

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

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

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Danielle Carlin: Well, welcome, everybody, to the first webinar in the Health Effects and Mitigation of Arsenic: Current Research Efforts and Future Directions workshop panel discussion webinar series. My name is Danielle Carlin and I'm one of the program administrators with the Superfund Research Program that's here at the National Institute of Environmental Health Sciences.

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. The topics will include exposure sources and mitigation, remediation, bioavailability, contributions of advanced techniques, and susceptibility.

These panel discussions today stem from the workshop that was held back in Research Triangle Park North Carolina on March 3 and 4, 2014. The panel discussions as they were originally scheduled to be held at that workshop but were postponed, as you probably all remember, due to inclement weather.

This webinar is the first in the series and is titled Contributions of Advanced Techniques to Understanding Arsenic in Health and the Environment and we invited our leading researchers to serve as panelists to support discussion on specific questions. You will hear more about the webinar format from our session moderator shortly, which is going to be Dr. Mike Waalkes from NIEHS.

We're grateful to the session moderator and the panelists for their time and effort in this webinar.

Our next webinar, I just want to remind everybody, will be held tomorrow, May 7th at 1:30 p.m. Eastern Time and you can register for that webinar as well as any of the other future webinars at the link, which is bit.ly/ArsenicSeries. And that's Arsenic with a capital A and Series with a capital S. The link is case sensitive, so you need to use capital A and S in the terms ArsenicSeries. And I just want to mention it's also -- our contractor here, Justin Crane, has also posted the link on the webinar.

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

We encourage you in the audience to submit your written questions and comments during the webinar as well. The session moderator will tell you more about the format of this webinar discussion. Information from the workshop from this webinar series will be captured in a report that's being written by Dr. Marisa Naujokas from MDB, Inc.

The session moderator for today's webinar, as I mentioned before, is Dr. Mike Waalkes. Mike is the group leader of the inorganic toxicology group and the Chief of the National Toxicology Program laboratory at the NIEHS. He is also currently an adjunct professor of molecular toxicology at Duke University.

Mike, I just would like to thank you for moderating this session and I'm going to turn it over to you.

Mike Waalkes: All right.

(Operator instructions)

NIEHS will collect all write-in questions and comments for consideration in the final report.

Panelists were tasked with a single question and their proposed answers will be shown in the slides to serve as a starting point for discussion. We can discuss changes to the proposed answer, and from this discussion we hope to capture data gaps in order to guide future research.

Now briefly review the questions and the panelists today for this session.

The first question is what are the most appropriate assessment methods for acute and chronic arsenic exposure in humans?

The panelist will be Miranda Loh who is of the University of Arizona and Badawi Dweik of Giner, Inc.

The second question will be what biomarkers are the best to predict human arsenic-induced diseases? And a secondary question, are there disease-specific biomarkers?

The panelists here will be Barry Rosen of Florida International University and Maria Argos of the University of Illinois at Chicago.

And the third question is what is the impact of the microbiome on arsenic and does the microbiome alter arsenic metabolism?

And this will be answered by Kun Lu of the University of Georgia.

And the fourth question is, what are other complex exposures that have been associated with arsenic and what data are needed to determine the effects of arsenic with these other exposures, such as metals and poly-aromatic hydrocarbons?

The panelist here completing the discussion will be Luz Maria Del Raso of the Mexican National Polytechnic Institute.

So, we can now proceed to the first question presented by Miranda Loh.

Miranda Loh: Okay so I'm going to talk us a little bit about assessment methods for acute and chronic arsenic exposure in humans. First, I wanted to say there's different ways that we can assess exposure. We can measure it and we can measure it in the environment. Different ways people can be exposed to arsenic would be through drinking water or food or accidental ingestion of soil and dust.

Another way that we can measure it is through biomarkers of exposure and these would be measurements that we take in, for example, urine -- is very commonly done as well as your toe nails or finger nails -- are another relatively commonly measured matrix for taking biomarkers of exposure.

Another way which we can also do for assessing exposure is to model it and modeling alone is usually obviously not good enough so you need some measurement. I just want to put that out there as three different types of methods that we can use.

The next thing would be to really define, what is acute exposure and what is chronic exposure? And this is maybe not as easy. As I was thinking about it, there isn't necessarily, as far as I know, some particular level that is said to be acute. There's lethal doses -- and minimum lethal dose has been defined as about 1 to 3 mg/kg, so that's 1 to 3 mg of arsenic per kg of body weight, which is a very high exposure.

Most people in general wouldn't be accidentally exposed to this by ingesting it. Certainly in occupation settings there may be higher risk of being exposed but we'll limit this to just general public exposures at environmental levels. In terms of measurement, you can measure arsenic in blood, however, because it has a very short half-life in blood, so only a couple of hours, you would have to be able to measure it very close to the exposure point.

For an acute exposure, which usually means a high short-term exposure, I would recommend urinary arsenic. Of course a 24-h sample would be better but I think some people found that spot samples are adequate. The question is what kind of exposure are we interested in? Usually inorganic arsenic is what we consider as to being more toxic. Things like seafood may have higher levels of organic arsenic and therefore you would want to be able to distinguish this by, for example, speciating arsenic.

In terms of what is an acute level – this is also difficult. I have here that total arsenic greater than 100 µg/L in concentration has been recommended for further examination for possible sources and species. I would probably actually move that to chronic.

I've seen different numbers. I've seen 50 µg/L. I've also seen that, you know, something that is close to two in arsenic – what might be acute poisoning might be closer to above 200 µg/L where you might consider treatment.

Again, what I'm trying to say here is you really have to decide what we consider as being an acute versus chronic exposure.

For chronic, I kind of use as a guideline what the EPA has currently in the IRIS database for their non-cancer risk assessment. This value of 0.3 µg/kg of body weight per day means that if you are exposed under this level, it's considered to be – you're at relatively low risk of any appreciable health effects. And above that, there's a higher risk. It doesn't mean that you necessarily will be getting some kind of health effect.

