Critical Aspects of EPA's IRIS Assessment of Inorganic Arsenic

Interim Report

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Critical Aspects of EPA's IRIS Assessment of Inorganic Arsenic

Interim Report

Committee on Inorganic Arsenic

Board on Environmental Studies and Toxicology

Division on Earth and Life Studies

NATIONAL RESEARCH COUNCIL
OF THE NATIONAL ACADEMIES

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Preface

The US Environmental Protection Agency's Integrated Risk Information System (IRIS) program has been working for several years on updating its toxicologic assessment of inorganic arsenic. The agency released an updated draft cancer assessment of inorganic arsenic in 2010. However, in 2011, Congress mandated an independent peer review of the assessment by the National Research Council before EPA takes any action to make the assessment final. In response to that mandate, EPA withdrew its draft cancer assessment and announced plans to redo the toxicologic assessment to include cancer and noncancer effects. The agency asked the National Research Council to provide a review in two phases. The first phase would involve providing EPA with guidance on key aspects of performing the toxicologic assessment (the focus of this report), and the second phase would be a review of the draft document after the agency completed its assessment.

In response to EPA's request, the National Research Council convened the Committee on Inorganic Arsenic, which prepared this report. The members of the committee were selected for their expertise in toxicology, epidemiology, carcinogenesis, mechanisms, genomics, physiologically based pharmacokinetic modeling, environmental medicine, risk assessment, and biostatistics (see Appendix A for biographic information on the members).

For the first phase of the project, the committee conducted a public workshop to evaluate critical scientific issues in assessing cancer and noncancer effects from oral exposure to inorganic arsenic. The workshop was held on April 4, 2013 (see agenda in Appendix B). The committee wishes to thank the invited speakers for their participation in the workshop and panel discussions. The workshop proceedings were used by the committee to inform its preliminary survey of the literature on inorganic arsenic.

The committee's report has been reviewed in draft form by persons chosen for their diverse perspectives and technical expertise in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of the independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards of objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We thank the following individuals for their review of the report: Thomas Burke, Johns Hopkins Bloomberg School of Public Health; Yu Chen, New York University; David Dorman, North Carolina State University; Molly Kile, Oregon State University; Roger McClellan, Toxicology and Human Health Risk Analysis; Louise Ryan, University of Technology Sydney School of Mathematical Sciences; Timothy Pastoor, Syngenta Crop Protection, Inc.; Craig Steinmaus, University of California, Berkeley; and Michael Waalkes, National Institute of Environmental Health Sciences.

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of the report was overseen by Joan Rose, Michigan State University, and David Eaton, University of Washington. Appointed by the National Research Council, they were responsible for making certain that an independent examination of the report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of the report rests entirely with the author committee and the institution.

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The committee is grateful for the assistance of National Research Council staff in preparing the report. It particularly wishes to acknowledge the support of Project Director Susan Martel, who coordinated the project and contributed to the committee's report. Other staff members who contributed to this effort are James Reisa, director of the Board on Environmental Studies and Toxicology; Keri Stoever, research assistant; Tamara Dawson, program associate; Norman Grossblatt, senior editor; and Mirsada Karalic-Loncarevic, manager of the Technical Information Center.

Finally, I thank all the members of the committee for their efforts throughout the development of this report.

Joseph H. Graziano, PhD
Chair, Committee on Inorganic Arsenic

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Critical Aspects of EPA's IRIS Assessment of Inorganic Arsenic

Interim Report

Summary

The US Environmental Protection Agency (EPA) Integrated Risk Information System (IRIS) program develops toxicologic assessments of environmental contaminants. IRIS assessments provide hazard identification and dose–response assessment information. The information is then used in conjunction with exposure information to characterize risks to public health and may be used in risk-based decision-making, in regulatory actions, and for other risk-management purposes. Since the middle 1990s, EPA has been in the process of updating the IRIS assessment of inorganic arsenic. Arsenic is a naturally occurring element in the environment that can occur in different forms. *Inorganic arsenic* refers to a set of arsenic-containing molecules that includes elemental arsenic and many other molecular structures that involve arsenic in combination with elements other than carbon. *Organic arsenic* consists of a broad set of arsenical compounds that include carbon in their structures; they include the relatively simple monomethyl arsenic and dimethyl arsenic that are metabolites of inorganic arsenic in humans and more complex molecules that are used as herbicides, pesticides, or animal feed additives. EPA's toxicologic assessment will focus on inorganic arsenic.

The development of the toxicologic assessment of inorganic arsenic is a considerable undertaking for the IRIS program. Much research has been performed on the chemical, and improved approaches to performing assessments have been recommended. Although the multiple challenges are clear given the history of the assessment, there are also opportunities to take advantage of the rich data on inorganic arsenic to apply evaluation tools and integrative approaches so as to conduct the assessment in a transparent manner. Many of the improved approaches were recommended by previous National Research Council committees that evaluated other chemicals, and it is clear from EPA's draft conceptual model and analysis plans that the agency plans to incorporate the recommendations. The task of the present committee was to focus on recommendations specific to the assessment of inorganic arsenic. (A different National Research Council committee is reviewing EPA's initiatives to improve the overall process and quality of IRIS assessments.)

In response to a congressional mandate for an independent review of the IRIS assessment of inorganic arsenic, EPA requested that the National Research Council convene a committee to conduct a two-phase study. In the first phase (the focus of the present report), the committee was to organize a workshop to evaluate critical scientific issues in assessing cancer and noncancer effects of oral exposure to inorganic arsenic and offer recommendations on how the issues could be addressed in EPA's IRIS assessment. The second phase of the study will begin after EPA completes its draft assessment; it will involve a review of the draft assessment to determine whether the committee's recommendations were appropriately addressed and also reflect recommendations for improving IRIS assessments made in other National Research Council reports. As part of the first phase, a workshop was held on April 4-5, 2013, to obtain input on critical aspects of interpreting and applying the scientific information on inorganic arsenic for the purposes of hazard identification and dose–response analysis. Having been informed by the workshop and its own preliminary survey of the scientific literature, the committee offers the following recommendations for performing the IRIS assessment as outlined in Figure 1 and described in Box 1. These recommendations are specific to inorganic arsenic and may not be applicable to the assessment of other chemicals.

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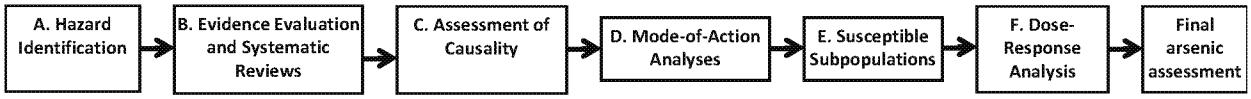

FIGURE 1 Steps of the toxicologic assessment of inorganic arsenic.

BOX 1 Committee's Guidance and Recommendations for Improving Steps of the Toxicologic Assessment of Inorganic Arsenic Illustrated in Figure 1

A. Hazard Identification. A broad literature search and screening process will be used by EPA to identify health effects that have been studied in relation to inorganic arsenic, and a preliminary draft of these efforts was provided to the committee. For the purposes of its review, the committee conducted a preliminary survey of the literature and identified categories of health outcomes that, in the end, overlapped with those identified by EPA. As a starting point, the committee attempted to prioritize these health end points on the basis of the perceived strength of evidence and the importance to public health (see Box 2). Chapter 4 provides some guidance on end point specific issues EPA should consider as it conducts a more comprehensive and systematic evaluation of the literature.

B. Evidence Evaluation and Systematic Reviews. EPA has indicated that systematic review will be used to support its toxicologic assessment of inorganic arsenic. To perform such reviews, the committee recommends searching for studies on specific outcomes (see Box 2) that meet the following criteria: individual measures of arsenic exposure, measurement of arsenic that precedes the outcome, and low to moderate exposure to inorganic arsenic (less than 100 µg/L in drinking water). It will also be important to organize the data from the individual studies into evidence tables. The example tables provided by EPA in its draft plans appear to capture the salient categories of information with respect to epidemiologic data. Meta-analysis should also be considered for priority end points if there are three or more peer reviewed studies on the outcome of interest. For dose-response meta-analysis, studies will need to have characterized three or more exposure levels. Chapter 3 provides general guidance for performing systematic reviews and meta-analyses, as well as for developing evidence tables on animal and in vitro data to inform causality determinations and mode-of-action analyses in the low exposure range.

C. Assessment of Causality. EPA's draft plans provide a causal determination framework that describes how it will categorize the evidence on different end points into five possible categories. The committee supports this five-category approach, and recommends that judgments are characterized with respect to the modified Bradford Hill criteria for causality. The assessment of causality will help EPA prioritize end points for subsequent analysis of mode of action and dose response.

D. Mode-of-Action Analyses. The committee supports EPA's plans to conduct mode-of-action analyses for end points it classifies as having a *causal* or *likely to be causal* relationship with arsenic. Consideration might also be given to performing mode-of-action analyses for end points with suggestive evidence for the purpose of determining whether there is sufficient evidence to support a stronger causal association and, if so, informing dose-response analyses. Guidance on performing mode of action analyses is presented in Chapter 6.

E. Susceptible Subpopulations. The committee's survey of the literature has identified several factors that could contribute to susceptibility to arsenic. Chapter 5 outlines the importance of considering these factors when interpreting the epidemiologic evidence. Although it is unclear that any of these factors can be evaluated quantitatively in the assessment, their existence is an important consideration when evaluating population risk.

F. Dose-Response Analysis. Epidemiologic data are expected to serve as the basis for the dose-response analyses performed for most end points. As outlined in Chapter 7, efforts should be directed at performing dose-response analyses in the range of epidemiologic observations. For some end points it may be possible to perform dose-response meta-analyses. Should the data in the range of observation be inadequate for developing risk estimates that meet EPA's needs, mode-of-action data should be used to the extent possible to extrapolate below the observed range. The committee concurs with EPA's draft plan that even if a mode of action cannot be determined with reasonable certainty, dose-response analyses should be performed on health end points deemed to have a causal or likely causal relationship with arsenic. In the absence of mode-of-action data, alternative statistical approaches, described in Chapter 7, are recommended.

- **Hazard Identification:** Overall, the committee agrees that documents provided by EPA have outlined an improved approach to determining the cancer and noncancer effects that may be associated with exposure to inorganic arsenic. The process involves screening the literature, evaluating studies, organizing the evidence, and using a causal determination framework to document how decisions are made about which health end points are linked to inorganic arsenic. Evidence tables are a useful means of organizing the epidemiologic and toxicologic information, and it will be important for the narrative of the IRIS assessment to explain clearly how judgments are made with respect to the causal determination framework. The committee conducted a preliminary survey of the literature on many of the health end points that EPA will consider, and key observations about those end points and the implications for dose–response modeling are provided later in this report. In Box 2, the committee has attempted to set priorities among the health end points. These categorizations will be refined by EPA after it conducts a more comprehensive analysis.

- **Systematic Reviews:** EPA has indicated that several systematic reviews of the scientific literature on inorganic arsenic will be used to support its toxicologic assessment of inorganic arsenic. The systematic reviews will be used to address questions raised during the hazard-identification process with respect to guiding the dose–response analysis. To facilitate and focus the systematic reviews with respect to epidemiologic studies, the committee recommends that in selecting the studies to be included in meta-analyses, EPA should consider proceeding as follows:
  - Systematically search for studies on specific outcomes (see suggested list in Box 2) that meet the following criteria: individual measures of arsenic exposure (preferably with biomarker measurements), measurement of arsenic that precedes the outcome, and low to moderate exposure to inorganic arsenic (less than 100 μg/L in drinking water).
  - Consider meta-analyses if there are at least three or more peer-reviewed studies available on the outcome of interest. For dose-response meta-analysis, studies will need to have characterized at least three or more exposure levels.
  - Review the quality of the evidence (risk of bias) using established guidelines for epidemiologic studies. Elements in the evaluation of study quality include the assessment of study outcome based on standardized definitions, participation rate, adjustment of associations for relevant confounders, and other considerations that will depend on study design.

BOX 2 Hierarchy of Health End Points of Concern for Inorganic Arsenic

- Tier 1: Evidence of a causal association determined by other agencies and/or in published systematic reviews
  - Lung, skin, and bladder cancer
  - Ischemic heart disease
  - Skin lesions
- Tier 2: Other priority outcomes
  - Prostate and renal cancer
  - Diabetes
  - Nonmalignant respiratory disease
  - Pregnancy outcomes (infant morbidity)
  - Neurodevelopmental toxicity
  - Immune effects
- Tier 3: Other end points to consider
  - Liver and pancreatic cancer
  - Renal disease
  - Hypertension
  - Stroke
  - Pregnancy outcomes (fetal loss, stillbirth, and neonatal mortality)

- **Mode of Action:** Mode-of-action analyses should be used to inform dose–response modeling with respect to the shape of the curve, particularly in the low dose region, and the understanding of interhuman variability. Even if the mode of action cannot be firmly established, the exercise can be used to guide modeling qualitatively in the low dose region and used in considering susceptibility. EPA has outlined a process of delineating a pathway that connects a molecular initiating event to an adverse health end point at a higher biologic level of organization. The process will be used to organize mechanistic information to determine how mode-of-action information supports low-dose extrapolation and to inform how dose–response analyses account for the uncertainty associated with susceptibility. The committee supports EPA's plans to perform mode-of-action analyses for health outcomes on which there is evidence to infer a causal or likely to be causal relationship with inorganic arsenic.

The analyses should follow a stepwise approach, starting with the questions to be answered. All the existing data should be explored, supportive and conflicting evidence should be examined, and it should be determined whether an exposure–response continuum for sequential progression and time dependence of the proposed key events can be established. Biologic plausibility and concordance of evidence from in vitro, animal, and human data should be explored. Consideration could also be given to how the mode of action could be modulated by other potentially causal agents.

- **Susceptibility:** Multiple factors can affect susceptibility to inorganic arsenic, including life stage, genetic factors, sex, nutritional deficiencies, health status, lifestyle (for example, smoking and alcohol consumption), and coexposures. The committee agrees with EPA's proposal to use probabilistic approaches in considering the uncertainty and variability associated with those factors. Susceptibility due to pre-existing disease is an important consideration, as arsenic has been shown to increase the risk of several major diseases prevalent in the United States (e.g., diabetes, cardiovascular disease). On the basis of the degree of evidence on a vulnerable population, consideration should be given to whether dose–response assessment should focus on the population as a whole or should involve separate assessments for the general population and susceptible groups. With respect to smoking—which appears to increase the risk of skin lesions, lung and bladder cancer, and maybe cardiovascular disease—it might be possible to apply sensitivity approaches to the dose–response relationship for the various end points to determine the degree to which smoking would change the potency calculation.

Consideration should also be given to the growing evidence from human and animal studies that suggests that early-life exposure to arsenic may increase the risk of adverse health effects and the risk of impaired development in infancy and childhood and later in life. Thus, the timing of exposure should be considered in evaluating epidemiologic studies for dose–response assessment. EPA's current approach of assessing less than lifetime exposures for cancer risk from nonmutagenic carcinogens by prorating the risk equally regardless of age needs to be critically evaluated to determine whether it is appropriate for inorganic arsenic.

There is clear evidence of sex differences in the metabolism of inorganic arsenic. Because arsenic metabolism is a recognized susceptibility factor, it seems likely that the toxicity of arsenic could differ between men and women. Indeed, evidence of such differential risks is growing.

- **Dose–Response Analyses:** Because many epidemiologic studies of cancer and noncancer end points now characterize risk associated with low to moderate concentrations of inorganic arsenic, in some cases approaching or including background concentrations, it may be possible to estimate risk directly from the range of observations in the literature. Background concentrations of arsenic vary, but the committee judged that urinary arsenic concentrations of 1–5 μg/L (summing inorganic, monomethyl, and dimethyl arsenic forms) is a reasonable estimate for the US population. The committee does not assume that those background concentrations are with or without health effects; rather, it assumes that the needs of assessing health risks can be facilitated by characterizing dose–response relationships down to the background concentrations by using observed data.

The committee recommends that EPA develop risk estimates across the array of health effects on which there is adequate epidemiologic evidence and then derive risk-specific doses to address the needs

of analyses that would typically use a reference dose (RfD). That approach would facilitate efforts to evaluate cumulative risks posed by exposure to multiple chemicals, conduct risk–benefit assessments, or to conduct other comparative analyses. An RfD might be selected from among the risk-specific doses on the basis of such factors as end-point severity, interhuman variability, and policy considerations so that it may be used by stakeholders until guidance on how to use risk-specific doses for noncancer end points is established.

If the health-assessment needs cannot be fully met by using modeling of the data in the range of observation, extrapolation to below the observed range may be necessary. The preferred approach would be to use data that define the individual and population risks and their associated uncertainty on the basis of analyses of adverse-outcome pathways or mode of action and human variability in susceptibility. That would describe human pharmacokinetics, biomarkers of exposure, tissue doses of relevant arsenic forms, and the multiple toxicodynamic processes that lead to the adverse outcomes. However, it is not clear whether such an approach would be feasible without further research and modeling. In the absence of adequate mode-of-action information, EPA could use modeled curve shapes that approach the low range of observation to extrapolate below that range. Fitting of alternative models could inform how far below the range of observation the extrapolation is essentially independent of model choice and thus provide greater confidence in the extrapolation to that point. Consideration should be given to whether mechanistic information can inform the process. Extrapolations will become increasingly uncertain as they go further below the observed range, so it would be reasonable for the IRIS assessment to stipulate the range over which the dose–response relationship derived for cancer and noncancer end points is useful for risk assessment. To assess uncertainties in such an extrapolation, EPA could use a form of sensitivity analysis, fit multiple models to the data in the range of observation, and assess how sensitive extrapolation below the range of observation was to the choice of model. The IRIS program should explore the possibility of performing dose–response meta-analyses for inorganic arsenic with standard methods that have been described in the environmental-health and public-health literature. Methods are available to evaluate both linear and nonlinear dose–response curves.

1

Introduction

The US Environmental Protection Agency (EPA) asked the National Research Council to help its Integrated Risk Information System (IRIS) program in developing a scientifically credible toxicologic review of inorganic arsenic. IRIS assessments include the hazard identification and dose-response assessment steps of risk assessment. The two other risk-assessment steps of exposure assessment and risk characterization are conducted separate from the IRIS assessment to support risk management decisions. In develoing the IRIS assessment for inorganic arsenic, the agency is faced with the challenge of evaluating a large body of scientific information on arsenic, addressing recommendations for improving aspects of its past analyses of arsenic (e.g., NRC 1999, 2001; EPA SAB 2007, 2011), and incorporating recommendations for generally improving hazard identification and dose–response analysis (e.g., NRC 2009, 2011). EPA has worked on updating its assessment of inorganic arsenic for many years and released an updated draft cancer assessment of inorganic arsenic in 2010. However, in 2011, Congress mandated an independent peer review of the toxicologic assessment of inorganic arsenic by the National Research Council before EPA takes any action to make its IRIS assessment final. In response to the mandate, EPA withdrew its draft cancer assessment and announced plans to redo the toxicologic assessment to include cancer and noncancer effects. The agency has asked the National Research Council to provide guidance in the early stages of developing the IRIS assessment and to review the draft document after the agency has considered the committee's recommendations and input from stakeholders. The statement of task for the project is presented in Box 3.

The National Research Council convened the Committee on Inorganic Arsenic, which comprises experts in epidemiology, toxicology, carcinogenesis, mechanisms, biologic modeling, environmental medicine, biostatistics, and risk assessment (see Appendix A for biographic information on the members). The committee held a workshop on April 4, 2013, to obtain input from arsenic researchers, risk-assessment professionals, and stakeholders on important aspects of hazard identification and dose–response analysis for inorganic arsenic (see Appendix B for workshop agenda). Information from the workshop was supplemented by materials provided by EPA, including a summary report of an internal EPA partner workshop, a summary report of a public stakeholder workshop held in January 2013, a planning and scoping summary for the toxicologic review of inorganic arsenic, EPA's draft plans for performing the review, and draft supporting documents that provide examples of how EPA has begun it hazard assessment. The committee also considered written and oral statements provided by stakeholders over the course of the study. A preliminary review of the arsenic literature was also used in conjunction with these materials to inform the committee's evaluations. The conduct of more comprehensive or systematic reviews of the literature was not part of the committee's charge.

This interim report addresses the first phase of the project by providing recommendations on key aspects of and controversies related to the performance of the hazard identification and dose–response analyses for inorganic arsenic. The committee's recommendations are specific to inorganic arsenic and may not be applicable for other chemical assessments. The committee considered materials describing EPA's continuing efforts to improve the development of IRIS assessments (EPA 2013a,b). It was outside the scope of the committee's task to comment on those materials.[1]

[1]Another National Research Council committee is reviewing EPA's initiatives to improve the overall process and quality of IRIS assessments. That committee expects to release its report in 2014.

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BOX 3 Statement of Task

An ad hoc committee will plan and conduct a public workshop to evaluate critical scientific issues in assessing cancer and noncancer effects from oral exposure to inorganic arsenic. The workshop will enable the committee to gather a variety of perspectives from stakeholders and others on the issues. Informed by the workshop, the committee will prepare an interim report providing recommendations on how those issues can be addressed in EPA's IRIS assessment. After EPA has revised the IRIS assessment, the committee will review it to determine whether the scientific literature on inorganic arsenic was adequately evaluated, whether appropriate methods were used to derive cancer risk estimates and noncancer reference values, and whether dose–response relationships between inorganic arsenic and cancer and noncancer effects were appropriately estimated and characterized. As requested by Congress, the committee will also determine whether the arsenic document reflects recommendations made in Chapter 7 of the 2011 NRC report on formaldehyde for improving descriptions of methods and criteria for selecting studies, approaches to evaluating critical studies, weight-of-evidence analyses, and justification of modeling approaches in IRIS assessments.

RECOMMENDATIONS FROM PREVIOUS NATIONAL RESEARCH COUNCIL COMMITTEES

EPA has made clear that two National Research Council reports will influence how it conducts its assessment of inorganic arsenic. The more recent one was an evaluation of EPA's draft IRIS assessment of formaldehyde (NRC 2011). Chapter 7 of that report outlined a "roadmap to revision" for future IRIS documents, and Congress has directed EPA to address the recommendations in its toxicologic assessment of inorganic arsenic. The recommendations are in four broad categories: improving (1) descriptions of methods and criteria for selecting studies, (2) approaches to evaluating critical studies, (3) weight-of-evidence analyses, and (4) justification of modeling approaches. Important elements in achieving improvements in those categories include standardization of the presentation of evidence, standardization of review and evaluation approaches (for literature review, hazard identification, dose–response modeling, and weight-of-evidence evaluation), guidelines for study selection, description and justification of assumptions and models used, and assessment of the sensitivity of derived risk estimates to model assumptions and end points selected.

EPA also plans to incorporate more general recommendations about assessment approaches outlined in *Science and Decisions* (NRC 2009), the second of the two reports mentioned above. A major recommendation of that report was that more attention be focused on designing the risk assessment in the formative stages, specifically in planning and scoping and in problem formulation. EPA was encouraged to involve risk managers, risk assessors, and stakeholders early in the process to help determine which major factors to consider, the decision-making context, and the timeline and depth needed to conduct the assessment. The report recommended that dose–response assessments be conducted similarly for cancer and noncancer effects so that risk-specific doses can be developed for both types of effects. Background exposures and disease processes, susceptible populations, and modes of action that may affect human dose–response relationships should be factored into the dose–response analysis. Suggestions were also made for planning the characterization of uncertainty and variability early in the process so that it can be developed to meet the needs of comparative evaluation of risk-management options.

The Natioanl Research Council also conducted two evaluations of EPA's drinking-water standard for inorganic arsenic (NRC 1999, 2001). Those reports were used by EPA to set a maximum contaminant level of 10 μg/L for arsenic (66 Fed. Reg. 6975 [2001]). The database on arsenic has expanded substantially since those reports were published and will have to be considered along with the guidance of the other National Research Council reports (NRC 2009, 2011).

THE ENVIRONMENTAL PROTECTION AGENCY'S DRAFT PLANS

EPA provided the committee with its draft planning documents for future IRIS assessments (EPA 2013a,b) and for inorganic arsenic in particular (EPA 2013c,d), which clearly reflect the agency's intent to address the recommendations of previous National Research Council committees (NRC 2009, 2011). The draft planning and scoping summary reflects input collected from program and regional offices of EPA and other stakeholders and provides a general description of the EPA plans to approach the toxicologic assessment. Important elements of the plan include

- Literature search and evaluation—Systematic review principles will be used to evaluate the scientific literature on inorganic arsenic, and studies will be judged according to defined criteria and such factors as bias and study quality.
- Temporal and exposure considerations—Factors will include subchronic and chronic exposures, exposures during different life stages, and evaluation of risks posed by oral, inhalation, and dermal exposures. Aggregate exposures will be considered only in the context of how they might affect exposure estimates in dose–response analysis. Exposure to metabolites will be considered only in mode-of-action analyses.
- Scope of hazard identification—The toxicologic assessment will include cancer and noncancer effects of exposure to inorganic arsenic. In addition to the temporal and exposure considerations described above, factors that might increase susceptibility to inorganic arsenic will be considered, and uncertainties associated with the hazard identification will be characterized.
- Scope of dose–response analysis—The dose–response analyses will examine potential health risks over a range of exposures, and probabilistic, linear, and nonlinear low-dose extrapolation will be included to the extent possible. Aggregate exposures, bioavailability, and speciation will be considered only in the context of how they might affect dose estimates in dose–response analysis. Uncertainties associated with those considerations and other aspects of the dose–response analysis will be considered qualitatively and quantitatively. Mode-of-action analyses will be conducted for health effects associated with direct exposure to inorganic arsenic.
- Communication and public outreach—EPA will seek public input from stakeholders throughout the development of the toxicologic assessment through formal workshops and meetings, webinars, and a public Web site.

STRUCTURE OF THE REPORT

Specific aspects of EPA's plans for the inorganic-arsenic assessment are reviewed in the remaining chapters of this report. Chapter 2 describes exposure issues that will be important to consider in the IRIS assessment, including sources and biomarkers of exposure to inorganic arsenic and the chemical's toxicokinetics; Chapter 3 provides guidance for performing systematic reviews and meta-analyses for the assessment. In Chapter 4, the committee provides an overview of relevant evidence on most of the major health effects that will be the focus of EPA's hazard identification process. The committee did not perform a comprehensive or systematic review of the literature but focused on identifying studies and issues that will be useful in guiding mode-of-action assessments and dose–response analyses. Chapter 5 discusses factors that increase susceptibility to the adverse effects of inorganic arsenic and their implications for the IRIS assessment. The committee's recommendations regarding the performance of dose–response assessments of inorganic arsenic are presented in Chapter 6.

2

Exposure Considerations

Arsenic is a metalloid, having chemical and physical properties intermediate between a metal and a nonmetal. The importance of considering the form of arsenic to which people are exposed, arsenic metabolites, and exposure metrics used in studies has been well documented. How epidemiologic studies have assessed exposure to inorganic arsenic is an important element of study selection, interpretation, and use in dose–response analyses. Several studies and review articles concern the strengths and weaknesses of various exposure measures, including biomarkers, of arsenic (e.g., Vahter 2002; Hough et al. 2010; Basu et al. 2011; Maull et al. 2012; Calderon et al. 2013).

NATURAL AND ANTHROPOGENIC SOURCES

There are numerous potential sources of exposure to inorganic arsenic. Inorganic arsenic occurs in the environment as arsenate (iAs[V]) and arsenite (iAs[III]). Human exposure can result from naturally occurring sources, such as inorganic arsenic in drinking water; from occupational exposure; from the ingestion of contaminated food products, such as rice; and from industrial sources sometimes referred to as "arsenic and old waste" (Gorby 1994). The US Geological Survey, in a study of all major aquifers in the United States, estimated that 7% of household wells have arsenic concentrations that exceed the Environmental Protection Agency's maximum contaminant level (MCL) of 10 ppb, leaving roughly 4.2 million people with the responsibility of taking corrective action themselves (Ayotte et al. 2011). In addition, an unknown number of public water supplies at times exceed the MCL. Moreover, of the 1,320 Superfund sites now on the national priority list (EPA 2013e), 901 name arsenic as a contaminant of concern (EPA 2013f).

FOOD SOURCES

There is increasing evidence that dietary inorganic arsenic makes an important contribution to total inorganic arsenic exposure, particularly when inorganic arsenic concentrations in water are low. In the United States, data from the National Health and Nutrition Examination Survey (NHANES) has proved to be extremely useful for the investigation of the food sources of inorganic arsenic and their relative contributions to overall exposure. Probabilistic exposure modeling that used NHANES dietary data from 2003–2004 coupled with duplicate diet and biomarker measurements estimated that the major contributors to dietary inorganic arsenic intake are vegetables (24%), fruit juices and fruits (18%), and rice (17%) (Xue et al. 2010). Indeed, the lay press has recently called attention to the issue of inorganic arsenic in fruit juices and rice (Consumer Reports 2012a,b), creating concern in the US public. Of all the food components of the US Market Basket Survey, rice—particularly rice grown in the south central United States—has the highest concentration of inorganic arsenic (Schoof et al. 1999; Williams et al. 2007). A recent evaluation of data on 2,323 children 6–17 years old who participated in 2003-2008 NHANES studies found that children who reported consuming rice had urinary arsenic concentrations that were nearly 40% higher than concentrations in those who did not consume rice (Davis et al. 2012). Rice consumption has also

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been associated with urinary arsenic concentration, including inorganic arsenic and its metabolites monomethyl arsenic (MMA) and dimethyl arsenic (DMA), in pregnant women (Gilbert-Diamond et al. 2011). A recent report also indicates that the meat of broiler chickens fed a diet that included roxarsone, an organic arsenical feed additive that promotes growth and feed use, is a source of dietary arsenic, although it is not clear that the meat arsenic is the inorganic form (Gul Kazi et al. 2013).

Given that a great deal of epidemiologic research concerning the health effects of inorganic arsenic exposure from drinking water has been conducted in South Asian and Taiwanese populations, for whom rice is a dietary staple, the committee recommends that the contribution of inorganic arsenic intake from rice be considered in interpreting findings from those regions. For example, Kile et al. (2007) conducted a duplicate diet study to estimate daily arsenic intake in a region of Bangladesh with varied concentrations of inorganic arsenic in household drinking water. The authors concluded that when drinking-water arsenic exceeded 50 ppb, water was the dominant source of inorganic arsenic intake. However, at water arsenic concentrations less than 50 ppb, dietary arsenic is a more important contributor to overall intake. In the United States, an analysis of 252 participants in the National Human Exposure Assessment Survey-Arizona and Arizona Border Survey with diet, water, and urinary arsenic data concluded that 75% of inorganic arsenic exposure was attributed to diet in households that had arsenic in drinking water at 10 μg/L or lower (Kurzius-Spencer et al. 2013). Thus, whenever possible, exposure to inorganic arsenic from rice, rice flour, and other rice-based foods should be considered in estimating total daily exposure, a practice that has largely been ignored. It is critically important because biomarkers of inorganic arsenic exposure, such as urinary arsenic, capture exposures from all sources. However, the estimate of arsenic intake from rice is problematic because arsenic concentrations in rice vary widely by the type of rice and cooking method and because of variation in per capita rice consumption. The committee suggests that EPA consider a probabilistic approach to estimate daily arsenic intake from rice to account for such variability in both arsenic concentrations in rice and rice consumption.

BIOAVAILABILITY

After ingestion, inorganic arsenic in drinking water is rapidly and almost completely absorbed from the gastrointestinal tract (Zheng et al. 2002). Inorganic arsenic in contaminated soils is more poorly absorbed and varies with the type of soil. In a swine model of soil arsenic bioavailability, the fraction of arsenic absorbed ranged from 7 to 75%, depending on soil type (Juhasz et al. 2007); this information could be of use in situations where the ingestion of soil via pica or ordinary hand-to-mouth activity in children is a concern. The absorption of inorganic arsenic in food is less well studied. However, using a similar in vivo swine model, a study of inorganic arsenic bioavailability in vegetables grown hydroponically in arsenic-contaminated water found that its bioavailability varied between 50 and 100%, depending on the type of vegetable (Juhasz et al. 2008). There is also evidence that the bioavailability of arsenic from ingested rice is high (Juhasz et al. 2006). Rice strains vary in arsenic content and in their relative proportions of inorganic arsenic and methylated arsenic species (Consumer Reports 2012b). A mass-balance study of the bioavailability of rice arsenic in humans involved two volunteers who ate a wheat-based diet and then switched for a week to a rice-based diet that had a known mass (and speciation) of arsenic (He and Zheng 2010). Of all of their dietary items, rice had the highest arsenic content, and 76% of the rice arsenic was inorganic arsenic. The mean urinary excretion fraction was about 58% in one subject and 63% in the other—indicating that a substantial fraction of the rice arsenic was absorbed. Several studies have identified increased concentrations of urinary arsenic metabolites after ingestion of rice, and studies that used more sensitive arsenic speciation methods have found elevated concentrations of urinary inorganic arsenic and DMA in people for whom rice is a dietary staple (e.g., Cascio et al. 2011). Thus, considerations of dose–response relationships derived from epidemiologic studies concerning the health effects of arsenic in drinking water should include the likelihood that the dose derived from drinking water alone does not represent the total arsenic dose.

ABSORPTION, DISTRIBUTION, METABOLISM, AND ELIMINATION

A summary of the evidence on the metabolism of arsenic and the implications for understanding arsenic's toxicity at low doses was provided at the committee's workshop by Thomas (2013). The metabolism of inorganic arsenic is complex and leads to the formation of various arsenic species that differmarkedly in toxicity, tissue distribution, and rate of elimination. Inorganic arsenic, both in the trivalent and pentavalent oxidation states, is easily absorbed in the gastrointestinal tract. Absorbed pentavalent arsenic is largely reduced to trivalent arsenic in the blood, particularly after low to moderate exposures (Vahter 2002). Trivalent arsenic is the main form taken up by the hepatocytes (Lerman et al. 1983) and subsequently metabolized to MMA and DMA, both of which may exist in the trivalent and pentavalent oxidation states. Arsenic methyl transferase (AS3MT) is the requisite enzyme, and *S*-adenosyl methionine serves as the methyl donor (Lin et al. 2002) (Figure 2). Arsenical thiols may also form (Fricke et al. 2005), but there is less evidence of their occurrence in human urine (Raml et al. 2007), perhaps in part because of their oxidation once eliminated (Currier et al. 2013). In short, human tissues, blood, and urine contain a mixture of arsenic metabolites that vary in acute and chronic toxicity.

