we can do, but from my point of view as a plant biologist, I read the Consumer Reports, we probably all have, on the investigation on rice, and everyone's calling for a standard to be set for rice, but it's not going to be that straightforward to figure out what that standard should be. We need a lot more toxicological work before I think we could do that. And then they also recommended, as I am, developing types of rice that take up less arsenic and then doing some immediate things like not using pesticides that contain arsenic, I already mentioned not using fertilizer that has arsenic, and certainly stop using arsenic drugs, some of which are still given to animals, and so to eliminate another input.

Because, let's face it, I mean, arsenic is present in the soil and water. We can't get rid of it. So we sort of have to manage how much of it ends up in the food we eat. And I think I'll just leave it there.

Danielle Carlin: So, David, we have time for about three to four minutes of questions.

David Thomas: Okay. I'd ask the panelists if anyone has a comment on these two presentations.

Mary Kay O'Rourke: This is Mary Kay O'Rourke, and my question would be I've heard somewhere that if you alter the arsenic uptake in rice that frequently it flips over and it will increase the cadmium uptake in rice. So if that's the case are you gaining anything by developing a hybrid that will retard arsenic, or are you just trading that for a new set of potential health risks?

Mary Lou Guerinot: This is Mary Lou. So the problem you're referring to is that arsenic is very mobile under anaerobic conditions, and then if we grow them without anaerobic conditions then cadmium is very mobile, because cadmium precipitates out in the paddy fields and the rice can't take it up. So there is a tradeoff, you're correct. So upland rice accumulates cadmium. So researchers are actually looking at cadmium uptake, as well, and we're going to try and manage both of those problems, because it is a real tradeoff.

Danielle Carlin: So, David, Michelle has --

Mary Kay O'Rourke: I'll do another --

Danielle Carlin: Oh, go ahead.

Mary Kay O'Rourke: I'd do another follow-up question, and now I forgot what it was. Forget it.

Danielle Carlin: Okay, while you're remembering I think Michelle has a few questions here.

Michelle Heacock: Okay, I've got a very specific question, so hold on. It's a long one. Does arsenobetaine degradation in (inaudible) environments follow that of its analog, glycine betaine? The latter is an osmoregulatory compound that degrades to acetate and trimethylamine, followed by TMA degradation to methane, carbon dioxide and ammonia. For TMA arsine, would arsine gas be produced in lieu of ammonia? If so, it could certainly be toxic, for instance, absorbed from human GI tracts.

Danielle Carlin: And I think that's a question direct to Dr. Juhasz.

Albert Juhasz: Thanks for that one.

Michelle Heacock: You're welcome.

Albert Juhasz: Can you repeat the question?

Michelle Heacock: I knew you were going to do that. All right, here we go. Does arsenobetaine degradation in (inaudible) environments follow that of its analog, glycine betaine? The latter is an osmoregulatory compound that degrades to acetate and trimethylamine, followed by TMA, or trimethylamine, degradation to methane, carbon dioxide and ammonia. For trimethyl arsine, would arsine gas be produced in lieu of ammonia? If so, it would certainly be toxic, for instance, it's absorbed by human GI tracts.

Albert Juhasz: Well, arsine gas is obviously toxic and is -- can be absorbed in the GI tract. To be honest, I don't know whether that would be the case. It sounds possible, but to be honest, I don't know.

David Thomas: I will say this issue about the resemblance of the metabolism to the TMA pathway is an idea that's been floating around for a while, and it does raise some concerns. I think that what's -- I think it sounds like it may be biochemically plausible, but whether it's actually biologically significant remains to be determined.

Michelle Heacock: Do we have time for one?

Danielle Carlin: We have time for one more question.

Michelle Heacock: And this question is to please comment on relative -- the relative contribution of arsenic in rice to total arsenic exposure, considering wide variability and exposure to arsenic in water. So I think they're asking what is the contribution of being exposed to arsenic from rice versus water.

Mary Lou Guerinot: So, this is Mary Lou. I can give you a number that our Superfund group at Dartmouth, I think it's in their PNAS paper, but they say that if you eat a half a cup of cooked white rice it's equivalent to drinking a liter of water that has the 10 parts per billion, the limit for arsenic in drinking water. They were just trying to get a comparison so that people could get an idea, like a half a cup of rice is the average amount of rice that's consumed per day in the US, so it'd be equivalent to drinking a liter of water that has 10 parts per billion of arsenic. But we just

don't have studies that addressing the risks associated when it's coming in via your food as opposed to via your water.