We'll talk about chronic long-term exposures the risks tend to be things like cancer and perhaps some of the other things that have been associated with arsenic exposure such as diabetes and cardiovascular disease. Again, we can use urinary arsenic and most people tend to use urinary arsenic as a form of exposure.

However, for chronic exposures, we have to remember that urinary arsenic reflects about two to four days of exposure and we would want to actually measure this over and over again over time. Another form of measurement and biomarkers that we use is toe nails. If you can cut your toe nail where it grows from the nail bed and maybe cut them as that moves forward out of your nail bed, we could say that your toe nails would be reflective of perhaps a couple months of past exposure, so not your immediate exposure.

The benefit for them is that they're easy to collect and they are usually thought of as a longer term average

measure. But of course there's a target. We don't have a guideline level for toe nail concentrations and some epi studies have started using them as a marker of exposure so perhaps there can be some analysis of this for certain levels. But it hasn't been used as much as urine in the past.

So neither one of these methods is perfect at really getting at your total exposure unless you really have a really constant level of arsenic in your body where you're taking in about the same amount every day. These are going to be subject to variations and time.

One suggestion I would have for chronic exposure, at least, is to perhaps supplement it with modeling if you have a sense of how much may be ingested either via drinking water and if that's a relatively constant source for drinking water with food, you can – we can use certain methods, for example, of probabilistic modeling to try and fill in the gaps, so to speak, between, for example, measurements -- biomarker measurements-- which is urine measurements -- that are done periodically.

That's kind of the quick summary of some things that I would like to say about arsenic exposure methods for chronic and acute methods. I'm not sure if the other panelists have something in particular to comment on this but these are sort of my recommendations.

Mike Waalkes: Well, let's move onto the other panelists who had this question then.

Badawi Dweik: Yes. Good afternoon, everybody. I think I agree with Miranda regarding the arsenic being used in the urine as the most relevant and widely accepted method so far to assess exposure in the detection of arsenic. However, it's very important for the toxicity of the arsenic in the body not only to find what's the total amount of the arsenic metal present but also it's very important to understand what forms that arsenic comes with. There is – as we know the exposure of arsenic comes from different ways and through dietary basically and drinking water.

What – you know—urine is used indicator for an exposure like maybe up to 24-h to 48-h and it's a good biomarker. It can correlate also for exposure. In general, [community] has used it in many of those studies to reflect on that but the life time of the arsenic species is relatively short in the blood as in urine. So if somebody had been exposed to urine before and would like to assess the previous history, urine might not be a good indication.

Understanding the urine from the chemistry point of view, there are several structure forms and oxidation states for arsenic because it kind of form sometimes alloys with other metals and sometimes covalent bonds with other existing compounds like hydrogen, oxygen, carbon and other elements.

If it comes in the inorganic forms, which means does not contain carbon, this compound is the most which is most of the concern. And within the inorganic also you can see it has in different forms and comes as arsenic three and arsenic five within inorganic. Example of those the arsenite, which is arsenic three and arsenic five. However, there is other forms which is less of a toxic form, which is organic arsenic and thus comes usually those being neutralized by bacteria in the plants and animals and they have to contain mostly carbon and hydrogen.

So if somebody would like to do an indication of what is the arsenic being exposed, he has to give some speciation with that form. However, there is some difficulties involved with that because this arsenic species itself is sensitive to the environment, which is like, for example, if you want to take the samples and ship it and move it from one place to another, there is a certain requirement you have to keep in place to keep that form of what you received the sample on to be able to be giving a good indication.

If this sample is been exposed to oxygen, because if you look at what is the difference between arsenite,

which is arsenic three and arsenate, which is arsenic five – I'm talking about the inorganic form—it's just an oxygen being added to the arsenic five. And this species itself if the water's been exposed to oxygen, if the form of the arsenic is not maintained, so maybe the speciation might not reflect what you have started with, if it was not kept properly.

Overall, in urine the two main groups that can be categorized is the total of inorganic and the total of organic. And within organic you have the two species which is the dimethyl arsenic acid, which is referred to as DMA and the monomethyl arsenic acid, which is the MMA.

Sometimes in the body as referred by the previous speaker, you may have a high concentration of arsenic which comes from the sea food and that might not be considered temporary, so might not reflect what has been as something to worry much about it– I mean for a long term.

So for example you can see in the body because I'm going back to the requirement from EPA. EPA has the maximum allowed arsenic in the drinking water not to exceed 10 parts per billion and this is including the total arsenic. In the case if you been exposed to sea food, you may come and seeing arsenobetaine, which is a form of organic arsenic and you may have a high concentration in the urine for the temporary period. That's why it's recommended not to have sea food taken if you would like to have a good assessment of what type of arsenic that you are being exposed to.

Again, the common method using the speciation is using [synthro] labs. It is very expensive, also costly to be able to look for those speciation of arsenic. So even though they are important but the test itself might be costly because they are relying on the current method.

From our point of view in the detecting and measurement, we are trying to work on providing new ways to measure those species by using a cheaper and lower cost and more of an easy to measure on-site devices. For example, if you would like to assess a certain exposurable population or a small area to arsenic, you may have to go through those logistic, collecting samples, sending them to the lab, [and to to] speciation that would be little bit more difficult and more expensive.

If you are able to have a device that you can be able to do this and perform it on-site, you could save a lot of effort and you could be really having new tools to know more about the people being exposed. So from the point of view that us, as a company working on this area, we're trying to provide a new way of working on sensors to assess the urine exposure or a different toxic form.

I can talk more but I will leave some time for questions because I think the time has been going.

Mike Waalkes:Are there comments from the rest of the panel?

Danielle Carlin:Mike? We only have time for one minute.

Mike Waalkes:Okay. Well, let me say that it seems to be a consensus then on the answer of this, if I can sum between the two, that at least with chronic arsenic exposure, the feel I get is that urinary arsenic levels are the best method for assessment. Is that what you both feel?