There is strong evidence from cell and animal-model systems that the trivalent species of inorganic arsenic, MMA, and DMA are far more toxic than the less reactive pentavalent species. MMA(III) has been shown to be particularly cytotoxic in human cell cultures (Styblo et al. 2000). The extent to which DMA(V) can be reduced to the more toxic DMA(III) is unclear although there is some evidence that DMA(III) is also found in human biologic samples (Valenzuela et al. 2005). The retention of arsenic in tissues is influenced by a host of factors, particularly methylation capacity. In that regard, recent work with the AS3MT knockout mouse has been extremely revealing in that it clearly demonstrates the importance of arsenic methylation in facilitating elimination and decreasing tissue retention (Drobna et al. 2009).

Collectively, inorganic arsenic and its metabolites have many targets of toxicity and carcinogenicity. For example, IARC (2012) lists the lung, urinary bladder, and skin as known targets and the prostate, liver, and kidney as three probable targets for carcinogenicity. Chapter 4 of the present report describes many other organ systems in which noncancer toxicity is manifested. Tissues vary extensively in their arsenic methylation efficiency (Kobayashi et al. 2007), which probably affects their susceptibility to toxic insult. However, the cellular uptake of the various arsenic metabolites and the intracellular distribution and extrusion of them might also vary extensively among tissues and contribute to the variation in toxicity (e.g., Dopp et al. 2010). Those variations imply that the mode of action of inorganic arsenic might depend on the type of tissue, as well as exposure factors. Predictions of tissue concentrations of inorganic arsenic and its metabolites may be obtained by using physiologically based pharmacokinetic models, although human data to parameterize such models is limited (El-Masri and Kenyon 2008).

The relative toxicity of the various arsenic metabolites is related to both their inherent reactivity and their circulating half-lives. The administration of a single oral dose of radioactive ^{74}As as the trioxide to human volunteers revealed half-lives of 2, 10, and 38 days (Pomroy et al. 1980); because some arsenic distributes to bone (Lindgren et al. 1982), it is conceivable that with chronic long-term exposure there is an even longer half-life at a steady state. Indeed, a recent report that describes a case of osteoresorptive arsenic intoxication in a 47-year-old woman who had osteoporosis and had been exposed to arsenic as a child provides support for earlier animal studies that indicated that bone can be a reservoir of inorganic arsenic (arsenate) from which arsenic can be released later in life (Dani 2013). Buchet et al. (1981a) administered 500 μg of arsenic as arsenite, MMA, or DMA to human volunteers. Within 4 days, urinary excretion totaled 46, 78, and 75% of the administered dose, respectively. Another study by the same investigators administered sodium meta-arsenite ($NaAsO_2$) to volunteers at various doses up to 1 mg/day for 4 days and reported that arsenic methylation was not saturable even at the dose of 1 mg/day (Buchet et al. 1981b).

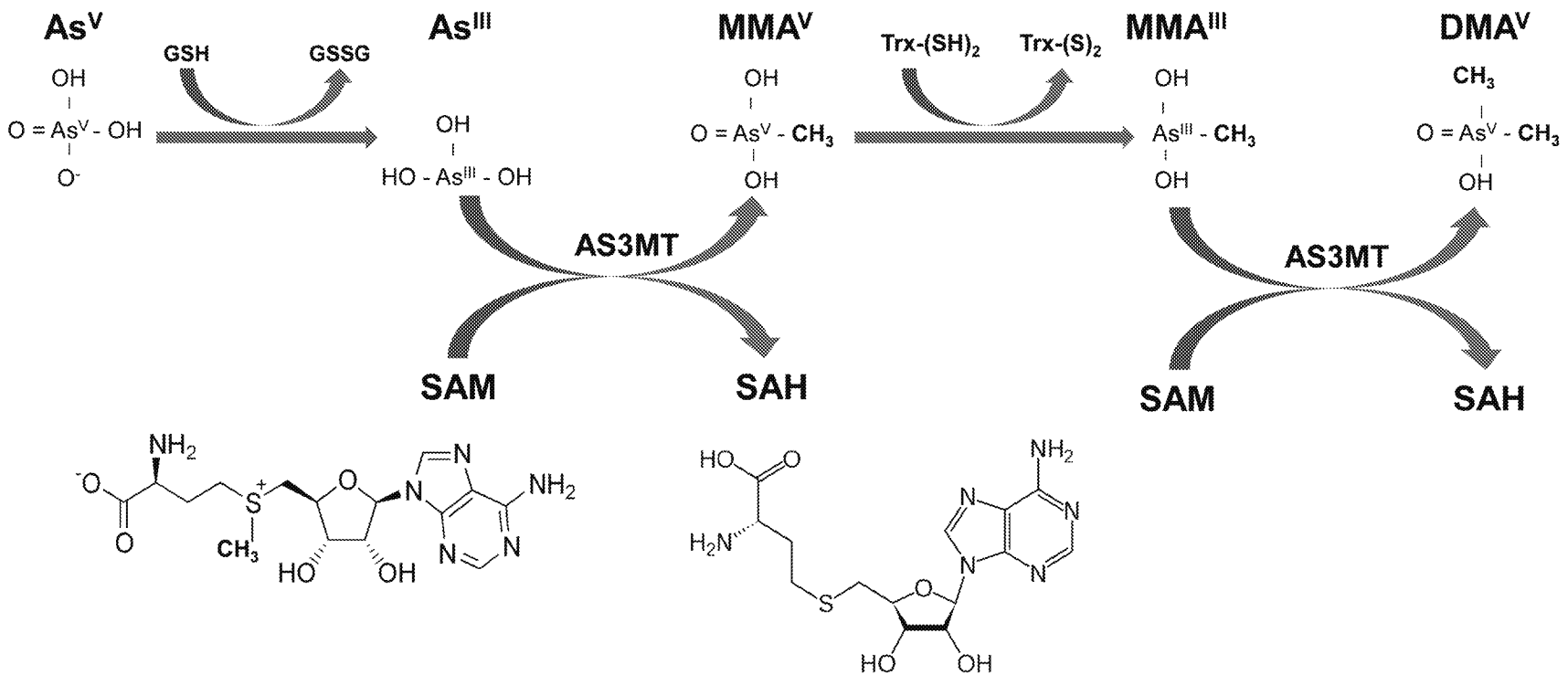

FIGURE 2 Arsenic metabolism: Inorganic arsenate (As^V) can be reduced by glutathione (GSH) or other reductants to yield inorganic arsenite (As^{III}) and glutathione disulfide (GSSG). As^{III} is converted to pentavalent monomethylarsonic acid (MMA^V), a reaction catalyzed by arsenic-3-methyltransferase (AS3MT), with *S*-adenosyl methione (SAM) serving as the methyl donor; in the process, SAM is hydrolyzed to *S*-adenosyl homocysteine (SAH). After the reduction of MMA^V to MMA^{III} by thioredoxin (Trx), a second methylation step results in the synthesis of dimethylarsinic acid (DMA^V). (Figure courtesy of Megan Hall.)

People vary widely in their ability to metabolize inorganic arsenic, as reflected by the widely varying proportions of inorganic arsenic, MMA, and DMA in urine and blood. Women are more efficient than men in converting inorganic arsenic to DMA, particularly during pregnancy, when arsenic metabolism is facilitated (Vahter et al. 2006), and children appear to methylate arsenic as well as adults (e.g., Wasserman et al. 2004). There is consistent evidence from epidemiologic studies in populations exposed to high arsenic concentrations in drinking water that people who can efficiently convert inorganic arsenic to DMA are at lower risk for arsenic-induced disease than those who are "poor methylators", who have higher proportions of MMA and inorganic arsenic in urine. In particular, in comparison with those who have a low proportion of MMA (III + V) in urine, those who have a higher proportion have been observed to be at higher risks for heart disease, atherosclerosis, peripheral vascular disease, hypertension, and bladder, lung, and skin cancer (reviewed in Steinmaus et al. 2010). Those findings are derived from studies in Taiwan, Argentina, and Bangladesh. There is compelling evidence that arsenic metabolism and toxicity vary because of genetic factors (see discussion in Chapter 5).

Thus, the hazard assessment of oral exposure to inorganic arsenic should take into account the fact that some people are more susceptible than others because of their relative inability to metabolize arsenic. Susceptibility factors, discussed more fully in Chapter 5, include life stage, genetics, sex, nutritional characteristics (such as folate, selenium, and body-mass index), pre-existing diseases, smoking, alcohol consumption, and exposure to mixtures of chemicals.

With regard to animal studies, it is important to note that the metabolism of inorganic arsenic varies widely among species (Vahter et al. 1995a; Vahter 1999; Loffredo et al. 2003). Dogs and mice, for example, are extensive methylators of inorganic arsenic and excrete 80% or more of a given dose as DMA in urine and less than 5% as MMA. Humans excrete more MMA on the average than other species, and this suggests that humans may be poorer methylators of inorganic arsenic. There is extensive evidence from cellular and animal models that MMA (III) and DMA (III) are more toxic than inorganic arsenite (e.g., Styblo et al. 2002), although the in vivo toxicities reflect the overall balance both among forms of arsenic and the extent and rate of its elimination. Arsenic excreted by humans tends to be 10-20% MMA, whereas that of dogs, hamsters, mice, rabbits, and rats is 1-5% MMA (Vahter 1999). Guinea pigs, marmoset monkeys, and chimpanzees do not methylate inorganic arsenic (Vahter 1999). Several species metabolize inorganic arsenic to a trimethyl form as well, but this form has not been observed in humans. After ingestion of DMA, however, a few percent of the arsenic excreted in the urine was in the form of trimethylarsine oxide (Marafante et al. 1987).

Finally, considerable work in mice has described the tissue distribution of various arsenic metabolites. For example, the oral administration of radiolabeled arsenate (^{73}As) led to accumulation in organs known to be targets for inorganic arsenic–induced cancers (bladder, kidney, skin, and lung). MMA was found in all organs except the bladder. In the kidney, inorganic arsenic was the predominant species (Hughes et al. 2003), possibly because arsenate undergoes renal tubular reaborption via the phosphate transporter (Ginsberg and Lotspeich 1963; Ginsberg 1965). Bladder and lung had the highest percentage of DMA; this is of interest in that mouse studies of DMA suggest that it might be a tumor promoter, albeit at concentrations of 10-100 ppm, concentrations that are not relevant to human exposures (reviewed by Hughes et al. 2003). It is notable that in AS3MT knockout mice, which have markedly impaired arsenic methylation, dramatic cytotoxic urothelial cell changes occur within hours after the administration of sodium arsenite (Arnold et al. 2013).

BIOMARKERS OF EXPOSURE

There are various ways to estimate exposure to environmental chemicals. Environmental sampling can be conducted in air, water, soil, house dust, and food to estimate exposure. To estimate exposure, statistical models can be used to relate environmental concentrations to internal dose, estimated over various time frames, but such processes rely on an assumption that environmental concentrations correlate with amounts of arsenic ingested and inhaled (e.g., Nasreddine and Parent-Massin 2002). A more direct way to

assess exposure to arsenic from all sources is to assess biomarkers of internal dose, although such measures also have limitations, as discussed below. The term *biomarkers* can refer to analytic measures that reflect mechanistic biologic *effects* of an environmental exposure or reflect an estimate of the *internal dose* of exposure to an exogenous agent. This section will concentrate on the latter type of biomarker with regard to arsenic. It should be noted that whether using environmental measures of exposure or biomarkers of exposure, similar issues arise regarding the constancy or variability of exposure, population mobility, the relevant period of exposure (e.g., long latency would make exposures long in the past the relevant information), and other factors, so repeated measures over time are valuable.

The most commonly used biomarker of arsenic exposure is total arsenic in urine. Arsenic measured in urine is derived from recent exposures, largely the previous day, so consideration of whether exposures are constant or changing over longer durations involved in the development of arsenic-induced toxicities is needed. Total arsenic includes some forms of organic arsenic commonly occurring in various seafoods, such as arsenobetaine, that have short half-lives and little or no toxicity. The speciation of arsenic metabolites in urine is thus necessary to separate the contribution of arsenobetaine from fish and seafood from the metabolites of inorganic arsenic. After exposure to inorganic arsenic, the proportions of arsenic metabolites excreted in human urine are typically 10-30% inorganic arsenic, 10-20% MMA, and 60-70% DMA (Vahter 2002). A limitation of the use of urinary arsenic is that altered renal function might influence the extent to which the various arsenic metabolites (and creatinine) are excreted. If the urine collection is not a timed collection over 24 hours, it is essential to adjust the measured urinary arsenic concentrations for variation in urine dilution, which may vary widely. In that respect, creatinine excretion has been associated with arsenic methylation efficiency in several studies (Gamble et al. 2005; Nermell et al. 2008; Basu et al. 2011). Barr et al. (2005) has pointed out that using urinary creatinine to create a ratio with the urinary chemical concentration can introduce bias because of the undue influence that high or low ratios can produce. In recent years, the most common practice is to use urinary creatinine concentration as a covariate in a regression analysis to reduce this effect. Creatinine excretion also varies with age, sex, meat intake, and muscle mass. Adjusting by specific gravity is a useful alternative to creatinine adjustment, in that it is less influenced by those factors (Nermell et al. 2008). Several studies have examined interindividual and intraindividual variability in urinary arsenic measurements and have concluded that total urinary arsenic is relatively stable but that the concentrations of urinary arsenic metabolites are not (e.g., Calderon et al. 1999; Hopenhayn et al. 2003a; Steinmaus et al. 2005; Kile et al. 2009). Such studies have shown that pregnancy, life stage, and disease can influence metabolism.

Several other types of exposure biomarkers often used in human population studies involve primarily inorganic arsenic (Button et al. 2009). Among them are hair arsenic and toenail arsenic. Because arsenic accumulates in tissue that are rich in the protein keratin, such as hair and nails (Mandal et al. 2003; Raab and Feldmann 2005), these tissues can be used as matrices for exposure biomarkers. Measurements taken from the tissues are generally indicators of past exposure to arsenic, in contrast with urinary measurements, which reflect more recent exposure, although the period of exposure captured by these tissues can still be short compared with the latency periods between exposure and some arsenic-induced effects. Internal deposition of inorganic arsenic into hair and nails occurs through blood flow, but these structures are themselves external, so environmental contamination must be considered. Various cleaning techniques are used to remove external contaminants. They are adequate for toenails, but removal may not be complete, particularly for hair samples, which are more porous, or in populations that wear open footwear or go barefoot. Unlike blood and urine, these biomarkers can provide information on the timing and dose of past exposure. Hair grows at an average rate of 1 cm per month, so the 1 cm of hair nearest the scalp should correlate with the integrated level of exposure over the last month. Segmentation of hair in 1-cm blocks can provide repeated measures of exposure at monthly intervals. Because of the growth rate of toenails, the lag time between the formation of living nail (found in the cuticle area) and the clipped nail at the end of the toe takes about 6-12 months; thus, measures of toenail arsenic represent past exposure (Karagas et al. 2000; Slotnick and Nriagu 2006). There is evidence that toenail concentrations were fairly consistent over a period of several years (Garland et al. 1993; Karagas et al. 2001a). Fingernails grow

faster and can yield information on exposure about 6 months in the past. Thus, consideration should be given to the exposure metric's relevance to health outcomes with respect to their latency.

Finally, since the advent of sensitive inductively coupled plasma mass spectrometry techniques, blood arsenic has been used in recent years but remains the least used biomarker. In particular, speciation of arsenic in blood is more complicated than that in urine. In at least two studies in Bangladesh, total arsenic in blood was found to be significantly associated with urine and water arsenic and with risk of skin lesions (Hall et al. 2006; Y. Chen et al. 2007a). Whole-blood arsenic would be most reflective of acute exposure; however, as with any biomarker of chemical exposure, chronic low-dose exposure would eventually lead to a steady state. Such a situation could exist in the case of drinking-water contamination or dietary exposure. Limitations of the methods of measuring blood arsenic include the relatively narrow range of values (in comparison with urine), its high cost, and its failure to exclude the relatively nontoxic species of arsenic derived from seafood. Its strength is related to its biologic proximity to organs of concern and a reduced risk of external contamination compared with hair and nails. Arsenic metabolites in blood differs from those in urine in that there is a substantially lower proportion of DMA (because of its shorter circulating half-life) and relatively more inorganic arsenic and MMA (Hall et al. 2006).

In summary, measurements of arsenic in urine, blood, toenails, and hair have been used as biomarkers of exposure to inorganic arsenic in various epidemiologic studies. The extent to which these biomarkers have been "calibrated" to external exposure to inorganic arsenic varies, and this sometimes poses a challenge in the interpretation of findings. Nevertheless, the Integrated Risk Information System assessment can benefit from careful examination of studies in which estimates of both external exposure (such as water concentrations) and biomarker data are provided.

3

IRIS Assessment Development Plans: Evidence Evaluation, Systematic Review, and Meta-analysis

EVIDENCE EVALUATION

The US Environmental Protection Agency (EPA) draft plans for the inorganic arsenic assessment indicate that a broad literature search and screening process will be used to identify health effects that have been studied in relation to arsenic (EPA 2013c), and a preliminary draft of these efforts was provided to the committee (EPA 2013d). The scientific literature will be organized into summary tables by health-effect category to get an overview of the types and numbers of studies available for each health effect. Evidence tables will be used to provide more specific information on exposures, outcomes, and evaluation methods. Overall, the committee found that the draft plans and example tables captured the salient categories of information with respect to epidemiologic studies. However, no descriptions or examples were provided for organizing the evidence from animal and in vitro studies. Such information will be particularly important for mode-of-action analyses (discussed in Chapter 6).

Evidence Tables for Animal and In Vitro Studies

Understanding exposure data for each health outcome across the dose range is important and is an acknowledged difficulty EPA will face with the existing arsenic database. The draft plans for the inorganic arsenic assessment describes Evidence Tables by Health Effect Category that record exposure and outcome information for human studies. Evidence tables for animal and in vitro studies are also needed for mode-of-action analyses, described in Chapter 6 of this report. Information for the mode-of-action analyses will come in part from studies to be entered in the evidence tables, and the committee recommends that EPA design these tables, or additional tables, early in its evaluation process, to avoid unnecessary duplication of effort in evaluating the literature and populating the tables.

Evidence tables for animal and in vitro studies are not described in EPA's draft work plan. Information recorded should include details of dosing or exposure (such as form of arsenic, concentration or dose, route of exposure, and duration of exposure); the species and strain of animal, tissue or cell line; and all end points measured. For mechanistic studies, end points related to mechanisms of effects or mode of action should be recorded for each exposure reported. For all studies, observations of morbidity and mortality or biomarkers of cell death or impaired cellular integrity should be recorded. Such information can facilitate comparing dose-response relationships among different routes of exposure, cell lines, and especially animal models, given the marked species differences in arsenic kinetics and toxicity discussed in Chapter 2 and in the committee's workshop.

Analysis of Gene–Expression and Genomic Data

The dataset for arsenic includes genomic data, such as gene expression data, variability based on DNA polymorphisms, and differences in epigenetic control of expression. Some of the studies have used

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high-throughput technologies. Microarray technology is typically used for assessing gene expression and specialized arrays are available for detecting polymorphic and isoform variation. Gene expression measured by next–generation sequencing (NGS), often referred to as RNA-seq, is becoming more affordable and therefore its use is increasing. RNA-seq has a higher dynamic range than microarray and allows detection of polymorphisms and splice variants. NGS can be used to study epigenetic regulation of DNA expression. In reviewing studies reporting microarray or NGS data, EPA should consider the strengths and limitations inherent to the different high-throughput methods.

In evaluating gene–expression and genomic data, EPA should give close attention to the data processing steps and how they influence the results. For both technologies, raw expression values, extracted from image files, need to be adjusted so that the expression values reflect the abundance of genes in the sample. This preprocessing of microarray data has been reviewed by Allison et al. (2006)and Bolstrad et al. (2006). Various techniques for normalizing the expression values among microarray chips in an experiment have been developed and have been subjected to comparisons by statisticians and bioinformaticians (e.g., Qin et al. 2013). Hansen et al. (2012) discuss RNA-seq preprocessing. EPA's review could also assess how the alignment of the reads to the reference genome would influence gene expression results; this is discussed by Garber et al. (2011). Because batch effects, such as daily variations in ozone effects on dye and drift of scanner performance or between sequencer flow cells, are not eliminated by normalization, EPA should consider whether the statistical analysis considers batch effects appropriately.

Both technologies measure the expression of tens of thousands of genes simultaneously. When there are tens of thousands of comparisons and a paucity of replicates relative to the number of comparisons, sophisticated techniques are needed to appropriately apportion variance among fixed effects and random effects and to control the type-1 error rate for the multiple comparisons. In addition, expressions of individual genes are not necessarily independent of each other; that is, a given gene can increase the expression of some genes and decrease the expression of others if they belong to the same biological pathway. Thus, analysis of gene networks are now commonly used. In general, classical procedures for controlling overall error are over strict and tests of false discovery rate (Benjamini and Hochberg 1995) are often used in identifying differentially expressed genes. Other approaches use information from all genes on the chip to calculate moderated t-statistics. Two widely used methods are linear models for microarray data (limma, a Bayesian method, Smyth 2004) and statistical analysis of microarrays (SAM, a permutation method, Tusher et al. 2001). These methods have been adapted for NGS and were compared by Sonenson and Delorenzi (2013). EPA should review the statistical analyses used, ensure that they are sufficient to support conclusions reached, and note in the evidence tables details of the statistical analysis and limitations on the conclusions.

Many scientists confirm findings from high-throughput methods using other techniques, such as polymerase-chain-reaction or protein-based measurements. For conclusions based solely on expression results, the committee recommends a detailed analysis of the data supporting the conclusion. This should include thorough review of the effectiveness of the preprocessing and the statistical analysis and will require access to the raw data.

SYSTEMATIC REVIEW AND META-ANALYSIS

EPA's draft plans indicate that the hazard-identification process will generate questions specific to the toxicologic review, and the questions will be addressed in systematic reviews. EPA expects questions to be generated about aspects related to adverse-outcome pathways and dose–response characterization. For example, EPA indicates that systematic reviews of dose–response analyses will be used to identify chemical and nonchemical stressors that might contribute to the health outcome under consideration and to identify underlying disease processes that might be added to or increased by exposure to inorganic arsenic. Those uses were recommended by the National Research Council committee that wrote *Science and Decisions* (NRC 2009) and were followed up on in Chapter 7 of *Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde* (NRC 2011).

Efforts are under way to develop EPA guidance on performing systematic reviews. EPA held a workshop on August 26, 2013, that was devoted to that topic. Another National Research Council committee is evaluating such improvements of the IRIS process and is expected to issue a report in the first quarter of 2014. In light of those activities, the present committee focused its efforts on identifying issues specific to inorganic arsenic in the performance of systematic reviews rather than on specific procedural aspects of such reviews.

Systematic reviews and meta-analyses are widely used in medicine, epidemiology, and public health to evaluate and synthesize scientific evidence objectively (Egger et al. 2001). Systematic reviews comprehensively identify and evaluate the available evidence with a transparent process. They are used to reach evidence-based conclusions, to clarify the need for additional research, and potentially to summarize the evidence quantitatively with meta-analysis (Egger et al. 2001; Porta 2008; Rooney 2013). Meta-analysis is a statistical technique that can be used to pool evidence from systematic reviews if various conditions are met. To minimize uncertainty in original research, systematic reviews follow objective criteria for collecting and evaluating evidence in a comprehensive manner. A priori decisions and a predefined protocol are critical during the systematic review process (Berlin and Colditz 1999; Dickersin 2002); the protocol should describe the following steps: the research question, the search strategy and data sources, the study inclusion and exclusion criteria, the data to be abstracted and derived from the original studies (such as sample size, exposure and outcome assessment methods, and confounders evaluated), the criteria and methods for pooling effect estimates and measures of variability among studies. Systematic reviews and meta-analyses need to be replicable; other investigators following the same steps should be able to identify the same articles, abstract the same data, and reach similar conclusions. Originally developed in the field of clinical trials, systematic reviews and meta-analyses are commonly used to summarize epidemiologic research, including risk factors for a disease and dose–response relationships, but can also be used to summarize disease mechanisms (Dickersin 2002). The use of systematic reviews to evaluate experimental evidence is increasing, and some aspects are still in development. For instance, there is no clear best practice for evaluating the risk of bias in in vitro studies (Rooney 2013). Chapter 7 describes in more detail the role of meta-analyses in the evaluation of dose–response relationships and how they can be used for developing risk-based estimates. The role of systematic reviews and meta-analyses in the regulatory process for drugs, foods, and medical devices has long been recognized (Berlin and Colditz 1999), and their relevance for environmental policy, including both epidemiologic and experimental research, is increasing (NRC 2009, 2011). The Office of Health Assessment and Translation of the National Toxicology Program has developed an initiative to incorporate systematic reviews and evidence integration for literature-based environmental health assessment (Rooney 2013).

Given the large number of studies, evaluating the epidemiologic and experimental literature on arsenic health effects can be a daunting task. To guide the process and ensure high quality, it is critical to establish clearly the research questions that need to be answered. Examples of research questions about epidemiologic and experimental data, using cardiovascular disease as the health outcome, are shown in Box 4. EPA plans to include systematic review principles in performing its Integrated Risk Information System (IRIS) assessment of inorganic arsenic will be a welcome improvement.

EPA has some experience with performing systematic reviews and meta-analyses for IRIS assessments of other chemicals, such as trichloroethylene (EPA 2011). Lessons learned in performing those analyses might guide the systematic reviews and meta-analyses for inorganic arsenic. Also, a number of systematic reviews have summarized the association between arsenic and several health end points, including cancer (Celik et al. 2008), cardiovascular disease (Navas-Acien et al. 2005; C.H. Wang et al. 2007; Moon et al. 2012), diabetes (Navas-Acien et al. 2006; Maull et al. 2012), hypertension (Abhyankar et al. 2012), and neurodevelopment (Rodriguez-Barranco et al. 2013). Only a few of those reviews have attempted to generate pooled estimates of the association between arsenic and disease (Navas-Acien et al. 2006; Moon et al. 2012; Rodriguez-Barranco et al. 2013). Some of the systematic reviews of arsenic and health end points can be useful starting points, including their search strategies, table format, data collection, and quality evaluation forms. It is likely, however, that none of them will be sufficient for EPA's purposes. First, the article

BOX 4 Examples of Research Questions about Epidemiologic and Experimental Data

Examples of research questions that can be used to guide systematic reviews for the evaluation of arsenic-related health effects with overall clinical cardiovascular-disease mortality as the effect. They include questions related to hazard identification, dose–response analysis, and mode-of-action evaluation. Once the research questions have been established, a protocol should be developed to ensure that all relevant articles answering each of the questions are identified. Although the questions were developed by using cardiovascular disease as an example, other health end points and mechanisms could have been selected.

1. Is arsenic exposure, measured before the development of cardiovascular disease at the individual level, associated with overall clinical cardiovascular disease mortality in populations exposed to arsenic in drinking water at less than 100 μg/L?
2. What is the dose–response relationship between arsenic and cardiovascular-disease mortality throughout the range of arsenic exposure that is relevant for human populations (for example, arsenic in drinking water ranging from less than 10 μg/L to over 100 μg/L)?
3. What is the dose–response relationship between arsenic exposure and mechanisms of generation of reactive oxidant species or inflammation in relevant animal models and cellular systems that can be used to evaluate the mode of action of arsenic-related cardiovascular disease?

search will need to be updated. Second, the reviews categorized the studies into two groups (studies with arsenic concentrations less than or greater than 100 μg/L). However, a few studies conducted in populations exposed to high arsenic concentrations in drinking water had some categories at concentrations below 100 μg/L, and the results for those categories can be included in the analyses at low to moderate arsenic exposure.

General limitations of the conduct and interpretation of meta-analyses for arsenic health effects have been the substantial heterogeneity among studies, methodologic limitations in outcome and exposure assessment, the use of different biomarkers, the temporality of an association, adjustment for relevant confounders, and the potential for publication bias. Heterogeneity, however, is relatively common in observational studies; it should be evaluated but should not necessarily impede data-pooling. Sources of heterogeneity could be evaluated with meta-regression and analysis of influential studies. Publication bias is a concern in the conduct of systematic reviews, and different strategies are available to evaluate it, including the use of funnel plots.

The increasing number of published prospective studies that have adequate exposure and outcome assessment (for example, see section "Cardiovascular Disease" in Chapter 4) provides an opportunity for EPA to apply meta-analysis techniques to estimate the association between arsenic and relevant health end points. In selecting the studies to be included in the meta-analyses, EPA should systematically search for studies with individual measures of arsenic exposure (preferably with biomarker measurements), measurement of arsenic that precedes the outcome, and low to moderate exposure to inorganic arsenic (less than 100 μg/L in drinking water). Meta-analysis can then be performed if there are three or more peer reviewed studies available on the outcome of interest. For dose-response meta-analysis, studies will need to have at least three or more doses tested. As part of the systematic review process, study quality (risk of bias) needs to be evaluated using established guidelines for epidemiologic studies (Viswanathan et al. 2012; Rooney 2013). Elements in the evaluation of study quality include the assessment of study outcome based on standardized definitions, participation rate, adjustment of association for relevant confounders, and other considerations that will depend on study design.

Meta-analyses of published studies are sometimes limited by what is available in the aggregated data. Individual-level meta-analyses—meta-analyses that use raw data from the original studies (Riley et al. 2010)—provide an opportunity to answer specific questions and to use common definitions across studies. Major limitations of individual-level meta-analyses, however, are the requirement of active participation by the original investigators, of standardization across studies, and of additional resources and fund-

ing to conduct the studies. For the purpose of the IRIS assessment, given limited resources and the number and quality of published studies of some health end points associated with low to moderate arsenic exposure, conducting meta-analyses of aggregated data from published studies is an appropriate alternative to collecting and analyzing raw data for individual-level meta-analysis.

4

Hazard Identification

The major goals of the Environmental Protection Agency (EPA) toxicologic review of inorganic arsenic are identification of the disease hazards associated with chronic exposure to arsenic and estimation of the potential disease risks when people are exposed for a portion of their lifetime. For hazard identification, EPA has outlined a process that involves approaches to screen and organize studies, a causality framework for evaluating cancer and noncancer effects, and an evidence framework for determining susceptible life stages and populations. Those approaches have been designed to incorporate recommendations made in other National Research Council reports that are applicable to all Integrated Risk Information System (IRIS) assessments; they are under review by another Research Council committee.

The present committee has focused on EPA's plans for hazard identification that are specific to inorganic arsenic. EPA has indicated that its assessment of causality will be based primarily on studies in humans under the assumption that any health effects observed in such studies are relevant to humans, regardless of country of origin and regardless of whether the mode of action is understood. Animal and mechanistic data will provide supporting evidence with respect to biologic plausibility. If particular effects are observed only in animal studies, mechanistic data will be used to address questions about the relevance of the data to humans. If mode-of-action data are insufficient for determining relevance, EPA will assume that the effects are relevant to humans.

The sections that follow contain a preliminary survey of the scientific literature on what appear to be the most affected organ systems and focus on the epidemiologic evidence. The strengths and weaknesses of epidemiologic studies of cancer and noncancer effects were discussed in the committee's workshop by Cantor (2013) and Steinmaus (2013), respectively. The committee also considered mode-of-action information that would inform dose–response analyses, particularly with respect to low to moderate exposure (see Box 5). Consideration was also given to factors that could increase susceptibility to the effects of inorganic arsenic. In approaching its task, the committee did not attempt to conduct systematic reviews of the literature for each organ system. That task lies ahead for EPA. Rather, the committee has attempted to focus on factors that will help to tailor such reviews and to inform decisions about performing dose–response analyses.

The committee notes that among the noncancer effects of inorganic arsenic exposure, particular attention should be paid to the category of diseases that occur with a high prevalence in the US population so that it can be determined whether exposure to inorganic arsenic may be contributing to the underlying burden of disease in the population. That category includes cardiovascular disease, respiratory disease, kidney disease, and diabetes. Adverse effects of early-life exposure should also be considered.

BOX 5 Concentration Descriptors Used in This Report

The terms used here to describe the degree of arsenic exposure are not necessarily the same as those used in the cited published studies. *High exposure* is used to denote exposure to concentrations of inorganic arsenic in drinking water at 100 μg/L or higher. *Low to moderate exposure* refers to water concentrations of less than 100 μg/L.

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SKIN DISEASES

Arsenic-related skin conditions include two related conditions: preneoplastic or nonneoplastic skin lesions (henceforth referred to as skin lesions) and nonmelanoma skin cancers. The types of skin conditions and their risks are determined by dose, duration, and susceptibility to exposure. The epidemiologic features of the two conditions, especially as related to those determining factors, are described below.

Skin Lesions

Arsenical skin lesions predispose a person to some skin cancers and serve as an indicator of sufficient arsenic exposure for (or increased susceptibility to) the occurrence of other cancer and noncancer diseases (Ghosh et al. 2007). Owing to differences in arsenic metabolism and toxicity among species, observational epidemiologic studies are preferred for determining the association between arsenic exposure and skin lesions.

Skin lesions have a well-established dose–response relationship with arsenic in drinking water. ATSDR (2007) based its chronic minimal risk level of 0.0003 mg/kg-day on skin lesions. Skin lesions were reported to occur at concentrations as low as 10 μg/L in cross-sectional (Ahsan et al. 2006) and prospective cohort (Argos et al. 2011) studies. The association was confirmed at concentrations of 50–100 μg/L (Guha Mazumder 2003; Guo et al. 2006; Argos et al. 2011). However, other studies have found skin lesions to occur only at high concentrations (e.g., Haque et al. 2003).

In a longitudinal study in Bangladesh, the odds of skin lesions were about 70% higher at water concentrations of 50–100 μg/L than at less than 10 μg/L. The odds nearly doubled in those exposed at 100–200 μg/L and nearly tripled in those exposed at 200 μg/L or higher (Argos et al. 2011). The association may be modified by sex (Chen et al. 2006), genetic variations (Ahsan et al. 2007), and diet (Pierce et al. 2011).