Michelle Heacock: Thank you. And I would just -- I had -- another question came in that was just a clarification from the first one. Basically it's asking so essentially not enough is known about anaerobic microbial degradation pathways for trimethyl arsine. So they're just asking for clarification.

Mary Lou Guerinot: I would say that's certainly true. We haven't even begun to really think about what the microbes are doing to the extent that we need to.

Danielle Carlin: Okay. David, we need to move on to the next question.

David Thomas: Okay. The third question is do we have satisfactory biomarkers to assess arsenic exposure in humans? And this question is Mary Kay O'Rourke.

Mary Kay O'Rourke: And, like many others, this is not my primary area. I'm not a biomarker toxicologist. What I am is a field-oriented person. So one of the big questions for me is how are these biomarkers going to be collectible in the field? And the key issue that I think we need to think of before we answer this question is what's the question the researcher is trying to answer? Because sometimes for certain types of studies, yes, our biomarkers are adequate. But for the complex studies, particularly around food, the answer for me is no, the biomarkers are not adequate.

And I'm going to step back to Margaret's talk, where she's essentially looking at the vast quantities of food that are out there and looking at the food intake, whether through, as modeled, using TDS or other approaches, and that's relative to a gold standard. So you're saying in one case that when you model it, one set of models will underestimate the measured arsenic whereas another set of models will overestimate the amount of arsenic. But the real question for me is that's all relative to the biomarker, so is the biomarker stable relative to the food that comes in, or what are the other parameters that influence those analytes you're detecting as biomarkers?

Then I move on to what Mary's talking about, and I know she also has -- Mary Lou -- also has information on other nutrient. We've also looked at some of these other nutrient, quote, unquote, "interferences" from my perspective. So if uptake of specific nutrients can alter the yield of the arsenic that's appearing in the biomarkers, then how does that tie together with the sufficiency of the biomarkers relative to the question being asked?

So we have some standard biomarkers that work. We have total arsenic that, because of methylation of some of the species or a direct passthrough of things like arsenobetaine, those are sort of confounded for the actual species from the environment that a person or an animal or a plant is uptaking. So that's my deal around questions. You've got to refine the question, consider the specific biomarker, and make sure that whatever the complexity of the biomarker production within the human group or body answers that question.

For me, the next thing I have to think about is the stability of that biomarker. How many samples

do I need to take in the field if we're talking about urinary biomarkers, and at what timelines, and can I reasonably ask a population of people if this is an epi study to produce those urinary samples on a schedule? And if I'm not doing that, am I collecting all the urine and looking at a 24-hour sample?

And how do I implement that with people who have real lives and go off and work and do other things? How do you get those samples? And are they stable enough in whatever mode you're collecting them so that you're not getting any changes among those species? And is the participant willing to provide those samples that way? So how large do those incentives need to be, and at what point do those incentives become coercive?

There's been a lot of discussion in the literature about how you standardize arsenic biomarkers. And some people want to look at the metabolism and standardize it relative to creatinine. But if you look at the creatinine-adjusted data you don't always end up with biomarkers that are related particularly well to some of your environmental measures. And so what's the best mechanism around standardizing those biomarkers relative to dilution?

Then the big thing for me is how is this going to vary by media within the population? So what we've been talking about today so far is ingestion, ingestion of water, ingestion of food. And when you ingest the food you're not just looking at a pure rice sample. You're looking at the other foods that are in there with that rice, other nutrients that are going to alter how that food may be methylated, what those interferences are. And, to be blunt, I have no clue about that, and I don't think anybody else really does, either. So these impacts of micronutrient ingestion on the production of the biomarkers will then alter the relationship seen between either the food estimates or what we're headed to next, the environmental samples, and how that's all going to relate.

So my big conclusion at the end is are the biomarkers the gold standard we think they are, or do we have to do more research about how different pathways of exposure, different items that are ingested, impact those biomarkers? And I'll wrap it up at that.

David Thomas: Okay. Thank you, Mary Kay. I will just open it first to the panelists to see if they have any comments or questions.

Mary Kay O'Rourke: And I'll chime in and say, Mary Lou, you made a statement that was really interesting to me at the workshop itself where we were starting to talk about the impact of folate and folate on uptake.