Badawi Dweik:I think for acute exposure, urine works, no problem well because this is – now for a chronic it depends. Urine works well if the population has been in a steady exposure to this environment, the food and the drink and if somebody have just one sea food or been exposed long time ago, the chronic exposure might not, using the urine, might not be the best, so you may go to the nail and toes– this is what I see. However, I'm not – our expertise comes in the measurement of the species that the medical community see, which is needed for assessing exposure.

Maybe Miranda would like to add more?

Miranda Loh: Yes, I agree with Badawi that for acute exposure, I think urine is probably the best way to go. For chronic I think you probably need a combination of methods. You could do urine but I think if you only do urine, you would need several measures over time and even that I think that's better -- and it depends how well you space them apart.

I think for chronic exposure it would be useful to have some measurement of external exposure. So, if you know that the exposure is most likely happening primarily through water, drinking water, or through diet, or both, if you can somehow get a measurement of that or do some combination of measurement and modeling of exposures, so trying to figure out how much people drink in their water and do they drink water from the same source.

For dietary exposures I really think unfortunately that modeling is the best way to go because it's very difficult to get good measures of dietary exposure that are consistent over time. It's just expensive and there's a lot of things to consider.

I would recommend for chronic actually doing a combination of biomarkers and monitoring if possible. And I think toe nails do have their value because they give you a sense of past exposures as well. So, that would be my answer for that.

Badawi Dweik: Did you think of (multiple speakers)

Mike Waalkes: (Multiple speakers) Over all then it's not one answer. It's kind of complex then?

Miranda Loh: For chronic, yes. I think so. Other people might have another opinion on that but I think it's not something that you can take one measurement and say well, we know.

Mike Waalkes: Well, okay.

Badawi Dweik: Have you used the hair? Did you? I don't know, nobody mentioned the hair as exposed because we are not working on that domain but is hair considered one of those you can assess for human exposure to arsenic? I almost think that (multiple speakers).

Miranda Loh: Yes, you can use hair but so, hair is usually thought of as more easily contaminated and so toe nails you think of as being less likely to be contaminated by external pollution and it may be less likely to absorb anything too. So that's why generally for arsenic, toe nails are the more accepted method.

Mike Waalkes: Well, we need to move on here. But that -- thank you very much.

Badawi Dweik: Well, thank you.

Mike Waalkes: So can we move to the next slide? So, Maria.

Maria Argos: So the question that I was asked to think about was, what biomarkers are best to predict human arsenic-induced diseases and I think my response to this question is that I think in the upcoming years we'll be identifying new biomarkers from emerging omic technologies that will substantially enhance our ability to predict arsenic-induced diseases.

But in order to put these new omic data into perspective I do want to mention a biomarker that's currently

used in epidemiologic studies and I think has proven to be useful for broadly predicting disease among arsenic exposed populations and that's the measurement of urinary arsenic species.

So as mentioned by our previous speakers, individuals with greater proportion of monomethylated arsenic species in their urine have been shown to be at increased risk of developing several arsenic-related clinical outcomes such as skin lesions, cardiovascular disease, cancers, and mortality. This existing biomarker has been broadly informative with respect to predicting arsenic-induced diseases and I would say it's reflective of both current exposure dose and arsenic methylation capacity.

Many of us can think of several limitations of this biomarker for the prediction of arsenic-induced diseases that might be overcome with the use of the newly emerging omic data as biomarkers. Some of the main limitations with urinary arsenic species as a biomarker of arsenic-induced diseases is that it really only reflects perhaps one domain of susceptibility to arsenic and that's the ability to metabolize arsenic whereas there may be other susceptibility domains not captured by this biomarker that may also increase an individual's risk for one or several arsenic-induced diseases.

And secondly we can think that urinary arsenic species for some populations may just be a short-term indicator of exposure. So for certain diseases with a longer latency period, these markers may not accurately capture or reflect risk for disease outcome. And this is particularly true for populations where exposure is more transient or historical.

So I think with the emergence of new omic technologies the possibility for the discovery of perhaps a number of new biomarkers that can predict arsenic-induced diseases is possible.

The most informative biomarkers may be early biological effect biomarkers and these biomarkers would reflect intermediate changes between arsenic exposure and disease onset. Human studies are beginning to emerge that are observing potential arsenic related alterations on the transcriptome, epigenome, metabolome, proteome, and microbiome.

Many of these studies to date have been focused on characterizing the molecular mechanisms of arsenic exposure. Few, if any, of these studies have subsequently evaluated whether these molecular alterations are actually associated with predicting disease risk.

In order for us to really answer the question of what biomarkers are best to predict human arsenic-induced diseases, molecular epidemiology studies still need to accomplish several things. Most importantly studies need to evaluate whether exposure-related effects predict arsenic related diseases.

This will be accomplished through longitudinal studies using stored biological specimens, which potentially could make important contributions in this area through using method keys-controlled designs using existing biological specimens that were collected prior to the onset or clinical diagnosis of disease. I also think it will be important to use repeated samples from individuals so that the reliability of these biological measures can be evaluated across time.

We still need additional studies that are more integrated. So, examining different types of omic data within the same study sample and by doing this I think this will provide us with a panel of biomarkers that has enhanced specificity that may serve as an overall better biomarker for arsenic-induced diseases.

Since populations are often exposed to multiple chemicals that similarly could affect some of these intermediate pathways, I also think that future studies need to adequately be powered to evaluate what, in many cases, is very moderate effect sizes. Studies also need to evaluate whether there are dose dependent effects. So there may be different biomarkers that are reflective of arsenic-induced diseases based on low-

to-moderate dose exposures as compared to high exposure levels.

Many of the existing omic studies have evaluated these biomarkers in blood and while I do think that this is a useful approach from a population health perspective, I think we should also consider the evaluation of tissue specific markers and instances when this is feasible -- so perhaps when you have biopsy tissues available.

