SOT ISSUE STATEMENT

The Complexities in Assessing the Risk to Public Health of Low-Level Arsenic Exposure

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This document was developed by members of the Society of Toxicology and is intended to be an overview of the issues and complexities associated with exposures to low-level arsenic. It is meant to provide a brief, up-to-date starting point for those scientists interested in arsenic toxicity and to provide links to more detailed information.

Introduction

Environmental exposure to arsenicals continues to be a major public health concern for hundreds of millions of individuals worldwide. As a significantly abundant element in the Earth's crust, arsenic is a ubiquitous metalloid, producing global human exposures from drinking water, soils, food, and industrial wastes.

The lethality of high-level arsenic exposures has been recognized for millennia, and expanding epidemiological evidence has found associations between continued high-level (e.g., >100 ppb or µg/l in drinking water) exposures and a range of chronic diseases and disabilities. There is some evidence suggesting increased risk of health effects from drinking water exposures below 100 ug/L. A 2013 National Research Council committee report on inorganic arsenic provided a hierarchical listing of the causal relationships between environmental arsenic exposures in humans and incidences of certain cancers (lung, bladder, and skin), cardiovascular disease, lung disease, and neurological and cognitive deficits. With support from both animal models and epidemiological studies, the report also recognized that exposures at any time throughout the life span—from preconception through old age—may enhance disease promotion by arsenic.

Basic research in animal models, which examines human-relevant exposures using both whole-life and adult exposure paradigms, as well as molecular epidemiological studies, have greatly advanced the understanding of the dose-responsive effects of arsenic on many organ functions in the body. Translational studies are revealing genetic and epigenetic associations between arsenic and disease, and advanced epidemiological studies demonstrate many confounding exposures that both promote (e.g., tobacco smoking) and reduce (e.g., folate, B vitamins, selenium) the pathogenic effects. However, these studies and those that evaluate the manner in

which arsenic moves through the environment and is metabolized in exposed individuals reveal the complexity in establishing the health risks and cause of disease from arsenic.

This complexity challenges current efforts by regulatory agencies, such as the World Health Organization (WHO), the US Environmental Protection Agency (US EPA), and US Food and Drug Administration (US FDA), to establish rules and guidelines that prevent or reduce arsenic-promoted adverse health outcomes. The result is a continued need for advanced and rigorous research into:

- the fate and transport of arsenic in the environment that results in exposure;
- the factors determining the metabolism and kinetics of arsenic in the body;
- genetic and epigenetic determinants of susceptibility and action; and
- the mechanisms of toxicity that may produce disease from chronic, low-level exposures.

This research will lead to an improved understanding of the exposure/dose-response relationship for arsenic and different disease states. Such understanding is critical to the development of well-supported, health-based regulatory limits that appropriately balance the benefits of risk-reduction activities with the associated costs of such activities.

Exposure Assessment

There are several important chemical properties of arsenic that need to be considered in reliably measuring arsenic for characterizing exposure. These include valence state (trivalent or pentavalent), organic or inorganic form, and other characteristics (e.g., sulfide or phosphate form in the case of arsenic in soils). These properties influence bioavailability (i.e., absorption of form of interest from the lungs or gut versus absorption of highly soluble forms of arsenic as found in drinking water). In addition, these properties also affect the toxicity of different arsenic species. Measuring total arsenic that includes both inorganic and organic arsenic, rather than speciated arsenic in biological media, will yield toxicologically inaccurate dose estimates. For example, total arsenic measured in seafood is not meaningful from a risk perspective since much of arsenic found in seafood is of a non-toxic organo-arsenic form. One must use caution in interpreting studies where only total arsenic is assessed in urine as this also includes organic forms of arsenic from food, as well as methylated arsenic metabolites. Some arsenic metabolites, in particular dimethylarsinic acid (DMA), also are found in food as well as being metabolites of other organic arsenicals such as arsenosugars and arsenolipids. Thus, DMA in urine, especially at low levels of arsenic in water, would not accurately reflect inorganic arsenic intake. Methylated trivalent arsenic species, which are toxicologically more relevant, are short lived, adding important uncertainties to the interpretation of findings.

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Quantifying human exposure to inorganic arsenic is critical to reliable interpretation of epidemiological studies and to their use in risk assessment, as well as guiding *in vivo* and *in vitro* studies. The two main approaches for quantifying arsenic exposure to humans are:

1) measurement of arsenic species in environmental media, including food, air, water, dust, and soil, and
2) use of biomonitoring data, most commonly arsenic in urine and in toenails.

Each approach can be used to quantify intake and is associated with different strengths and limitations. Failure to adequately consider these limitations can result in over- or underestimates of total exposure to inorganic arsenic and the misidentification of the relative importance of different sources, creating potential biases when extrapolating dose-response relationships across or within populations.