Mary Lou Guerinot: I did not talk about folate that I remember.

Mary Kay O'Rourke: All right, it must have been somebody else.

Danielle Carlin: That was Mary Gamble, from Columbia University.

David Thomas: Well, certainly the folate status is going to affect the methylation profile for ingested inorganic arsenic. That's clear from the work in Bangladesh. So I guess that's a

micronutrient issue there, interaction.

Mary Kay O'Rourke: So the issue for me is in the research we're doing around food, and if we are assessing what is in food, then how are we going to model that if we don't have knowledge of the micronutrients and how that will impact the biomarkers?

David Thomas: So you're suggesting that that would presumably be useful to be a component of any food -- study that's focused on food as to how the --

Mary Kay O'Rourke: Absolutely. And I think it's going to have to be more broad than just folate. It's going to have to be a number of these micronutrients, because I think there are many that are in there and we have no idea how they potentially impact the biomarkers.

Margaret Kurzius-Spencer: I agree with you there -- this is Margaret -- Mary Kay, that it is important to look at the micronutrient intake as well as the dietary intake. I was going to suggest that there seems to be some indication that different biomarkers might be better at assessing exposure from food versus water, and certainly better at assessing long-term versus more acute exposure.

Mary Kay O'Rourke: And I'd agree with that. One of the things you have to then think about, for instance, they were talking -- some papers talk about hair as a better integrated sample to give you a monthly view. If you go with hair, then you've got cultural issues. How much hair can you get from people? We found along the US-Mexico border a real reluctance of women to cut their hair and provide that 1-cm scalp sample. And so you're cutting the underlayer of hair, hoping it'll work out. But there's a subject reluctance in some of the biomarkers that might be ideal to collect, but a population may not be as excited about providing them.

David Thomas: So in terms of longer term exposure histories, things like hair and toenails are probably a better choice. But the question is whether you're giving up information about speciation by using hair and toenails, because I don't think we have a good understanding, at least for toenails, about what the species -- how the species in toenails might reflect the metabolic profiles of individuals.

Mary Kay O'Rourke: And I completely agree with that. We've done some work with toenails, and it's been problematic.

Margaret Kurzius-Spencer: Do -- this is Margaret again -- do we even know how the arsenic gets into the toenails and the hair?

David Thomas: Well, presumably it's in some sort of an equilibrium with blood. I mean, that's the model that people assume. But I have to say there's not a whole lot of -- as far as I know not a whole lot of data for -- unlike mercury, there's not a whole lot of data for arsenic on the (inaudible) state between hair, blood and toenails.

Danielle Carlin: David, Michelle has a couple of questions.

David Thomas: Sure.

Michelle Heacock: And actually this ties in really well with what you are discussing right now, and the question is what's the distribution of arsenic species across different tissue samples collected, and what types of samples give a history of arsenic accumulation?

Mary Kay O'Rourke: And I'm going to punt that to David. I'm punting that directly to David.

David Thomas: Well, I mean, I think there's actually -- actually, there's sort of a dearth of human tissue data just in general. We don't have -- I mean, we have a lot of -- a good bit of animal tissue data, but we don't have a whole lot of human tissue data that can be tied to environmental exposure. It's actually quite a problem.

Michelle Heacock: So, David, do you have any reason to believe that it would be much different in humans versus animals?

David Thomas: I don't know, because humans have -- I mean, if you look at urinary metabolic profiles, humans have one that differs from most other species in the distribution of inorganic monomethyl and dimethyl in terms of the fractional amounts. So whether or not animal data is a good predictor or not really depends on having enough human data to test that out.

Michelle Heacock: Okay. And I've got another question that ties in with what we're talking about, is how long, or what is -- do we know what the difference is in the half-life between the various arsenic species?

Mary Kay O'Rourke: I certainly don't. Once again, I'll punt that to David.

Michelle Heacock: Poor David.

David Thomas: Well, there is one human study that was done by John [Bouchet] and his associates back in the early 1980s with human volunteers, and it does show that if you administer orally arsenate dimethyl, or monomethylarsonic acid, which is the +5 oxidation state, or dimethyl arsenic acid to volunteers and collect their urine, that as you add a methyl group you increase the rate of clearance in the urine. So methylation does promote clearance. There's no question about it.

Danielle Carlin: Okay, so, David, we need to move on to the next question.