The clear dose–response relationship persists when exposure is measured by way of urinary concentration of monomethyl arsenic (MMA), a metabolic intermediate of arsenic. A case–control study in Bangladesh found a dose–response relationship between the percentage of total urinary arsenic that was MMA and skin lesions in 594 cases and 1,041 population-based controls (Ahsan et al. 2007). A small Taiwanese case–control study of 26 skin-lesion patients with sex- and age-matched controls indicated that participants who had a high percentage of MMA (more than 15.5%) had an odds ratio (OR) of 5.5 compared with those who had a low percentage of MMA, and those with low dimethyl arsenic (DMA), less than 72.2%, had an OR of 3.25 compared with those who had high DMA (Yu et al. 2000).

In a Mexican cross-sectional study of 104 people who lived near mining operations and contaminated groundwater (76 exposed at 50 μg/L or more, 28 exposed at 10 μg/L or lower), residents who had skin lesions had urinary MMA concentrations of 7.5 μg/L whereas residents who did not have lesions had 4.8 μg/L (Valenzuela et al. 2005). By using pathway analysis to address the collinear relationships among urinary arsenic metabolites, Kile et al. (2011) have also shown that urinary MMA concentration is associated with an increased risk of skin lesions in a case–control study.

Skin Cancer

Arsenic is an established skin carcinogen, first classified by the International Agency for Research on Cancer (IARC) in 1987 on the basis of observations of patients treated with the arsenical Fowler's solution. A causal relation between arsenic in drinking water and skin cancer was confirmed in the 2012 IARC publication based on ecologic studies in Taiwan, primarily in the southwest region, where geologic arsenic is endemic (Wu et al. 1989). To date, almost all published studies linking arsenic exposure to skin cancer have found supportive evidence of nonmelanoma skin cancers (basal-cell and squamous-cell carcinoma).

The evidence of skin-cancer risk posed by high arsenic exposure appears to be well supported by cohort, case–control, and ecologic studies, with increasing arsenic exposure associated with increasing skin-cancer risk. A US population-based case–control study of skin cancers also reported evidence of an increase in invasive squamous-cell carcinoma in those who had the highest concentrations of toenail arsenic (Karagas et al. 2001b).

At lower arsenic exposure, some studies have been able to estimate the effect of arsenic exposure on skin-cancer risk although in general the findings from these studies have been less conclusive. The ASHRAM case–control study in Hungary, Romania, and Slovakia found an association between histologically confirmed basal-cell carcinoma of the skin and drinking-water inorganic arsenic concentrations greater than 100 μg/L (Leonardi et al. 2012). The study suggested that at lower concentrations an OR of 1.18 was associated with each 10-μg/L increase in average lifetime water concentration, with the odds of basal-cell carcinoma increasing with increasing concentrations of inorganic arsenic. A case–control study in Slovakia showed statistically significantly higher concentrations of urinary arsenic metabolites in people who had nonmelanoma skin cancer than in controls (Ranft et al. 2003). Arsenic exposure was primarily from residential proximity to a coal-burning power plant. The urinary arsenic concentrations in the study were all less than 50 μg/L but showed an association with nonmelanoma skin-cancer status.

Other studies of lower arsenic exposure have been unable to measure skin-cancer risk at the lower concentrations precisely because of methodologic and sample-size limitations. A Danish ecologic study of low exposure (primarily arsenic at less than 2 μg/L) was conducted with geographic information system (GIS) methods. No association was observed between water arsenic and nonmelanoma skin-cancer incidence (Baastrup et al. 2008). Aside from the extremely low concentrations, the study was limited by lack of histologic specificity in that basal-cell and squamous-cell carcinomas were grouped as one outcome. A small case–control study in Lagunera, Mexico, with 42 cases and 48 controls suggested an increased risk of nonmelanoma skin cancer, which was modified by the presence or absence of human papillomavirus (Rosales-Castillo et al. 2004). Exposure in the study was measured as cumulative exposure as determined by measurement of urinary arsenic and extrapolation to cumulative exposure after assessment of the participant's residential history.

A more recent US case–control study of squamous-cell carcinoma used sensitive methods for urinary arsenic detection. It reported a dose-related increase in total urinary arsenic after arsenobetaine was subtracted out (median = 4.76 μg/L) and a trend in each urinary fraction (inorganic arsenic, MMA, and DMA), with the association strongest for MMA (Gilbert-Diamond et al. 2013).

A US case–control study of melanoma skin cancer found an increased risk starting at an toenail arsenic concentration of 0.04 μg/g and showed evidence of a dose-related increase in risk above that concentration (Beane Freeman et al. 2004). The risk was particularly strong in those who had a prior skin-cancer diagnosis. However, the study was limited by the use of colon-cancer cases as controls.

Mode of Action

Few studies have been able to elucidate the molecular or cellular basis of skin lesions or cancer directly in skin tissues in human populations exposed to arsenic. Several in vitro and animal studies have attempted to examine molecular and cellular alterations in response to external exposure to arsenic at various doses. Those studies have identified immune-related, oxidative stress–related, apoptotic, stem-cell, mitochondrial, and genomic alterations that may potentially underlie basal-cell and squamous-cell cancer development in response to arsenic stimuli (Yu et al. 2006; Kitchin and Conolly 2010; Lee et al. 2011; Liao et al. 2011; Tokar et al. 2011a; Zhao et al. 2012; Huang et al. 2013; Lee et al. 2013; Pei et al. 2013). The findings from the experimental studies are difficult to extrapolate directly to understand how arsenic induces skin lesions or tumor development in humans. However, to the extent that the studies involved exposures at relevant dose ranges and are compatible with the epidemiology, the evidence might be worth considering in the IRIS assessment to strengthen or refute results of analyses that are relevant to skin pathology.

Although this section integrates discussions of skin lesions and skin cancers, the committee notes that although skin lesions are clinical markers of susceptibility to arsenic-related skin and internal cancers and other health outcomes, it is not clear whether skin lesions are direct precursors of most skin cancers in arsenic-exposed people. Despite the existing dogma, no published longitudinal studies have documented the transition from skin lesions to skin cancer in humans. Thus, it is important to evaluate modes of action underlying skin lesions and skin cancer carefully to understand how they are similar and different and their potential implications for dose–response analyses for these seemingly related end points.

Key Considerations for the IRIS Assessment

The committee recommends that EPA consider skin studies that have histologic specificity and that allow the separation, for example, of basal-cell carcinoma and squamous-cell carcinoma because current data suggest that they are influenced by arsenic exposure and could have different dose–response relationships.

RESPIRATORY EFFECTS

Lung Cancer

Arsenic exposure via drinking water is an established lung carcinogen in humans. Associations have been consistently observed in highly exposed populations in Taiwan, Japan, Chile, Argentina, and the United States (Guo 2004; IARC 2004, 2012). And ecologic analyses in Chile suggest that in utero or early-life exposure increases the risk of arsenic-related lung-cancer mortality (Smith et al. 2006).

Case–control and cohort studies in those and other regions examined risks at lower levels of exposure (reviewed in EFSA 2009; Steinmaus et al. 2013). For example, a hospital-based case–control study in Chile (151 cases and 419 controls) detected increased risks beginning with the category of average water concentrations of 10–29 μg/L which became statistically significant at 30–39 μg/L compared with 10 μg/L or lower (Ferreccio et al. 2000). In a population-based study of 306 lung-cancer cases and 604 controls, the ORs were specifically increased in those who drank water during the peak exposure periods (1958–1970), which was about 40 years or more before the diagnostic period of the cases (2007–2009); however, no test of latency or dose–response effects was performed (Steinmaus et al. 2013).

In a preliminary case–control study in Argentina, there was evidence that the percentage of MMA and polymorphisms in cystathionine beta-synthase modified the risk of arsenic-induced lung cancer (Steinmaus et al. 2010).

In Taiwan, a cohort study (139 lung-cancer cases) found a trend of increasing lung-cancer risk beginning at an arsenic concentration of 100 μg/L; less than 10 μg/L in water was the reference concentration, and there was no observable association with 10-99 μg/L (Chen et al. 2004).

In Bangladesh, a trend of an association between well-water arsenic concentration and risk of malignant lung tumors, in particular squamous-cell tumors, was found in a pathology-based case-control study of smokers (3,223 cases, 1,588 controls); a limitation is that the study used people who had suspicious lung lesions (for example, inflammatory, tubular, or other disease) as controls (Mostafa et al. 2008).

Each of those studies examined trends in estimates of relative risk associated with specific categories of drinking-water arsenic, however, and did not model continuous dose–response relationships. The one study that did examine a linear dose–response relationship was conducted on the basis of GIS exposure models in Denmark and found no association with lung cancer (409 cases) (Baastrup et al. 2008); however, the concentrations of arsenic in drinking water were less than 2 μg/L for most participants. Likewise, in an ecologic analysis in a population of Mormons in Utah (34 lung-cancer deaths) (Lewis et al. 1999), there were no observable associations compared with the US general population. However, such ecologic comparisons at low exposure are not likely to be informative, because of misclassification and confounding. A case–control study in New Hampshire that measured people's toenail arsenic as a biomarker (223 cases, 238

controls) found an increased OR for small-cell or squamous-cell carcinoma of the lung at higher concentrations of arsenic, especially in those who had a history of lung disease (Heck et al. 2009a). In the Strong Heart Study of 3,932 American Indians 45-74 years old, baseline urinary arsenic concentrations were related to an increased hazard ratio for lung-cancer mortality of 1.56 (95% confidence interaval [CI] 1.02-2.39) over almost 20 years of followup; 78 lung-cancer deaths were observed. There was a linear trend of increasing lung-cancer mortality with increasing urinary arsenic concentrations with no evidence of a threshold (p = 0.04) (Garcia-Esquinas in press). Most recently, a large prospective cohort study in Japan observed a dose-dependent increase in lung-cancer incidence in relation to arsenic intake in food as measured through a food-frequency questionnaire (Sawada et al. 2013). The increase in risk was particularly pronounced in cigarette-smokers.

Mode of Action

The mode of action of arsenic in lung carcinogenesis is not completely understood, but some patterns appear to be emerging. Although arsenic is not directly mutagenic, it has been shown to affect several oncogenic pathways that are relevant to lung cancer, including epigenetic, microRNA, gene expression, and mitochondrial DNA alterations. In particular, three oncogenic pathways have been implicated in lung cancer, including those of the epidermal growth factor receptor (Biscardi et al. 1999; Simeonova and Luster 2002; Andrew et al. 2009a; Li et al. 2011; Stueckle et al. 2012; Sung et all. 2012), phosphoinositide 3-kinase/Akt (Dong 2002; Gao et al. 2004; Zhang et al. 2006; Beezhold et al. 2011; Chen et al. 2012; Z. Wang et al. 2012), and Nrf2/Keap1 (X.J. Wang et al. 2007; Andujar et al. 2010; Zheng et al. 2012).

Key Considerations for the IRIS Assessment

There is evidence of a trend of increasing risk at higher levels of arsenic exposure, but relatively few studies at lower levels of exposure have examined dose–response relationships with lung cancer. It may be possible to model the dose–response relationships from the estimated relative risks associated with categories of exposure. Risks may involve cofactors (including genetic factors) and be modified by timing of exposure (for example, early in life) and may necessitate assessment of potential confounding by cigarette-smoking. Associations could be specific to histologic types of lung cancer. The ability to detect associations at low levels of exposure requires measurement not only of water concentrations of arsenic but of biomarker concentrations that encompass all sources of exposure, including diet, that may be important especially when drinking-water arsenic concentrations are relatively low.

Nonmalignant Respiratory Outcomes

Arsenic's noncancer pulmonary effects have been less well studied than its lung-cancer effects, but results of a number of human epidemiologic studies suggest deleterious effects of arsenic on a variety of nonmalignant pulmonary outcomes, including respiratory symptoms, airway epithelial damage, impaired pulmonary function, chronic obstructive pulmonary disease (COPD), and tuberculosis (Mazumder et al. 2000, 2005; Milton and Rahman 2002; Milton et al. 2003; Smith et al. 2006, 2011; Parvez et al. 2008; Rahman et al. 2011).

Cross-sectional studies in Bangladesh and India report increased risks of clinical respiratory symptoms in people who have skin lesions and are exposed to arsenic at concentrations greater than 500 μg/L (Mazumder et al. 2000; Milton and Rahman 2002). A prospective evaluation of the participants (11,746) in the Health Effects of Arsenic Exposure Longitudinal Study in Bangladesh revealed that those in the second quintile of water arsenic (7-40 μg/L) had a 27% increase in risk of respiratory symptoms compared with those in the lowest quintile (Parvez et al. 2010). In a subset of the same cohort (950), arsenic exposure was associated with significantly lower forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) in people exposed to water arsenic at concentrations above 97 μg/L than in those

exposed at less than 19 μg/L (Parvez et al. 2013). The greatest effect of arsenic was evident in male smokers and people who had skin lesions. A handful of earlier studies in India and Pakistan also observed decreased pulmonary function in those exposed at greater than 250 μg/L (De et al. 2004; von Ehrenstein et al. 2005; Nafees et al. 2011).

A retrospective cohort study in Chile reported that in utero arsenic exposure was associated with a decline in FEV_1 and FVC in adulthood (Dauphine et al. 2011). In utero exposure to arsenic has also been linked with increased risk of acute respiratory infections in infants in a large cohort (1,551) in Bangladesh (Rahman et al. 2011). A cross-sectional study in India reported a 10-fold increase in the prevalence of bronchiectasis, a specific type of COPD, in people who had skin lesions (108) compared with those who did not (150) (Mazumder et al. 2005). An earlier ecologic study in the arsenic-endemic areas of Chile reported an excess of mortality from COPD in people 30–39 years old (especially women) who were exposed to a weighted average arsenic concentration of 570 μg/L during 1955–1969 (Smith et al. 1998). Higher mortality from chronic bronchiectasis was observed only in those who were exposed to arsenic during early childhood or in utero (Smith et al. 2006). The ecologic analysis captured mortality in 1989–2000 in the two cohorts that were exposed to arsenic in early childhood or in utero before (1950–1957) or during (1958–1970) times when the peak exposure reached as high as 1,000 μg/L. Significantly higher risks of death from bronchiectasiswere observed in those who were exposed to arsenic during early childhood (standardized mortality ratio [SMR] = 12.4; 95% CI 3.3–31.7) or in utero and early childhood (SMR = 46.2; 95% CI 21.1–87.7) (Smith et al. 2006). An ecologic study in Taiwan also reported higher mortality from bronchitis in people living in areas with a high prevalence of blackfoot disease than in a reference population or the rest the country during the peak arsenic exposure (median 780 μg/L, 1971-1994) (Tsai et al. 1999).

In addition to COPD and associated outcomes, arsenic exposure has been linked to chronic lung infections, especially pulmonary tuberculosis. In the same population in Chile discussed above, Smith and colleagues reported significantly higher mortality from pulmonary tuberculosis (RR = 2.1; 95% CI 1.7-2.6) resulting from arsenic exposure (Smith et al. 2011). The risk mimicked the exposure history trend in the population (rising risk after chronic exposure and then falling back to normal after cessation of exposure). Although that novel finding is consistent with potential immunosuppression effects of arsenic, it has yet to be replicated in other large population studies.

A recent study in the United States has reported an association between prenatal inorganic arsenic exposure and increased risk of respiratory disease, such as upper respiratory tract infections and colds (Farzan et al. in press).

Mode of Action

As discussed in the committee's workshop by Lantz (2013), a substantial body of literature from both epidemiologic and animal studies of arsenic suggests impaired immune function, aberrant wound repair, and disrupted matrix and barrier function. A major consideration in elucidating the mode of action should be epidemiologic and animal studies that identify potential pathogenic mechanisms in response to low to moderate arsenic exposures. As indicated above, results of prospective epidemiologic studies suggest that arsenic exposures are linked to chronic loss of lung defenses, such as secretion of CC16 protein from airway cells (Parvez et al. 2008, 2010). Subchronic or in utero exposures of mice to low to moderate concentrations of arsenic in drinking water (10–100 ppb) led to a decrease in immune gene expression and aberration in inflammatory protein expression (Kozul et al. 2009a; Ramsey et al. 2013a) that may make mice more susceptible to airway inflammation (Kozul et al. 2009b). However, other mouse studies report that genes that sustain lung and vascular matrix, wound repair, and barrier function are compromised by arsenic exposure (Lantz et al. 2007, 2009; Hays et al. 2008; Petrick et al. 2009). Those changes in matrix and wound repair observed in mice correlate to changes observed in exposed humans and isolated human cells (Josyula et al. 2006; Lantz et al. 2007; Olsen et al. 2008). Increased matrix metalloproteinase-9 (MMP-9) expression and activity may be important biomarkers of inhibitory effects of arsenic on lung function (Josyula et al. 2006;

Olsen et al. 2008), although they may also be portions of the overall change in inflammatory gene expression. Another important consideration with respect to mode of action is whether in utero or perinatal exposure poses a significant risk of lung dysfunction or disease. Recent animal studies suggest that that may be the case (Lantz et al. 2007, 2009; Hays et al. 2008; Kozul et al. 2009b; Ramsey et al. 2013b), but few epidemiologic studies have examined affects of in utero arsenic exposure on lung disease. The underlying mechanism of chronic inflammation, impaired immune function, and aberrant matrix maintenance is not clear but may involve arsenic-induced oxidative stress (Lantz and Hays 2006). Protection from oxidative stress can reduce arsenic-induced pulmonary inflammatory responses and injury in mouse models (Zheng et al. 2012; Tao et al. 2013).

Key Considerations for the IRIS Assessment

Despite a large number of studies that have demonstrated nonmalignant lung disease after exposure to a wide range of arsenic (40-1,000 μg/L), little information is available to determine a full dose-dependent relationship, especially at low to moderate doses. For the IRIS assessment, the challenge is to integrate information from different studies that focus on different types of nonmalignant respiratory outcomes in populations exposed at different doses and for different periods. Critical synthesis of the human population studies coupled with a coherent integration of mode of actions underlying different nonmalignant respiratory outcomes and phenotypes should be a focus for consideration by EPA.

CARDIOVASCULAR DISEASE

In 2012, a systematic review and meta-analysis of epidemiologic reports of arsenic-related cardiovascular disease concluded that evidence from different countries consistently shows an association between high chronic arsenic exposure and cardiovascular disease (Moon et al. 2012). (See Chapter 3 for comments on the usefulness of this systematic review for EPA's IRIS assessment.) Until recently, the evaluation of the evidence on low to moderate exposure in drinking water (less than 100 μg/L) has been limited by the low quality of the available studies that support the role of arsenic as a cardiovascular-disease risk factor (Moon et al. 2012). However, a number of epidemiologic studies of arsenic exposures in large populations report dose–response relationships that are useful to evaluate if there is an increased risk of cardiovascular disease at low to moderate arsenic concentrations (Sohel et al. 2009; Wade et al. 2009; Y. Chen et al. 2011a) (see Table 1). The joint evaluation of the dose–response relationship across those studies can provide useful information for performing the IRIS assessment. Cardiovascular disease may be the most important noncancer disease risk posed by environmental arsenic exposures given the high burden of cardiovascular disease worldwide.

Although the current studies suggest that cardiovascular disease risk is increased by low to moderate arsenic concentrations in drinking water and possibly food, there is a need to confirm the relationship. Inclusion of the most recently published data should provide an opportunity for establishing better dose–response relationships in the low-dose region. Ample human studies are available to establish dose–response relationships. However, results of a number of animal studies support the identification of cardiovascular disease risk and provide valuable information on mode of action that reduces the uncertainty regarding causality (see below). The focus should be on human studies that investigated coronary arterial disease, myocardial infarction, cardiovascular disease, and overall cardiovascular-disease mortality because risk of these disease outcomes appears to be increased by low to moderate exposure. Peripheral arterial disease (such as blackfoot disease) can be excluded from the focus because few studies have investigated the association with low to moderate arsenic exposure and the association with high exposure seems to be limited to populations that have poor nutrition (Tseng et al. 2005). Cerebrovascular disease can be included, but there may be few conclusive epidemiologic studies that had adequate exposure and

TABLE 1 Large Cohort Studies of Overall Cardiovascular Disease and Arsenic Exposure Measured at the Individual Level and Reported in Two or More Arsenic Categories in Populations with Arsenic in Drinking Water at Less than 150 μg/L

Reference	Population	End Point Ascertainment	Outcome(s)	Arsenic Assessment	Exposure Categories	No. Cases	Relative Risk	95% CI	Adjustment Factors	Evaluation of Effect Modification
Y. Chen et al. 2011a[a]	Araihazar, Bangladesh Ages 18-75 y	Verbal autopsy, medical records	CVD mortality	Baseline individual well water	<12 μg/L	43	1.00	Reference	Age, sex, education, BMI, smoking status	Stronger associations in current smokers
					12.1-62.0	51	1.21	0.80-1.84		
					62.1-148.0	41	1.24	0.80-1.93		
				Baseline urine arsenic (μg/g of creatinine)	<105.9 μg/g	44	1.00	Reference		
					106.0-199.0	48	1.15	0.77-1.72		
					199.1-351.8	54	1.56	1.03-2.38		
Sohel et al. 2009[a]	Matlab, Bangladesh Ages ≥15 y 50% men	Verbal autopsy	CVD mortality	Household well levels	<10 μg/L	129	1.00	Reference	Age, sex, education, asset score (SES)	No differences by sex. Other subgroups were not reported.
					10-49	153	1.03	0.82-1.29		
					50-150	476	1.16	0.96-1.40		
Wade et al. 2009[b]	Inner Mongolia (one village) Ages: 0 to >80 y 50% men	Verbal autopsy, medical-record review	CVD mortality	Household, shared, or community well levels	<5 μg/L	44	1.00	Reference	Age, sex, education, smoking status, drinking, farm work	Not reported.
					5.1-20	26	1.07	0.64-1.78		
					20.1-100	72	1.22	0.82-1.82		
Moon et al. 2013	Arizona, Oklahoma, N/S Dakota (Strong Heart Study) Ages: 45-64 40.8% men	Hospitalization and death records, adjudication by physician panel	CVD incidence	Baseline urine arsenic (μg/g of creatinine)	<5.8 μg/g	265	1.00	Reference	Age, sex, education, BMI, smoking status, LDL-chol	Stronger associations in participants from Arizona, participants with diabetes, and participants with DMA above the median.
					5.8-9.7	297	1.14	0.95-1.35		
					9.8-15.7	291	1.05	0.87-1.26		
					>15.7	331	1.24	1.02-1.50		
			CVD mortality	Baseline urine arsenic (μg/g of creatinine)	<5.8 μg/g	86	1.00	Reference		
					5.8-9.7	95	1.12	0.83-1.52		
					9.8-15.7	115	1.26	0.92-1.73		
					>15.7	143	1.65	1.20-2.27		

This table is provided as an example that can be useful also for other end points (such as ischemic heart disease).

Abbreviations: BMI, body-mass index; CI, confidence interval; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; LDL-chol, low-density lipoprotein cholesterol; NR, not reported; SES, socioeconomic status.

[a]Data on exposures greater than 150 μg/L are not presented.

[b]Subset of residents exposed since 1990.

outcome assessment at low to moderate exposure. Even in studies that had high exposures, the relationship of arsenic to cerebrovascular disease appears inconclusive. Finally, hypertension, although shown to have some relation to arsenic exposure (Abhyankar et al. 2012), is less of a priority inasmuch as there are few studies at low to moderate exposure, most of the evidence available is cross-sectional, and it is not a clinical cardiovascular end point.

Mode of Action

As with arsenic-induced cancers, there may be several plausible modes of action for arsenic-related cardiovascular conditions. If determination of the mode of action is needed, EPA should consider evidence and reviews that suggest that chronic inflammation and reactive oxygen species (ROS) formation are central to the pathogenesis of arsenic-induced cardiovascular disease. Chronic vascular inflammation is suggested by a mechanistic epidemiologic study (Wu et al. 2012) within a large prospective study of cardiovascular-disease mortality associated with arsenic (Y. Chen et al. 2011a). Chronic inflammation may be a derivative of a chronic increase in ROS, a known risk factor for cardiovascular disease. Animal studies have been effective in demonstrating that vascular remodeling occurs in response to low to moderate arsenic exposures (10-100 ppb) (reviewed by States et al. 2011) and that ROS generation, especially from NADPH oxidase activation, is essential in promoting vascular pathogenesis (Straub et al. 2008). Atherosclerosis is a primary mechanism in the etiology of arsenic-related ischemic heart disease, and arsenic-induced atherogenesis has been demonstrated in a genetic mouse model of atherosclerosis after low to moderate exposure to arsenic, as discussed by Lantz (2013) in the committee's workshop (also see Lemaire et al. 2011). In addition, atherogenic potential in mice may be enhanced by in utero arsenic exposures (Srivastava et al. 2007). Alternatively, other modes of action—such as interactions with protein thiols to change protein function, disruption of cardiac and vascular matrix after increased expression of matrix-degrading proteases (such as MMP-9) (Soucy et al. 2005; Hays et al. 2008; States et al. 2009; Wu et al. 2012), or interference with transcriptional regulation of metabolic and inflammatory genes (Padovani et al. 2010; States et al. 2011)—cannot be ruled out. However, those changes in gene regulation may act through or derive from upstream activation of ROS signaling.

Key Considerations for the IRIS Assessment

Ample epidemiologic reports find a causal association between arsenic exposure of humans and increased risk of cardiovascular disease and mortality. A review of current literature, including recent meta-analyses, provides support for identification of arsenic as a hazard for cardiovascular disease. The recent literature includes large prospective studies that provide an opportunity to establish dose–response relationships for arsenic-induced cardiovascular disease. In evaluating the causal relationship between arsenic exposure and cardiovascular diseases, however, EPA should address potential uncertainties resulting from differences between the study population and the general population. Animal data and data that reveal potential modes of action demonstrate sensitivity of the cardiovascular system to arsenic exposures relevant to humans and support identification of modes of action. That can be useful for supporting causality in the epidemiologic studies, help to identify sensitive populations, and inform the dose–response analysis.

BLADDER EFFECTS

Arsenic is a known bladder carcinogen (IARC 2004, 2012). Assessment by IARC included ecologic studies of highly exposed populations in Taiwan, Chile, and Argentina and indicated higher mortality from bladder cancers in exposed than in nonexposed populations. In addition, ecologic studies in Taiwan reported increasing SMRs with higher categories of exposure on the basis of village median water concentrations of arsenic, and associations with well-water arsenic concentrations were supported by evi-

dence from cohort and case–control studies in Taiwan (IARC 2004, 2012). More recently, a case–control study in Chile found evidence of a dose-related increase in bladder-cancer incidence (Steinmaus et al. 2013). Those studies were important for establishing a causal relationship between arsenic exposure and bladder cancer, and, as described later in this report, those from Region II in Chile signal the importance of exposures early in life. Those studies, however, did not estimate the shape of the dose–response curve and cannot inform estimates of the dose–response relationship at lower exposures.

There is supportive evidence that drinking-water arsenic could be affecting bladder-cancer occurrence in the United States; this evidence should be carefully evaluated by EPA, particularly with respect to smoking, which is a known risk factor for bladder cancer (see Chapter 5). The studies include an ecologic study of the proportion of private-well users that found correlations in New England where high arsenic concentrations in bedrock wells are known to occur (Ayotte et al. 2006). Case–control and cohort studies that detected effects at lower exposure tended to have poor statistical precision and mostly examined dose–response relationships by using categories of exposure. For example, in a categorical analysis in a nested case–control study of male smokers (Michaud et al. 2004), the OR was over 2 in the highest tertile of toenail arsenic in long-term smokers (older than 45 years old), but this was not statistically significant. Likewise, a case–control study in New Hampshire found about a 2-fold risk of transitional-cell carcinoma of the bladder in the highest exposure category in smokers but with wide confidence intervals (Karagas et al. 2004). However, an analysis of the New Hampshire data that treated exposure as a continuous variable observed a dose–response relationship with increasing exposure in smokers, and a case–control study in Nevada reported more than a 2-fold OR of bladder cancer in smokers in the highest category of estimated cumulative intake of arsenic via drinking water, but it was evident only for an exposure period 40 years before diagnosis (Steinmaus et al. 2003).

In an earlier US National Bladder Cancer Study, a linear trend in the ORs for bladder cancer was observed in smokers on the basis of index of arsenic exposure (drinking-water concentrations multiplied by number of years consumed), a trend present for the exposure window of 10–19 years before diagnosis (Bates et al. 1995). Fifty years or more of well-water consumption in a region of Argentina that had high contamination was associated with bladder cancer in a case–control study of smokers (Bates et al. 2004). Estimated drinking-water arsenic exposure (limited to up to 40 years before diagnosis), however, was unrelated. A cohort study in Taiwan detected risks at arsenic concentration below 50 μg/L but was based on only a few cases (two men and five women exposed at less than10 μg/L and one man and three women at 10–49 μg/L) (Huang et al. 2008); thus, a dose–response analysis could not be performed. No excess risks were observed in association with the time-weighted average arsenic exposure from drinking water, even among smokers, in a case–control study in Michigan (Meliker et al. 2010). No association was also found in an ecologic analysis of a cohort study of bladder-cancer mortality in Mormons (a nonsmoking population) in Utah (Lewis et al. 1999) or in an analysis of cancer incidence in an Australian cohort (Hinwood et al. 1999). It is conceivable that lack of associations in studies at low exposure could be due to weak statistical power to detect the more modest effects that might be expected, especially in the cohort studies that had few bladder-cancer cases. Studies based on bladder-cancer mortality are inherently biased toward the null because bladder cancer is typically survivable.

Mode of Action

An emerging body of literature suggests that susceptibility to arsenic exposure may involve genetic factors, and studies have helped to inform understanding of the mechanism of action. Findings include interactions between arsenic exposure and polymorphisms in genes involved in DNA repair, cell cycle, xenobiotic and arsenic metabolism, and metal transport (e.g., Hsu et al. 2008; Andrew et al. 2009b; Karagas et al. 2012; Lesseur et al. 2012), and differences in risk depending on percentages or ratios of urinary metabolites (IARC 2012). These studies are discussed in greater detail in Chapter 5 (section on Genetics and Arsenic Metabolism and Toxicity).

There is a vast literature on mechanistic studies related to arsenic tumorigenesis. Experimental designs range from detailed probing of specific molecular pathways to broad studies of genetic differences

associated with carcinogenesis or cancer risk, including gene-expression studies and population-based studies of genomic changes. Potential modes of action have been proposed, such as cytotoxicity and regenerative repair, formation of reactive oxygen species and resulting oxidative stress and DNA damage, impaired DNA repair, dysregulation of signaling pathways that control the fate of cells (cycle progression, proliferation, differentiation, and apoptosis), and perturbations of gene structure. Epidemiologic and experimental studies have shown genetic differences and epigenetic changes in various processes. A mode-of-actin analysis will need to evaluate the results of mechanistic studies. It will need to consider that, just as bladder cancer is not a single disease, there may be multiple means by which arsenic can cause bladder cancer. Which mode of action applies may depend on many factors, including the populations or species in question (in the case of experimental studies), individual genetic differences, nutritional differences, magnitude of arsenic exposure, and the period of exposure (e.g., in utero, adult).

Key Considerations for the IRIS Assessment

There are a number of considerations in assessing the potential relationship between arsenic exposure and bladder cancer, especially at levels of exposure relevant to the US population. Evaluation of dose–response relationships may necessitate conversion of categories of exposures to fit continuous dose–response curves as was done in the European Food Safety Authority Assessment (EFSA 2009). Complications in evaluating lower exposures include diet's possible importance as a contributor to exposure and the need to incorporate considerations regarding smoking and other cofactors. It is crucial to assess exposure on an individual level and to include a biomarker and relevant cofactors where possible. A limitation of early ecologic studies of highly exposed populations that relied on mortality data is that bladder cancer is not typically fatal. In addition, timing and duration of exposure can influence the magnitude of the effects of arsenic on bladder-cancer incidence. Associations were observed with more recent exposure in some case–control studies and exposure in the distant past in others. In summary, if possible, the IRIS assessment should focus on the studies of both recent and past exposure that examined bladder-cancer incidence (rather than mortality) and that examined susceptible groups of the population (on the basis of cofactor exposures or genetics) and dose–response relationships.

RENAL EFFECTS

Adverse effects on the kidneys have been found in different arsenic-exposed populations around the world, including the United States. Renal cancer is relatively rare, but chronic renal disease is common. Although reports vary in study design, have different extents of exposure, and use different outcome measures, they appear to concur that arsenic exposure causes renal disease in humans. Some examples are discussed below, but they are not meant to limit EPA's evaluation of the literature.

Renal Cancer

Arsenic has been shown to cause cancer of tissues in the urinary system other than the bladder; for those, there may be different modes of action for cancer development. Broadly speaking, cancer of the urinary system is either renal-cell carcinoma or cancer of the urothelium (which occurs in the renal pelvis and ureters as well as the bladder). In *International Classification of Diseases, Ninth Revision (ICD-9)* diagnosis codes commonly used in epidemiologic studies of cancers associated with arsenic exposure, urothelial cancer is coded as a subtype of renal cancer rather than bladder cancer. As noted in the committee's workshop by Cohen (2013), most cancers of the urinary system occur in urothelial tissues, especially the bladder.

Increased mortality from cancer of the kidney, including the renal pelvis and ureters (*ICD-9*, code 189), has been reported in studies in Argentina (Hopenhayn-Rich et al. 1998), Chile (Yuan et al. 2010),

and China (Tsai et al. 1998; Yang et al. 2004). In Chile, mortality increased by about 10 years after high exposure began, peaked around 25 years later, and then declined; the decline occurred later in women (Yuan et al. 2010). The incidence of urothelial or transitional carcinoma increased with arsenic exposure (Ferreccio et al. 2013). In the committee's workshop, the study was described as having excellent exposure assessment (Cantor 2013). Yang et al. (2004) calculated cancer as a moving average over the study years of 1971–2000 and found that renal, renal pelvis, and ureteral cancer mortality decreased as the time since the end of exposure increased. They noted that smoking rates remained constant during that time, and this strengthened the case for a causal role of arsenic in these cancers. Chen et al. (2010) examined the incidence of urinary cancer in northeast Taiwan, where arsenic concentration in well water ranged from less than 10 μg/L to more than 300 μg/L. Urothelial-cancer incidence—adjusted for age, sex, education levels, cigarette-smoking, and alcohol use—increased with well-water arsenic concentration. (During the committee's workshop, it was pointed out that concentrations were based on the well used by the subjects at the time of enrollment in the study.) Although relatively few cancers were observed, the numbers of both total urinary system cancers (45 cases) and urothelial carcinoma (36 cases) were significant and showed positive dose–response relationships.