I think these are the studies that are needed using these new omic data sources to begin to identify and validate new biomarkers that predict arsenic-induced diseases suitable for use in population studies.

Perhaps another approach which would be considered for biomarkers is those indicating genetic susceptibility. So I think that there are likely to be genetic susceptibility markers of arsenic-induced diseases that we still need to identify.

For example, arsenic methyltransferase has been shown consistently to be associated with arsenic metabolism, which in turn has been shown to influence arsenic related diseases. However, through new statistical methods such as genome-wide interaction scans, we may actually be able to still identify other genetic variance that modify the effect of arsenic in relation to specific disease outcomes. I think this may prove to be useful for identifying high risk individuals in relation to specific arsenic-induced diseases within exposed populations.

So Daniell if you want to advance to the next slide.

So the second question I was asked to think about was are there disease-specific biomarkers?

With respect to this next question, I would say there likely are to be disease-specific biomarkers but they still need to be identified from human studies. We know arsenic is associated with a variety of disease conditions, epidemiologic evidence has supported associations to varying degrees of arsenic with cancers, cardiovascular disease, respiratory function, diabetes, neurologic, and cognitive outcomes. But the major underlying pathway or pathways for these diseases have not been yet well-established based on their association with arsenic.

It's possible that we may be able to identify biomarkers that reflect common underlying pathways but also biomarkers that may reflect disease-specific pathways. In terms of which of these biomarkers we need to prioritize, I think we need to consider about what our research objectives and what our public health goals are and this will I think lead us to which we should prioritize.

So from a public health perspective, I think we may be more broadly interested in biomarkers that are reflective of common underlying arsenic toxicity pathways. These will generally serve to identify higher risk populations for developing broadly, arsenic-induced diseases. These may be biomarkers that are blood-based or even genetic markers.

I think these types of biomarkers may be more suitable to situations where we have interventions that are primarily focused on exposure remediation or reduction for high risk populations. Whereas from an etiologic perspective I think we would be more interested in the disease specific biomarkers.

These may be either blood-based or tissue-based and these would primarily serve to enhance etiologic studies evaluating arsenic exposure in association with specific disease outcomes, perhaps in situations where we are evaluating the effects of arsenic exposure at low exposure doses.

I think these biomarkers would be better suited to interventions that are being used to inhibit biological

processes underlying specific disease pathways or where the biomarkers themselves are being used for screening or early diagnosis in clinical interventions.

So, I'll stop there.

Mike Waalkes: Okay, that's great. Can we move onto Barry then?

Barry Rosen: Okay, well, we've been hearing about the different biomarkers that are currently in use, which include blood, urine, sometimes more invasive analysis and biomarkers, and I was thinking about the title of our session which is contribution of advanced techniques. I was thinking that we need to develop new methods to look at exposure, new types of biomarkers.

As a microbial biochemist, I'm very interested in the microbiome that we'll hear about in the next question and it occurred to me that we could use the microbiome to tell us about exposure. I propose that we can use the genes of the human microbiomes, not just the gut microbiome but the dermal microbiome, the respiratory microbiome, the urogenital microbiome as diagnostic procedures to quantitatively assess exposure.

This would allow determination not only of how much exposure there is but also the roots of exposure. So we could tell the difference, for example, between respiratory exposure through something like coal dust or exposure through contact, from exposure in the diet.

I've listed here more – about a dozen genes that have been identified in my lab and others and these are all genes that are inducible by both inorganic and organic arsenicals. They're inducible down to levels of 10 to the minus 8th molar or even lower. Well below the 10 ppb that's the MCL for the EPA.

These genes are present in every organism, in different combinations, in every organism of all of the microbiomes. That means that they can be used as ways to detect exposure in the different roots through use of the different microbiomes.

Not only the gut microbiome but they're present in the fungi of the urogenital microbiome. They're present in the respiratory microbiome and for example in immunocompromised individuals that would have fungal infections these are present in those individuals. This could possibly be a new way of determining exposure in a noninvasive way.

These could all be detected through fecal specimens for oral exposure, swabs for skin exposure, nasal swabs, urogenital swabs. They can be done very sensitively down to about 1 ppb or less. They're very rapid and relatively inexpensive. It doesn't require any speciation. It doesn't require ICPMS and you can in fact design tips that would allow determination of multiple genes through the different roots of exposure at the same time. So, (multiple speakers)

Mike Waalkes: Can I ask a question here?

Barry Rosen: Certainly.

Mike Waalkes: Would you be able to look at say turnover of the microbiome for loss of induction of these – arsenic to tell whether or not you have an acute or chronic exposure?

Barry Rosen: This would not depend on which organisms are present in the microbiome because all of the organisms of the microbiome of all the microbiomes have different combinations of these genes. So, it might be possible to determine which species are doing this by the choice of the sequences that we use.

But to tell the difference between chronic and acute is possible because this is quantitative.

So we should be able to design ways to tell how much the exposure is – the level of exposure.

Mike Waalkes:Okay. Great. Sounds good.

Barry Rosen:So that's basically what I wanted to say and I'd be happy to talk about it – to discuss it.

Danielle Carlin:Mike, we have two more minutes.

Mike Waalkes:I think this is a fascinating concept of quantitation of exposure and if any of the other panelists have something to say, that's great.

Barry Rosen:Well, I think we need new technologies that are faster, cheaper, and better and this is a possible way to do it.

Danielle Carlin:So, Mike, Michelle Heacock here has some questions that have come in from our webinar audience.

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Mike Waalkes:Okay.

Michelle Heacock:So the question is, how do you differentiate arsenic modified specifically by humans from that by the microbiome?

Mike Waalkes:Barry?

Barry Rosen:Well, these genes would be expressed through exposure to environmental arsenicals. The ones that I've listed here are induced by inorganic arsenic or by organic arsenic and the exposure itself is what's inducing it. Not the metabolism by the microbiome itself. So you're looking at environmental exposure.