David Thomas: Okay, we're now on Question No. 4. Is understanding arsenic speciation in the environment more relevant for exposure/risk assessment or for determining fate and transport? And the discussant is Matthew Polizzotto, from North Carolina State.

Danielle Carlin: Matt's not with us.

David Thomas: That's right. So are we doing this as a group?

Danielle Carlin: So, David, if you want to just maybe say a few things about his slide, and then we can open it up for discussion.

David Thomas: Okay. Well, you can see Matt has three bullet points on his slide. And I think the first one, understanding speciation is critical for both evaluating the potential exposure and determining fate and transport. And if you think about it in more detail, exposure is related to fate and transport in the environment.

And speciation does control the mobility of arsenic in the environment as we were hearing earlier with the issue about flooded rice fields changing these conditions in the soil that affects the uptake of arsenic by rice plants. And that's clearly a good example of how mobility -- speciation affects mobility. It affects the entry into the food chain, or, conversely, the amount that's [immobilized] in the soil or sediment on the (inaudible) soil.

And the question, there's (inaudible) about mitigating exposure is a question about whether you can control the geochemical environment to limit the bioavailability of arsenic as it's related to mobility and transport in the -- both in the soil and in the transfer that might occur from soil to organisms.

And can we -- do we have some comments on this from the panelists?

Mary Lou Guerinot: This is Mary Lou. So I'm not a soil scientist, and I've already sort of addressed the anaerobic versus aerobic. So basically you're talking about arsenic(III) being really mobile under anaerobic conditions in the paddies and then arsenic(V) is really the [piece] that the plant is taking up under aerobic conditions. And then the question is regardless of which form they take up, what happens to it when it gets in the organism? Is it getting metabolites? Is it getting methylated?

But as far as mitigating exposure, I'll just say that in addition to thinking about aerobic versus anaerobic in the case of plants, people are also thinking about fertilizing with a competitor, so, for example, if the arsenite's coming in via the silicate pathway, does fertilizing with silicate then as a competitor decrease the amount of arsenite that would be taken up? So people are thinking that way also.

David Thomas: Right. Right, And, Arthur, excuse me, Albert, do you want to say something about these issues about remediation efforts to reduce the bioavailability of arsenics in soils?

Albert Juhasz: Yes, well, I'll start out by saying that speciation is extremely important, whether you're looking at speciation within rice, other plants or foods and also contaminated soils and dust. When you're undertaking bioavailability or bioaccessibility assessment, you need to have other lines of evidence, as well. So if you have an idea of the speciation with a contaminated soil, then that will give you an idea of potentially soluble phases. And if you can link that up to the bioavailability or bioaccessibility data, it just gives you a little bit more strength in terms of the interpretation of that data.

In terms of minimizing bioavailability through amendments, there has been a little bit of work

done on that, namely looking at the influence of amorphous iron additions into soils to minimize arsenic bioaccessibility and bioavailability. But also the point that Mary Lou made, that, well, you can add other things into the system that might compete for uptake, phosphate in the case of arsenite. So if you have an arsenic-impacted soil that's got (inaudible) phosphate in the system, potentially you can decrease the arsenic relative bioavailability.

But if you're assessing it using an in vitro methodology, you may actually see the opposite result. You may see an increase in arsenic bioaccessibility, because it's out competing arsenic (inaudible) on the iron oxide, so you're releasing more into solution, therefore your bioaccessibility determination goes up. So, again, in terms of remediation efforts, there's still a little bit of work that needs to be done in terms of having both bioavailability and bioaccessibility methodologies match up so you can use your surrogate methodology with some confidence.

David Thomas: I think it might be interesting to point out --

Mary Kay O'Rourke: This is Mary Kay O'Rourke. The other --

David Thomas: Sure. Go ahead.

Mary Kay O'Rourke: The other thing I would point out is you've also got to consider this as part of an aggregate exposure. So what happens when the dust is inhaled? What's the bioavailability that way as opposed to what's been ingested? So you've got whole organisms here. The uptake is a lot easier when we talk about plants, but we have multiple media, multiple pathways for humans that we have to discuss, and that's going to be different depending on each of those pathways.