Only a few papers have reported on cancer of the kidney excluding urothelium (*ICD-9* code 189.0). Ferreccio et al. (2013) did not find renal-cell cancer incidence to be increased in Chile, and Chen et al. (2010) reported that renal-cell cancer was not increased in northeastern Taiwan. In southwestern Taiwan, renal-cancer mortality increased 6-fold (in males) to 16-fold (in females) at the highest arsenic concentrations (over 600 ppb) (Chen et al. 1992). Huang et al. (2011) conducted a case–control study of the incidence of pathologically verified renal-cell cancer in the Taipei area of Taiwan, where arsenic concentrations in drinking water range from undetectable to 4 μg/L (average 0.7 μg/L). They reported that the OR for renal-cell cancer was increased (age- and sex-adjusted OR = 2.31) in the top one-third of urinary arsenic (greater than 20.95 μg/g of creatinine) compared with the referent group (greater than 12.3 μg/g of creatinine). In a followup study, the group that had the highest urinary arsenic also had significantly higher urinary 8-hydroxydeoxyguanosine concentrations (also corrected for creatinine), a biomarker of DNA damage that has previously been associated with risk of renal cancer; this highlights a potential mechanism of cancer formation (Huang et al. 2012).

Mode of Action

Potential modes of action for renal cancer include those described in Chapter 6: oxidative stress (production of reactive oxygen species and diminished antioxidant reserves), DNA damage, cell proliferation and accumulation of mutations, and dysregulation of cell-cycle control. Recent studies by Tokar et al. (2012a) specific to the kidney found that an increase in renal-cell carcinoma resulted from in utero exposure to inorganic arsenic followed by DMA after weaning. In a followup study (Tokar et al. 2012b), rat kidney stem cells exposed to arsenic for 10 weeks displayed characteristics of cancer cells—a suggestion that early-life effects may be key to the development of renal cancer.

Renal Disease

Significant higher mortality from renal disease (*ICD-9* codes 580–589) has been found in regions that have high arsenic concentrations, such as Taiwan (Tsai et al. 1999) and Chile (Smith et al. 2012), than in regions that have low arsenic. In Taiwan, SMRs for renal diseases decreased from 1971, when use of low-arsenic water became common, to 2000 (Chiu and Yang (2005); this finding strengthens the association with arsenic. In Antofagasta, Chile, chronic renal diseases (chronic renal failure, unspecified renal failure, chronic glomerulonephritis, and sclerosis) were increased in those who were exposed in childhood or in utero and in childhood (Smith et al. 2012).

Renal disease may not be the cause of death, so studies of renal-disease incidence or changes in biomarkers of impaired renal function may reflect the true impact of arsenic exposure on the kidneys better.

The incidence of renal disease (chronic glomerulonephritis, nephrotic syndrome, nephritis and nephropathy, and chronic or unspecified renal failure) was increased in southwestern Taiwan (Wang et al. 2003). In a cross-sectional study of a population exposed to moderate arsenic concentrations in central Taiwan (geometric mean urinary arsenic 70.6 μg/L), urinary arsenic was associated with an estimated glomerular filtration rate (eGFR) of less than 90 mL/min/1.73 m^2 but not with an eGFR of less than 60 mL/min/1.73 m^2, the standard definition of reduced eGFR (J.W. Chen et al. 2011). The association of arsenic with measures of glomerular filtration rate has not been evaluated in populations exposed to arsenic at low to moderate concentrations. However, a study in southeastern Michigan, a region that has a population-weighted mean water arsenic concentration of only 11 μg/L reported increased SMRs for renal disease over expected values in both males and females in association with water arsenic (Meliker et al. 2007). The appearance of protein or albumin in the urine can indicate increased permeability of the glomerular filtration barrier, impaired reabsorption of filtered proteins, or release of tubular proteins into the urine as tubular cells die. Increased rates of proteinuria or albuminuria have been reported after low to moderate arsenic exposures in the Strong Heart Study in the United States (Zheng et al. 2013) and after high exposures in Bangladesh (Y. Chen et al. 2011b). Moreover, proteinuria improved with the change to a water supply that was lower in arsenic. Impaired tubule function, indicated by excretion of β-2-microglobulin and *N*-acetyl-beta-glucosaminidase (NAG), has been found in populations exposed to arsenic at moderate to high concentrations (J.P. Wang et al. 2009; J.W. Chen et al. 2011); alterations in NAG activity in a Korean population that apparently had lower exposure (geometric mean urinary arsenic 8 μg/g of creatinine) have also been reported (Eom et al. 2011). Experimentation in rats showed that NAG excretion was increased a month after exposure to arsenic in drinking water at 30 mg/L (J.P. Wang et al. 2009).

Arsenic is associated with diabetes mellitus in populations exposed to arsenic (see section "Diabetes" below). Diabetes mellitus results in nephropathy, and this complicates the interpretation of the association between arsenic and renal disease. Several studies explicitly considered diabetes and found that the association between arsenic and impaired renal function remained after control for diabetes or in stratified analysis in participants who had and did not have diabetes. In a study in Bangladesh, the association was maintained after adjustment for hypertension and HbA1c; this supports the idea that proteinuria was independent of diabetes (Y. Chen et al. 2011b). Similarly, the association between arsenic and albuminuria was independent of diabetes in people who had low to moderate arsenic exposures in the United States (Zheng et al. 2013). J.P. Wang et al. (2009) reported that increases in urinary NAG were more pronounced in diabetic people than in nondiabetic people in a high-exposure area. Results of experimental studies support that interaction. Kidney weight (absolute and relative to body weight), oxidation of renal proteins, renal malondialdehyde, and measures of glomerular filtration (serum creatinine and urea nitrogen) were more severely affected by arsenic in rats that had been rendered diabetic with alloxan treatment (Patel and Kaila 2010). EPA should consider whether arsenic and diabetes have interactive effects on renal function.

Mode of Action

There have been few mechanistic studies of renal function. Processes described in Chapter 6 on modes of action might apply to kidneys. Studies have provided evidence of mitochondrial toxicity (Peraza et al. 2003) and increased mesangial proliferation secondary to hexokinaseII expression in the mesangial cells of the glomeruli (Pysher et al. 2007). The investigators noted that mesangial cells play an important role in the control of glomerular filtration and are affected by diabetes and that their proliferation decreases GFR. Differences in mechanisms of arsenic elimination could contribute to differences between species and strains in its effects on the kidney. For instance, lack of upregulation of the MRP1 transporter renders BALB/c mice more sensitive to the nephrotoxic effects of arsenic than C57Nl/6J mice (Kimura et al. 2005). Oxidative stress, inflammation, and other mechanisms of cell damage may also apply to kidney cells.

Key Considerations for the IRIS Assessment

There is mounting evidence that exposure to arsenic causes cancer of intrarenal urothelial tissues and renal-cell carcinoma in humans and mice. In humans, the incidence of renal cancer (urothelial and renal-cell carcinoma), renal-disease mortality, and proteinuria decreased after the arsenic concentration in water was reduced in Taiwan and Chile, and this supports a causal role. Results of studies in mice suggest that prenatal and early-life exposure confer susceptibility to renal-cell cancer. Renal-cell cancer is infrequent (about 3%) and has a low death rate (lower than 10%) in the United States (Pili and Rodriguez 2008). Thus, it is unlikely to be detected by mortality-based studies. Chronic kidney disease is more common (about 13% and an additional 0.16% with end-stage disease) (C.Y. Hsu 2011). Evidence is available from US populations that arsenic at low to moderate concentrations in drinking water is associated with proteinuria. In the case of other chronic renal diseases, there is only limited cross-sectional evidence at high exposure. Experimental studies support a role of arsenic exposure in kidney damage. In evaluating the literature on arsenic effects, EPA should consider chronic kidney disease and whether arsenic and diabetes have interactive effects on kidney function.

PREGNANCY OUTCOMES

Fetal Exposure to Arsenic

Early-life development is recognized as a potentially critical window of vulnerability to effects of toxic agents. It is essential to evaluate the potential adverse effects of fetal and postnatal exposure to inorganic arsenic. Experimental and human studies have shown that both inorganic arsenic and its methylated metabolites readily pass the placenta (Concha et al. 1998a; Jin et al. 2006; Hall et al. 2007). An important feature is the increase in the efficiency of maternal arsenic methylation during pregnancy (Concha et al. 1998a; Li et al. 2008; Gardner et al. 2011), which results in lower exposure of the fetus to inorganic arsenic and MMA, the most toxic arsenic metabolite, with advancing gestation. The maternal urinary fraction of MMA decreases markedly in the middle of the first trimester (Gardner et al. 2011), whereas the fraction of inorganic arsenic decreases slowly throughout gestation. Those findings are supported by studies in mice that show mainly DMA in blood and tissues of the newborn in spite of high maternal exposure to inorganic arsenic (10–85 mg/L in drinking water) during gestation (Devesa et al. 2006; Jin et al. 2006). Thus, the timing of urine sampling during pregnancy may have implications for the evaluation of arsenic methylation efficiency.

Birth Weight

Arsenic is embryotoxic and teratogenic in experimental animals that are given high doses (Wang et al. 2006; Hill et al. 2008). Because the kinetics and toxicity of arsenic vary among animal species, the extrapolation of data to humans is difficult.

With respect to human studies, a number of small, mainly cross-sectional studies have had inconclusive results concerning birth weight in relation to arsenic exposure, probably mainly because of the small samples. Of the larger studies, two ecologic studies in northeastern Taiwan and northern Chile found arsenic-related decreases in birth weight whereas a study in Inner Mongolia found an opposite association. In Taiwan, the authors compared birth weights in an area (four townships with 18 villages) that had well-water arsenic concentrations of 0.2–3,600 μg/L (30% of wells had over 50 μg/L) with those in matched townships (based on urbanization level) that had arsenic in public water supplies of less than 0.9 μg/L; the birth weights in the latter were an average of 29.0 g higher (95% CI 13.6–44.6 g), after adjustment for maternal age, education, marital status, and infant sex (Yang et al. 2003). In the Chilean study, the authors compared birth weights in the town of Antofagasta, which had arsenic in the public water supply at an average of 42 μg/L (33–53 μg/L) with those in Valparaiso, which had arsenic at less than 1 μg/L (Hopen-

hayn et al. 2003b). The multivariable-adjusted model showed that birth weights in the latter were an average of 57 g higher (95% CI -9 to 123 g).

A study in Inner Mongolia compared mean birth weight (9,890; from prenatal-care records) in four groups of "subvillages", categorized according to the average of available well-water arsenic concentrations: less than 20, 21–50, 51–100, and over 100 μg/L (Myers et al. 2010). However, wells in a village were measured for arsenic only if the well in one of five randomly selected households exceeded the local standard of 50 μg/L, and this might have influenced the finding that birth weights in the village group that had the highest mean arsenic concentration averaged 50 g higher than those of the group that had the lowest mean arsenic concentration. In addition, well-water screening for arsenic took place in 1991–1997 whereas the pregnancies studied occurred from December 1996 to December 1999.

A recent population-based prospective study involving 1,578 mother–infant pairs in rural Bangladesh and measurements of arsenic in maternal urine collected in early and late gestation (median 80 μg/L, 10th–90th percentiles 26–400 μg/L) found a significant inverse association between size at birth and urinary arsenic concentration (Rahman et al. 2009). The dose-dependent difference in birth size was obvious mainly at maternal urinary arsenic concentrations below 100 μg/L; each increase of 1 μg/L was associated with a 1.68-g reduction in birth weight. Arsenic exposure was also associated with smaller head and chest circumferences in a similar manner. In the same cohort of pregnant women, a longitudinal evaluation of fetal growth characteristics in early and late gestation, as measured with ultrasonography, supported a weak association between maternal urinary arsenic concentrations and fetal size, mainly in boys (Kippler et al. 2012).

Fetal and Infant Loss

A few studies have probed the associations between prenatal arsenic exposure and fetal and infant loss. The associations with fetal loss are less convincing, particularly at low-dose exposure, but there seem to be fairly consistent results concerning infant mortality. Several of the few available human studies are, however, ecologic in design. Evaluation of trends in pregnancy outcomes in Antofagasta, in northern Chile, indicated an increased rate of late fetal loss (overall 3%) during the period that had the highest water arsenic concentrations (about 800 μg/L during 1958-1970) relative to mortality in Valparaiso, which had essentially no arsenic in the drinking water (Hopenhayn-Rich et al. 2000). The rate ratio was 1.7 (95% CI 1.5-1.9) for stillbirth, 1.53 (95% CI 1.4-1.7) for neonatal mortality, and 1.26 (95% CI 1.2-1.3) for postneonatal mortality after adjustment for location and calendar time. In an ecologic study in Bangladesh, outcome data on 30,984 pregnancies in 600 villages, grouped geographically in 16 centers, were related to the average water arsenic concentrations in the centers (each based on seven to 14 wells) (Cherry et al. 2008). After adjustment for socioeconomic and health factors, the OR for stillbirth (overall 3.4%) was 1.80 (95% CI 1.14-2.86) at 50–80 μg/L compared with that at less than 10 μg/L. Arsenic-related increases in risk of spontaneous abortion, stillbirth, neonatal death, and preterm birth have also been reported by cross-sectional studies in Bangladesh (192 and 533 women) and West Bengal (202 women), in which retrospective data about drinking-water sources and outcomes of previous pregnancies were collected (Ahmad et al. 2001; Milton et al. 2005; von Ehrenstein et al. 2006). The risk ratios were 2–3 for both spontaneous abortions (two studies) and stillbirths (all three studies) in high-exposure groups; but even in the largest of the studies, the number of cases was small. In the largest Bangladeshi study (Milton et al. 2005), the OR for neonatal death was 1.8 (95% CI 0.9-3.5) for water concentrations above 50 μg/L (70 cases) compared with concentrations below 50 μg/L (16 cases).

A few population-based studies in Bangladesh with individual exposure data have been conducted. Kwok et al. (2006) reported pregnancy-outcome data on 2,000 women in three areas that had known high arsenic concentrations in drinking water. A weak but statistically significant association between arsenic concentrations in drinking water and birth defects (OR 1.005 for all defects combined, 95% CI 1.001–1.010) was found, but no other adverse effects on pregnancy outcomes. Another study collected fetal and infant mortality data on 29,134 pregnancies in Matlab, Bangladesh (Rahman et al. 2007). Data on indi-

vidual arsenic exposure were based on interviews about history of drinking-water sources, carried out in a parallel study, which also measured arsenic concentrations in all functioning tube wells in Matlab. Women who were using water that had arsenic at 277-408 μg/L (fourth quintile) had a significant increase in the relative risk (RR) of fetal loss of 1.14 (95% CI 1.01–1.30) and of infant death of 1.29 (95% CI 1.08–1.53) (Rahman et al. 2007). There was a significant dose–response relationship between arsenic exposure and risk of infant death. In a later population-based, prospective cohort study of 2,924 pregnant women in the same area, the OR of spontaneous abortion was 1.4 (95% CI 0.96-2.2) in women who had urinary arsenic concentrations in the fifth quintile (249-1,253 μg/L, median 382 μg/L) compared with women in the first quintile (less than 33 μg/L) (Rahman et al. 2010). However, both the second and third quintiles showed similar ORs, so the dose–response relationship was not convincing. Infant mortality increased more clearly with increasing maternal urinary arsenic concentrations; a hazard ratio of 5.0 (95% CI 1.4–18) was calculated in the fifth quintile of maternal urinary arsenic concentrations (268–2,019 μg/L, median 390 μg/L) compared with the first quintile (less than 38 μg/L). Most of the infant deaths occurred in the first week after birth. Because the passage of arsenic into breast milk is limited, breastfeeding protects the infants from arsenic exposure (Concha et al. 1998b; Fangstrom et al. 2008).

Mode of Action and Susceptibility Factors

Fetal growth is influenced by multiple factors, including genetic predisposition, maternal nutrition, and environmental exposures. The mechanisms by which arsenic might affect birth size are not well understood but may involve oxidative stress or perturbation of oxidative defense that leads to placental insufficiency (Ahmed et al. 2011). Arsenic-associated changes in gene expression in cord blood related to stress, inflammation, and apoptosis have been reported (Fry et al. 2007). And experimental studies have shown that arsenic, even at low doses, may act as an endocrine-disrupting chemical (Bodwell et al. 2004; Davey et al. 2007, 2008) and influence the insulin growth-factor system, glucose homeostasis, and cellular growth. Recently, arsenic has also been associated with altered DNA methylation in cord blood and with histone modifications (Cronican et al. 2013). The immunosuppressive effect of arsenic (see section "Immune Effects" below) may contribute to infant mortality.

Key Considerations for the IRIS Assessment

It seems clear that all metabolites of inorganic arsenic easily cross the placenta to the fetus. A number of epidemiologic studies, including prospective cohort studies, have provided evidence that arsenic exposure through drinking water during pregnancy may cause dose-dependent impairment of fetal and infant growth and infant survival. Data on the effects of arsenic on fetal loss are inconclusive. For some outcomes, particularly birth size, the effects of arsenic seem to start at low-level exposure. There appear to be available data that can be used for dose–response characterization concerning birth size and infant growth and possibly infant mortality.

NEUROTOXICITY

Arsenic has traditionally been categorized as a peripheral neurotoxicant; it produces a clinical picture of severe polyneuropathy after severe poisoning. Occupational exposure, as can occur in copper smelters, has been associated with peripheral neuropathies and related poor motor function (Sińczuk-Walczak et al. 2010). However, recent animal and human studies suggest that arsenic neurotoxicity, even after environmental exposures, can include the central nervous system.

Human Population Studies

Studies of Children

Epidemiologic reports on neurotoxicity are largely consistent with the experimental studies described above. Low to moderate concentrations of arsenic in drinking water have been associated with neurocognitive deficits in children (see Table 2). Tsai et al. (2003) conducted an ecologic study of adolescents and found lower neurodevelopmental test scores, after adjustment for socioeconomic factors, in Taiwanese children who lived in areas that had high concentrations of arsenic in drinking water. Calderón et al. (2001) found that urinary arsenic concentrations were inversely associated with verbal IQ in 80 Mexican children (6–8 years old) who lived near a smelter even after adjustment for blood lead concentrations. Mean concentrations of urinary arsenic per gram of creatinine were 62.91 μg/L-g (27.54–186.21 μg/L-g) in the exposed population and 40.28 μg/L-g (18.20–70.79 μg/L-g) in the nonexposed population. Urinary arsenic also predicted subscales of verbal comprehension and long-term memory formation. Data from a small pilot study conducted in the United States show an inverse association between hair arsenic and IQ in adolescents (11–13 years old) (Wright et al. 2006) and suggest that arsenic toxicity may occur at exposures lower than those found in Bangladesh. The median concentration of arsenic in hair was 18 ppb.

Several large cross-sectional studies have been conducted in areas of endemic arsenic exposure. Wasserman et al. (2004) conducted a cross-sectional cohort study of 201 Bangladeshi 10-year-old children that demonstrated a strong dose-dependent adverse effect of increased drinking-water arsenic on IQ after adjustment for covariates. The mean arsenic concentration was 118 μg/L, and the range was wide, 0.1–790 ppb. Individual urinary arsenic concentrations did not significantly predict IQ ($p = 0.09$ for full-scale IQ), but the direction of the effect was the same as for arsenic in water. In a followup study conducted in Bangladesh by the same team in children whose water arsenic ranged from 0.1 to 464 μg/L, whole-blood arsenic in 8- to 11-year-old children was significantly negatively related to several Wechsler Intelligence Scale for Children, 4th Edition (WISC-IV) subscale scores, including verbal comprehension. Urinary arsenic (per gram of creatinine) was significantly negatively associated with verbal comprehension scores (Wasserman et al. 2011). In another large study, Von Ehrenstein et al. (2007) assessed 351 children in West Bengal and found inverse associations between urinary arsenic and vocabulary scores, object assembly, and picture completion. Reconstructed cumulative and peak exposures were estimated from drinking-water arsenic concentrations and were not predictive of test scores.

TABLE 2 Urinary Arsenic Concentrations in Studies of Neurotoxicity

Study	Urinary Arsenic, μg/L
Rosado et al. (2007)	58.1 ± 33.2
Hamadani et al. (2011)	Median = 51; mean = 100; 10th-90th percentiles 200-238 (adjusted for specific gravity)
Roy et al. (2011)	Median = 55.2; IQR = 39.7
Wasserman et al. (2004)	116.4 + 148.8
Wasserman et al. (2007)	120.1 + 134.4
Wasserman et al. (2011)	43.3 + 73.7
Calderón et al. (2001)	Exposed group[a]: 62.91 (range 27.5-186.2) (adjusted for Cr) Nonexposed group[a]: 40.8 (range 18.2-70.8) (adjusted for Cr)
Von Ehrenstein et al. (2007)	78 ± 61 (range 2-375)

[a]Table 2 in source paper provides incorrect values of standard deviation, so this table uses the mean and the range.
Abbreviations: Cr, urinary creatinine; IQR, interquartile range.

Early-life exposure (during pregnancy and infancy) was not associated with infant development as assessed at the age of 7 or 18 months by the Bayley Scales of Infant Development in a large cohort study conducted in Bangladesh (Tofail et al. 2009; Hamadani et al. 2010). However, in a followup study of the same children (more than 1,700), inverse associations between maternal urinary arsenic in pregnancy and verbal/full-scale IQ (Wechsler Preschool and Primary Scale of Intelligence) were found at the age of 5 years of age but only in girls (Hamadani et al. 2011). Associations were stronger with the cross-sectional measures of urinary arsenic, again only in girls. Results of a study of mice support that sexually dimorphic finding: female pups were more vulnerable than males to the developmental effects of arsenic exposure with regard to locomotor activity and depletion of dopamine in the nucleus accumbens (Bardullas et al. 2009). Another study in Bangladesh indicated effects on motor function. Parvez et al. (2011) assessed motor function in 8- to 11-year-old children with the Bruininks-Oseretsky Test of Motor Proficiency and found an inverse association of total composite scores with water arsenic and with biomarkers of arsenic exposure. Whether the effects are peripheral or central nervous system effects is not clear.

With respect to behavioral studies, Roy et al. (2011) conducted a cross-sectional study of school-age children (median age 7 years) in Mexico and found that urinary DMA concentrations in boys were associated with higher scores scores on the oppositional, cognitive problems and ADHD subscales of the teacher ratings on the Conners Comprehensive Behavior Rating Scales, although adjustment for cognitive-test results diminished the association. That might suggest that the behavioral findings were driven largely by cognitive deficits.

Studies in Adults

The relationship between arsenic exposure and neurotoxicity in adults is less well studied than in children. A community-based participatory research study conducted in rural elderly adults found that GIS-based groundwater arsenic exposure was significantly related to poorer scores in language, visuospatial skills, and executive functioning. Long-term low-level exposure to arsenic was also associated with poorer scores in global cognition and memory (O'Bryant et al. 2011). No biomarkers were collected. The effects of arsenic in elderly populations is a particular research need.

Animal and in Vitro Experimental Studies

A study by Rodríguez et al. (2002) showed learning and behavioral deficits in rats exposed prenatally to arsenic via maternal drinking water. Increased spontaneous locomotor activity and increased errors in delayed alternation tasks (a test of memory and executive function) were found in arsenic-exposed offspring compared with nonexposed controls. Animals in the exposed groups consumed arsenic at 2.93–4.20 mg/kg per day, far more than any human study has reported except Dekeish et al. (2006) in Japan. (That report described the consequences of ingesting arsenic-contaminated infant formula, which led to a mass poisoning and reports of 130 fatalities. An IQ score of less than 85 was reported in 20.6% of survivors.)

Animal studies have also shown a dose–response relationship between arsenic in the brain and arsenic in drinking water—a demonstration that arsenic crosses the blood–brain barrier to the central nervous system (Rodríguez et al. 2002; Luo et al. 2009; Xi et al. 2009, 2010). In each of those animal studies, doses of arsenic were probably in the range of milligrams per kilogram per day, although not all studies reported water consumption rates. All studies involved concentrations of inorganic arsenic greater than 1,000 μg/L. Nagaraja and Desiraju (1994) found delayed acquisition and extinction of operant behaviors in rats exposed to sodium arsenate at 5 mg/kg per day in drinking water for 3 months. Increased concentrations of arsenic in cerebellar tissues have been associated with impaired performance on the Morris water-maze test (working and spatial memory) in mice (Y. Wang et al. 2009). Those deficits might be due to increased oxidative toxicity and changes in neurotransmission.

Increased neurotoxic oxidative stress (lowered concentrations of reduced glutathione and increased lipid peroxidation) in mouse brains after oral administration of arsenic trioxide was reported by Rao and Avani (2004) and Chaudhuri et al. (1999). An in vitro study demonstrated that neurite outgrowth was suppressed by sodium arsenite possibly because of induced neuronal apoptosis (Aung et al. 2013). Changes in concentrations of neurotransmitters—such as acetylcholine, dopamine, serotonin, and norepinephrine—have been found in the central nervous system after arsenic exposure (Nagaraja and Desiraju 1993, 1994; Chattopadhyay et al. 2002a,b). In studies using brain explants and neuronal cell cultures, neural networking and increased reactive oxygen intermediates were altered after exposure to arsenic (Chattopadhyay et al. 2002a). In rats exposed to arsenic during pregnancy, fetal brain neurons underwent apoptotic changes and neuronal necrosis (Chattopadhyay et al. 2002b). However, not all studies have demonstrated adverse effects of developmental exposure to inorganic arsenic, and null findings have been reported (Gandhi et al. 2012).

There is a growing literature on inorganic-arsenic toxicity that uses doses more commonly encountered in human exposure scenarios. The most commonly used drinking-water concentration is 50 μg/L, which is within the range seen in many epidemiologic studies. Martinez-Finley et al. (2008) focused on perinatal exposure to inorganic arsenic (defined as pregnancy through weaning at the age of 23 days). They reported that inorganic arsenic at 50 μg/L in drinking water was associated with increased depressive behaviors. (This period of exposure is parallel to a prenatal-exposure paradigm in humans, and preweaning mice are developmentally equivalent to a third-trimester human fetus.) Plasma corticosterone concentrations were about two-fold higher in the exposed group compared with controls. Hippocampal corticotropin-releasing-factor receptor binding was increased, and serotonin 5HT1A receptor binding was reduced. Overall, the results suggest that affected offspring might be predisposed to depressive-like behavior because of disrupted regulatory interactions between the hypothalamic–pituitary–adrenal axis and the serotonergic system in the dorsal hippocampal formation. In a followup study by the same group (Martinez-Finley et al. 2009), mice exposed to inorganic arsenic perinatally took longer to acknowledge a novel object and demonstrated deficits in spatial memory on an eight-arm radial-maze task. Arseni- treated animals had lower concentrations of glucocorticoid receptors in the brain coupled with higher plasma corticosterone; this suggests that the lower glucocorticoid receptor concentrations are a primary event that is followed by a compensatory increase in blood concentrations of corticosterone. A third paper by the group (Martinez-Finley et al. 2011) reported glucocorticoid-receptor–mediated transcriptional deficits in the mitogen-activated protein kinase/extracellular signal-related kinase pathway. That pathway plays a critical role in learning and memory formation, and consideration should be given to whether it could be an underlying cause of learning deficits associated with inorganic arsenic.

Key Considerations for the IRIS Assessment

A growing body of literature on both animals and humans suggests that low to moderation concentrations of arsenic are associated with neurologic deficits, particularly in IQ tests in children. Animal studies have demonstrated deficits in executive function, motor function, and spatial memory. Mechanisms appear related to oxidative-stress–induced apoptosis and effects on neurotransmitters. Human studies on arsenic neurotoxicity have been conducted primarily in children. Prenatal exposure did not predict performance on general developmental tests at the age of 18 months in Bangladeshi children but did predict performance at the age of 5 years; this either suggests a latent period for arsenic exposure in utero or suggests that subtle effects of arsenic exposure cannot be detected in the relatively insensitive psychometric tests used in toddlers and can be adequately assessed only at higher ages. Most studies have used IQ tests or other tests of general cognitive function and their subscales. No clear pattern has emerged with respect to nonverbal skills associated with inorganic arsenic exposure; however, deficits in verbal cognitive skills are the most common finding reported in epidemiologic studies (see Table 3).

TABLE 3 Most Sensitive Neurodevelopmental End Points in Human Studies

Study	End Point
Verbal Skills	
Hamadani et al. (2011)	Verbal IQ
Wasserman et al. (2011)	Verbal IQ
Von Ehrenstein et al. (2007)	Vocabulary
Wright et al. (2006)	Verbal IQ, verbal learning, story memory
Calderón et al. (2001)	Verbal IQ
Nonverbal Skills	
Wasserman et al. (2004, 2007)	Performance IQ, processing speed
Von Ehrenstein et al. (2007)	Object assembly, picture completion
Rosado et al. (2007)	Visual–spatial abilities, digit span, memory, sequencing
Parvez et al. (2011)	Scores on Bruininks-Oseretsky Test of Motor Proficiency, including total motor composite, body coordination, fine manual control

There is evidence of a dose–response relationship in animal studies, but the dose range used is orders of magnitude higher than what is typically assessed in human studies. A small body of literature that used 50 ppb during perinatal exposures has demonstrated measurable effects, but there has not been an attempt to delineate the dose–response relationship. Epidemiologic studies are not uniform in how they assess exposure. Studies have used a wide variety of exposure metrics, including concentrations in water, urine, hair, and blood. A compounding difficulty is that few studies are prospective and the dose–response relationship may depend on the timing and duration of exposure. One study that showed a clear dose–response relationship between water arsenic and IQ is the one by Wasserman et al. (2004) in Bangladesh. Lower full-scale and performance IQ scores were observed for each increase in the quartile of water arsenic. Exposure was similar to that in the United States in the lowest quartiles (less than 5.5 ppb and 6–50 ppb). Water arsenic concentrations of 10 ppb and 50 μg/L were associated with raw score losses of 3.8 and 6.4 points, respectively, but these are not equivalent to IQ points, because the WISC-III has not been standardized in Bangladesh. Results of studies of behavior and more domain-specific tasks (such as executive function and behavior) suggest possible adverse effects, but this aspect of neurotoxicity has been relatively understudied. Overall, more epidemiologic studies are needed to confirm animal findings. Motor function has also been understudied, as have the effects of arsenic exposure on cognitive function in adults, especially the elderly.

DIABETES

Several epidemiologic studies have evaluated the association between arsenic exposure and the risk of diabetes. In 2011, the National Toxicology Program (NTP) comprehensively evaluated the epidemiologic and experimental evidence on arsenic and diabetes end points (Maull et al. 2012). After considering consistency among populations, the strength and temporality of the associations, and biologic plausibility, the NTP review concluded that existing human data provided "limited to sufficient" support of an association between chronic exposure to arsenic at high concentrations (150 μg/L or more in drinking water) and diabetes. Below 150 μg/L, concentrations that are more relevant for arsenic risk assessment, the NTP review judged that the evidence was "insufficient" to conclude that arsenic was associated with diabetes because of the lack of prospective studies, limitations in exposure and outcome assessments, and lack of adjustment for relevant confounders.

Some studies, however, characterized by better measures of outcome and exposure were supportive of an association, including several cross-sectional studies in Mexico (Coronado-Gonzalez et al. 2007; Del Ra-

zo et al. 2011). For dose–response considerations, the Mexican studies included water arsenic concentrations of less than 10 μg/L to greater than 100 μg/L (geometric mean 24.4 μg/L) (del Razo et al. 2011). Another relevant study was conducted in pregnant women who lived in an area of Oklahoma in which at least 25% of drinking-water sources had arsenic concentrations greater than 10 μg/L (Ettinger et al. 2009). In that study, arsenic measured in blood and hair samples at delivery was associated with impaired glucose tolerance during pregnancy, but the association with hair arsenic was not statistically significant (Ettinger et al. 2009). Evidence from National Health and Nutrition Examination Survey (Navas-Acien et al. 2008, 2009a; Steinmaus et al. 2009), although directly relevant to the US population, was considered limited in the NTP review because of challenges in interpreting urinary arsenic biomarkers in the presence of seafood intake (Navas-Acien et al. 2011).

Since the publication of the NTP report (Maull et al. 2012), several studies have been published, including one cross-sectional study (Gribble et al. 2012) and two prospective studies (James et al. 2013; Kim et al. 2013) conducted in the United States. They were conducted in rural communities that had stable populations and low migration rates from Arizona, Colorado, Oklahoma, North Dakota, and South Dakota. Arsenic was measured at the individual level either in urine or in drinking water, diabetes was measured by using standardized outcome definitions, and the associations were adjusted for relevant risk factors, including body-mass index and sociodemographic factors. Table 4 provides the evidence from those studies as an illustration of important features of the studies that should be considered. Some recent studies have also reported that genetic variation in several genes (such as AS3MT, NOTCH2) could increase the risk of type 2 diabetes (Drobna et al 2013; Pan et al. 2013). Overall, recent epidemiologic studies support an association between arsenic at low to moderate concentrations (10–50 μg/L) and diabetes risk. They are directly relevant to evaluating a dose–response relationship for US populations and should be carefully reviewed by the IRIS program in evaluating diabetes as a relevant end point to be included quantitatively for arsenic risk assessment.

Mode of Action

An increasing number of experimental and mechanistic studies have evaluated the potential mode of action of arsenic in causing diabetes. The NTP review of the experimental evidence concluded that although as a whole the experimental literature was inconclusive, recent studies designed to evaluate diabetes-related end points generally supported a link between arsenic and diabetes (Maull et al. 2012). Diabetes could be related to the inhibition of insulin production by pancreatic β cells or the inhibition of basal or insulin-stimulated glucose uptake. Relevant mechanisms by which arsenic could affect β-cell function and insulin sensitivity include oxidative stress, glucose uptake and transport, gluconeogenesis, adipocyte differentiation, calcium signaling, and epigenetic effects (Diaz-Villasenor et al. 2007; Maull et al. 2012). In a genomewide study of peripheral blood lymphocytes in northern Mexico, moderate to high arsenic exposure was associated with hypermethylation and hypomethylation of several diabetes-related genes (Smeester et al. 2011). Additional coexposures, especially to a high-fat diet, could be particularly important for US populations: experimental evidence on rodents suggests that arsenic interacts with a high-fat diet to induce glucose intolerance without changes in plasma insulin concentrations (Paul et al. 2011).