The primary exposure sources for arsenic are food, water, soil, dust, and ambient and workplace particulates in air. It is not uncommon for arsenic to be measured in only one or two sources, leading to potential underestimates of total arsenic exposure. This may not be very significant when a single source, typically water, predominates; however, at lower levels of arsenic in water, other sources, such as food, soil, and dust, may predominate. Measurements of arsenic in food, either from the food itself or added via cooking, are rarely made in epidemiology studies and often are not available from other relevant studies for integration into exposure estimates. Moreover, as noted above, measuring arsenic in food is complicated, with the need to speciate arsenic since certain types of food arsenic, such as arsenobetaine or other organo-arsenicals, are of limited toxicological relevance. In contrast, inorganic arsenic found in certain foods, such as particular cultivars of rice and rice products, will add to total inorganic arsenic intake. In this example, failure to consider arsenic in food will underestimate exposure. In addition, certain foods, such as seafood, contain DMA, which also is a metabolite of inorganic arsenic, as well as other organic arsenicals, such as arsenosugars. These complexities in source assessment add uncertainty to the interpretation of studies of urine arsenic in which inorganic arsenic and its metabolites, monomethylarsonic acid (MMA) and DMA, are quantified and seafood consumption is not included in analysis. In this example, attributing DMA in urine to metabolism of ingested inorganic arsenic would overestimate exposure to inorganic arsenic if urinary DMA is from organic arsenicals in food and not derived from inorganic arsenic.

Biomonitoring studies (typically urine or toenails) are most useful in risk assessment when they can be translated to dose estimates. Concomitant measurements of arsenic in environmental media can facilitate the use of biomonitoring studies in dose estimates. The role of modifying factors must be considered in interpreting findings. For both urine and toenails, collection of multiple samples from an individual, as well as dietary survey information, can enhance the

interpretation of findings. For analysis of arsenicals in urine, in addition to understanding the sources of methylated metabolites of inorganic arsenic, such as DMA, appropriate methods for normalization (volume versus creatinine versus specific gravity) must be considered. Basing comparisons on urinary creatinine levels also can pose difficulties. This is especially critical for investigations of cardiovascular diseases, diabetes mellitus, and hypertension, which can significantly alter renal function and metabolism, affecting creatinine levels. In contrast to urine arsenic, toenail arsenic integrates exposure over longer periods of time (months versus days for urine arsenic); however, toenail arsenic is measured as total arsenic, and translating findings of toenail arsenic to inorganic arsenic intake is even more complicated than with urine arsenic. In addition, separating internal arsenic exposure from external contamination is difficult.

Models for Arsenic Toxicity Testing

A significant amount of what is known about the adverse health outcomes following high environmental exposures to arsenic is derived from human studies, but *in vivo* and *in vitro* model systems also have been invaluable to the present understanding of arsenic toxicity.

A number of whole animal models have been used to study the effects of arsenic and provide a bridge between *in vitro* mechanistic studies and human responses to arsenic. The ability to introduce genetic modifications in mice can provide further information. In addition, mice can be used to test intervention strategies for reducing the adverse outcomes of arsenic exposure. Use of mouse strains with differing responses to arsenic can provide additional information of susceptibility and toxic responses, but care needs to be taken when extrapolating between species.

As research moves toward an understanding of the effects of arsenic at or below 100 ppb, the use of model systems, such as those that mimic the diversity of human populations, will be necessary to provide disease endpoints and biomarkers of effect that can be validated in humans to assess health risks and to develop protective strategies. Several modes of action and associated biomarkers of effect have been identified and continue to be studied. These include:

- cytotoxicity with regenerative proliferation (evidence of inflammation and hyperplasia) following binding of trivalent arsenicals to sulfhydryls of critical cellular proteins;
- oxidative stress (increased DNA oxidation and decreased ability to handle reactive oxygen species);
- decreased DNA repair (inhibition of repair enzymes by binding to zinc finger protein locations);
- alterations in cell signaling pathways;
- alterations in extracellular matrix (alterations in matrix metalloproteinases and inhibitors);

- and certain types of endocrine disruption (such as interference with the hypothalamic/pituitary axis and glucocorticoid receptors)

Among other things, mode-of-action understanding is relevant to the selection of dose-response models for arsenic risk assessment. The modes of action noted above are consistent with a non-linear dose-response model for purposes of arsenic risk assessment.

Arsenic and Metabolism

Inorganic arsenic is predominantly metabolized in the body after ingestion or inhalation by a specific enzyme, arsenic (+3 oxidation state) methyltransferase (As3MT). This enzyme is highly expressed in the adrenal glands and liver and, to a lesser extent, in many other tissues. As3MT methylates inorganic arsenic and generates methylated intermediates (predominantly mono- or di-methylated arsenite or arsenate). This process has been described to enhance excretion of arsenic and facilitate removal of arsenic from the body. However, some of the trivalent methylated intermediates may be more toxic than the parent inorganic arsenic, suggesting they may play a role in the negative health effects of arsenic.

Susceptible Populations

It is clear from studies of populations ingesting high arsenic levels in drinking water in excess of 100 ppb that life-long exposure to arsenic is associated with multiple diseases. However, even at the same exposure/dose, not everyone exposed to arsenic develops disease. Thus, it is important to understand the basis for variations in susceptibility to disease. While some of this variability is likely random, it also is correct that host factors (e.g., genetics, age, or nutrition) influence likelihood of disease.