Albert Juhasz: That's absolutely right. And I think another important point when you're looking at soils and dust is the particle size. So if you're talking about inhalation, well, if you're inhaling the less than 10 micron then it may not go into the lungs, but it may be then transported out and swallowed. And so then you're looking at not an inhalation, per se, but through (inaudible) ingestion. So particle size is extremely important, and also the other things that go with that, where there's the potential for enrichment of arsenic in smaller particle sizes, so your exposure might actually be higher with those as you decrease (inaudible) particle sizes.

Mary Kay O'Rourke: Absolutely agreed.

David Thomas: I think it's interesting to point out that there's really -- there's two aspects of this, what's happening in the environment. One's really is the physical chemical properties of soils that Albert's talking about that have the potential for affecting the (inaudible) out of the soil matrix. And the other thing is really the microbial or whatever, microbial component of the environment in the soil that, for instance, seems to play a role in the dimethyl -- formation of dimethylarsenate that's taken up by rice plants. So you have speciation being driven by strictly physical chemical properties and also by biological processes.

Danielle Carlin: So, David, unless there's any other discussion from the other panel members, we have not received any specific comments regarding this question, so, again, I think if the

panelists want to say anything else, if not, we can move on to the next question.

David Thomas: Okay, all right. Without any other comments we have the fifth question, which is do available models adequately represent aggregate exposure to arsenic? What is limiting -- the model or the data? And this is open for full panel discussion, so I'll ask people to chime in here and we'll see what comments we get online.

Mary Lou Guerinot: Well, I'm not a modeler, but I would say based on our discussion so far we are slowly racking in data for the modelers to use.

Margaret Kurzius-Spencer: I would second that.

David Thomas: So do you think we have enough information about -- I think -- I guess -- is there a consensus there's a lack of information about the amount of information about chemical forms of arsenic in foods? Do we have a reasonable understanding of what the right (inaudible)?

Mary Kay O'Rourke: I would agree with that, David. I think we know very little about foods. We only know a little about a very limited number of foods. And one of the things we found with some of the work Roberge did with the apple juice and apples in general, sometimes we think we know something about foods but we're not -- until you analyze it you do not know it, and that has to be examined more extensively, for even those foods that are not currently listed as arsenic-bearing foods.

David Thomas: Let me extend that a little farther. In the first question there was a discussion about using the Total Diet Study data versus the Schoof -- 1999 Schoof data and getting these what you think are overestimates or underestimates of the intake. What do you think that reflects? What does that tell us about the data?

Margaret Kurzius-Spencer: It may -- this is Margaret -- it may in part reflect that the variability that I was also discussing and the fact that what people eat isn't necessarily the same as what has been measured in samples collected by TDS or Schoof, which is why I think that it is important to do duplicate food sampling to see exactly what people are eating -- maybe not exactly but approximately what people are eating and what the arsenic content, including species, are in the food that they eat, including how they prepare it, which has an impact.

Mary Kay O'Rourke: And the complexity of the other foods that they eat with it.

Margaret Kurzius-Spencer: That's true.

Margaret Kurzius-Spencer: Mary Lou, I have a little bit of a question for you. Basically, how well do you think that the factors involved in the variability of arsenic in rice are understood? I mean, you discussed differences in cultivars, in soils and the water regime and such, and can you differentiate basically the causes of arsenic speciation in any particular cultivar based on those factors?

Mary Lou Guerinot: No, we're at a very descriptive point. We're really just screening. And then

this is one of the points I wanted to make, when we're screening we're usually screening total arsenic. And then if you really want to find out like inorganic versus methylated forms, we have almost no data on that. There's a study by Williams where there's alarming high levels of total arsenic in US rice, higher than rice from Bangladesh, but when they went in and looked at inorganic, then US rice had some of the lowest values.

Margaret Kurzius-Spencer: Yes.

Mary Lou Guerinot: So we can say that, but we don't understand why.

Margaret Kurzius-Spencer: Yes.

Mary Lou Guerinot: And that's, you know, that's a very long-term goal is to figure that out. Because if we can't get rid of arsenic in the soil and water, then we have to find plants that don't take it up or that don't metabolize it if it is getting metabolized.

Margaret Kurzius-Spencer: Do you have any knowledge or sort of an idea of what might happen to arsenic in other plants that may not accumulate it but that appear to have relatively high concentrations, at least in some of the samples?

Mary Lou Guerinot: I mean, there really isn't a lot out there. I mean, grasses are better at taking up arsenite, because they tend to take up silicate. But any plant that is going to take up phosphate has the potential to take up arsenate. The transporters don't tend to discriminate. They -- it looks a lot like phosphate, and they'll take it up.