Michelle Heacock:And we've got another question that's come through as well – I think this might be for you – directed at you as well, Barry?

What about also looking for respiratory arsenate reductase aureus too -- in the GI tract? So, too, as in as well.

Barry Rosen:Yes, I think that each of the microbiomes would have all of these genes. So for example, respiratory organisms and both normal and pathogenic for example pseudomonas has most of the genes that are listed under bacterial genes and so it would be very easy to design primers that would be specific for pseudomonas infections.

I'm not sure if I answered the question.

Mike Waalkes:I think so, yes.

Daniell Carlin:[inaudible] I think in the sake of time we need to go on to the next question?

Mike Waalkes:Dr. Lu? Are you there?

Kun Lu: Good afternoon. Okay, the next question is what are other complex exposures that have been

associated with arsenic? What data are needed to determine the effects of arsenic with other pollutants?

This is important to have in mind that the presence of arsenic in high concentration combined with other pollutants, especially if the contamination is natural contamination (Speech accented word-indiscernible) a source of (Speech accented word-indiscernible) activities mineral dissolution (Speech accented- phrase indiscernible) is common to have the presence of other elements as Mg, Ca, . . . , Fe, Mn Cr, Ba, (speech accented word-indiscernible) fluoride, for example, (Speech accented phrase-indiscernible) especially in Latin America, as Argentina, New Mexico (Speech accented phrase-indiscernible) . . .

. . .the co-exposure with arsenic and other pollutants. And it's too common the level of these pollutants are elevated in drinking water, in general there is very good correlation the amount of arsenic with other pollutants. The most frequently and the most frequently positive correlations have been reported by barium, by vanadium, and also by fluoride.

Especially (Speech accented phrase-indiscernible) . . . we are especially interested in the exposure to arsenic and fluoride because both of these contaminants have very common—multiple actions. For example, the multiple action of arsenic is associated with the production of (Speech accented- phrase indiscernible) . . . and also fluoride is inhibitor of many enzymes associated with preserve redox homeostasis and it is too common that other contaminants as barium also and fluoride alter the homeostasis of the redox systems and can produce some interactions.

Other important source of combination of these pollutants is associated with anthropogenic or occupational activities. In this case it is common to have an exposure to arsenic present with other metals as lead and cadmium especially in places where they are exposed by (Speech accented word-indiscernible), for example what happens in Mexico, places where it is too common the combination of exposure to arsenic and this metal especially exposure to air or exposure to soil more than the water.

Also it is common the exposure to arsenic with the use of pesticides especially in areas with existing endemic problems with drinking water contamination and the area is an agricultural area that frequently uses pesticides. This is important to evaluate what happens with this combination of co-exposure or also the exposure with other pollutants as they (Speech accented phrase-indiscernible).

In most cases it is recognized the presence of arsenic will have the opportunity to evaluate other pollutants it's hard to know how to attribute the other pollutants effects with the effects that are generally attributed to arsenic. There are some cases that effects that are produced by the other contaminates—back to the case of fluoride for example—the exposure to fluoride produces several of the symptoms that are normally has been attributed to arsenic. As they produce (speech accented word-indiscernible) cardiovascular disease (Speech accented phrase-indiscernible) sickness. It is hard to know the contribution of only the fluoride, only the arsenic, and the combination of both of them.

The recommendation in this case is if the exposure is by drinking water the recommendation is to evaluate the other possible metals. If the people have the possibility to evaluate by using ICP-mass, for example, to know if there are present other metals that we need to consider by the effects in this area.

If the sources are anthropogenic or occupational, also to try to know what other metals or other pollutants will be co-exposure or will be in combination with arsenic. If we have the possibility to evaluate what is the presence for other contaminants we can look at the association in the environment and to look (Speech accented word-indiscernible) of the cellular process that will be affected.

In most of the case, the arsenic in combination with other pollutants could share many of the cellular processes that are associated, for example with metal, it is common to have inflammatory response that is

one of the effects that arsenic produces when it is alone.

It is common that a simultaneous exposure can induce oxidative stress and affect the (Speech accented word-indiscernible) system. Especially affected the levels of (Speech accented word-indiscernible). It is important to know if the co-exposure with other pollutants can affect the arsenic biotransformation.

We recognize that it is not enough to evaluate the exposure to arsenic alone—the total arsenic-- (Speech accented- phrase indiscernible) to evaluate the profile of the arsenic species. And some of these pollutants—possible pollutants— can (interrupt) the biotransformation of arsenic.

In the concrete case of co-exposure with fluoride we have preliminary results that we would find that the combination of arsenic with fluoride can reduce the ability of the methylation of arsenic.

It's important in case of population exposed to arsenic and other pollutants—for example, the case of fluoride—to also evaluate the biotransformation of arsenic evaluate during (Speech accented phrase-indiscernible) the profile of arsenic methylation.

Also it's important to have in mind that the presence of arsenic with other pollutants can alter all the process of absorption, distribution, (Speech accented-indiscernible), and in general toxicokinetics of the arsenic will be modified. And in consequence, the effects that generally have been associated only for arsenic and are present with other pollutants will be modified.

There is some information in (Speech accented word-indiscernible) that shows the combination of fluoride with arsenic, pesticides with arsenic, can produce interactions that affect the (Speech accented word-indiscernible), will produce (Speech accented word-indiscernible) effects or produce a (Speech accented work-indiscernible) of the effects. Especially if we can share some metabolic (Speech accented word-indiscernible) for example.

That is my intervention. Have in mind that if the contamination of arsenic by drinking water is by natural contamination—geological contamination—it is possible to have (in the presence) other methods or other elements that can contribute with the effects that have normally only been attributed to arsenic.

That's all.

Daniell Carlin: Mike, we have about four minutes.

Kun Lu: Thank you.