Differences in the distribution of arsenic metabolites in human urine have been associated with the risk of arsenic-associated disease. It may be possible to predict variable arsenic metabolism using genetic markers, although molecular epidemiology studies are required to define genetic variations that correlate with susceptibility. As3MT gene variants have been associated with differences in metabolism of arsenic to the more potent As(III) methylated metabolites and may influence likelihood of disease. Nevertheless, currently validated genetic markers explain only a fraction of the variability in arsenic metabolism and in disease risk.

Because specific dietary micronutrients play a role in arsenic metabolism, dietary deficiencies may enhance susceptibility to arsenic. For example, deficiency of the micronutrients folate and methionine is associated with reduced arsenic excretion, while sufficiency is associated with increased excretion. Moreover, the efficiency of arsenic methylation by the AS3MT enzyme is influenced by nutritional status. While limited in number, epidemiology studies provide evidence that sufficiency of certain micronutrients is associated with reduced risks for health

effects, such as skin lesions, bladder cancer, and cardiovascular disease. These issues are particularly relevant to populations with low socioeconomic status and dietary deficiencies. The potential for nutritional interventions to reduce the toxicity of arsenic represents an active area of research.

At the same exposure level, smokers may be at greater risk from arsenic than non-smokers. Such associations have been found, for example, in studies of arsenic-associated bladder and lung cancer and cardiovascular disease. As with the nutritional factors, associations between arsenic and disease in smokers may be influenced by polymorphisms and arsenic exposure level.

Life stage also is an important factor for disease susceptibility. Early life (either *in utero* or in early childhood) exposure to arsenic has been associated with disease later in life (e.g., lung disease and cancer). Animal studies are being used to provide mechanistic understanding of the findings from the epidemiological studies. Defining biomarkers and critical windows of exposure could help identify those individuals most at-risk to develop arsenic-associated disease. There is a need for additional research regarding pre-conceptional exposures.

Policy and Regulation

WHO recommends the maximum contaminant level (MCL) for arsenic in drinking water be no more than 10 ppb (μg/l). This guideline is designated as provisional because of measurement difficulties and the practical difficulties and costs in removing arsenic from drinking water. Where it is difficult to achieve the guideline value, member states may set higher values as standards, taking into account local circumstances, resources, and risks from low-arsenic sources that are contaminated microbiologically.

In the United States, the US EPA lowered the MCL for arsenic in drinking water from 50 ppb (μg/l) to 10 ppb (μg/l) in 2001, and in 2006, the new MCL went into effect for municipal water supplies or systems serving more than 25 individuals. While the US EPA monitors municipal water supplies and systems, it does not regulate or normally monitor the amount of arsenic in private wells. Monitoring private wells falls to the individual. Many state or local health departments have information for testing arsenic levels in wells and even programs to help individuals have their water tested.

Recent research into the adverse effects of arsenic is raising concerns regarding the adequacy of public health protection afforded by the MCL of 10 ppb (μg/l) with respect to cancer and non-cancer endpoints. However, an additional issue of concern is whether water utility companies can realistically decrease arsenic levels below 10 ppb (μg/l) without driving the costs of water treatment to financially unsustainable levels, making it necessary to consider both the health

effects and the financial costs of reducing the levels in municipal systems when setting the MCL.

In addition to ingestion via water, ingestion of inorganic arsenic in food and drink also may lead to increased exposures. For example, elevated levels of inorganic arsenic have been found in random samples of apple juice and rice. The US FDA has set 10 ppb (µg/l) as the maximum level of arsenic in apple juice. Because early life exposures to arsenic have been associated with diverse health outcomes in adults, the US FDA also is currently recommending an action level of 100 ppb (µg/kg) inorganic arsenic in infant rice cereals, which is similar to the regulated limit in the European Union. The European Union adopted regulatory limits for arsenic in rice and rice-based products in 2015. However, the role of inorganic arsenic in rice and disease in humans is unclear. WHO is currently evaluating the global burden of disease (i.e., cancer and non-cancer) that can be attributed to arsenic in food in order to provide global regulatory recommendations. Many other countries, including China, Australia, Japan, and Canada, have already moved forward with limits for a range of food stuffs.

While the US EPA categorizes arsenic as a hazardous air pollutant, there is no ambient air standard for arsenic.

Conclusion

The mechanisms by which arsenic induces a broad range of health effects continues to challenge those in the disciplines of environmental health sciences, toxicology, and public health. Despite many millions of years of human exposure to arsenic and it being generally recognized as a poison for millennia, the understanding of the health risks posed by arsenic exposures in different developmental windows and the policies that protect global population health from arsenic exposures in the essential media of air, water, and food continue to evolve. With more than 19,000 peer-reviewed publications on arsenic in the past 25 years, it is arguably one of the most well-studied and important toxicants. However, there is still a great need for additional research and discovery to support innovative strategies that protect public health from arsenic and to develop risk management strategies to reduce health risks.

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*The Complexities in Assessing the Risk to Public Health
of Low-Level Arsenic Exposure*

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