Mary Kay O'Rourke: Okay. And, David, I'm rereading the question here and looking at the question about what is limiting, the model or the data. I think when it comes to the data inputs, certainly we don't have enough data. By the same token, I'm going to ask you this question. The model from a pharmacokinetic standpoint, one of the things I think I heard you say is that we have a paucity of human data in terms of what the human body does at various tissue types, what those exposures are at various tissue levels. And I'm assuming, then, we're using the animal data to project into that model. How effective and accurate do you think that actually is?

David Thomas: Well, that's a concern, because you don't have -- you're lacking a whole lot of human data to confirm the estimates of the model. So you can -- the models are based on sort of essentially scaling an animal-based model to the human physiology, physiological conditions, and then you would like to have -- so that model will give you predictions of the profiles of different metabolites in different tissues, including the tissues that you think are the target tissues for toxicity.

But what you would really like to have is a body of human data to confirm or to evaluate the quality of those estimates of concentrations in your tissues. And that really is lacking. You have scattered data from some very high exposures, some of which are homicidal or suicidal uses of inorganic arsenic. You have a few samples from environmental exposures at typically fairly high levels. So you're really not -- that gives you concern. I mean, you would like to have validation of those estimates.

Mary Kay O'Rourke: So the models themselves are potentially flawed until we get more data.

David Thomas: Well, I don't know if they're flawed. They may -- it seems to -- you might say they're not fully developed. They're not --

Mary Kay O'Rourke: Yes, that's fine.

David Thomas: I don't think they're conceptually flawed. They may just lack the level of validation that you would like to have to be fully confident about the estimate.

Margaret Kurzius-Spencer: David, this is Margaret. How do you think we could get more data or information on arsenic distribution in human tissue?

David Thomas: Well, you just have to get tissues from exposed individuals, and which actually means you need to know their exposure, which is -- you just -- going in and just collecting (inaudible) tissue from random donors doesn't tell you much, because you're lacking the exposure dimension on those individuals, so what you measure, you can't really tie it back to a specific exposure.

So it'd be nice to have a study where you had a population where you aggregate exposure and you were at least getting something like blood samples from those people. That would be a useful piece of information that you could --

Margaret Kurzius-Spencer: By the same token, could you do something with autopsy data where you are using the hair as a biomarker, so you would have an idea what the exposure was over the last month, and then you could look at the tissue-specific -- or are the items we're looking at in these tissues transient in a way that an idea like that would not work?

David Thomas: I don't know, I mean, the question is what the degradation of species or the alteration or the postmortem alteration of species and tissues would be. That's something, again, in humans I don't think we have adequate data to even -- to evaluate that. But if you could tie some integrated measure of exposure like toenail arsenic to your tissue analysis it might give you at least some estimate of the level of exposure. It's actually surprising when you go to look for human tissue arsenic data how scattered the data and how little data there are available.

Mary Kay O'Rourke: And that would be interesting, because you could actually get the nail at the nail bed, and so you could have a greater temporal correlation between the nail and the tissue than you would have necessarily in a standard study where the nail is growing out at, oh, I don't know, a couple of millimeters per month and you've got to back calculate what that exposure was at the time that the nail formed.

David Thomas: Yes, I don't know what the temporal resolution across the nail would be. It's just like the question about what the temporal resolution is on hair. Mercury is probably the gold standard for temporal resolution in hair. I don't know. There's even -- I don't know whether there's enough data out there on arsenic to see -- so you can reconstruct exposure that way.

Danielle Carlin: David, we have a few questions that have come in. Michelle, do you want to ask them?

Michelle Heacock: Yes. This actually ties in really nicely with what you were just discussing, and that is, could you comment on the correlation between a mother's arsenic level and her newborn's arsenic level? How does one measure the level in a newborn? Would arsenic show up in toenails or in the hair of the baby?

David Thomas: Well, there are maternal (inaudible) cord blood studies from -- I know from -- I think from Bangladesh and possibly from other populations. And I'm not fully -- I don't remember the details, but I think the cord blood and the maternal bloods were actually quite well correlated, indicating this arsenic's crossing the placental barrier, and the exposure of the fetus is based on the -- I mean, mimicking the exposure of the mother. But there are data out there that you can look at that.