Key Considerations for the IRIS Assessment

Results of recent epidemiologic evidence from studies conducted in Mexican and US populations, including prospective studies conducted in Arizona (Kim et al. 2013) and Colorado (James et al. 2013), support the association between low to moderate arsenic exposure and diabetes risk. The studies provide useful information for the IRIS assessment to use in evaluating the dose–response relationship at arsenic concentrations relevant for US populations. Mechanistic and animal evidence constitutes information that is relevant for evaluating the mode of action in arsenic-related diabetes. The NTP review, systematic reviews of the literature, and the possible conduct of dose–response meta-analyses should guide the inclusion of diabetes as a relevant health end point for quantitative arsenic risk assessment.

TABLE 4 Recent Studies of Diabetes and Arsenic Measured at the Individual Level That Reported Two or More Arsenic Categories[a]

Reference	Design	Population	Diabetes Definition	Arsenic Assessment	Exposure Categories, µg/L	C/NC	Relative Risk	95% CI	Adjustment Factors	Evaluation of Effect Modification
Gribble et al. 2012	CS	Arizona, Oklahoma, N. and S. Dakota (Strong Heart Study) Ages: 45-64 years 40.8 % men	Prevalent diabetes (FPG ≥ 126 mg/dL, OGTT ≥ 200 mg/dL, HbA1c ≥ 6.5%, or diabetes medication)	Baseline total arsenic in population with low seafood intake	<7.9 7.9–14.1 14.1–24.2 ≥24.2	413/558 492/507 503/467 531/454	1.00 1.26 1.38 1.55 p trend	Reference 1.14–1.39 1.25– 1.52 1.39– 1.72 <0.001	Age, sex, BMI, education, smoking, alcohol, urinary creatinine	Association was found mostly in those with poor diabetes control and was stronger in former smokers and in N/S Dakota
Kim et al. 2013	Nested CC	Arizona (Southwestern American Indians) Ages: ≥25 years No sex data	Incident diabetes (OGTT ≥ 200 mg/dL)	Baseline total urine arsenic in population with low seafood intake	6.6–15.3 15.3–21.0 21.1–29.3 29.4–123.1		1.00 1.3 2.2 1.3 p trend	Reference 0.12	Age, sex, BMI, urinary creatinine	Not evaluated (small sample)
				Baseline inorganic arsenic (µg/g of creatinine)	0.1–4.5 4.6–6.9 7.0–9.4 9.5–36.0		1.00 2.4 2.3 1.8 p trend	Reference 0.06		
James et al. 2013	Case-CO	San Luis Valley, Colorado Ages: 20-74 years 46% men	Incident diabetes (FPG ≥ 140 mg/dL, OGTT ≥ 200 mg/dL, or self-reported diagnosis and diabetes medication)	Time-weighted average arsenic in drinking water	1–3 4–7 8–19 ≥20	120 148 139 141	1.00 1.11 1.42 1.55 p trend	Reference 0.82–1.95 0.94– 2.48 1.00– 2.51 0.04	Age, sex, race, income, BMI, physical activity, smoking, alcohol, family history	Not evaluated (small sample)

[a]The studies were conducted in populations with drinking water concentrations of arsenic less than 100 µg/L in the United States and were published after the NTP (2011) review.

Abbreviations: BMI, body-mass index; CC, case–control; CO, cohort; CS, cross-sectional; C/NC, cases/noncases; FPG, fasting plasma glucose; HbA1c, hemo-hemoglobin A1C; OGTT, oral glucose tolerance test.

EFFECTS ON LIVER, PROSTATE, AND PANCREAS

Liver

Arsenic is known to have potential carcinogenic and toxic effects on the liver (IARC 2004, 2012). Ecologic epidemiologic studies of populations in Taiwan and Chile have reported increased liver-cancer mortality in relation to arsenic exposure. In studies conducted in southwest Taiwan, Chen et al. (1986) reported an age- and sex-adjusted OR of 2.67 for liver-cancer mortality in those who had used well water that had high arsenic concentrations for 40 years or more compared with a relatively nonexposed area. In another study, they reported a dose–response relationship between arsenic concentrations in artesian well water and mortality associated with a number of cancers, including liver cancer in males, but the trend in females was not significant (Wu et al. 1989). In southwest Taiwan, in an area that had a median arsenic concentration in drinking water of 780 μg/L, Tsai et al. (1999) reported an increase in mortality due to liver cancer compared with local and national reference groups. Rivara et al. (1997) in a study in Antofagasta and Calama-Chuquicamata, Chile, determined that in an area that had high concentrations of arsenic in both the soil and water, some as high as 800 μg/L of drinking water, there was an increase in mortality (RR = 2.2) due to liver cancer in comparison with a region that had low concentrations of arsenic. Smith and co-workers (Smith et al. 1998; Liaw et al. 2008; Smith et al. 2012) also studied that population in Antofagasta, Chile. In their initial study (Smith et al. 1998), they observed increases in mortality associated with bladder and lung cancer but no increase in overall liver-cancer mortality from arsenic. In a later study, they found increased liver-cancer mortality in those who were exposed in utero and when young during peak exposure periods (before a change in water treatment). Specifically, they found higher childhood mortality from liver cancer than expected (RR = 10.6) (Liaw et al. 2008) and increased liver-cancer mortality in young adults (SMR = 2.5) (Smith et al. 2012), albeit on the basis of a small number of cases.

Not all epidemiologic studies have identified increases in liver cancer in relation to arsenic exposure. In a study of cancer mortality associated with arsenic in the drinking water in Cordoba, Argentina, although increases in lung and renal cancer incidence were found to be dose-dependent, mortality from liver cancer was similar in all three arsenic-exposed (low, medium, and high) groups (Hopenhayn-Rich et al. 1998). Guo (2003) used data from the Taiwan National Cancer Registry on over 40,000 patients in 243 townships who had liver cancer to determine the distribution of hepatocellular carcinoma and cholangiocarcinoma. No difference in the distribution of cancer cell types was found between the townships with endemic arsenic intoxication and the other townships. The study also found no association between arsenic and hepatocellular carcinoma. Baastrup et al. (2008) also found no increase in liver-cancer cases associated with arsenic in a Danish cohort study that had low drinking-water concentrations of arsenic (0.05–25.3 μg/L). However, none of those studies examined timing of exposure.

In studies of liver toxicity, Islam et al. (2011) measured arsenic concentrations in the hair and nails of 200 people in Bangladesh (which correlated with drinking-water concentrations) and found that indicators of liver toxicity (serum alkaline phosphatase, aspartate transaminase, and alanine transaminase) were significantly higher in the high-exposure group (over 50 μg/L in drinking water). Similarly, in a study of people in West Bengal, India, Das et al. (2012) found higher concentrations of bilirubin, alanine transaminase, aspartate transaminase, and antinuclear antibodies (an indicator of autoimmune status) in the serum of people who lived in an area that had an average arsenic concentration of 203 μg/L in drinking water than in serum of nonexposed people.

Arsenic and liver cancer have been studied in experimental animals. Waalkes et al. (2003, 2004a,b, 2006) found that male mice from dams exposed to sodium arsenite (0, 42.4, and 85 ppm) in drinking water on gestation days 8–18 had dose-dependent increases in hepatocellular-carcinoma incidence and multiplicity. An increase in hepatocellular carcinomas was also found in male and female mice exposed to arsenic (0, 6, 12, and 24 ppm in drinking water) in utero and over their lifetime (Tokar et al. 2011b).

Key Considerations for the IRIS Assessment

There is some epidemiologic evidence that liver cancer may be associated with arsenic exposure, in particular with exposure early in life, but the total body of work is not completely coherent. There is also evidence of hepatocyte damage, but overall the studies are not conclusive, especially with respect to low to moderate arsenic exposure. Thus, liver disease is unlikely to have high priority for the IRIS assessment.

Prostate

The prostate is among the tissues potentially associated with arsenic toxicity, including cancer (IARC 2004). In a number of studies of prostate and other cancers in Taiwan, increased prostate-cancer mortality was noted with a dose-related gradient of arsenic in drinking water from wells (Chen et al. 1985; Wu et al. 1989; Chen and Wang 1990; Yang et al. 2008). Notably, in a cohort study of mortality from malignant neoplasms in 314 precincts and townships, an age-adjusted increase in mortality from prostate cancer was associated with arsenic concentrations in well water (Chen and Wang 1990). The authors also found that mortality from prostate cancer declined when the contaminated water sources were replaced (Yang et al. 2008); this supports a causal link. Lewis et al. (1999) examined the relationship between arsenic in drinking water and prostate cancer in an ecologic analysis of a cohort of Mormon residents of Utah and found an SMR of 1.45 and support of a dose–response relationship. In an ecologic study in Canada that evaluated areas that had soil concentrations of arsenic greater than 100 mg/kg and water concentrations greater than 10 μg/L, Hinwood et al. (1999) found an increase in prostate cancer (standardized incidence ratio 1.14) that was the only cancer significantly increased after stratification into exposure categories. In American Indians in Arizona and North and South Dakota who participated in the Strong Heart Study, baseline urinary arsenic concentrations (median 9.7 μg/g of creatinine; interquartile range 5.8–15.6) were associated with increased prostate-cancer mortality over almost 20 years of followup (Garcia-Esquinas et al. in press).

However, associations with prostate cancer have not been consistently found in epidemiologic studies. Rivara et al. (1997) examined mortality from prostate cancer in residents of two regions in Chile, one of which was exposed to high concentrations of arsenic in drinking water (some as high as 800 μg/L), and found an RR of 0.9. Baastrup et al. (2008) found no increase in mortality from prostate cancer due to arsenic in a Danish drinking-water cohort study in which exposures ranged from 0.05 to 25.3 μg/L, although most participants were exposed at below 2 μg/L.

Mode of Action

A series of studies that used cell culture have also suggested that arsenic has an ability to transform normal cells into a cancer-like state. Achanzar et al. (2002) incubated human prostate epithelial cells (RWPE-1) in 5 μM arsenic and found a malignant transformation of the cells that produced epithelial-cell tumors when placed in nude mice. Benbrahim-Tallaa et al. (2005) found that that transformation was associated with aberrant genomic DNA methylation and K-ras oncogene activation at the same arsenic concentration. It was associated with Ras signaling activation (Benbrahim-Tallaa et al. 2007). Similarly, Tokar et al. (2010a) found that arsenic at 5 μM transformed the human prostate epithelial/progenitor cell line WPE-stem to a malignant cancer stem-cell–like phenotype. Singh et al. (2011) found that exposure of human prostate epithelial cells (RWPE-1) to arsenic caused nuclear DNA damage and mutations in mitochondrial DNA and resulted in increased cell survival when arsenic concentrations were as low as 8 pM. They also found (Treas et al. 2012) that arsenic in combination with estrogen altered epigenetic regulatory-gene expression, global DNA methylation, and histone modification in those cells.

Key Considerations for the IRIS Assessment

There is some, albeit modest epidemiologic evidence of an association between arsenic and prostate cancer, including two studies in the United States. In vitro studies of arsenic suggest that a change in phenotype may occur in isolated prostate cells exposed to arsenic. Given that evidence and the burden of prostate disease in US men and its associated health-care costs, the committee believes that the IRIS assessment of inorganic arsenic should at least consider the issue of prostate cancer.

Pancreas

Increased incidence of or mortality from pancreatic cancer in relation to arsenic-contaminated drinking water has not been reported in populations of Taiwan, Argentina, Chile, or Bangladesh that were exposed to high concentrations of arsenic (IARC 2004). However, increased pancreatic-cancer incidence was found in an ecologic comparison of children born before and after a 1955 episode of arsenic-contaminated milk powder (used for bottle-fed infants) in Japan (Yorifuji et al. 2010, 2011). Excess mortality from pancreatic cancer was observed in children younger than 5 years old during the contamination episode. The study was ecologic, and exposure occurred only during a limited period early in life, so an evaluation of a dose–response relationship was not possible. Recent studies at low to moderate exposure have also reported an association between arsenic and pancreatic cancer. In a hospital-based case–control study in Spain, the OR for pancreatic cancer when the highest and lowest quartiles of toenail arsenic concentrations were compared (>0.106 vs ≤0.052 μg/g) was 2.02 (95% CI 108–3.78) (Amaral et al. 2012). In American Indians in Arizona and North and South Dakota who participated in the Strong Heart Study, baseline urinary arsenic concentrations (median 9.7 μg/g of creatinine, interquartile range 5.8–15.6) were associated with increased pancreatic-cancer mortality over almost 20 years of followup (Garcia-Esquinas et al. in press). Although limited because of uncertain exposure assessment, GIS analysis in Florida also noted higher pancreatic-cancer incidences in relation to residential proximity to wells that had arsenic concentrations above 10 μg/L (Liu-Mares et al. 2013). Although the mechanism has not been characterized, one hypothesis is that arsenic increases the risk of diabetes (see section "Diabetes: above), which is a risk factor for pancreatic cancer.

Mode of Action

Studies of arsenic as a causative agent of pancreatic cancer are few. Results of studies of arsenic trioxide by Xue et al. (2009) indicated a dose-dependent effect on AR42J cells (rat pancreatic epithelial cell line). At low concentrations, it caused apoptosis; but at high concentrations (8 μM), it caused oncosis as identified by an increase in malignant changes in the cells detected with laser scanning confocal microscopy.

Key Considerations for the IRIS Assessment

Although there is emerging evidence of potential carcinogenic effects of arsenic on the pancreas, it may be difficult for EPA to establish risks on the basis of available evidence.

IMMUNE EFFECTS

In the hazard assessment of inorganic arsenic, it is important to consider effects on the immune system. The immune system is required to protect against infection. Dysregulation of the immune system may influence cancer and cardiovascular-disease risk. Both innate and adaptive immune responses should be considered. Epidemiologic, rodent, and in vitro data all support a relationship between inorganic arse-

nic exposure and immune system effects. The immunologic effects could include sensitization to exposure to viruses and bacteria; such effects have been seen in human populations exposed to arsenic.

It is also important to consider the relation of adverse effects on the immune system to periods of developmental susceptibility, when the thymus plays a substantial role. In early pregnancy, for example, the immune system changes toward Th2 cytokine response, which protects the fetus from being recognized as foreign (Calleja-Agius and Brincat 2008). As a result, Th1-dependent cytokine production is suppressed, and the child is born with Th2-biased immune responses. After birth, the immune system requires maturation of the Th1 cytokine response to achieve effective resistance to diseases (Dietert and Zelikoff 2008). That series of prenatal and postnatal events seems to be more sensitive to toxic insult than is the adult immune system (Dietert 2009). Suppression of theTh1-dependent function increases susceptibility to infections and reduces response to childhood vaccination (Heilmann et al. 2006; Vorderstrasse et al. 2006).

The hazard assessment of inorganic arsenic should include the following body of literature and integrate mode-of-action information where possible.

Epidemiologic Evidence

Results of several cross-sectional studies in both children and adults indicate that arsenic may be immunosuppressive. Results of recent studies indicate that arsenic is immunotoxic early in life when the thymus is an essential organ of the immune system. In a mother–child cohort in rural Bangladesh, thymus size, measured with ultrasonography, was inversely associated with maternal exposure to arsenic through drinking water, measured as the concentration of arsenic metabolites in urine in early and late pregnancy (range 5.5–1150 μg/L; n = 1,556) (Moore et al. 2009), which corresponded to similar concentrations in the drinking water (Vahter et al. 2006). Maternal urinary arsenic was also positively associated with diarrhea and acute respiratory infections in the infants (n = 1,552) (Rahman et al. 2011). The findings suggest that in utero arsenic exposure impaired child thymus development and increased morbidity, probably via immunosuppression. The results of those studies are supported by those of a recent study conducted in the United States in which low prenatal inorganic arsenic exposure was associated with an increased risk of respiratory disease, such as upper respiratory tract infections and colds (Farzan et al. 2013). With respect to mode of action, it is important to consider the relationship of dose and time dependence of exposure to key immunologic events.

In a followup of the Bangladeshi study (Ahmed et al. 2011), immune and inflammatory markers, measured with immunohistochemistry and multiplex cytokine assay, were assessed in 130 women. Significant positive associations between maternal urinary arsenic and placental markers of 8-oxoguanine (8-oxoG) and proinflammatory cytokines—such as interleukin-1beta (IL-1β), tumor necrosis factor-alpha (TNFα), and interferon-gamma (IFNγ)—were found. The 8-oxoG measured in the placenta was also positively associated with the placental proinflammatory cytokines. Urinary arsenic in early gestation was inversely associated with CD3+ T cells in the placenta.

In a study in Thailand, researchers examined the extent to which maternal arsenic exposure affects gene expression in the newborn by assessing cord-blood genomic signaling. The researchers monitored gene-expression profiles in a population of 32 newborns whose mothers experienced varied arsenic exposure during pregnancy. The investigators used genomewide gene-expression analyses to identify genes that were predictive of prenatal arsenic exposure in a later test population. Those genes could be reduced to a set of 11 transcripts that maintained the maximal predictive capacity to classify prenatal arsenic exposure. Analyzing the genes in the context of their protein–protein and protein–DNA interactions demonstrated that prenatal arsenic exposure was associated with genes involved in stress, inflammation, metal exposure, and apoptosis in the newborn (Fry et al. 2007). Many of those genes are key players in the immune response. Exposure in the study was determined on the basis of arsenic in toenail samples. A toenail-water comparison suggested that the cohort was exposed to high concentrations of inorganic arsenic as evidenced by toenail measurements of up to 68.63 μg/g. A dose–response relationship was observed

between arsenic in toenails and the expression-level changes in the identified set of genes. Moreover, the genes shared binding sites for transcription factors that are known to be metal-responsive (such as metal-responsive transcription factor 1).

Experimental Evidence

Researchers examined mRNA and protein-expression changes in the lungs of mice that were chronically exposed to arsenic in food or drinking water to evaluate whether immune modulation contributes arsenic-related disease risk in the lung (Kozul et al. 2009a). Groups of C57BL/6J mice were exposed to arsenic in drinking water or food at 10 or 100 ppb for 5–6 weeks. A whole-genome transcriptome profiling assay of the lungs revealed substantial alterations in the expression of genes involved in innate immune response and in cell adhesion and migration, channels, receptors, differentiation, and proliferation. The investigators further demonstrated that genes for IL-1β, IL-1 receptor, a number of toll-like receptors, and several cytokines and cytokine receptors were also altered (Kozul et al. 2009a).

Following up on that work, the researchers investigated the effects of arsenic exposure on respiratory influenza A (H1N1) virus infection. C57BL/6J mice were exposed to arsenic at 100 μg/L in drinking water for 5 weeks and were then infected with influenza A/PuertoRico/8/34 (H1N1) virus by intranasal inoculation. Increased morbidity was observed in the mice, and higher titers of influenza were found in whole-lung homogenates. Several alterations in immune response were also found. For example, a decrease in dendritic cells in the mediastinal lymph nodes was observed early in infection relative to nonexposed controls (Kozul et al. 2009b).

Acharya et al. (2010) investigated arsenic-induced carcinogenesis and effects on the immune system in a mouse model. Ethylnitrosourea was used to induce tumors in mice, and arsenic was used as a promoter. Effects on the immune system were assessed on the basis of cytokine (TNFα, IFNγ, IL-4, IL-6, IL-10, and IL-12) production of lymphocytes and the specific apoptotic cascade in lymphocytes. Arsenic, especially in combination with ethylnitrosourea, induced marked neoplastic changes. Those findings were supported in histologic studies and by evidence of severe immune suppression that resulted from cytokine modulation and lymphocyte death.

Key Considerations for the IRIS Assessment

Taken together, the evidence of a relationship between exposure to inorganic arsenic and altered immune function warrants consideration of the immune system in the IRIS assessment. The immunologic changes seem to increase the risk of respiratory symptoms in particular. Those effects have been observed after both lower and higher exposures to inorganic arsenic, although few studies are available on low to moderate exposure. It is conceivable that such effects may contribute to impairmet in fetal and infant health and to detrimental health effects in adults.

SUMMARY

To help EPA set priorities among its efforts, the committee has created the following hierarchy of health end points of concern:

- Tier 1: Evidence of a causal association determined by other agencies and/or in published systematic reviews.
  - Lung, skin, and bladder cancer (Celik et al. 2008; IARC 2012).
  - Ischemic heart disease (Navas-Acien et al. 2005; C.H. Wang et al. 2007; Moon et al. 2012).
  - Skin lesions (ATSDR 2007).
- Tier 2: Other priority outcomes.

  - Prostate and renal cancer.
  - Diabetes.
  - Nonmalignant respiratory disease.
  - Pregnancy outcomes (infant morbidity).
  - Neurodevelopmental toxicity.
  - Immune effects.
- Tier 3: Other end points to consider.
  - Liver and pancreatic cancer.
  - Renal disease.
  - Hypertension.
  - Stroke.
  - Pregnancy outcomes (fetal loss, stillbirth, and neonatal mortality).

The first tier consists of end points that have been identified by other agencies or in systematic reviews as having a causal association with inorganic arsenic. The second tier consists of other high-priority outcomes of concern. The last tier consists of end points for which the evidence appears to be less strong. These categorizations will be refined by EPA after it conducts its more comprehensive analysis.

EPA's draft plans indicate that a causal determination framework will be used to categorizethe evidence on the different end points into five possible categories: causal relationship, likely to be a causal relationship, suggestive of a causal relationship, inadequate to infer a causal relationship, and not likely to be a causal relationship. The committee supports that five-category approach and recommends that strength-of-evidence judgments be characterized with respect to the modified Bradford Hill criteria for causality. In evaluating the studies, it will be important to consider the timing of exposure with respect to life stage, duration of exposure, and the latent period for the health outcome. The assessment of causality will help EPA to set priorities among end points for later analysis of mode of action and dose–response relationships.

5

Susceptibility Factors

Several factors are known to influence susceptibility to the adverse effects of arsenic. This chapter addresses susceptibility factors discussed in the committee's workshop by Beck (2013) and Hansen (2013)—life stage, genetics, nutrition, and pre-existing disease—and the additional factors of sex, smoking, alcohol consumption, and exposure to mixtures. The sections on these factors reflect a preliminary survey of the literature that the US Environmental Protection Agency (EPA) should consider more comprehensively and systematically as it conducts its toxicologic assessment of inorganic arsenic.

LIFE STAGES

Life stages refers to different ages and developmental stages throughout life, including prenatal development (pregnancy) and aging. There is increasing evidence that arsenic exposure early in life affects fetal and child health and development and health later in life. However, there seems to be a lack of data on the influence of aging.

Early-life development is a critical window of susceptibility for multiple toxic agents. The main focus has been on developmental neurotoxicity—recently reviewed by Bellinger (2013)—but there is increasing evidence that other organs and functions may be particularly susceptible during development, such as the immune system (Dietert 2011) and the lungs (Ramsey et al. 2013a). Thus, it is essential to evaluate whether early-life exposure to arsenic may affect the risk of the numerous arsenic-related health effects observed in adults and to consider this question in the health risk assessment.

Concerning prenatal arsenic exposure, it seems clear that all metabolites of inorganic arsenic easily cross the placenta to the fetus. Strong correlations between arsenic in maternal blood and in cord blood have been found in women exposed to arsenic-contaminated drinking water (Concha et al. 1998a; Jin et al. 2006; Hall et al. 2007). Epidemiologic studies, including prospective cohort studies, have provided evidence that arsenic exposure through drinking water during pregnancy may cause dose-dependent impairment of fetal and infant growth and survival (see section "Pregnancy Outcomes" in Chapter 4). In particular, the developing immune system and central nervous system seem to be susceptible to arsenic exposure, and adverse effects seem to appear even after relatively low arsenic exposure (see sections "Neurotoxicity" and "Immune Effects" in Chapter 4).

Besides the prenatal and perinatal stages, early childhood may be a period of susceptibility to inorganic arsenic exposure. A few epidemiologic studies have indicated that continued exposure after birth may impair children's growth. A cross-sectional study in China suggested that increased water arsenic concentrations were inversely associated with body weight of children (720) 8–12 years old (S.X.Wang et al. 2007). In a prospective cohort study in Bangladesh, measures of 2,372 infants were related to concentrations of arsenic metabolites in the urine of mothers in early and late pregnancy and of the children at the age of 18 months (Saha et al. 2012). The study adjusted for age (within each age group), sex, maternal body-mass index, socioeconomic status, and birth weight or length. Observed inverse associations of maternal urinary arsenic with children's weight and length at the age of 3–24 months were markedly attenuated after adjustment for relevant covariates. However, the associations of urinary arsenic at the age of 18 months with weight and length at the age of 18–24 months were more robust, particularly in girls. The

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effects seemed to appear after low arsenic exposure. Compared with girls in the first quintile of urinary arsenic (less than 16 μg/L, adjusted for specific gravity), those in the fourth quintile (26–46 μg/L) were almost 300 g lighter and 0.7 cm shorter. In a followup of 1,505 mother–infant pairs on whom there were data on concentrations of arsenic, cadmium, and lead in maternal and child urine, the effects of the combined exposure to these metals on children's weights and heights up to the age of 5 years were evaluated (Gardner et al. 2013). The investigators adjusted for family socioeconomic status, maternal tobacco chewing during pregnancy, cooking with indoor fires, maternal education, season of birth, parity, and exposure to cadmium and lead. In the longitudinal analysis, the multivariable-adjusted attributable difference in children's weight at the age of 5 years was -0.33 kg (95% CI -0.60 to -0.06) for high arsenic exposure (95th percentile or higher) compared with the lowest exposure (5th percentile or lower). The corresponding multivariable-adjusted attributable difference in height was -0.50 cm (95% CI -1.20 to 0.21). As in the earlier study, the associations were apparent primarily in girls

Those findings are supported by experimental studies in which mice were exposed to very low concentrations of arsenic (10 μg/L in drinking water) prenatally via the dams' drinking water or postnatally (Kozul-Horvath et al. 2012). Birth outcomes, including litter weight and number of pups, were unaffected, but exposure in utero and postnatally resulted in impaired growth of the offspring. The growth deficits resolved afer cessation of exposure in male mice but not in female mice up to the age of 6 weeks.

Adverse effects of arsenic on child health, especially increased risk of infectious diseases and signs of immunosuppression, have been reported in several cross-sectional epidemiologic studies (see section "Immune Effects" in Chapter 4). Whether the underlying effect was initiated before birth or during childhood is not clear. Similarly, impaired cognitive function has been reported in several epidemiologic studies and indicates that children, especially of preschool age, are susceptible to arsenic-induced neurotoxicity (see section "Neurotoxicity" in Chapter 4).

The increasing evidence that early-life exposure to inorganic arsenic increases the risk of adverse health effects later in life was discussed in the committee's workshop by Waalkes (2013). Important evidence comes from the studies in northern Chile, showing considerably increased risk of cancer and noncancer effects in young adults who were born in Antofagasta during or shortly before the period (1958–1970) of high arsenic concentrations in drinking water (Smith et al. 2006; Yuan et al. 2007; Dauphine et al. 2011; Smith et al. 2012). In particular, the risk of death from chronic bronchiectasis was higher in those who had been exposed in utero and during early childhood and higher in those who had been exposed in utero compared with the rest of Chile (Smith et al. 2006) (see section "Respiratory Effects" above). The increased susceptibility to respiratory effects of early-life exposure has support from experimental studies that indicated impairment of lung development caused by in utero exposure to arsenic (Ramsey et al. 2013a,b). Recent epidemiologic studies of arsenic-related developmental immunotoxicity have also indicated long-term consequences for susceptibility, for example, to infections (Moore et al. 2009; Ahmed et al. 2012).

The human evidence of later-life effects of low-dose early-life arsenic exposure is supported by a series of experimental studies in mice that were given drinking water that contained arsenic at 50 μg/L (mainly arsenate) during gestation (or before mating) and thereafter until weaning. That exposure regimen resulted in marked dysregulation of the hypothalamic–pituitary–adrenal axis in male mice at the age of 35 days (Goggin et al. 2012), interactions with the glucocorticoid receptor and associated signaling pathway (Martinez et al. 2008; Martinez-Finley et al. 2011), and impaired learning and memory (Martinez-Finley et al. 2009).

Perhaps the most striking animal studies are those of Waalkes and co-workers, who demonstrated that arsenic exposure in utero, with no additional postnatal exposure, leads to the development of liver, lung, ovary, and adrenal tumors in rats (Waalkes et al. 2003). They have gone on to show that prenatal arsenic exposure increases the carcinogenicity of other agents in the offspring when they reach adulthood (Tokar et al. 2011a).

A few recent studies have indicated that maternal arsenic exposure during pregnancy may influence epigenetic markers, specifically those related to DNA methylation, in DNA isolated from the blood cells of newborn children. Two studies in Bangladesh of 113 and 101 mother–newborn pairs reported positive

associations between maternal arsenic exposure and global methylation (methyl-incorporation or LINE-1 assays) in DNA isolated from cord blood (Kile et al. 2012; Pilsner et al. 2012), whereas no significant difference in in cord blood LINE-1 methylation was observed between arsenic-exposed (55) and -unexposed (16) newborns (as assessed by arsenic in hair and nails) in a cross-sectional study in Thailand (Intarasunanont et al. 2012). The study by Pilsner et al. (2012), which also included the Alu and LUMA assays, indicated that the effects on DNA methylation were largely sex-specific, with arsenic-related increased methylation in newborn boys but decreased methylation in girls. Evaluation of methylation in cord-blood DNA of 134 infants in a prospective birth cohort in New Hampshire using the Illumina Infinium Methylation 450K array found evidence of differential patterns of DNA methylation in relation to fairly low maternal urinary arsenic concentrations in late pregnancy (median 4.1 μg/L, interquartile range 1.8–6.6 μg/L, maximum value about 300 μg/L) (Koestler et al. 2013). Among the 100 loci that had the strongest association with arsenic, those in CpG promoter islands (44) showed mostly (75%) higher methylation in the highest-exposed group (>6.6 μg/L) than in the lowest-exposed group. However, cg08884395 (associated with estrogen receptor 1, ESR1) and cg27514608 (peroxisome proliferator–activated receptor-γ coactivator 1-α [PPARGC1A]) showed inverse associations between arsenic exposure and methylation and had statistically significant trends. Although changes in DNA methylation have been observed and associated with arsenic exposure, their biologic effects and meaning at a functional level in the cell and as related to health effects are unknown.

Key Considerations for the IRIS Assessment

Collectively, the highly suggestive evidence that early-life exposure to arsenic, even at low concentrations, increases the risk of adverse health effects and impaired development in infancy and childhood and later in life leads the committee to suggest that the timing of exposure, particularly early-life exposure, be considered in evaluating epidemiologic studies for dose–response assessment. The increasing body of data on mechanisms, including hormone interactions and epigenetic alterations, in support of those effects warrants consideration.

GENETICS OF ARSENIC METABOLISM AND TOXICITY

Genetic factors probably confer susceptibility or resistance to inorganic arsenic exposure. Genetic variants may have biologic effects of their own that act synergistically with inorganic arsenic or may have normal function in the absence of inorganic arsenic. Genetics may interact with inorganic arsenic ion multiple ways. Three possible interactions are as follows:

- Genetic variants may increase or decrease the absorbed dose of inorganic arsenic or alter the excretion of inorganic arsenic or its metabolites. The committee knows of no examples of this type of susceptibility.
- Genetic variants may alter the metabolism of inorganic arsenic independently of excretion or absorption. Most of the arsenic literature deals with this type of susceptibility.
- Genetic variants may increase or decrease the organ or cellular toxicity of inorganic arsenic. There is a small body of literature on this type of susceptibility.

As described below, there are many examples in the literature regarding genetic susceptibility factors, but their role in risk assessment is still unclear; the prevalence of these factors will vary in different populations that have different underlying ethnicities. Furthermore, the function of the genetic variants will probably vary with dose and may not be relevant in some exposure scenarios. Finally, most of the published reports are of case–control studies that provide few or no data on timing of exposure. An often overlooked underlying aspect of genetic susceptibility to environmental factors is the timing of exposure.

Regardless of the type of environmental factor (for example, chemical, social, or nutritional), the timing of exposure probably plays a critical role in whether a genetic factor will interact with it.

Critical Developmental Windows and Gene–Environment Interactions

The field of gene–environment interactions is surprisingly underdeveloped. Most environmental health studies of gene–environment interactions have used a small-scale candidate-gene approach and have not planned for replication in independent populations. Underlying the problem is an often neglected aspect of genetic epidemiology studies: gene expression changes from one life stage to another. Genetic variants that produce gene–environment interactions might do so only when the exposure corresponds to a critical developmental window during which the gene is highly expressed (Wright and Christiani 2010). Most gene–environment interaction studies are case–control studies and cannot address critical developmental windows.

Genomewide Approach vs Candidate-Gene Approach

Most human studies of genetic variation and arsenic have focused on a few candidate single nucleotide polymorphisms (SNPs) that may modify arsenic toxicity or predict its metabolism. Although such an approach has strengths, including biologic plausibility and clear a priori hypotheses, there are limitations in selecting only a few SNPs for a study (Rebbeck et al. 2004). The potential functional effect of many polymorphisms is sometimes undefined or even controversial. In addition, it is unlikely that one SNP of one gene can account fully for the complexity of function of an entire biologic pathway. Finally, some genes or SNPs that have important roles in arsenic pathogenesis may not have been identified yet, and a candidate approach would not be able to identify such genes. Thus, bias is a concern in the selection of SNPs in a candidate approach (Wright and Christiani 2010).