Mike Waalkes: Are there other points that the panelists would like to make? So you mentioned oxidative stress as other metals as one of the major things you might see with arsenic? (Multiple speakers)

Kun Lu: Yes, for example in the case of cadmium it is possible the combination of arsenic and cadmium, also the exposure to cadmium alone is associated with oxidative stress. And the combination with arsenic can increase this oxidative stress and can reduce the pool of glutathione (Speech accented word-indiscernible) and affect the profile for arsenic methylation.

Mike Waalkes: And you also see that other metals interfere with arsenic methylation?

Kun Lu: With Arsenic methylation is only especially with cadmium. With the cadmium I had reports that in combinations with cadmium also altered the biotransformation of inorganic arsenic.

Barry Rosen: One thing that's clear is that inside of cells there is no free arsenic. All the arsenic is tied up with glutathione, which means the pools of glutathione are greatly perturbed by binding to arsenic.

Kun Lu: Yes. Okay. Thank you.

Mike Waalkes: It would be a factor in oxidative stress certainly or the potential for oxidative stress certainly.

Kun Lu: Yes because (speech accented-indiscernible) (technical difficulty)

Daniell Carlin:[inaudible] so Michelle any call casts any more questions coming in from our audience?

Michelle Heacock: One question that has come in is you mentioned that concurrent exposure to arsenic with other elements can alter the toxicokinetics. Is there any research that you know of or any modeling that's been done that can key the part the difference between being exposed to arsenic say and fluoride versus cadmium or would studies be required to actually look at each separately?

Kun Lu: Unfortunately there is no time model. I have information in the co-exposure especially in mice for example and when the animals are exposed to the combination of fluoride, for example, and arsenic. The total concentration of arsenic decrease in presence of fluoride for example. In that situation and it is possible that the distribution of accumulation of arsenic will be altered. But using models especially for toxicokinetics will be (Speech accented word-indiscernible) but we will need more information about the experimental results.

Mike Waalkes: This seems to be one, if I could interject, that has many data gaps. Is it time to move on?

Danielle Carlin: Ah yes, yes, let's move back then to Dr. Kun Lu.

Mike Waalkes: Okay.

Kun Lu: Okay, okay. (speech accented-indiscernible) I am going to talk about the question about the impact of the microbiome on the arsenic because Dr. Barry Rosen already talked about some very exciting perspectives on the impact of arsenic on the microbiome so I have (Speech accented word-indiscernible) perspective.

So over the last ten years we have been frequently talking about the gut microbiome or other microbiome because they are important in human health. In terms of arsenic certainly microbiome especially the gut microbiome affects the biotransformation for arsenic. I think this is a well ('cited' or 'started'(speech accented-indiscernible)) direction. It dates back to many years ago. From the early studies by Dr. Rowland and Barry, it shows eventual metabolism of inorganic arsenic using the gut microbiome from the rats. And also Dr. (Merack-Tableau) and Dr. David (___) did a phenomenal job on the (speech accented word-indiscernible) of the metabolism by the gut microbiome.

And you (speech accented word-indiscernible) almost all the biotransformation reaction of the arsenic can be catalyzed by the gut bacteria, including the reduction from the (speech accented- word indiscernible) and (speech accented- word indiscernible) of arsenic and also the methylation including the mono-methylated and the di-methylated arsenic species. Also the de-methylation can be performed by the gut bacteria.

And in particular, the thiolation reaction of arsenic attracts a lot of attention because of the nature of the highly reducing (speech accented- word indiscernible) and the high sulfur concentration in the gut. And

the thiolated species could be mono-(thiolated) di-(thiolated) or tri-(thiolated).

Also some thiolated arsenic species are highly toxic and it can be efficiently uptake into the cell. That causes some concern about the (evolving) of the gut bacteria in the arsenic toxicity.

So in summary for the first point the concern is the microbiome effect on biotransformation for arsenic that is well done using some of the (speech accented- word indiscernible) models.

And another perspective, microbiome can influence the arsenic on the bioavailability. I see this is actually a two directional question. So in fact is it how microbiome and arsenic bioavailability (is impacted). So far not too many studies have been conducted, but I think that's a gap and it should be better addressed for the future study.

The study by Dr. (speech accented- word indiscernible) from the University of (speech accented- word indiscernible) show the gut microbiome actually can increase the bioaccessible arsenic (direction) about three-fold. And when dealing with the sample from the (speech accented- word indiscernible) using the (speech accented- word indiscernible) stimulator of human gut ecological systems.

And also I think the important thing is the bioavailability for arsenic may be particularly important for arsenic in complex matrix, such as the soil, (speech accented- word indiscernible), and food. Because of the increase gut microbiome and arsenic interaction.

So it's unlike the arsenic used from the drinking water, arsenic in other complex matrix can potentially change the profile of arsenic metabolites because of changes of biotransformation.

With the biotransformation and the bioavailability, could it be affected by the microbiome? So, I would like to put some perspective on this. So let me consequently impact the toxicity of arsenic and the individual susceptibility because we all know it's arsenic metabolite that's important for the arsenic toxicity and (has been widely used) as a biomarker for the (toxicity) susceptibility for the human population.

So if we recognize the microbiome can affect the biotransformation and the bioavailability of arsenic, we may also expect the microbiome to also affect the toxicity of arsenic and individual susceptibility in human population.

Currently, this answer has not been adequately addressed and that represents a current gap for the future study.

So, I'm going to move on to the next slide about does the microbiome alter the arsenic metabolism? I actually already talked about this previously. I'm going to highlight some other components.

There is no doubt that the microbiome alters the arsenic metabolism, and so almost all the reactions can be changed by the gut bacteria. And this could be because the gut bacteria are directly involved in arsenic metabolism including the methylation, the (thiolation), and also the reduction.

So the current question is, which species are actually involved in those reactions? Have we identified those species? Have we (modulated) the gut bacteria (speech accented- word indiscernible) function that changes the arsenic metabolism? I think that represents an exciting direction for futures studies.