Michelle Heacock: And one -- another question that's related to measuring arsenic in hair is a clarification that can you measure -- can the hair length give a temporal plot of arsenic exposure?

David Thomas: Yes, that's this issue of temporal resolution along the length of the hair, because the hair is an extruded product and grows out from the root to the tip, so that gives you a timeline. And with -- the example, again, is with mercury, where you can actually get a pretty good reconstruction of exposure in methyl mercury exposed fish eaters of their exposure by looking at the changes in concentration along the length of the hair. And I do not know if that has been done for arsenic with any degree of resolution.

Michelle Heacock: Okay. And I just wanted to comment that audience members seem to be agreeing with the panel on this question that it seems like we need more sort of adjustment of the model and more data. So a few comments have arrived. And the first one is we need TDS or (inaudible) to regularly analyze both total and inorganic arsenic in foods. The key would be obtaining low enough detection limits.

And the question -- I had another question come in as I was looking at that other one. I've lost the second one. Here we go. And then the second comment is a concerted effort to measure and compare biomarkers of different types in conjunction with a complete dietary analysis might provide the data needed for adequate modeling. I don't know if you wanted to comment, anyone wanted to comment on that.

David Thomas: Well, I mean, I would certainly think those are ideas that would not be hard to find people to endorse them, because it provides the sorts of information we've been talking about today.

Mary Kay O'Rourke: And I would add to that if we have a known scenario where you have differential uptake up arsenic and cadmium, and I think Margaret has done a little work along these lines, you can't look at arsenic when you're thinking about these dietary things in and of itself, because you're going to have these tradeoffs. And if you have the tradeoff within the plant,

and I have no data to back this up, it would not surprise me if you have tradeoff within a human body.

Michelle Heacock: And along the lines of modeling, we had a question on what are your thoughts on in silico modeling of arsenic accumulation?

David Thomas: Sorry, what was it, in silico?

Michelle Heacock: Yes, computer modeling.

David Thomas: Yes, well, I mean, there are examples of that I think in the literature trying to build a functional model to look at cellular uptakes, cellular response. I think it has value. I think probably the limiting factor is always this issue of having data to -- actual data to confirm the predictions of the model and to test the predictions of the model so you can actually go back and see whether your model is adequately representing what you want -- the process, the phenomenon that you want to represent.

Michelle Heacock: And another question that came through was are there any biomarkers of biological effects of the consequence of exposure to arsenic?

Margaret Kurzius-Spencer: This is Margaret. We've found a relationship between arsenic exposure and MMP9 in both sputum and blood. And I think other people -- I know other people have found effects on DNA methylation and probably also some oxidative effects.

Mary Kay O'Rourke: And I think another segment of our group has also been looking at relationships with osteoporosis or sarcopenia.

David Thomas: There are probably a lot of studies that have identified a range of biomarkers of effect. The question is whether you can organize them into types. Are there -- is there a class of biomarkers that relate to oxidative changes in cells or a class that's related to altered DNA function, and can you -- are they both sensitive and specific when you want to apply them to studies?

Michelle Heacock: Another question is at the molecular level are there data on where arsenic is accumulated in the cell?

David Thomas: Actually, there's not a whole lot of data on that. There's some data on subcellular distribution. But the molecular -- the cellular molecular target, or molecular targets in cells of arsenic are really not well characterized. There's not a real good cellular budget for arsenic showing how it's distributed among different components, compartments of the cell.

Michelle Heacock: There's a question on inhalation of arsenic. So how good are the data on the bioavailability of arsenic through inhalation of dust? So, for instance, air in environments for off-road vehicle drivers.

David Thomas: Well, that sounds like a question for the people from Arizona.

Margaret Kurzius-Spencer: Well, it sounds like a good question. There's more data on inhalation of arsenic from burning coal in China, and I don't know of any information on the effects of off-road vehicle driving in Arizona or places where there's naturally occurring high arsenic.

Mary Kay O'Rourke: I don't know of anything with ORV data, but part of our group with our Superfund site is doing some work specifically with dust in children in a contaminated location, and those data are not ready for prime time yet, but I'd expect something soon.

David Thomas: And there is a little occupational data with, I guess, probably smelter workers showing a pretty strong correlation between air arsenic levels and urinary arsenic levels over -- although, again, pretty high levels of exposure. And they're usually typically mixed exposure to arsenic and other metals and metalloids in that environment.