An alternative approach is to use genomewide scans, which capitalize on advances in high-throughput technology and on the data from the completed HapMap Project (Hirschhorn 2005; Hirschorn and Daly 2005). Genomewide scans allow screening of the genome in an unbiased manner with respect to genetic risks factors (Kronenberg 2008). The greatest strength of such an approach is that it allows new biologic relationships to be discovered. Its primary weakness is that well-known biologic associations are ignored. When genetic associations, or gene–environment interactions, are simply ranked, a gene that may have greater biologic plausibility a priori is considered equal to all other genes—even genes that may not be expressed in the target tissue (Wright and Christiani 2010). To date, genomewide association studies have been conducted to identify genetic risk factors for diseases as varied as age-related macular degeneration (Haines et al. 2006), cardiac diseases (Cupples et al. 2007; Vasan et al. 2007), diabetes (Meigs et al. 2007; Wellcome Trust Case Control Consortium 2007), amyotrophic lateral sclerosis (van Es et al. 2007), rheumatoid arthritis (Plenge et al. 2007; Thomson et al. 2007; Wellcome Trust Case Control Consortium 2007), and cancer (Broderick et al. 2007; Murabito et al. 2007; Spinola et al. 2007; Zanke et al. 2007). Whole-genome sequencing is an approach that has the advantage of discovering rare variants that may modify arsenic toxicity. Overall, the field of genomics has undergone a paradigm shift from a hypothesis-driven approach to a hypothesis-generating approach. Because genomic approaches generate multiple comparisons on a grand scale, tests of statistical significance need to take into account that genes are not necessarily expressed independently of each other and still control for overall experimentwide error. One procedure is a correction for multiple comparisons, such as Bonferroni correction or control of false discovery rates. Such methods are purely statistical, however, and ignore biology. Another approach is to conduct the research in multiple populations sequentially. Researchers generate hypotheses in one population and then test them in separate populations as a second-line screening and validation or replication method (Rodriguez-Murillo and Greenberg 2008). At least one whole-genome interaction study of inorganic arsenic has been conducted (Pierce et al. 2012). The region proximal to the arsenic 3+ methyltransferase enzyme (AS3MT) gene was identified as containing variants associated with the percentage of

monomethyl arsenic (MMA) and dimethyl arsenic (DMA) metabolites and is associated with the risk of a disease outcome (skin lesions). Replication in an independent population is still needed, however.

Recently, a linkage analysis was conducted in the Strong Family Heart Study, a family-based study of mostly American Indian families in Arizona and Oklahoma (Tellez-Plaza et al. 2013). Urinary arsenic and its metabolites were considered as quantitative traits. Linkage studies differ from association studies (typically of case–control design) in studying family members and estimated heritability (the percentage of variance in urinary arsenic metabolites due to genetics). Regions in the genome that have suggestive logarithm of the odds of disease (LOD) scores (over 1.9) were described on chromosomes 5, 9, and 11. AS3MT is found on chromosome 10, and a locus that had an LOD score of 1.8 was found proximal to this site. Like genomewide-association studies, linkage studies identify genomic regions of interest, not specific genes. Further study, typically deep sequencing and the identification of functional variants, is needed before any genes can be considered as susceptibility factors. Finally, in any study of genetic contributions to arsenic metabolism, estimates of heritability are probably influenced by the degree of exposure in the underlying populations (that is, in the absence of arsenic exposure, a genetic trait cannot exhibit heritability), and this influence may explain the modest LOD score for the region proximal to AS3MT.

Studies of Genetic Susceptibility to Arsenic

Arsenic Metabolism: Role of Methylation

The metabolism of inorganic arsenic is critical for its toxicity and has been studied extensively in humans and animals. Two processes are involved: reduction and oxidation reactions that interconvert arsenate and arsenite and methylation reactions, which convert arsenite to MMA and DMA (Tam et al. 1979; Buchet et al. 1981a,b; Marcus and Rispin 1988). Inorganic arsenic is methylated, mainly in the liver, to form monomethylated and dimethylated metabolites (Vahter and Marafante 1987; Hopenhayn-Rich et al. 1993). There is also evidence of genetically determined differences in arsenic methylation (Vahter et al. 1995b; Engström et al. 2013; Harari et al. 2013; Schlebusch et al. 2013). The biomethylation process involves both the arsenic 3+ methyl transferase gene and and the glutathione *S*-transferase (GST) enzyme systems (particularly the omega class) (Styblo et al. 1995). Further upstream, several genes related to one-carbon metabolism (such as folate metabolism genes, which provide methyl groups as substrate for methyl transferase enzymes) are also involved. Once arsenic is absorbed into the blood, it undergoes reduction to arsenite and then methylation to yield MMA and DMA, which, in their pentavalent forms, are more readily excreted in urine. Studies have shown that animal species and individuals vary markedly with respect to arsenic methylation efficiency and that efficient methylation of inorganic arsenic is associated with a high rate of excretion (Vahter 2002). Gamble et al. (2005) reported associations between one-carbon donor status and urinary methylated arsenic species in Bangladeshi adults. The percentage of DMA in urine was positively associated with plasma folate, although the percentages of inorganic arsenic and MMA were negatively associated with folate. In a followup study, folate supplementation led to enhanced arsenic methylation and excretion and lower blood arsenic concentrations (Gamble et al. 2007).

Glutathione *S*-transferase

Glutathione could be a necessary catalyst for arsenic methylation through its role in the reduction of arsenate to arsenite. The GST-omega isozyme family is critical in this reaction although purine nucleoside phosphorylase can also reduce arsenate. There are eight classes of GST in mammalian cells, each of which may have subclasses of isozymes. These enzymes catalyze the nucleophilic attack on electrophilic chemicals, including xenobiotics, by glutathione. Various studies have addressed polymorphisms in GST isozymes and either arsenic speciation or toxicity. Chung et al. (2013) found an association between GST null variant (GSTM1 MspI) with increased risk of urothelial cancer. A GST-omega nonsynonymous SNP (Ala140Asp) modified the association between hair arsenic and metabolic syndrome (Wang et al. 2007).

Lesseur at al. (2012) assessed polymorphisms in five GST isozymes (P, M, O, T, and Z). A coding SNP in GST P1 (Ile105Val) modified the association of bladder cancer with toenail arsenic concentrations. Main effects were also noted in the risk of bladder cancer and GSTO2 SNP Asn142Asp and GSTZ1 SNP Glu32Lys. In a Vietnamese population, GST P1 Ile105Val was associated with lower MMA; this suggested a protective effect of the SNP (Agusa et al. 2012). Such a finding would be counterintuitive given the study by Lesseur et al. (2012) of this SNP with respect to toenail arsenic and bladder cancer; however, Lesseur et al. did not report arsenic speciation. L.I. Hsu et al. (2011) reported that the GSTT1 null variant modified the association between cumulative arsenic estimated by water concentrations and bladder cancer. Polymorphisms in other GST genes (GSTO1, GSTO2, GSTP1, and GSTM1) did not modify that association. Similarly, Hsieh et al. (2011) did not find an association or evidence of effect modification between arsenic exposure and GSTO1 and O2 polymorphisms and carotid atherosclerosis but did find evidence of effect modification between a haplotype of purine nucleoside phosphoralase SNPs and well-water arsenic. Finally, Chung et al. (2011) reported that subjects who had the GSTO2 Ala140Asp SNP had lower proportions of MMA in urine than subjects who were homozygous for the Ala allele; this suggests a protective effect of the SNP. That SNP was also assocated with a reduced risk of urothelial carcinoma. Collectively, those studies suggest that the genetic variability of the GST enzymes may influence susceptibility to arsenic toxicity, but the ability to use this information in the IRIS assessment is not clear.

Arsenic 3+ Methyl Transferase

The enzyme AS3MT catalyzes the methylation of arsenite by using *S*-adenosyl methionine as the methyl donor. The AS3MT gene has been the target of several candidate-gene studies of genetic susceptibility to arsenic. A nonsynonymous SNP M287T and an intronic SNP in the AS3MT gene were both associated with reduce methylated arsenic species in urine in American Indians (Gomez-Rubio et al. 2012). Lower levels of methylation of arsenic also predicted higher risk of metabolic syndrome in women in that study. Beebe-Dimmer et al. (2012) did not find an association between AS3MT SNPs and bladder cancer, but they found evidence of an interaction between arsenic in drinking water and the Met287Thr SNP with an odds ratio of 1.17 per 1-μg/L increase in water arsenic concentration restricted to people who had at least 1 copy of the Thr allele. The previously described study by Lesseur et al. (2012) did not find evidence that interactions between AS3MT SNPs and arsenic exposure affected bladder-cancer risk. Agusa et al. (2011) found relationships between urinary arsenic methylation and two noncoding SNPs. Engström et al. (2011) genotyped a suite of SNPs in the AS3MT gene in Bangladesh and Argentinian Andean populations that had high water arsenic exposures. They found a haplotype (series of SNPs on the same allele) that was associated with lower urinary percentage of MMA and one that was associated with higher urinary percentage of MMA in both populations. The haplotype with the higher percentage of MMA was the major allele in Bangladesh (52% allele frequency), whereas the haplotype associated with the lower percentage of MMA was the major allele in Argentina (70% allele frequency). In addition, four polymorphisms in DNA methylation enzymes DNMT1a and DNMT3b were associated with a higher urinary percentage MMA in the pregnant women in Bangladesh.

Genomic Screens

At least two larger-scale genomic screens have been conducted in populations characterized for arsenic exposure. These studies differ from the previously described linkage study in that they were not conducted in related people and used SNPs rather than short tandem-repeat markers to tag genomic regions of interest. Karagas et al. (2012) screened 10,000 nonsynonymous SNPs in a subset of subjects in a case–control study of bladder cancer conducted in New Hampshire (832 cases and 1,191 controls). The top hits were then validated in the larger case–control study. Variants in the FSIP1 gene (for a fibrous sheath interacting protein) and the SLC39A2 gene (for solute carrier protein 39A2, a metal transporter) modified the association between arsenic in drinking water and bladder cancer.

In a genomewide genotyping analysis conducted in 1,313 Bangladeshi subjects, a significant association between arsenic metabolism and toxicity phenotypes and five SNPs proximal to the AS3MT gene was found (Pierce et al. 2012). An expression array conducted in a subset of 950 subjects demonstrated increased AS3MT expression associated with the SNPs, and this suggests that these are *cis*-quantitative trait loci. Finally, one of the SNPs modified the association between urinary arsenic and premalignant skin disorders in a case–control study in Bangladesh (Pierce et al. 2012).

Key Considerations for the IRIS Assessment

A number of studies have demonstrated that genetic factors can modify the response to inorganic arsenic. Most have been candidate-gene studies that focused on arsenic-metabolism genes. A small number have also addressed whether these genes modify the relationship between inorganic arsenic exposure and health effects but only for cancer, metabolic syndrome, cardiovascular disease, and skin lesions. There has been one genomewide scan (of 300,000 SNPs) in a population characterized for inorganic arsenic exposure, and it validated previous research on AS3MT gene variants as potential risk factors (Pierce et al. 2012). A family-based linkage study provided moderate evidence that the same region modifies arsenic metabolism (Tellez-Plaza et al. 2013). Although results have not been validated in independent populations and deep sequencing of the region has not been performed, this information, when considered with the results of candidate-gene studies of AS3MT, suggests that this gene is a modifier of inorganic arsenic metabolism. Nonetheless, further work is needed to characterize genetic susceptibility to arsenic. If the methods for incorporating genetics into risk assessment are sufficient at this stage, AS3MT would be a logical choice for incorporation. Candidate-gene studies have identified genetic variants that modify arsenic toxicity in base-repair genes (Applebaum et al. 2007; Breton et al. 2007; Ebert et al. 2011). These studies were specific to cancer end points. Overall, genetic risk factors for inorganic arsenic toxicity are still not fully understood, and for most genes the genetic risk probably varies too greatly with inorganic arsenic dose, age at exposure, and ethnicity of the underlying population to allow reasonable estimates of increased or decreased risk.

SEX DIFFERENCES IN ARSENIC METABOLISM AND HEALTH EFFECTS

There is evidence of marked sex differences in the metabolism and toxicity of arsenic. It is essential to evaluate such differences in the IRIS assessment to protect the most susceptible people in the population. Evaluation of sex differences may also provide information on mechanisms and modes of action of arsenic.

It is well documented that the biotransformation of arsenic differs by sex. Other factors that may influence arsenic metabolism, to be considered in evaluating the sex-dependent metabolism, include magnitude of exposure, age, pregnancy, liver diseases, and smoking (Vahter 2002, 2009). In general, women excrete a greater percentage of DMA and a lower percentage of MMA in urine than do men after similar exposures (Hopenhayn-Rich et al. 1996; Hsueh et al. 2003;Lindberg et al. 2007, 2008a; Tellez-Plaza et al. 2013). That is probably related to women's having more efficient one-carbon metabolism than men, especially a potential of remethylating homocysteine via the choline–betaine pathway (Zeisel 2011). In addition, arsenic methylation efficiency increases during pregnancy (Concha et al. 1998a; Gardner et al. 2011).

Results of a number of studies have indicated that men are more affected by arsenic-related skin effects, including skin cancer, than women (Watanabe et al. 2001; Kadono et al. 2002; Chen et al. 2003; Rahman et al. 2006; Ahsan et al. 2007; Lindberg et al. 2008b; Leonardi et al. 2012). That does not seem to be the case for all arsenic-related cancers, however, and should be studied in more detail. As discussed in the committee's workshop by Cantor (2013), the indicated differences might be influenced by the background prevalence of the end point under study, and epidemiologic studies have rarely evaluated the differences in relative or absolute risk of arsenic-related health effects. Recent data indicate that girls may

be at higher risk for developmental effects after early-life arsenic exposure (Hamadani et al. 2011; Saha et al. 2012; Gardner et al. 2013) and boys after prenatal exposure (Kippler et al. 2012).

There may be several mechanisms of the observed sex difference in arsenic toxicity. It may be mediated at least partly by a difference in arsenic metabolism, inasmuch as the reduced form of the monomethylated metabolite, MMA(III), is highly toxic. Efficient methylation to DMA increases the excretion of arsenic from the body (Vahter 2002). Arsenic-related skin lesions, for example, are influenced by methylation efficiency in a sex-dependent manner (Lindberg et al. 2008b). There may also be sex differences in toxicodynamics. For example, when pregnant mice were exposed to arsenic in drinking water at 42 or 85 mg/L on gestation days 8–18, female offspring developed ovarian and lung tumors and uterine and oviduct hyperplasia whereas male offspring had a highly increased incidence of liver and adrenal tumors later in life (Waalkes et al. 2003). Liver tumors were induced in female offspring only when the in utero arsenic exposure was combined with skin application of a tumor-promoting phorbol ester, 12-*O*-tetradecanoylphorbol-13-acetate (Waalkes et al. 2004a; Liu et al. 2006; Tokar et al. 2010b). Gene-expression analysis of the liver of male mice with hepatocellular carcinoma induced by exposure to arsenic in utero showed overexpression of ER-α and cyclin D1 and a feminized expression pattern of several cytochrome P450 genes (Waalkes et al. 2004b). In addition, hepatic DNA from male offspring showed a significant reduction in methylation in GC-rich regions (Xie et al. 2007). Interaction of arsenic with ER-α and estrogen-associated functions has previously been reported (Lopez et al. 1990: Chattopadhyay et al. 1999; Stoica et al. 2000; Chen et al. 2002; Du et al. 2012; Treas et al. 2012). Mice that were exposed postnatally showed fewer sex differences in cancer development; arsenic increased lung adenocarcinoma and hepatocellular carcinoma in both sexes, but gallbladder tumors occurred only in males (Tokar et al. 2011b).

In studies of mice exposed to more environmentally relevant doses of arsenic (0.05, 0.5, 5.0, or 50 mg/L in drinking water for 4 months), monthly assessment of locomotor activity showed that female mice in all treatment groups exhibited hyperactivity at every test. In contrast, male mice exhibited hyperactivity in the group exposed at 0.5 mg/L and hypoactivity in the highest-dose group after 4 months of exposure (Bardullas et al. 2009).

Key Considerations for the IRIS Assessment

There is clear evidence of sex differences in the metabolism of inorganic arsenic. Because arsenic metabolism is a recognized susceptibility factor, it seems likely that the toxicity of arsenic could also differ between men and women. However, only a few arsenic-related health outcomes have been evaluated by sex. It is essential to evaluate sex differences in the IRIS assessment to provide protection for the most susceptible people in the population.

NUTRITIONAL DEFICIENCIES

As discussed in the committee's workshop by Beck (2013), there is considerable evidence from studies in animal models and in humans that nutritional factors may influence arsenic metabolism and toxicity. For example, studies of animals fed diets deficient in methionine, choline, or proteins have demonstrated decreased methylation and increased tissue retention of arsenic (Vahter and Marafante 1987). In West Bengal, India, poor nutritional status (low body weight) was associated with an increased risk of skin lesions (Mazumder et al. 1998). Similarly, in Bangladesh, lower body-mass index has been associated with an increased risk of arsenicosis skin lesions (Milton et al. 2004; Ahsan et al. 2006). In addition, using a validated dietary food-frequency survey in the large prospective Bangladesh Health Effects of Arsenic Longitudinal Study, Heck et al. (2009b) found that higher intakes of protein, methionine, and cysteine were associated with 10-15% greater urinary arsenic excretion after controlling for numerous covariates. Those are but a few examples of the extensive literature identifying poor nutrition as a susceptibility factor in arsenic toxicity.

Essentially, the most important dietary factors aside from protein and amino acid intake appear to fall into two categories—vitamins and nutrients that are involved in one-carbon metabolism and the synthesis of SAM, the universal methyl donor; and selenium, an element whose antagonism of arsenic toxicity has long been known (Levander 1977; Zheng et al. 2005).

Nutritional Effects on One-Carbon Metabolism

Nutritional effects on one-carbon metabolism on arsenic metabolism and toxicity were discussed in the committee's workshop by Gamble (2013). The synthesis of SAM, which is required for each of the two methylation steps of arsenic metabolism, is influenced by many nutrients that directly or indirectly contribute methyl groups to the one-carbon metabolic pathway (such as folate, choline, and betaine) or serve as cofactors for enzymes in the pathway (such as vitamins B_2, B_6, and B_{12}) (reviewed in Hall and Gamble 2012). In particular, deficiencies of folate and the B vitamins appear to exacerbate the associations between inorganic arsenic exposure and its health effects in humans. For example, in a population-based cross-sectional study in Bangladesh, the association between arsenic exposure and hypertension was stronger in participants who had lower than average dietary intakes of folate and other B vitamins (Y. Chen et al. 2007b). A nested case–control study of incident skin-lesion cases and matched controls (Pilsner et al. 2009) in Bangladesh and a case–control study in India (Mitra et al. 2004) reported that folate deficiency is a risk factor for arsenic-induced skin lesions. That folate deficiency might modify the risks of arsenic-induced health outcomes can be explained by the observation that people who are folate-deficient are poor methylators of arsenic and have more of the more toxic inorganic arsenic and MMA metabolites in blood and urine than those who are folate-replete (Gamble et al. 2005). Moreover, folic acid supplementation of folate-deficient study participants facilitated the synthesis and urinary elimination of DMA (and total arsenic) and lowered blood MMA and total arsenic concentrations (Gamble et al. 2007). Another study of the influence of nutritional status on arsenic metabolism, carried out in pregnant women in Bangladesh, found only a marginal effect of plasma folate (after adjustment for the degree of arsenic exposure), which influences the methylation markedly (Li et al. 2008). That effect was due at least partly to the strong effect of pregnancy on arsenic methylation, starting very early in pregnancy and possibly related to the betaine-mediated induction of one-carbon metabolism in early pregnancy (Gardner et al. 2011).

Influence of Selenium Status on Arsenic Toxicity

Selenium and arsenic antagonize each other's toxicity, probably because they facilitate the excretion of one another in bile (Levander 1977; Zeng et al. 2005). A selenium–arsenic–glutathione conjugate has been identified in rabbits and mice (Gailer et al. 2002; Burns et al. 2008) but has not been validated in humans. Epidemiologic studies have described inverse relationships between blood or plasma concentrations of selenium and the percentage of MMA in blood or urine or both (Basu et al. 2011; Pilsner et al. 2011). A case–control study in Bangladesh also reported inverse relationships between blood selenium concentrations and urinary arsenic and the risk of arsenic-induced skin lesions (Y. Chen et al. 2007a).

Key Considerations for the IRIS Assessment

It is clear that folate and selenium nutritional status have important effects on arsenic metabolism and toxicity. Therefore, the IRIS assessment should consider the nutritional status of study populations when examining dose–response relationships reported in the epidemiologic literature. Although a nutritional deficiency should not exclude an epidemiologic study from inclusion in a systematic review, nutritional factors could be qualitatively noted when the weight of evidence is considered. However, concerns about deficiencies affecting arsenic toxicity in the United States should be muted because of the nutrition-

al status of the population with regard to folate and selenium. The United States mandated the fortification of foods with folic acid in 1998, thereby decreasing the prevalence of folate deficiency (and rates of adverse birth outcomes associated with deficiency during pregnancy). However, some segments of the population rely on maize rather than wheat and thus do not benefit from supplemented foods. People exposed to arsenic in Bangladesh and much of South Asia have a high prevalence of folate deficiency, which exacerbates arsenic toxicity (Gamble et al. 2005, 2007; Hall and Gamble 2012). The selenium content of foods varies with the selenium content of soils in which they are grown. In the United States, soil selenium concentrations vary widely (USGS 2012). However, comprehensive reviews of blood and serum selenium concentrations in the United States (e.g., Combs 2001) have led to the conclusion that "the risk of selenium deficiency in the general US population is negligible" (Laclaustra et al. 2009).

PRE-EXISTING DISEASE, SMOKING, AND ALCOHOL CONSUMPTION

Pre-existing Disease

Risk assessment is increasingly concerned about the vulnerability that stems from interaction between a chemical's mode of action and disease processes that can occur in segments of the population. With respect to inorganic arsenic, the evidence summarized in Chapter 4 that arsenic increases the risk for several major diseases—including cardiovascular, respiratory, and renal disease and diabetes—suggests that this source of vulnerability (pre-existing disease) may be particularly pertinent, especially given the high rates of these diseases in the United States. Because this is an emerging field of vulnerability assessment, several underlying principles and implications for quantitative risk assessment are outlined below in relation to considerations for inorganic arsenic. Chemical interactions with background disease processes may be of several types as shown in Figure 3:

- The disease alters chemical action by altering toxicokinetics so as to change internal dose materially.
- The disease alters chemical action by affecting host defense mechanisms, as can occur when a pathologic condition is associated with chronic inflammation and oxidative stress.
- The chemical increases the likelihood of disease by damaging systems that are also affected by the disease process.

In the first two interactions, the presence of the disease process in an exposed person can be expected to shift the dose–response curve because the chemical is more effective in a diseased person. In the third, the disease is more likely to occur in an exposed person because of the chemical's contribution to the pathologic process.

Those interactions may occur in many exposed people, but at low dose they are most pertinent in those who are furthest along the disease spectrum and thus make up a group that is highly vulnerable both to the disease and to chemical toxicity. The interactions are most likely to be important on a population level if the vulnerable group constitutes a sizable fraction of the population. For example, if the disease is rare, few people are at heightened risk; this might be important for these few, but the population risk may not be greatly affected. In contrast, if the disease is common, many people will have it, and many others will have risk factors and subclinical biomarkers indicative of it that can in time lead to it. That implies a vulnerability distribution in which healthy members of the population are resistant to the chemical and those on the threshold of disease are most vulnerable to a chemical-induced shift to clinical disease. From a dose–response perspective, such a distribution may cause effects to be seen at exposures well below the threshold in a typical person because sensitive members of the population will respond at lower doses and

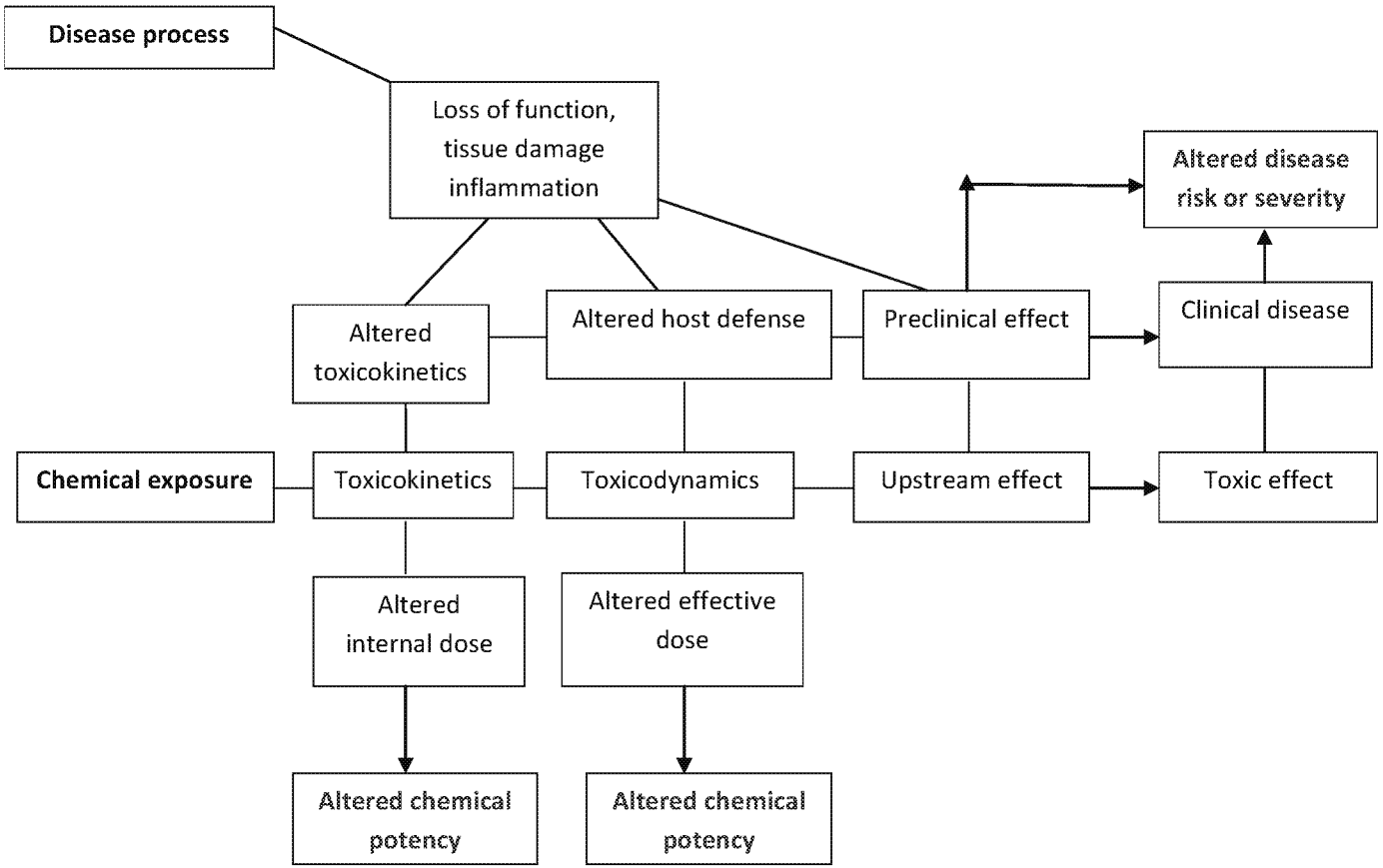

FIGURE 3 Potential interaction between chemical exposure and disease process. Interaction could lead to altered chemical potency as disease process affects host toxicokinetics or defense mechanisms (downward arrows) creating a vulnerability to chemical effect. Interaction could also affect disease risk especially if the chemical and disease have similar upstream pathways and clinical outcomes (upward arrows). In this case, chemical exposure creates an additional risk factor for disease to occur.

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highly sensitive members at even lower doses. Such variability in threshold related to disease or other host factors may make it difficult to identify a population threshold at levels of exposure commonly encountered by the population even if a threshold exists at the individual level (Schwartz et al. 2002; NRC 2009). This is a likely explanation for the linear-appearing relationship between fine particulate matter (PM) and cardiovascular mortality (White et al. 2009). Inhalation of fine PM is a source of inflammation and oxidative stress that is associated with a variety of cell-signaling and physiologic changes that are common to the pathogenesis of cardiovascular disease. Alterations in heart-rate variability and telomere shortening are two such mechanisms whereby some members of the population (such as the elderly and those with impaired cardiopulmonary function) have values of these risk biomarkers in the direction of clinical disease and thus have a lowered threshold for PM-induced clinical effects relative to healthy people (Pope et al. 2004; Grahame and Schlesinger 2012; Langrish et al. 2012). Although fine-PM–induced cardiovascular effects may be widespread in the population at high dose, they might be experienced only by those who have preclinical risk biomarkers at low dose; the presence of these vulnerable people tends to push the observable dose–effect relation to lower doses (NAS 2009). If the dose–response relationship appears linear on that basis, it may have a different slope at low doses, at which the chemical is primarily adding to underlying disease, and at high doses, at which the chemical can exert its adverse effects even in healthy people.

Thus, the factors most important in evaluating the potential for arsenic to interact with background disease processes on a populationwide basis are these:

- Overlap between toxic mode of action and disease mechanisms in terms of similar target organs, similar upstream signaling pathways, common biomarkers (a biomarker of chemical effect is also a preclinical biomarker of disease), and common defense mechanisms involved in fending off the effect.
- Prevalence of the overlapping disease.
- Prevalence of preclinical disease.

Regarding disease prevalence, it is important to take note of whether particular segments of the population that may be exposed to arsenic are also more susceptible to an interacting disease; for example, black Americans have higher risk of hypertension and heart disease (Kurian and Cardarelli 2007).

Assessment of the potential for background disease interaction starts with evaluation of arsenic itself, its toxic outcomes, upstream indicators of effect, other relevant mode-of-action information, and governing toxicokinetic factors. The first avenue to explore is whether arsenic's toxic outcomes are peculiar to arsenic or are similar to acute or chronic illness experienced by the population. For example, arsenic exposure is associated with skin lesions that are peculiar to arsenic overdose and are uncommon in the general population. Although it is possible that arsenic interacts with other skin conditions, it has not been documented except in the case of psoriasis, in which arsenic has a therapeutic effect (Farber 1992).

In contrast, epidemiologic studies show that exposure to arsenic increases the risk of cardiovascular disease, diabetes, renal disease, respiratory disease, neurodevelopmental impairment, and cancer (Naujokas et al. 2013). That is evidence that arsenic can contribute to some diseases that are prevalent in the US population. In some cases, the data indicate that arsenic affects subclinical markers of those diseases, and this provide mechanistic support for the epidemiologic associations. For example, a study in two Romanian towns found that people exposed to arsenic at 40 μg/L (on the average) in drinking water were more likely to have abnormal blood-pressure responses to psychologic or cold-induced stress (Kunrath et al. 2013). Those end points are predictive of hypertension (Wood et al. 1984). Arsenic exposure is associated with increases in plasma adhesion molecules that are indicative of endothelial dysfunction, vascular inflammation, and increased risk of cardiovascular disease (Y. Chen et al. 2007c). Thus, arsenic appears to influence cardiovascular-disease risk by at least two pathways at relevant doses. Comparable evidence on other disease outcomes (such as renal dysfunction, diabetes, and cancer) would be helpful in establishing the nature and extent of the interaction between arsenic and the disease process. Given that those diseases are prevalent in the US population, consideration should be given to whether many people may be vulnerable to the ef-

fects of arsenic exposure because the disease processes impair defense mechanisms or otherwise act in concert with arsenic's mode of action.

The potential for arsenic to contribute to ongoing disease processes caused by other factors means that it has the potential to shift the distribution of a clinical biomarker toward a disease cutpoint so that a greater percentage of the population will be over the cutpoint and classified as having the disease. That effect is illustrated in Figure 4, in which the dose–response relationship for cadmium-induced decrease in renal function (glomerular filtration rate, GFR) is shown as a leftward shift in the baseline GFR distribution and more people are classified as having chronic renal disease (GFR <60 mL/min per 1.73 m^2) (Ginsberg 2012). Cadmium risk can then be expressed as the increase in population risk of chronic renal disease rather than merely as a quantitative change in GFR or protein leakage. That is similar to how small decreases in IQ caused by lead exposure can appear minor on an individual level but be best understood on a population level by analyzing the effect on the IQ distribution of the population (Schwartz 1994; Grosse et al. 2002; Jakubowski 2011). It is important to consider that inferences about how changing the mean of the distribution affects the number of people in the tails are subject to a number of conditions related to population variability and heterogeneity (Bellinger 2007).

Given the evidence that arsenic affects the rate of various diseases, as well as upstream preclinical biomarkers of the diseases, it may be possible for a noncancer assessment to describe the increased disease risk associated with any particular arsenic dose. If a reference dose (RfD) is derived, it can be described as the dose associated with a de minimis increase in disease risk, whose statistical bounds are based on the degree of uncertainty and variability in the analysis. The determination of what is de minimis risk can take into consideration the severity of the end point and the size of the uncertainty distribution associated with the risk at the RfD (NRC 2009). That information will help risk managers to understand the implications of arsenic exposure and the meaning of the RfD if one is derived.

The practical implications of arsenic's interaction with background disease are as follows:

- Those who have pre-existing disease (such as cardiovascular disease, diabetes, or renal dysfunction) may be more vulnerable to arsenic toxicity. Evaluating the evidence of susceptibility on the basis of pre-existing disease requires sufficient disease and nondisease subjects who have well-defined arsenic exposures and assessment of arsenic-related end points. Most epidemiologic studies, however, have not evaluated differences in arsenic susceptibility, so it is difficult to evaluate the importance of the interaction between arsenic and background disease.