And on the other hand, we understand the gut microbiome actually has the systematic effect in the host. So, I mean this may not be only the question of gut bacteria composition but also (speech accented- word

indiscernible) the systematic response induced by the microbiome change.

So we know if we change the microbiome it can also trigger the (speech accented- word indiscernible) expression (change or chain) and change the gene expression in the brain and also can influence the host's metabolic activity.

So the effect of the microbiome actually is far beyond their location so that's likely because other biome or microbiome change can induce the other systematic (speech accented- word indiscernible) which also contribute to alter the arsenic metabolism, as we observed from the different models.

So, I mean in summary so I think that's no question. I mean the microbiome alters the arsenic metabolism, and we do have some current gaps for future studies including identifying species, (modulation) the gut microbiome to change the arsenic metabolism and understanding the mechanisms for those (modulations).

So maybe anything to add by other panelists and audience?

Danielle Carlin: Mike, we can open up (multiple speakers).

Miranda Loh: I have a question. This is Miranda Loh. I was asking -- can -- Barry -- so would the microbiome and other parts of the body like Barry had mentioned, nasal or the skin, would they possibly also do some transformation to arsenic?

Barry Rosen: Well, yes. (multiple speakers)

Kun Lu:(speech accented-indiscernible) Okay. I think this is a good question. I think the majority of studies focus on the gut microbiome. I'm not clear if other microbiome is also involved in the arsenic metabolism.

Barry Rosen: We can make some predictions based on the genomics of different organisms and that is that the arsenic reductases are probably the most common arsenic genes of arsenic biotransformation. Organisms in any of the microbiomes, I'm certain, would be able to reduce arsenic to arsenite. The methyltransferase is less common but the reduction is universal.

Mike Waalkes: So you're saying methyltransferases are less common in the microbiome?

Barry Rosen: In general, if you look at the distribution of arsenic genes, the most common gene is RSR of the transcriptional repressor and then practically every arsenic resistance operand has a [permease] gene like RSB or ACR3 and a reductase gene like RC or ACR2. So, in terms of biotransformations, reduction is the most common.

Mike Waalkes: Now, I have -- there has been some work with the thiolated metabolites and toxicity or not? Or is that still an open question, if they're toxic --

Barry Rosen: I don't know maybe Kun Lu can answer that question I haven't studied (multiple speakers).

Mike Waalkes: There's a lot of data on the methylated species.

Kun Lu: So what's your question? I can barely hear you.

Mike Waalkes: The thiolated species of arsenicals that the gut bacteria produces, is there any evidence

that these might be toxic?

Kun Lu: Yes. Yes. There's a number of studies showing that the thiolate species is very toxic. Okay. So some are even more toxic than the inorganic and methylated arsenic. Some are as toxic as the trivalent methylated arsenic like the (DMA³) or something like that. So that is highly toxic. That is also (speech accented phrase-indiscernible) thiolated arsenic by the gut bacteria.

Barry Rosen: So, the gut bacteria can create thiolated arsenicals but are those taken-up? Do they go into circulation? Do they reach the liver?

Kun Lu: So you mean for the thiolated species?

Barry Rosen: Yes.

Kun Lu: I'm not aware of in the liver, but in the urinary samples the thiolated species can be detected. So that's maybe some indication of systematic circulation of those metabolites.

Mike Waalkes: Well, it must reach to circulation then one way or the other.

Danielle Carlin: Okay, Mike?

Mike Waalkes: That's very interesting.

Danielle Carlin: Michelle has some questions.

Michelle Heacock: This is a two part question that came in. The first part of it is do chronically exposed humans have a different microbiome population than non-endemic areas?

Kun Lu: So you mean for the human population study, right?

Michelle Heacock: Yes. So in those populations that are chronically exposed, is there any difference between those that are chronically exposed versus a population that isn't? Is there a difference in the microbiome?

Kun Lu: I'm not aware of any study how – being conducted on this, especially on the chronic larger study. This summer I read some study using the limited sample size but it didn't find a statistical difference in the exposed population and the control samples. But that could be because of a limited sample size and the limited statistical power for that. So that's what I think is for the human population study and that represents a future direction and the current gap for that.

Mike Waalkes: Is that looking at just flora or is that looking at genomics or, or what?

Kun Lu: It look for the -- actually the abundance of the competition.

Mike Waalkes: Okay.

Michelle Heacock: And the second part of the question, what are the arsenic metabolic products in an arsenic endemic microbiome? So, are there any different arsenic metabolites present in those microbiomes of people who are exposed to arsenic?

Kun Lu: That's actually a tough question because for the (speech accented word-indiscernible)

environment for the arsenic metabolism in humans you always actually combine the effects of the metabolism from the host and the bacteria. So for me it is hard to distinguish which one is the (speech accented word-indiscernible) source okay because the reaction is always similar-- methylation, thiolation—so I guess for this question I assume that the metabolic reaction should be the same.

Michelle Heacock: Okay, this is a clarification question. Does the thiolation happen only by the microbiota?

Kun Lu: Some people believe so but several studies show the thiolation can also happen without any bacteria. I think that is a very controversial debate.

Danielle Carlin: Mike? I think we can probably open it for other questions. Michelle can read off some of the other questions that have come regarding the other – for the other speakers.

Mike Waalkes: I think we have a couple minutes.

Michelle Heacock: Okay, great. We have a few questions that came for the first question and we have one so far from the second. If you have any additional questions, please send them through.

The first one is, what is the best adjustment method to correct urine dilution for urinary arsenic and this is directed, again, to the first questions – panelists of the questions.

Miranda Loh: We found that in general that you can correct it for creatinine or specific gravity and historically people always used creatinine but people seem to be moving away from it now just because creatinine itself can vary based on different things such as personal characteristics, etc. and what you eat and such.