Mary Kay O'Rourke: Oh, God, I'd forgotten. We did some work back 2000, 2003, where we were working in a smelter community and we were looking specifically at arsenic and dust, and we sampled, oh, I don't remember how many, maybe 150 homes and ended up with urine samples. And the only place where we found a high occurrence was actually a house where the people were remodeling, and they ripped up the carpet, which liberated vast quantities of high-arsenic dust, and then we saw it in the urinary biomarkers. But with day in, day out low-dose experience that we would've attributed to inhalation, and we did no control for ingestion, we did no control for water concentrations, at that point we were attributing it virtually all to inhalation. We found very little association with urine except in this one high-exposure scenario.

Albert Juhasz: I think some data like that should be viewed with a little bit of caution, because it may not necessarily be the inhalation exposure. It may still be an ingestion exposure pathway.

Mary Kay O'Rourke: Absolutely.

Albert Juhasz: And try to differentiate -- to try to differentiate between the two is extremely difficult.

Mary Kay O'Rourke: Absolutely agree.

Danielle Carlin: I think we have time for maybe one or two questions, and then we need to wrap up.

Michelle Heacock: Okay, we've got a question looking back to early life exposures again. Has anybody looked at meconium, arsenic and cord blood relationships along with the mother's -- the exposures of the mother?

David Thomas: I don't think meconium has been sampled, but I may be corrected on that. I don't recall that being (inaudible) that was being collected in those mother-child exposure studies. It would be interesting, actually, to know.

Margaret Kurzius-Spencer: Can I -- this is Margaret -- can I ask you a question, Dave, whether

fecal samples have been evaluated even in mice in terms of arsenic exposure?

David Thomas: Yes, there's data on fecal arsenic in mice in relation to some oral bioavailability studies. Human data, I'm not actually aware of anything offhand.

Margaret Kurzius-Spencer: Is arsenic speciated in feces?

David Thomas: There's some data on that. There's a lot of inorganic and a lot of -- and dimethyl, as I recall, in mouse feces. So the metabolites are getting -- there's some biliary excretion of metabolites, so we think some of it comes back that way.

Danielle Carlin: Okay, so, David, we have to wrap it up then.

David Thomas: Well, just let me thank the panelists for doing this, and NIEHS and Danielle for putting this session together and the support staff for making it all work for us, and turn it over to Danielle to finish up.

Danielle Carlin: Thank you so much, David. You did a wonderful job at moderating today's session.

So I just want to conclude this webinar with a few closing remarks. First, our next and last webinar -- yes, unfortunately, our last webinar -- is scheduled for June 3, 2014, at 3:00 p.m. Eastern Time, and it's titled Prevention and Remediation Strategies for Arsenic Exposure, and that's going to be moderated by my colleague, Dr. Heather Henry, who's with the Superfund Research Program. So I highly encourage you to register for this webinar, and you can do so by going to, again, the link at the very bottom in green, bit.ly/ArsenicSeries. You can also go to the NIEHS Arsenic Workshop website for more information and to find the registration link.

I also wanted to mention I've received several questions about these webinars being recorded, and they are being recorded, and we are working on trying to get transcripts available. There's quite a bit of logistics that go into posting these webinars onto our website, but we are actively trying to get these webinars onto the website. So stay tuned. It's going to probably take a little while longer.

And I also want to mention that if you're interested in more information about all of our speakers you can find their full biographies on our website. And then I also -- for those of you that have questions and comments that are in the audience that were not addressed in our session today, we will send those to the speakers, and their responses will be captured in the final report from the workshop.

So now can we go to the next slide? Okay, so of course all of the acknowledgements. I'd like to just start out by thanking Dr. David Thomas again, did a really great job at being a very interactive moderator; our panelists, especially Dr. Albert Juhasz, for calling all the way in from Australia, so we hope we didn't keep you up to late; and, again, to all of our other panelists for just having a great conversation today.

I'd like to thank all my colleagues with the Superfund Research Program, especially Michelle Heacock, who has done such a great job at fielding all of the questions today; our contracting team, MDB, Inc., both Justin Crane and Maureen Avakian for keeping everybody on top of things; and then, finally, Marisa Naujokas for helping us out with the Workshop Publication.

So the final slide. And I think that pretty much concludes everything. Again, thank you to our audience and to everybody that joined us today and our moderator and our speakers. And I think we'll end and see you all again on June 3.