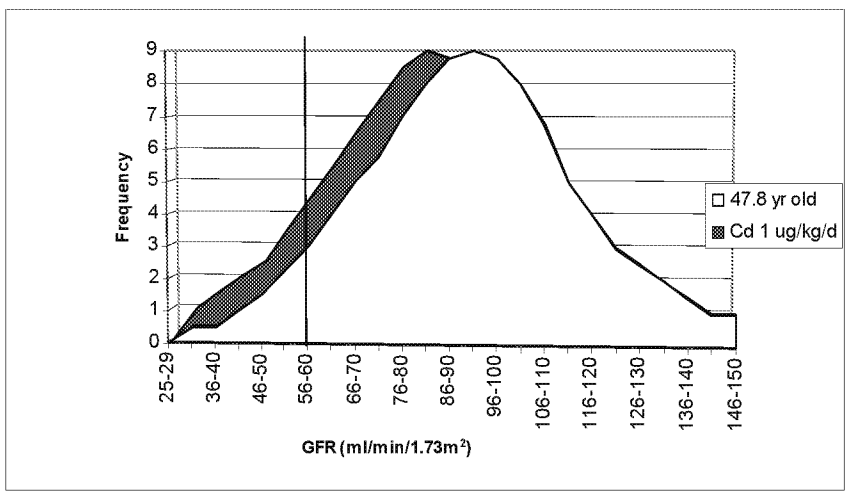

FIGURE 4 Cadmium-induced shift in GFR distribution at a chronic exposure of 1 μg/kg per day relative to a 47.8-year-old female baseline. Source: Ginsberg 2012. Reprinted with permission; 2012, *Journal of Toxicology and Environmental Health, Part A: Current Issues.*

- Those who are at high risk for a disease (they are genetically predisposed or preclinical markers are present) may be more likely to get the disease if exposed to arsenic. Evidence of that would ideally involve prospective studies that include assessment of a preclinical biomarker, arsenic exposure, and disease incidence over time. Without such evidence, it may still be possible to estimate the increased risk of disease (such as chronic renal disease or myocardial infarction) by evaluating how arsenic shifts the population distribution of preclinical biomarkers (such as GFR or heart-rate variability).
- It may be impossible to find a threshold of an arsenic-induced increase in disease in the population because a large number of people are in a preclinical state and would be sensitive to the low end of the dose–response curve. For that to be evident, the epidemiology data would need to characterize the relationship between arsenic exposure and risk of disease in a broad cross-section of the population (or look at precursor lesions or key events) and allow a robust examination of a low–dose slope.
- Whether the potential for arsenic interaction with disease processes differs between the populations from which the dose–response data are derived and the US population as a whole needs to be considered.

On the basis of the degree of evidence on a vulnerable subpopulation, consideration should be given to whether dose–response assessment will focus on the population as a whole or will involve separate assessments for the general population and susceptible subgroups. If it is the population as a whole, the traditional approach is to address variability with uncertainty factors; it may also be possible to analyze the effect of variability on risk by evaluating how the risk distribution of the disease shifts in response to arsenic. In essence, the risk distribution based on a subclinical biomarker is an expression of toxicodynamic variability that can be captured in dose–response assessment (NRC 2009; Ginsberg 2012).

The alternative approach is to address vulnerable subpopulations as separate from the general population and assign them unique potencies via dose–response modeling specific to the groups that might be based on actual–dose response data for the groups, on adjustments for specific toxicokinetic or toxicodynamic factors, or on more generic adjustment or uncertainty factors. An example of a generic approach is the application of age-dependent adjustment factors for heightened cancer potency in young children compared with adults in the case of mutagenic carcinogens (EPA 2005a). For arsenic, if it is known that a particular age group, disease (or disease-related end point), genetic variant, or coexposure creates unique vulnerability, efforts should be made to estimate the potency differences relative to the general population and on that basis to consider developing separate potency values or basing a single IRIS value on the most sensitive group or on the overall population with adjustments for vulnerable groups.

Potential Interaction with Smoking

Several epidemiologic studies have assessed the interaction between arsenic ingestion via drinking water and tobacco-smoking in Bangladesh (Chen et al. 2006; Melkonian et al. 2011), in Taiwan (Chen et al. 2004), or in occupational settings (Hertz-Piccioto et al. 1992). The studies have reported a synergistic interaction in which the occurrence of skin lesions (Chen et al. 2006; Melkonian et al. 2011) and the occurrence of lung cancer (Hertz-Piccioto et al. 1992) have been found to be greater than additive. Chen et al. (2004) found a dose–response trend for arsenic and lung-cancer risk that was more prominent among smokers. There is also evidence of an interaction between arsenic and smoking for cardiovascular disease (Y. Chen et al. 2011a; Moon et al. 2013) and bladder cancer (Karagas et al. 2004). The most detailed interaction study was done prospectively in nearly 4,000 Bangladeshi men with a 6-year followup. The synergistic effect between tobacco-smoking and arsenic exposure on skin lesions was most evident in the higher quintiles of arsenic exposure (Melkonian et al. 2011). For lung cancer, a reanalysis of six studies that reported on occupational arsenic exposure, smoking status, and lung cancer found a synergistic interaction that was also most evident in the higher arsenic exposure categories (Hertz-Piccioto et al. 1992). As summarized in Chapter 4, several lines of evidence suggest an interaction between smoking and bladder-cancer risk in which the arsenic-related bladder-cancer increase is more evident in smokers. No data

are available for determining whether exposure to environmental tobacco smoke interacts with arsenic with respect to skin, lung, or other end points. There are numerous potential mechanisms for the synergism, including inhibition of DNA repair by arsenic, which increases smoking-induced genetic damage. Most of the abovementioned studies of interactions between arsenic and smoking in Bangladesh and in occupationally exposed people involved men. There appears to be much less information on women. It was reported, however, that tobacco-chewing, which is prevalent in women in Bangladesh, was associated with a considerably higher risk of skin lesions in women than that in women who did not use tobacco (Lindberg et al. 2010). It was particularly obvious in women who had the lowest efficiency of arsenic methylation.

Because smoking is still relatively common, the finding of synergism with arsenic for at least four disease outcomes will be an important consideration. Indeed, of all susceptibility factors evaluated, smoking is probably the one on which the findings at both high and low to moderate arsenic exposure were most consistent. However, several uncertainties and data gaps with respect to this interaction could be acknowledged in attempting a quantitative assessment. The data gaps include lack of smoking–arsenic interaction studies for other end points, limitations of the information on the effects of secondhand tobacco smoke, the incomplete description of interaction dose–response relationships, and the lack of mechanistic understanding. Regarding interaction dose–response relationships, the interaction between two agents will depend on the relative size of the doses: the nature of the interaction may change as the ratio of exposures varies (e.g., see Chou and Talalay 1984). For arsenic, the interaction matrix for skin lesions suggests that the best chance to see synergism is at higher doses of arsenic (Melkonian et al. 2011), and this might suggest that the interaction is less likely to affect the dose–response relationship at low arsenic doses. However, that is uncertain given the limitatins of the statistical power in the epidemiologic data to find low-dose arsenic effects and interactions. For cardiovascular disease, for instance, there is evidence of effect modification at low to moderate concentrations of arsenic (Y. Chen et al. 2011a; Moon et al. 2013). Furthermore, the literature has generally evaluated smoking status (current, former, or never) without defining the amount of smoking needed to begin to see an arsenic interaction.

A plausible quantitative approach is a sensitivity analysis in which the smoking-interaction synergism size effect reported for skin lesions, lung cancer, bladder cancer, and cardiovascular disease is applied to the dose–response relationship for these end points to determine the degree to which it would change the potency calculation. If that degree is found to be influential, consideration can be given to providing a separate potency estimate for smokers. A separate estimate could be useful in standard-setting and particularly in risk–benefit analyses when the burden of disease related to arsenic exposure is assessed in the general population in light of the proportion of people who smoke. Public education regarding radon includes the cancer risk in nonsmokers vs smokers because of the synergistic interaction between these agents. If the data are sufficient, perhaps a similar approach could be used for public education concerning arsenic.

Potential Interaction with Alcohol Consumption

Although it is likely that many people have dual exposure to arsenic and ethanol, the potential for an interaction has not been extensively studied (Bao and Shi 2010). Results of a study of rats indicate that ethanol increases arsenic retention in the liver and kidneys and increases arsenic-induced hepatotoxicity (Flora et al. 1997). The epidemiologic data indicate higher concentrations of arsenic in urine in conjunction with alcohol ingestion, and this suggests a toxicokinetic interaction, higher arsenic concentrations in alcoholic drinks, or increased water intake after alcohol intake (Tseng et al. 2005). Ethanol may augment arsenic-induced oxidative stress and induction of angiogenic factors that would promote tumor growth (Klei and Barchowsky 2008; L. Wang et al. 2012). There are additional mechanistic avenues through which an interaction could occur and potentially affect the outcome of epidemiologic studies (Bao and Shi 2010). That may prove to be a subject of productive research but has not been sufficiently developed to understand the nature and dose–response relationship of coexposure interaction on key end points.

Key Considerations for the IRIS Assessment

Interaction with some disease processes or chemical coexposures, particularly smoking, may increase vulnerability to the effects of arsenic. Evidence that arsenic alters disease incidence or interacts with upstream disease biomarkers shows which end points are most likely to involve disease-related vulnerability. Evaluation of the size and nature of vulnerable population groups will help to determine whether available epidemiologic studies adequately capture these groups. That consideration will also show how the response at doses below the range of observation might be affected with respect to the feasibility of defining a population threshold or dose-dependent transition. Quantitative approaches may involve separate analysis of vulnerable groups if such group-specific data are available or adjustment of the overall population response to account for specific chemical interactions or vulnerability factors.

MIXTURES AND COEXPOSURES

Coexposures or mixtures that include inorganic arsenic complicate the risk evaluation of arsenic in at least two ways: arsenic may interact with other agents that potentially modify the effect of arsenic, and there may be a combination effect of arsenic with other similarly acting chemicals (such as other metals).

Arsenic may interact with other agents because it perturbs pathways and end points shared by the agents. The effect can be a "zero interaction" called additivity (for example, anticholinesterase pesticides have a cumulative effect on key enzymes [Timchalk et al. 2005]), synergism (greater than additive, such as arsenic or radon plus smoking), or antagonism (less than additive, such as the antagonistic relationship between arsenic and selenium). Those interactions are typically dose-dependent for each agent, so assessing how one chemical alters the response to another can be complicated. Thus, the nature of the interaction may depend on the dose ratio and require testing multiple dose combinations of the interacting chemicals to explore fully the types of interaction possible (Jonker et al. 2005); or interactions that may occur at high doses of one or both chemicals may not occur at lower doses (an interaction threshold; Yang and Dennison 2007). The main concern at low doses is that an exposure to a chemical that is well tolerated in the population (for example, below its threshold dose in most or all people) can become an important risk contributor through additive or synergistic interaction. Zero interaction, or additivity, is typically seen as shifting the dose–response curve to the left (more potent) according to simple dose additivity, whereas a synergistic interaction may have a different dose–response curve. Dose additive or synergistic interactions are likely to be highly variable in the population because exposure to the agents is not uniform and leads to a range of responses and vulnerabilities. That added variability may be important to capture in a risk assessment for chemicals with which prominent interactions are likely to occur at doses experienced in the population or with which coexposure to multiple similarly acting chemicals is likely. Additional uncertainty factors are possible, but background interaction with similarly acting chemicals can be a reason that thresholds can be difficult to define at the population level and can lead to the potential for linear-appearing low-dose slopes and related modeling (NRC 2009).

Inorganic arsenic has varied modes of action and numerous end points, so the potential for chemical–chemical interaction or combination effects is large (Hong et al. 2004; Hore et al. 2007; Islam et al. 2011; Naujokas et al. 2013). Two types of interaction that have been identified beyond those previously discussed with selenium and tobacco smoke are interactions with other metals and with polycyclic aromatic hydrocarbons.

Coexposure and Potential Interaction with Other Metals

Like arsenic, a number of metals are pro-oxidants and share underlying effects on proteins, signaling pathways, and genetic instability; thus, numerous arsenic–metal interactions are plausible (Hartwig 2013).

Candidates for such interaction with arsenic include lead, mercury, cadmium, chromium, nickel, and cobalt (Yao 2008; Valko et al. 2005). For example, cadmium and arsenic appear to have cumulative effects on renal-tubule leakage, as evidenced in humans exposed at moderate environmental doses (Huang et al. 2009). Those effects appear to be mediated by oxidative stress inasmuch as oxidative biomarkers increased in a pattern that appears additive among these agents. Interactions involving arsenic and other metals have not been well researched.

Coexposure and Potential Interaction with Polycyclic Aromatic Hydrocarbons

Another possible interaction is between inorganic arsenic and carcinogenic polycyclic aromatic hydrocarbons (PAHs). PAH-related DNA adducts and mutagenesis are increased by coexposure to arsenic, and this interaction appears synergistic in at least some experimental systems (Maier et al. 2002; Fischer et al. 2005). The most plausible mechanism is that arsenic-induced inhibition of DNA repair allows the PAH adducts to be longer-lived and thus to build up in host DNA. Several studies that have tested that hypothesis, however, have failed to find impaired removal of PAH–DNA adducts in the presence of arsenic (Maier et al. 2002; Chiang and Tsou 2009). Other mechanisms are possible, including arsenic-induced impairment of the p53 response to DNA damage (Chen et al. 2005; Shen et al. 2008). The fact that arsenic also potentiates ultraviolet-induced genetic damage (Wiencke et al. 1997; Chen et al. 2005) demonstrates the potential for interactive effects beyond PAHs. Although those interactions remain to be explained, they raise the possibility that at least some of the arsenic-induced tumors seen in human studies are comutagenic effects. That theoretically points to an alternative cancer-potency estimate based on the dose–response relationship in which arsenic potentiates the toxicity–genotoxicity–carcinogenicity of other agents. However, that may be somewhat speculative given limitations in quantitative dose–response data and in the mechanistic information needed to explore such possible potentiation fully. Therefore, this is a subject worthy of mention by EPA as an additional mechanistic consideration that may help to explain some of the end points associated with arsenic exposure.

For interactions in which there is arsenic coexposure with other carcinogens, the standard risk-assessment assumption of dose additivity is reasonable.

Key Considerations for the IRIS Assessment

In its evaluation of inorganic arsenic, EPA should consider coexposure to other metals and PAHs because these are the most documented. In accumulating the evidence on mixtures that include arsenic, it would be helpful to take note of which chemicals arsenic tends to occur with in the environment. Coexposure to some metals (such as lead and cadmium) is particularly likely because of their similar sources, persistence, and retention in topsoil (ATSDR 2007; Wang and Fowler 2008). Arsenic in drinking water sometimes occurs with manganese, uranium, radon, and other elements (Ayotte et al. 2011). (Arsenic, cadmium, chromium, lead, and mercury are among the most common metals on the basis of site frequency as tabulated by the Agency for Toxic Substances and Disease Registry (ATSDR 2009). Arsenic also occurs with other environmental chemicals, including PAHs (Mori et al. 2011).

Three cases of possible interactions could guide EPA's assessment of coexposures in the context of their affecting the interpretation of dose–response relationships in key epidemiologic studies.

Case 1. The key epidemiologic study does not have subjects who are more vulnerable as a result of coexposure to interacting metals or PAHs. For example, some Superfund sites might have hot spots of metal contamination involving arsenic and other metals. That could represent a special exposure scenario that is not captured by statistical sampling of a general population. By not counting or identifying such vulnerable people, an epidemiologic study might not capture the risk in this subgroup, and that could lead

to potential underestimation of risks to those who are subject to the coexposure. The concern is partially mitigated by recommendations made later in this report (see Chapter 7) to calculate potency values for multiple end points, not just the most sensitive one. The risk of each end point associated with arsenic could then be considered cumulatively with similarly acting metals that co-occur in that exposure groups.

Case 2. The key epidemiologic study has arsenic as a covariate (it is positively correlated) with other important metals and PAHs, but they are not measured. In such a study, the health effects might be completely (and incorrectly) attributed to arsenic without considering the contribution of other chemicals. That could lead to overestimation of the effects of arsenic.

Case 3. The key epidemiologic study has interacting metals or PAHs distributed by chance across the arsenic exposure groups; this would increase the unexplained variability in each group. The additional variability would tend to obscure or weaken the effect of arsenic.

In considering those scenarios, it would be helpful for EPA to assess, to the extent possible, how interacting metals or PAHs might co-occur in the epidemiologic study populations in comparison with the target population of the risk assessment. That could lead to a better understanding of potential underestimation or overestimation of risk and a description of how coexposures might contribute to overall potency on the one hand and uncertainty on the other and of whether cumulative risk posed by metals, PAHs, or other chemicals is important to consider.

SUMMARY

As described above, populations may be susceptible to the effects of arsenic because of their sex, life stage, nutritional status, metabolism-related polymorphisms, disease status, smoking status, and exposure to other chemicals that could interact with arsenic to produce a cumulative or synergistic effect. Consideration of susceptible subpopulations and life stages in deriving risk-based values is a complex task. Developing a potency adjustment for such populations is feasible if the appropriate dose–response data are available for a given subgroup in comparison with the general population. With respect to inorganic arsenic, it is plausible that those in pre-existing or high-risk categories for chronic diseases (such as cardiovascular disease, diabetes, and renal disease) could be more vulnerable to arsenic toxicity inasmuch as arsenic has been associated with increased risks of those conditions in at least some cases or has been shown to act on upstream biomarkers of disease risk (e.g., Wu et al. 2012; Kunrath et al. 2013). Thus, the available literature should be examined to assess whether such information is available in existing epidemiologic studies or systematic reviews. Windows of susceptibility appear to occur in connection with in utero or early-life exposure with respect to ensuing cancer, cardiovascular, and respiratory risk. Nutritional factors (such as low folate intake), genetic deficiency in arsenic methylation, and tobacco smoke might also predispose some people to arsenic toxicity.

The committee supports EPA's plans to use systematic reviews to evaluate susceptibility factors. For many of the factors considered in this chapter, it is unlikely that adequate data will be available to incorporate the considerations quantitatively in estimating risks. The factors that appear to have the strongest evidence are in utero and early-life exposures, sex differences, and smoking. If adequate data are found, consideration could be given to determining a separate potency calculation for a vulnerable subgroup that would be carried through the IRIS process to the extent possible and to determining whether the vulnerability is appropriately represented in the study population relative to the target (US) population. In the absence of adequate data, the size of the relevant subgroup (if large, likely to contribute to population risk) and whether the mechanism for vulnerability (if known) is likely to be operable at low dose could be evaluated. At high dose, both vulnerable and less vulnerable people can be affected; but at low dose, only the most vulnerable are likely to be affected. If that is a small percentage of the population, there might not be enough people to permit recording a statistically significant effect at low dose. The larger the fraction of the population that has a particular vulnerability, the greater the chance that the population will continue to have a significant response at low dose and thereby extend the dose range of ob-

served effects. That is the apparent explanation of the linear-appearing population response to particulate matter and cardiovascular mortality in spite of the likelihood that a threshold for the effect occurs at the individual level (NRC 2009). As the dose is lowered, fewer people are responsive, so statistical significance is harder to achieve, but this does not mean that the risk at low dose is zero. In cases in which a sizable subpopulation is vulnerable to a particular effect, it is reasonable to extend the dose–response relationship below the range of observation by a modest extrapolation.

6

Mode of Action

The Environmental Protection Agency (EPA) should evaluate the biochemical and systems-biology information available on arsenic to determine how it can help to explain the pattern of adverse health outcomes and the mode of action that best matches the underlying biology. Similarly, variability and its effect on the low end of the dose–response curve and the distinction between adaptive changes and adverse responses can be addressed in the context of mode of action.

Mode-of-action analysis provides a framework for data integration around a common theme for an agent and a specific health outcome (Andersen et al. 2000; Carmichael et al. 2011). The strategic vision for mode-of-action analysis in risk assessment has been laid out in EPA's cancer-risk guidelines (EPA 2005b) and has been successfully applied to the assessment of chloroform (EPA 2001) and trichloroethylene (EPA 2011). EPA's draft plans indicate that this mode-of-action framework will be included in its analysis of adverse-outcome pathways. Mode-of-action data can be extended to a number of uses beyond the cancer-risk guidelines, including noncancer health outcomes and sensitive population assessments. Mode-of-action analysis is a systematic approach to understand the relationship between exposure to an agent and biologic outcomes and can affect interpretation of events at the low end of the dose–response curve. It uses the identified key instigating events to drive dose–response analyses. An acknowledged challenge for EPA in using this approach is that many of the existing mode-of-action data have been developed at relatively high exposure to arsenic and the objective is to understand attributable risk at lower exposures. In addition, strong data on actual systemic exposure to the various forms of arsenic after ingestion are often difficult to obtain. Identifying those data gaps and their potential effect on the ability to extrapolate to potential effects at low exposures wil be an important part of the IRIS assessment process.

The mode-of-action framework (Boobis et al. 2006, 2008; Carmichael et al. 2011) in conjunction with the human-relevance framework (Meek et al. 2003) provides a transparent method of organizing information for hazard identification and risk assessment that includes exposure information, dose–response information, a clear conclusion, identified data gaps, and potentially susceptible populations. It permits the integration of cancer and noncancer risk assessment (Carmichael et al. 2011) and provides a transparent mechanism to share results with stakeholders. A similar approach that uses a graphical presentation of how epidemiologic and toxicologic data intersect is described by Adami et al. (2011). Similar discussions of new approaches to hazard identification and risk assessment are provided in *Science and Decisions: Advancing Risk Assessment* (NRC 2009) and *Toxicity Testing in the 21st Century: A Vision and a Strategy* (NRC 2007) and are meant to extend the concepts provided in the "Red Book" (NRC 1983).

IMPORTANT ASPECTS OF MODE-OF-ACTION ANALYSIS

The key aspect of mode-of-action analysis is that it provides an evidence-based assessment, integration, and synthesis of all the available data on a chemical, its adverse health effects, and its biology. The data integration and synthesis of a mode-of-action analysis incorporate various types of data, including epidemiologic data, standard toxicity-testing data, data from mechanistic biochemical and metabolism studies, data from gene-expression studies, and data on epigenetic changes. As discussed in the committee's workshop by Clewell (2013), those data can come from in vivo or in vitro studies involving a variety

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of species, systems, and cell types whose relevance to humans could be assessed. A chemical can modulate numerous upstream events, and determining whether these converge to form one or more modes of action for a specific adverse outcome can be challenging. Therefore, mode-of-action analysis endeavors to provide a plausible hypothesis for an adverse outcome (Andersen et al. 2000; Meek et al. 2003); there can be uncertainties in the sequence of events, in the causal nature of the events, and in whether more than one pathway is involved.

Mode-of-action analysis is distinct from a detailed understanding of mechanism of action and places into perspective all relevant scientific data that link key events and pathways to an adverse health outcome that is consistent with the underlying biology of the target tissue. It is that aspect of mode-of-action analysis that places available data into a context that can be used to interpret vulnerability among species and among individuals so that changes in dose–response relationships in going from high to low exposures can be understood. Thus, mode-of-action analysis is an essential step in Integrated Risk Information System (IRIS) assessment, and the committee applauds EPA's efforts to organize the in vitro and in vivo mechanistic data and its plan to use this analysis in interpreting dose–response relationships and intersubject variability in the inorganic arsenic assessment.

Mode-of-action analysis should be applied to organize and synthesize data and to harmonize cancer and noncancer end points, especially in the case of arsenic, on which there are large data gaps in regions of the dose–response relationship in key areas and low-dose exposures of regulatory concern. Although association studies alone are not sufficient for quantitative risk assessment, they provide a qualitative guide for hazard identification. One should rigorously examine each (epidemiology) study in the context of the modified Bradford Hill criteria to determine appropriateness for risk assessment (Boobis et al. 2006, 2008). Coexposures are particularly important to take into account because differences in cancer tumor spectrum or other health outcomes (such as diabetes, cardiovascular disease, immune effects, and developmental effects) between populations may be due to the presence of different contaminants (such as lead, mercury, cadmium, and silica) or different dietary inadequacies (such as zinc, selenium, folate, and protein). Similarly, toxicology studies are often performed to detect hazards and may not be designed for description of mode of action. Mechanistic studies performed in vitro and in vivo can inform understanding of the mode of action. An emphasis on hazard characterization is warranted and can be achieved by using a mode-of-action approach in which the necessary key events for the pathogenesis of each health outcome are examined in a dose-dependent and time-dependent manner.

Not all proposed modes of action will be supported by adequate data, and there will not always be complete data on the health outcomes under consideration, but the mode-of-action framework should be worked through for the proposed modes of action for each adverse health outcome of concern. It is understood that for many of the health outcomes, a detailed mode-of-action analysis will not be possible, because of lack of information. Providing the available data and identifying data gaps and the effect of the lack of information on confidence in the assessment of risk, particularly at low exposures, will be important parts of the evaluation. When approached in this manner, a proposed mode of action provides a unifying hypothesis for interpreting and integrating multiple studies and for explaining differences between the sexes, among populations, among different ages, and among species. Several test cases for cancer mode of action have been provided, and this framework has been extended to noncancer end points (Sonich Mullin et al. 2001; Meek et al. 2003; Boobis et al. 2006, 2008; Rhomberg et al. 2011). The mode-of-action framework provides a transparent method for examining the weight of scientific evidence separate from policy considerations but with an eye to informing science policy (Carmichael et al. 2011).

In beginning a mode-of-action analysis, the committee recommends that the IRIS program use an approach similar to that followed in the IRIS assessments of chloroform (EPA 2001) and trichloroethylene (EPA 2011). The process needs to begin with the question to be answered about the health effects of exposue to arsenic. Identifying the adverse outcomes of concern and determining the strengths and weaknesses of the available data are important steps in this process. Toxicokinetic data are essential for understanding how exposure corresponds to internal dose and how they vary among individuals. Exposure assessment will influence the dose-dependent and time-dependent response to an agent and is important for dose–response analysis and for risk assessment. Understanding exposure data related to each

health outcome throughout the dose range is a particularly important aspect of the process and is an acknowledged difficulty that IRIS assessors will face in light of the existing arsenic database.

EPA's draft plan for the inorganic arsenic assessment describes proposed evidence tables that will be organized by health category and will capture exposure and outcome information in human studies. A modified form is needed for animal or in vitro studies to record such information as the form of arsenic used, the species and strain, and the tissue or cell line. Understanding how the assessment was performed will be greatly facilitated by the use of additional tables that list modes of action for each health outcome (both cancer and noncancer) and include exposure, tissue dose (if possible), and key events. The analysis should further demonstrate how the documented exposure and time course of exposure lead to the key events. The tables should cite literature that was used to construct the analysis and discuss supporting and conflicting evidence. Linkage of adverse health outcomes to the key events in a mode-of-action analysis—when coupled with exposure, metabolism, tissue accumulation of parent and metabolites, and rate-limiting steps (such as protein binding, reactive oxygen signaling, cytotoxicity, proliferation, apoptosis, receptor effects, and immune suppression)—provides a strong hazard identification and characterization analysis. This evidence map of the exposure–response relationship, exposure frequency, and duration—when coupled with the time dependence of the sequential progression and time course of the observed health effects—should match the proposed key events predicted by the mode of action. Such a mode-of-action assessment can be particularly helpful in the analysis of potential confounding effects, of the role of simultaneous exposure to other agents or actions, and of individual risks and population susceptibilities.

Mode-of-action analysis is a transparent method for examining data and interpreting data gaps and for providing an integrated assessment of hazard identification and risk characterization. The linkage of in vitro to in vivo animal to human dose and dose duration response to temporal onset of key events is necessary (Andersen et al. 2000; Tsuda et al. 2003; Slikker et al. 2004a,b). The data can then be compared to see whether they are qualitatively and quantitatively similar. That comparison could be facilitated by creating concordance tables in the range of observations for in vitro, in vivo animal, and human data. The tables should take into account the number of cases and the incidence, the actual exposure (on the basis of a common unit, such as micrograms per liter of plasma, micrograms per gram of tissue, current exposure, total cumulative exposure, or tissue concentrations), species or population (and subpopulation) differences, coexposures, and the timing and route of exposure in the analysis. It would be helpful to document intervention studies that perturb the adverse-outcome pathway either upstream or downstream of the proposed effect and thus provide evidence of the relative importance to the outcome in question. In addition, it would be useful to document how the observed health outcomes and mode of action attributed to arsenic could be modulated by other potentially causal agents, such as micronutrient deficiency, other metal excess, host vulnerability factors, life stages, pre-existing disease, diet, sex, genetic background, and smoking. Box 6 lists the major steps of mode-of-action analysis.

BOX 6 Steps of Mode-of-Action Analysis

1. Provide problem formulation statement
2. Tabulate adverse outcomes with supporting and conflicting data
3. Provide pharamacokinetic data throughout the exposure and temporal range for each adverse health outcome and its precursors
4. List modes of action for each adverse outcome, linking pharmacokinetic and pharmacodynamic information to health outcomes in an exposure and temporal manner
5. Construct a concordance table to provide strengths and weaknesses of each proposed mode of action for each species, population, and subpopulation

POTENTIAL MODES OF ACTION OF ARSENIC

An important aspect of the IRIS assessment of inorganic arsenic will be provision of a proposed mode of action for each observed health outcome, including the associated supporting and contradictory evidence. If after execution of a mode-of-action framework analysis a cohesive mode of action is not apparent or it is clear that multiple modes of action may be involved, a mode-of-action summary statement should indicate that while elaborating the data and hypotheses assessed. Determination of the key events in an adverse-outcome pathway is in the context of the exposure and duration of exposure leading to the key intermediary steps in the pathogenesis of the health outcome. The key events must be linked in an exposure and temporal pattern for each of the steps in the pathway (cf Andersen et al. 2000; Tsuda et al. 2003; Slikker et al. 2004a,b). Where possible, data from multiple studies should be integrated to link tissue dose and biologically effective dose with the pathogenesis of the adverse outcome of concern. Thus, both dose response and temporal response must be consistent for a given hypothesized mode of action. For each primary health outcome, the precursor events (the rate-limiting or key events) can be determined as a function of time and exposure relative to the health outcome. Defining the key events consists of describing the absorption, metabolism, accumulation, and retention of arsenic or its metabolites in conjunction with any biochemical or functional change during the pathogenetic process that leads to the health outcome of concern (Boobis et al. 2009). For arsenic, defining the exposure at which tissue metabolism and accumulation occur at levels sufficient to trigger the next key event is important and could be informed by pharmacokinetic and pharmacodynamic models. The temporal and exposure relationship for the next key event for each health outcome in pathogenesis would permit linkage to the adverse outcome. The time course and dose dependence should be coherent with respect to dose, time, species, age, sex, and population for each adverse health outcome of concern.

The IRIS assessment should consider the plausible modes of action for each adverse outcome that might result from oral exposure to arsenic. Several modes of action have been described in the literature were discussed in the committee's workshop by Cohen (2013) and detailed in Chapter 4 as potentially associated with arsenic action. They include binding to protein sulfhydryl groups, reactive oxygen generation, reactive oxygen species (ROS) signaling and the oxidative stress that ensues, and perturbation of DNA methylation (epigenetic factors) (Kitchin and Wallace 2008; Kitchin and Conolly 2010). Tissue-specific binding to protein sulfhydryl groups could be explored as a mode of action that can lead to inhibition of a specific protein activity (Kitchin and Wallace 2008). Sulfhydryls (such as glutathione), vicinal thiols (such as pyruvate dehydrogenase and some zinc finger proteins), or selenium may be the cellular target of arsenic and its metabolites.

An example of how the arsenic mode-of-action analysis could be structured is provision of what is known about the dose-dependent interactions of arsenic with protein sulfydryls, vicinal dithiols, and zinc-containing proteins and how the interactions might lead to biochemical changes (such as ROS generation), functional changes (such as mitochondrial damage), and epigenetic changes as a function of exposure. How those effects could culminate in the development of a specific health outcome can then be described to the extent possible on the basis of the underlying data (see, for example, Snow et al. 2005), especially with respect to their dose dependence. That approach is supported by the temporal associations that are observed in mice exposed to a high concentration of arsenic in drinking water (Kitchin and Conolly 2010). The temporal analysis is useful and when coupled with a dose-dependent and exposure-dependent profile may provide an integrated sequence of events that define arsenic adverse-outcome pathways. Such a framework may highlight the induction of ROS and redox imbalance as an integrative mode of action for lung cancer, bladder cancer, and cardiovascular disease.

Arsenic's action is complex, and many mechanisms of action and several possible modes of action have been assessed in the context of exposure of humans to high doses through drinking water. It is generally accepted that arsenic is not directly mutagenic but rather acts via an indirect mechanism that involves secondary mediators (such as oxidant damage, modified proteins, and immune suppression). Other research has provided data to support proliferation as a mode of action of bladder carcinogenesis associated with high-dose exposure to inorganic arsenic. In adult rats given arsenic directly, cytotoxicity is ob-

served and is followed by regenerative repair (Nascimento et al. 2008; Suzuki et al. 2008a,b, 2009a,b, 2010, 2012; Yokohira et al. 2010). Similarly, an increased incidence of bladder neoplasms is observed in adult mice exposed to high doses of arsenic during the period of rapid cell proliferation in utero (Tokar et al. 2010b). Another hypothesized mode of action is epigenetic modulation that may influence arsenic-induced signaling response. The most studied aspects of the epigenome are DNA methylation, covalent posttranslational histone modifications, and microRNAs, and they have been associated with both cancer and noncancer effects. Epigenetic modifications are believed to influence signaling events and phenotypic variation in cells. DNA methylation is the most widely studied epigenetic modulation in humans and other mammals; it can influence gene silencing by directly affecting the affinity of transcription factors for their DNA binding sites. Arsenic exposure has been associated with decreased *S*-adenosyl methionine concentrations (and increased concentrations of *S*-adenosyl homocysteine), altered methyltransferase activity, and changes in DNA methylation patterns in animals and humans (Reichard and Puga 2010). Dose and temporal association of DNA methylation changes with key initiating events would need to be derived to support its role in mode of action analyses.

Gene-expression and other -omic data can support the determination of mode of action. Several groups have performed gene expression in cell lines, in primary human cells, and in tissues from animals treated with arsenic, and the results need to be organized by health outcome and dose, exposure duration, and time course of response as reviewed by Ankley et al. (2010). Analysis of the quality of the data and the normalization methods need to be considered. Several additional caveats need to be considered in that driver genes may be expressed at low levels and not detected, the relevant change may not be at the gene transcription level, many changes are simply responses and not causal mechanisms, and not all changes are adverse. In addition, the genomic profile should be obtained of the tissue type of origin (specifically, primary cells from this tissue for in vitro studies) for the health outcome of concern. Because genes are not independent variables, one should focus on rate-limiting steps for the proposed mode of action and key events. Integration of -omic data permits linkage among tissues, and the Bayesian network-analysis approaches may extend to comparison of populations (Subramanian et al. 2005; Geneletti et al. 2011; Schadt and Björkegren 2012). Pathway mapping can be a useful place to start in such qualitative analyses (Clewell et al. 2011; Yager et al. 2013), but current datasets need to be critically assessed with regard to normalization procedures and statistical control of batch effects and multiple comparisons. In addition to pathway mapping, one might focus on ligand through receptor to signaling to transcription-factor analysis or on the transcription-factor set (for example, zinc finger proteins with vicinal thiols). It is important to remember that genes are not independent variables and that statistical significance is not equivalent to biologic relevance (Moggs et al. 2004; Mootha et al. 2004). Several papers provide a framework for the use of gene-expression data to inform understanding of key events (Bercu et al. 2010; Gentry et al. 2010); however, they have focused on perturbation of pathways, not on approaches to determining mode of action or key events (Moggs et al. 2004; Mootha et al. 2004). Gene-expression analyses permit hypotheses—such as selenium depletion, zinc finger protein inactivation, or interruption of mitochondrial respiration—to be explored to support assessment of mode of action and key events.