We have decided that usually specific gravity or just not necessarily correcting it would be good. I think specific gravity tends to give you values that are not as widely different from your uncorrected values and of course though within specific gravity you want to be within a certain range that is considered a little more robust so that someone isn't overly hydrated or under hydrated. We generally think that specific gravity is a better way to do it and I think more people are doing it this way too.

Michelle Heacock: Okay, thank you. The second question for the same panelists for question one is, has anyone measured any other fluids, for example sputum? What about tissue levels? And the last part, would biopsies be appropriate under certain circumstances?

Miranda Loh: Is this for question one too? Sorry.

Michelle Heacock: This is for question one too but if anyone else in the panel also has something to contribute, please feel free to speak up.

Miranda Loh: Could you – sorry – could you repeat the question again? I didn't catch the whole thing.

Michelle Heacock: Sure. Has anyone measured any other fluids, for example sputum and what about tissue levels and what biopsies would be appropriate under – what circumstances would a biopsy be appropriate?

Miranda Loh: So I can talk a little bit about that. I've heard of other people using sputum but I think more measuring certain biomarkers of affect in sputum and then relating that to environmental levels.

We've thought about doing them and we've piloted using nasal, so, Barry had talked about this actually, but actually trying to measure metals in nasal mucus as well. So that's another possibility.

I don't know much about other tissues such, I don't know, fat or whatever else. I don't think you would do it in lipids for example but I don't know if anybody else has had any experience with other tissues.

Michelle Heacock: Okay, did any other panelists want to weigh-in on that one?

Okay, so this is directed for the question 2 – the panelists for question two and again if any of the other panelists want to weigh-in, please do so.

Would repeated measures of arsenic in epidemiology studies be necessary for all individuals in the study or are you suggesting repeated measures on a sub-set of individuals for establishing general patterns, a person's variability over time? And this is directed specifically for Maria actually.

Maria Argos: Danielle, can you hear me?

Danielle Carlin: Yes, I can hear you.

Maria Argos: I think the importance of using repeated samples for disease-specific biomarkers is more just a validation process for determining the reproducibility and validity of the biomarker. So it's not something I would necessarily make a recommendation would be needed for an entire study but maybe for just a sub-set of participants in order to just validate the biomarker and make sure that it is an appropriate biomarker for what we're proposing to actually use it for. So I would say that's something that would be great to have in sub-set but not necessary for the entire sample.

Michelle Heacock: We haven't had any other questions come in. If anybody else has a question they would like to submit, we've got a little bit of time.

Maria Argos: The one point I actually wanted to raise at the end of question one but we ran out of time, is that there's been some recent research indicating that potentially fish sources or other food sources may actually have a direct contribution of DMA to human exposure. So I think potentially when we're assessing exposure within human populations, we need to consider whether food sources of DMA need to somehow be partitioned from DMA that arises from metabolism of inorganic arsenic. Because I think potentially their toxicity could be very different in humans.

So I would say an additional consideration in terms of assessment methods for both acute and chronic exposure to arsenic in humans would be trying to isolate some of these DMA sources that are directly coming from food sources in humans. Potentially their toxicity profile could end up being a little bit different I think.

Miranda Loh: I think Maria has a really good point. We don't very often speculate when we sample anything other than urine really and for food especially because we know that different foods may have different compositions in terms of organic and inorganic. That's something that I think we really should start doing more of.

Danielle Carlin: Mike, this would be a time where there's any unresolved issues to discuss further?

Mike Waalkes: Well, I don't know that you've got a consensus on any of the questions and I'm not sure we can do it in five minutes.

I guess the one that was the closest to consensus was question one with regard to urinary arsenic being the metric to measure for exposure and then question three that the microbiome did impact arsenic metabolism. But I think the other questions definitely had complex answers and clear data gaps. I don't know if anybody else on the panel wants to give their two cents on those issues but that's my take home on this session.

Miranda Loh: Can I just actually follow up on what you just said and what we just said with Maria for – because I think it seems to me in this whole program one of the things we talk about is that we don't really have a good understanding of not just the intake sources of – the exposure that we get and that we take into our bodies and then how they're transformed. Because now it seems there's a lot more work on microbiome and that can have a huge impact on what we, you know, what happens to the arsenic in our body and then how it's excreted and we typically look for the same metabolites in the same species. Expanding that would be a research area is what we need in terms of better understanding arsenic as a whole.

Mike Waalkes: I think we kind of look at what we take in and look at what ends up in the urine then what's in the body is kind of a black box to a certain extent. But, anyway, maybe that's a data gap too.

Danielle Carlin: Okay Mike, I think we need to go ahead and wrap up.

Mike Waalkes: Okay.

Danielle Carlin: I'm going to take over, so once again I would just really like to thank first of all our moderator, Dr. Mike Waalkes, for doing an excellent job at moderating this session. I'd also like to mention our webinar audience, thank you so much for all the questions that have come in and if your question was not addressed, we will make sure to get it to the speakers and perhaps either respond to you either off-line or through the report that's going to be generated from this overall series of webinars as well as the work shop.

I'd like to thank all of our panelists. I think you all have done a wonderful job. All of my colleagues with the Superfund Research Program, especially Dr. Michelle Heacock, who has done a great job at moderating all of the questions.

Thanking our webinar logistics team, Justin Crane, Maureen Avakian, for keeping us on time and making sure that everything was technically working and then finally, Marisa Naujokas who's helping us out with our workshop publication.

So finally, I just want to reiterate for you to visit either our NIEHS arsenic workshop website, which contains more information about these webinars or again you can go to the link, which is bit.ly/ArsenicSeries.

We've received many questions about providing these slides as well as a recording of this on our website and we are working towards that but for right now, just tune into the webinars, because that's really where you're going to get most of the information.

Right now, I think we will go ahead and close up and we just hope that tomorrow again that you'll join us for our other webinar, which is going to be at 1:30 p.m. Eastern time. So please register for that and we'll also have announcements again about the other webinars coming in late May and June.

So with that, I think we're going to sign off. Thank you again.