Modified Bradford Hill criteria for assessing causality have been used to provide a structure for assessing causality systematically in toxicologic and epidemiologic studies (Adami et al. 2011). Causality assessment is a key defining principle in mode-of-action analyses and is an integral part of a toxicologic assessment. The application of the modified Bradford Hill criteria in the integration of mechanistic, toxicologic, and epidemiologic data is necessary to determine which adverse health outcomes are causally associated with a specific exposure, exposure duration, and timing of exposure. The application of these criteria to combined mechanistic and epidemiologic data has been conceptualized by Adami et al. (2011), and they have been applied to the effects of atrazine on breast cancer by Simpkins et al. (2011). EPA proposes the use of adverse-outcome-pathway analysis in a separate construct that is similar to mode-of-action analysis but does not consider that some changes are physiologic or even adaptive and not adverse. The utility of mode-of-action and adverse-outcome-pathway analysis lies in the fact that their dependence on the modified Bradford Hill criteria for causality assessment relies on exposure and temporal dependence for coherence of any observed effects; thus, this emphasizes attributable risk.

SUMMARY

Mode-of-action analyses permit a transparent assessment of the data supporting or refuting the human health effects of exposure to oral inorganic arsenic in the US population. Both cancer and noncancer effects can be integrated through a mode-of-action analysis. Such analysis permits an unbiased use of all the available data to examine the effects of arsenic exposure and exposure duration at different doses. A mode-of-action analysis needs to be compiled for each health outcome that has been ascribed to arsenic exposure to inform the risk assessment. Numerous mechanisms of action have been proposed for arsenic-associated health outcomes (including protein binding, ROS generation, epigenetic effects, and cytotoxicity), and it is unlikely that a single mode of action is responsible for all the observed adverse health outcomes. Mode-of-action analysis permits the integration of data to advance understanding of the coherence, biologic plausibility, and human relevance of findings throughout the exposure–response continuum and provides a transparent means of synthesizing the data. Mode-of-action analysis may be particularly useful in understanding low-dose exposure and dose-dependent transitions that may occur with increasing dose. The exposure (the tissue or biologic effect level) is considered in the context of metabolism, transport, and accumulation in the tissues of interest. Where nonlinearities in the pharmacokinetics or pharmacodynamics are identified, they can be used to understand the time and dose dependences of the key rate-limiting events and of the proposed adverse health outcome. The mechanistic, toxicologic, and epidemiologic data can then be interpreted in a comprehensive mode-of-action framework that facilitates an improved understanding of exposure–response relationships and interhuman variability of response.

7

Dose–Response Analysis

In comparison with most other chemicals evaluated by the Integrated Risk Information System (IRIS) program for hazard identification and dose–response assessment, there is a wealth of epidemiologic evidence on both cancer and noncancer end points after oral exposure to inorganic arsenic. The focus of this dose–response analysis chapter is on specific issues around the use of the epidemiologic data. As noted previously, the scope of the present National Research Council study did not include exposure by inhalation or dermal pathways, but issues addressed here could be applicable to epidemiologic studies involving those routes. On the basis of the extensive epidemiologic data, the committee expects that the results of animal and in vitro mechanistic studies will facilitate the understanding of the biologic plausibility or mechanisms of arsenic causation of effects observed in epidemiologic studies and interpretation of low-dose effects rather than be the focus of the dose–response analysis. Due consideration of the marked species differences in arsenic kinetics and toxicity would have to be factored into analyses using animal data.

The draft Environmental Protection Agency (EPA) development plan for the IRIS assessment of inorganic arsenic indicates that multiple cancer and noncancer end points will be evaluated and that dose–response analyses for the end points will be developed when feasible. The committee supports that approach, particularly in light of the substantial growth in epidemiologic information about a variety of noncancer end points. The implications of arsenic exposure for public health are much broader than the historical focus on cancer and skin lesions.

The following sections provide perspectives and recommendations on topics that the committee judged would play important roles in the dose–response analyses for health end points associated with inorganic arsenic. These considerations are specific to analyzing inorganic arsenic and may not be applicable or appropriate for other chemical assessments.

DOSE–RESPONSE ANALYSIS FOR NONCANCER AND CANCER END POINTS

The committee recognizes that quantitative dose–response analyses are one of the major functions of the IRIS program and that the extrapolations involved in this process are among the most controversial aspects of arsenic risk assessment. There has been a substantial expansion of epidemiologic studies of associations between arsenic exposure and a variety of health outcomes, including cardiovascular disease, diabetes, nonneoplastic respiratory changes, skin lesions, pregnancy, child development, skin cancers, bladder cancers, and lung cancers; the studies increasingly characterize risks of the health outcomes at low to moderate arsenic exposures.

Therefore, the committee recommends that EPA evaluate data on multiple outcomes to assess whether they are appropriate for direct estimation of risks of health outcomes in the range of epidemiologic observations. Such analyses would be undertaken as appropriate given the methods in the epidemiologic studies and as consistent with EPA technical guidance for evaluating dose–response relationships in the range of observations, such as with model-fitting approaches described in the EPA *Benchmark Dose Technical Guidance* (EPA 2012). Existing data may allow risk close to the range of background exposures for inorganic arsenic to be estimated directly from the epidemiologic findings, although limitations of the epidemiologic data will need to be considered; analyses by the European Food Safety Authority may constitute a useful example

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(EFSA 2009). Given the importance of maximizing the understanding and credibility of the health risks associated with arsenic and moving protection of public health beyond debates over the shape of the dose–response curve below the range of observation of positive findings for selected cancer end points, the committee believes that a critical review of the existing data and a focusing of attention of the IRIS program and its many stakeholders on the observed evidence for arsenic health effects is essential.

Background concentrations of arsenic exposure vary, but the committee judged that urinary arsenic at 1–5 μg/L (summing inorganic, monomethyl, and dimethyl arsenic forms) was a reasonable estimate for the US population for purposes of the present report (Karagas et al. 2001b; Navas-Acien et al. 2009b; Zheng et al. 2013). That range was estimated on the basis of published epidemiologic studies, current work that committee members were aware of, and values that are obtained from the National Health and Nutrition Examination Survey (NHANES) that exclude populations that consume substantial amounts of fish (to eliminate a large contribution by arsenobetaine and other seafood arsenicals in the NHANES total urinary arsenic measurements) (Navas-Acien et al. 2011). Background concentrations would exclude substantial exposure to arsenic from drinking water and so would arise from a variety of sources that potentially include very low concentrations in water, diet, dust, and other sources. The committee does not assume that the background concentrations are with or without health effects; rather, it assumes that the needs of assessing health risks can be facilitated by characterizing dose–response relationships down to the background concentrations by using observed data.

The committee recommends that EPA develop risk estimates for the variety of health effects on which there is adequate epidemiologic evidence and then derive risk-specific doses to address the needs of analyses that would typically use a reference dose (RfD). A risk-specific dose for a noncancer end point is an estimate of the dose associated with a degree of risk based on the dose–response function for that end point (NRC 2009). In the committee's workshop, Sande (2013) noted that stakeholders, such as state agencies, have used risk-based estimates only for cancer end points and would need some guidance in implementing the use of risk values for noncancer end points. Thus, EPA should provide guidance on how an RfD might be selected from among the risk-specific doses on the basis of such factors as end-point severity, interindividual variability, and policy considerations (NRC 2009). Candidate values for multiple end points will provide the basis for conducting cumulative risk assessment of chemicals that have common adverse outcomes, even when the end points are not the most sensitive. By providing both the dose–response function and a series of risk-specific doses, the IRIS assessment for inorganic arsenic will go beyond typical IRIS assessments, which develop a single RfD, and will facilitate an array of analyses, including cumulative risk assessment, benefit–risk ratios, and other comparative and economic analyses.

For both cancer and noncancer end points, consideration of the methods used in the epidemiologic study analyses will be important in developing the dose–response analysis in the observed range. For example, it may be preferable to consider exposure–response relationships by using categorical associations rather than the continuous data because they allow flexible evaluation of the dose–response relationships and do not simply assume that they are linear. Selection of the model should consider appropriate statistical methods for alternative models and biologic considerations. The IRIS program will also need to consider the extent to which published information can be used and whether it would be beneficial to collaborate with study authors to obtain study data. Choices made throughout these processes need to be clearly explained to provide transparency.

In the range of lower drinking-water exposures, the contribution of dietary sources of inorganic arsenic can be important or even dominant, so the total oral dose of inorganic arsenic needs to be considered regardless of source, as discussed previously. The committee recognizes that that is a difficult challenge. To address it, the committee recommends that EPA consider study-selection options to facilitate the dose–response analyses. Preference would be given to studies that best characterize exposures in the low to moderate range by measuring arsenic-exposure biomarkers (such as urinary inorganic arsenic and its methylated metabolites), which reflect exposures regardless of pathway or source, while considering standards for such biomarkers (Altar et al. 2008). Lower preference would be given to studies that characterize only water exposures although effects observed at moderate to higher exposure, in which water dominates, could be used by carefully considering likely dietary exposures and their influence on the lower end of the dose–response

curve. For example, a National Research Council (NRC 2001) report carried out a sensitivity analysis of the effect of alternative assumptions concerning dietary exposures on risk estimates. A challenge that the IRIS program will need to address in using these studies is that they have characterized arsenic exposures by using different metrics (such as urinary arsenic or water concentrations), but a common exposure metric is needed to integrate the information across epidemiologic studies or with in vitro or animal data to inform dose–response assessment. Measures of internal doses would be desirable and could be obtained from pharmacokinetic models, but it may be necessary to use a simpler analysis based on oral dose. The IRIS assessment will need to provide a clear description of the approach that is used to convert reported exposures in the studies to whatever common metric is employed. Other factors will also affect which lower-dose studies are ultimately chosen for dose–response assessment and how they are used, including numbers of subjects, methods of end-point assessment (such as odds ratios and standardized mortality ratios), control for confounders, potential for exposure misclassification to lead to underestimation or overestimation of risks, and other factors that could affect study interpretation and sensitivity to detect a low-dose effect. Finally, the committee notes that EPA will consider epidemiologic studies that involve various exposures, some of which will be fairly high and others of which will span a broad range; some studies will focus on low to moderate exposures. The quality of the studies—assessed on the basis of their exposure assessments, their potential for bias or confounding, and many other methodologic details—will determine which ones are used in the dose–response analyses. Although the committee sees new opportunities in the growing database of studies that include low to moderate exposures, characterizing the dose–response relationship over the broad range of exposures available might be useful, particularly for assessing sensitive populations and interaction with other risks factors (as discussed in the committee's workshop by Cantor [2013]).

If health-assessment needs cannot be fully met with modeling of the data in the range of observation, the committee recommends that EPA take one of the following approaches and provide the rationale for its choice:

- The preferred approach would be to extrapolate from the dose–response relationship in the range of epidemiologic observations by using mechanistic data and models to define the individual and population risks and their associated uncertainty on the basis of analyses of adverse outcome pathways or modes of action and human variability in susceptibility to arsenic-induced effects (see Chapter 6). This approach could describe human pharmacokinetics, biomarkers of exposure (such as urinary or toenail measures), tissue doses of relevant arsenic forms (such as monomethyl arsenic), and the multiple toxicodynamic processes that lead to the adverse outcomes for which extrapolation below the range of observation is desired. It would appropriately account for any dose-dependent transitions in the mechanisms of toxicity that exist (Slikker et al. 2004a,b).
- If the preferred extrapolation approach is judged to be unfeasible without additional data and modeling, the basis for that judgment needs to be provided. As an alternative, the committee recommends that EPA evaluate the dose–response relationship in the range of epidemiologic observation for a given health end point by using, if it is feasible, multiple reasonably fitted models and then extrapolating at most over a short distance below that range by using the modeled curve shape approaching the low range of observations (see Box 7 for example). Fitting of alternative models could indicate how far below the range of observation the extrapolation is essentially independent of model choice and provide greater confidence in the extrapolation to that point. Model averaging approaches might also prove useful (Morales et al. 2006; Wheeler and Bailer 2009). Nonmechanistic extrapolations further from the range of observation may be increasingly likely to overlook dose-dependent transitions that would substantially alter risk estimates (Slikker et al. 2004a,b). It should be considered whether mechanistic and biologic information can inform this process, and all choices in the process should be clearly explained. It would be reasonable for the IRIS assessment to stipulate the range over which the dose–response relationship derived for cancer or noncancer end points is useful for risk assessment and ranges in which it is considered inapplicable. Because epidemiologic observations have extended to lower doses in recent years, their utility for some end points will extend into the range of background exposure, so extrapolation may not be necessary.

BOX 7 Illustration of Proposed Strategy for Estimating Risk at Low Doses

The various approaches presented and implications for risk assessment are for illustrative purposes only, and no risk estimates should be inferred from the examples. To illustrate a strategy for estimating risk at low doses, an example is provided on the basis of hypothetical data on cardiovascular-disease mortality in relation to arsenic exposure (Table A). In this example, the relative risks (RRs) based on the dataset were adjusted for appropriate covariates, but additional data are not available.

TABLE A Summary Data on Cardiovascular-Disease Mortality from a Hypothetical Cohort Study

Urinary Arsenic, μg/g of creatinine	Midpoint	Relative Risk
<5	3.5[a]	1.0
5-10	7.5	1.05
10-15	12.5	1.2
>15	18	2.2

[a]Without additional information about the urinary arsenic concentrations below 5 μg/g of creatinine, a midpoint was set to $5/\sqrt{2}$, following guidelines for adjusting for limits of detection.

The committee's proposed strategy follows four steps:

Step 1: Fit a general nonlinear model to observed data. The nonlinear model can accommodate a linear fit if supported by the data. Evaluate the goodness-of-fit of the model to the observed data graphically or more formally with a statistical test.

Step 2: Test the hypothesis for linearity.

Step 3: Extrapolate below the observable range to the range of interest (roughly an order of magnitude in this example).

Step 4: Obtain an estimate of RR at low doses within the range of extrapolation.

In the hypothetical data, the reference group (Table A) had urinary arsenic concentrations below 5 μg/g of creatinine. The model is first fitted with this group represented at a "midpoint" value similar to what is commonly used for values below the limit of detection ($LOD/\sqrt{2}$). A comparison is made with the risk estimate when the model did not include the reference group with a value of RR = 1; this results in a negligible change. The results of models without the reference group are presented below.

Step 1: Fit a nonlinear exponential model. The model is fitted to the mean RR as a function of arsenic (x) constrained to a mean of RR of 1.0 as x approaches 0:

$$\mu = 1 + \exp(\beta_0 + \beta_1 \log(x)) \quad (1)$$

On the basis of visual inspection, the predicted model adequately fitted the observed RR estimates (Figure A). Two vertical reference lines are included in the figure to denote the region of observed data and the extrapolation region. The slope parameter was positive and significant ($p < 0.001$), indicating that RR increases as baseline urinary arsenic increases.

A nonlinear logistic model was fitted to the same data for comparison (results not shown). There was a negligible difference in the prediction of RR in the extrapolation region when an exponential or logistic nonlinear model was used. The estimated model and resulting confidence intervals presented herein are based only on the RR estimates and not the variability around the estimates. When the numbers of cases and controls are available for each group, biologic variability can be incorporated into the analysis.

Step 2: Test for linearity. The nonlinear model in equation (1) is linear with an intercept of 1.0 and a slope of $\exp(\beta_0)$ when $\beta_1 = 1$. Thus, a test of linearity is based on comparing the model in (1) to the (reduced) linear model by using a likelihood-ratio statistic compared with a chi-squared distribution with one degree of freedom. The hypothesis of linearity was rejected ($p < 0.001$).

(Continued)

BOX 7 Continued

Step 3: Extrapolation below the range of observation. The predicted nonlinear model in the observable range can be used for extension of the curve to the region of interest (Figure A). For comparison, the figure provides a benchmark-dose (BMD) analysis (green bar) of the data as represented by the point of departure and the use of a benchmark response of RR = 1.1.

Step 4: Estimate low-dose risk. To obtain an estimate of RR with low doses (as represented by the reference group), one could use this committee's recommended approach of extending the dose–response curve below the range of observed data points and into the "reference" population exposure levels. If the predicted model of equation (1) is used, the estimated RR at a baseline urinary arsenic concentration of 0.75 µg/g of creatinine is 1.0000003. Thus, the extrapolation curve provides a probabilistic estimate of arsenic-induced RR of cardiovascular-disease mortality for exposures between 0.75 and 7.5 µg/g of creatinine that is consistent with the data in the observed range and allows the development of a candidate RfD on the basis of a risk-specific dose within the extrapolation range. If an RfD already exists, EPA would be able to estimate the RR associated with it (assuming that it falls within this range) and the benefit of mitigating exposure in the range of the RfD.

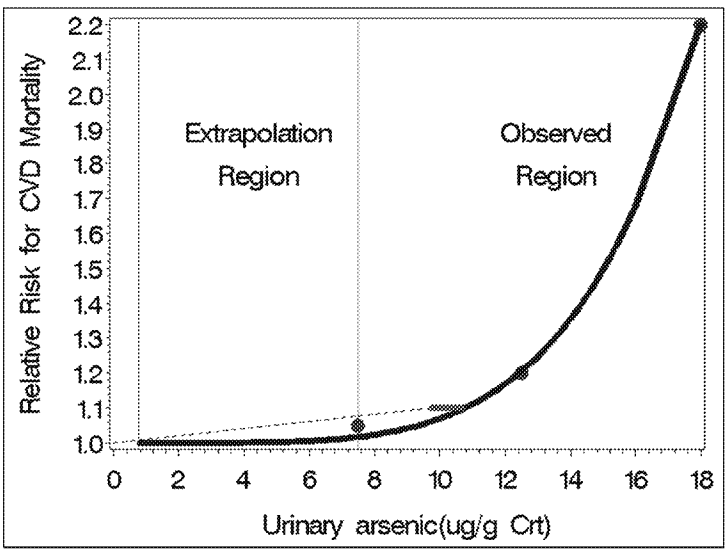

FIGURE A Hypothetical observed (red dots) and model-predicted mean relative risk (RR) for cardiovascular-disease mortality (blue curve) as a function of urinary arsenic (data presented in Table A) on the basis of a nonlinear exponential model. The model is parameterized to approach a mean RR of 1.0 as urinary arsenic approaches 0 but is extrapolated only 1 order of magnitude from the midpoint of the lowest group (excluding the reference group). The green bar represents the 95% one-sided confidence interval on the BMD associated with RR = 1.1. The dashed line connects the BMDL with the origin, in accordance with the traditional low-dose linear-extrapolation approach for cancer.

Those recommendations differ from some of the approaches outlined in EPA's draft development plan for the toxicologic review of inorganic arsenic. EPA proposes to use linear low-dose extrapolation as the default for cancer and noncancer effects. The committee proposes limited extrapolation by using the modeled shape of the dose–response relationship to provide a data-informed estimate of the potential dose–response relationship below the range of observation. Risks posed by background concentrations of arsenic should be characterized to the extent feasible, but in the absence of a sound mechanistic understanding of dose–response relationships, further extrapolation to lower exposures does not appear useful or pragmatic. EPA could use different models and present the risk estimates that would result to illustrate the magnitude of the impact of selecting one model over another. Justification should be provided for the chosen model, including any policy considerations that were factored into the decision.

EPA's plan discusses additivity to background disease processes, which the National Research Council report (NRC 2009) also discussed. Interpretation of this issue requires careful scientific justification and involves an understanding of adverse outcome pathways and their interactions with multifactorial disease processes at a level that would probably allow extrapolation based on a quantitative mode-of-action description (the preferred option noted above). Finally, EPA's plan focuses on characterizing uncertainty in the selected dose–response model rather than acknowledging that much greater uncertainties are generally associated with the issues of selection of alternative models. As noted in the second extrapolation option above, comparisons of results of alternative models are useful for learning how model-dependent the extrapolation results are and the uncertainty in them.

The committee is aware that there will be continued publication of epidemiologic studies of low to moderate exposures over the next several years. By focusing on the dose–response relationship in the range of observation, it will be easier for EPA to update selected components of the dose–response assessment as additional information becomes available. Substantial time is required to draft an IRIS assessment and complete the review process, so inevitably an assessment will become out of date. The committee recommends that EPA update selected health outcomes as new information becomes available. It is likely that within the 2–3 years that it takes to develop the assessment, updating will be useful.

The committee's recommendations are made specifically for the IRIS assessment of inorganic arsenic and should not be interpreted as applying to other IRIS assessments, given the robust epidemiologic data available related to exposures approaching background. Animal and in vitro studies have important roles to play in evaluating arsenic health risks by providing controlled experimental findings relevant to causality, mode of action, tissue dosimetry of specific metabolites, and other aspects of arsenic toxicology; they are not the focus of the dose–response analysis in the way that they are for many other chemicals. In addition, given the number of populations that are exposed to arsenic, epidemiologic data are increasingly available on concentrations directly relevant to the risk assessment. That is the case for a number of other chemicals, such as lead, ozone, and particulate matter. But in other cases, the available epidemiologic studies may involve exposures substantially higher than typical environmental exposures (for example, high occupational exposures), so analyses in the range of observations would not provide an adequate dose–response characterization for the needs of the IRIS program. Thus, some of the approaches recommended here for evaluating inorganic arsenic would not be feasible for many other chemicals.

DOSE–RESPONSE META-ANALYSIS

A critical issue in pooling epidemiologic studies for arsenic risk assessment for US populations is the relevance of arsenic concentrations in drinking water of the study populations and the evaluation of dose–response relationships. Meta-analytic approaches can be used to summarize reported results related to different magnitudes of exposure and to investigate the shape of the dose–response relationship (Rota et al. 2010). Available meta-analyses for arsenic-related disease have generally categorized the estimation of pooled estimates by dividing the studies of populations exposed to high arsenic concentrations (mean in drinking water above 50 μg/L, above 100 μg/L, or even above 150 μg/L) from studies of populations exposed to low to moderate concentrations (mean in drinking water less than 100 μg/L). That is a relatively simple technique that allows estimation of the association between arsenic and disease at relevant magnitudes of exposure, but it does not allow investigation into the shape of the dose–response curve across the range of exposures found in human populations. No dose–response meta-analysis has been conducted for arsenic-related disease. Multiple dose–response meta-analyses are, however, available in the environmental and public-health literature. For instance, dose–response meta-analyses have been conducted to evaluate occupational exposure to benzene and the risk of leukemia (Vlaanderen et al. 2010), the association of alcohol with esophageal squamous-cell carcinoma (Rota et al. 2010), and the association of selenium intake with cardiovascular disease (Flores-Mateo et al. 2006). Those meta-analyses could serve as examples for estimating the dose–response relationship of arsenic with relevant health end points, such as cancer and cardiovascular disease.

A number of methodologic issues need to be considered in conducting a dose–response meta-analysis. For studies reporting at least three category-specific relative risks and confidence intervals, the standard method of analysis is to fit a linear regression through the origin (reference category) weighted by using the estimated inverse variance of the log-relative risk. Alternative methods are used when the assumption that all relative risks in each study are independent is not met (Greenland and Longnecker 1992; Berlin et al. 1993; Orsini et al. 2012). In addition to linear dose–response meta-analyses, methods have been developed to evaluate nonlinear dose–response relationships by incorporating flexible splines, such as natural splines or restricted-cubic splines (Vlaanderen et al. 2010; Orsini et al. 2012). Those flexible methods provide excellent opportunities to evaluate the shape of the dose–response curve in epidemiologic studies across a wide range of arsenic exposure concentration although uncertainties may become greater at lower exposures, so careful consideration should be given when applying the methods. Although the direct incorporation of mode-of-action data into the analysis of dose–response data from human studies is challenging, biologic-response data could potentially inform the conduct and interpretation of the dose–response analysis on the basis of data from epidemiologic studies.

SENSITIVE POPULATIONS AND LIFE STAGES

IRIS assessments have long considered sensitive populations and life stages for noncancer end points but less frequently for cancer, although the *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* provided guidance on chemicals acting through a mutagenic mode of action (EPA 2005a). Epidemiologic studies of arsenic provide some evidence concerning sensitive life stages and populations in part owing to the unusual "natural experiments" that have resulted in relatively short well-defined periods of high exposure, as in northern Chile, or relatively well-defined times of using arsenic-contaminated water, such as the wells in Bangladesh. *Life stages* refers to different ages and development stages throughout life (such as infancy, pregnancy, and old age), whereas *populations* refers to groups of people who may have diverse or shared characteristics (such as genetic variations and phenotypic variations). The objective of IRIS assessments is to protect the general population, including those who may be more sensitive to toxicity induced by a chemical, particularly in comparison with those who were evaluated in the available epidemiologic studies. That implies assessing the potential health risks posed by arsenic in both men and women and considering the evidence of sex differences in both kinetics and susceptibility. As described throughout the health-effects sections presented earlier in this report, a number of studies and end points suggest that exposures during specific life stages need to be considered in the IRIS assessment and in the use of IRIS dose–response values in risk assessments.

The approach to assessing less than lifetime exposures for cancer risk from nonmutagenic carcinogens is typically to prorate the risk equally regardless of age; if exposure were for 10 years of a lifetime of 70 years, the lifetime cancer risk would be reduced by a factor of 1/7. There is an alternative approach for chemicals that have a mutagenic mode of action, which may not be the case for arsenic (Nesnow et al. 2002). Some findings with arsenic in humans and animals suggest that the appropriateness of the prorating approach needs to be considered particularly for early-life exposures. Although the Chilean studies may not be the best for characterizing the dose–response relationship, because they involve essentially a single high-concentration exposure for a specific duration, the committee recommends that these studies and others that yield evidence of life-stage sensitivity, including mechanistic studies, be considered in determining an appropriate approach for evaluating less than lifetime exposures. Although extremely high water concentrations were used in the early-life cancer studies of rodents, the consistency of the finding with the findings of epidemiologic studies provides at least supportive information (Tokar et al. 2010c, 2011b). The IRIS program needs to consider carefully the studies used to evaluate the cancer dose–response relationship, as well as the evidence of differential life-stage sensitivity, to ensure that both are appropriately addressed without inadvertently "double-counting". For example, if the epidemiologic dose-response relationships are characterized for populations exposed throughout life, including pregnancy and early life, the higher risks encountered in that period would be built into the overall dose–response relationship.

Some reports of early-life sensitivity to some noncancer effects—such as those found in studies of cardiovascular disease, infections, lung function and disease, and body size—have been discussed previously (Smith et al. 2006, 2011; Dauphiné et al. 2011; Rahman et al. 2011). Depending on the dose–response analysis for the different noncancer end points, the approach for addressing sensitive populations in the risk estimates may vary. The IRIS program could provide approaches for addressing those subpopulations given the derivations used for the IRIS toxicity values. For example, if risks arise from a specific window of susceptibility, they could indicate the appropriate period for the exposure assessment to be consistent with using a particular toxicity value.

IRIS USABILITY

The success of the IRIS assessment of inorganic arsenic will depend in part on its usefulness to IRIS partners in EPA and to stakeholders, including state, local, and tribal agencies throughout the country and internationally. The committee recommends a number of approaches that are not typical of IRIS assessments, such as providing risk estimates for noncancer outcomes for which a risk-specific dose could be derived. To ensure that a wide array of users can understand and apply the values derived in the inorganic arsenic assessment, it will be essential to implement recommendations offered by previous National Research Council committees, notably the report on the draft IRIS assessment of formaldehyde (NRC 2011).

The IRIS program has to address two major needs beyond the general need to create a well-organized document with clear summaries and supporting materials placed in technical appendixes to provide detail. The first is to advance the ability of users of the IRIS assessment to understand the scientific basis of its decisions so that they can evaluate them from the perspectives of the programs that partners and stakeholders are addressing. The second is to provide examples of how values might be used if they are different from those typically derived by the IRIS program; these would be particularly valuable if nonlinear analyses for cancer or dose–response characterization of risk for noncancer end points are used in the IRIS assessment. As stated above, the committee judges that a continuous dose–response function for noncancer end points based on the epidemiologic database is feasible and scientifically defensible for inorganic arsenic. For users unfamiliar with measures used in epidemiologic studies, ib contrast with toxicologic studies, it will be valuable to provide careful explanations and reference materials to ensure understanding of the dose–response analysis. An acceptable daily exposure level, such as an RfD, may be needed in some settings. Thus, the committee recommends that EPA derive a series of risk-specific doses based on the dose–response relationship for a given end point and provide guidance for choosing an RfD. Furthermore, EPA should stipulate the range over which the dose–response slope is reasonable for use in risk assessment and the exposure range over which the slope might be different or stipulate that the uncertainty is too great to allow a slope estimate. These features should, on the one hand, improve the flexibility and utility of IRIS values (for example, use for multiple end points, use for cumulative risk assessment, use in risk–benefit analysis, and use in traditional RfD calculations) and, on the other, show the limitations of the assessment (that is, the range in which the slope is highly uncertain and not recommended).

The hyperlinking capabilities of the Web present tremendous opportunities for the IRIS program to enrich the usability of its assessments. Summary presentations of cancer and noncancer analyses for oral or other routes of exposure could readily be linked to technical appendixes or examples as suggested above.

In closing, the committee recognizes the challenges that lie ahead for EPA in responding to the issues raised in this report and looks forward to reviewing a new draft IRIS assessment of inorganic arsenic. The new IRIS assessment, conducted with EPA's proposed approaches to improve analysis and transparency, will provide a critical data-based assessment of the health risks posed by exposure to inorganic arsenic, facilitate better understanding of the basis of decisions, and provide stakeholders with a valuable and user-friendly new resource.

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Appendix A

Biosketches of the Committee on Inorganic Arsenic

Joseph H. Graziano (Chair) is professor of environmental health sciences and pharmacology at the Columbia University Mailman School of Public Health. His research career has been devoted to understanding the consequences of exposure to metals on both the molecular and population levels. Dr. Graziano's past research was devoted to lead poisoning and has contributed to understanding of the adverse effects of lead exposure on childhood development. His laboratory developed the oral drug that is now widely used around the world to treat childhood lead poisoning. More recently, his research has aimed to understand the consequences of arsenic exposure on the US and Bangladeshi populations and to devise strategies to reduce toxicity and provide arsenic-free drinking water. Dr. Graziano received his PhD from Rutgers University. He is a member of the National Research Council Committee on Potential Health Risks from Recurrent Lead Exposure of DOD Firing-Range Personnel and a past member of the Committee on the Superfund Site Assessment and Remediation in the Coeur d'Alene River Basin.

Habibul Ahsan is Louis Block Professor of Health Studies (Epidemiology), Medicine (Genetic Medicine), and Human Genetics at the University of Chicago. He also holds appointments as director of the Center for Cancer Epidemiology and Prevention and associate director for population research at the university's Comprehensive Cancer Center. He studies the relationships between environmental and genomic factors in cancer and other diseases. He has published extensively on the molecular epidemiology and prevention of health effects of arsenic exposure and on the molecular and genetic epidemiology of breast and other cancers. Dr. Ahsan received his MD from Dhaka University and his MMedSc in epidemiology from the University of Western Australia.

Sandra J.S. Baird is a human health toxicologist with the Massachusetts Department of Environmental Protection Office of Research and Standards. She supports the air toxics and drinking-water programs through the development of toxicity values, evaluation of the implications of new toxicologic information and guidance, evaluation of site-specific toxicity and exposure-assessment issues, and development of guidance in support of risk-based decision-making. Her research interests include combining quantitative methods and toxicologic data to characterize uncertainty and improve understanding in the process of extrapolating human health risks from animal bioassay data. Dr. Baird received her MS and PhD in toxicology from the University of Rochester School of Medicine and Dentistry. She was a member of the National Research Council Committee to Review the Draft IRIS Assessment of Formaldehyde.

Aaron Barchowsky is professor in the Department of Environmental and Occupational Health at the University of Pittsburgh. His research interests are in investigating the cellular and molecular mechanisms underlying cardiovascular and lung diseases caused by environmental exposures to metals and chronic changes in redox status. In vivo and cell-cultured–based studies focus on the molecular pathology and etiology of vascular disease caused by chronic exposure to low concentrations of arsenic in drinking water. The cell-signaling pathways that mediate arsenic-stimulated pathogenic changes in endothelial cells and perivascular progenitor cells are being investigated. Dr. Barchowsky is an active member of the Soci-

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ety of Toxicology and recently served as president of the Metals Specialty Section and chair of the Education Committee. He received his PhD in pharmacology from Duke University.

Hugh A. Barton is an associate research fellow with Pfizer, Inc., working on mechanistic modeling of pharmacokinetics and pharmacodynamics. He is a member of the global Translational Research Leadership Team that is providing scientific and managerial oversight of application of modeling and biomarkers in drug discovery. He specializes in the use of physiologically based pharmacokinetic and mechanistic pharmacodynamic modeling to address low-dose, interspecies, and interroute extrapolations in estimating risks. He was a toxicologist in consulting companies and in the US Environmental Protection Agency before joining Pfizer. Dr. Barton received his PhD in toxicology from the Massachusetts Institute of Technology.

Gary P. Carlson is professor emeritus of health sciences at Purdue University. His research has focused on the relationship between the metabolism of chemicals and their toxic actions, including an interest in activation and detoxification pathways in the liver and other target organs. His work has involved using a variety of techniques ranging from in vitro assays to animal bioassays to examine the biochemical mechanisms by which chemical agents exert their toxic and carcinogenic actions. Dr. Carlson received his PhD in pharmacology from the University of Chicago. He is a national associate of the National Academies, having served on many National Research Council committees, most recently as a member of the Committee on Tetrachloroethylene and as current chair of the Committee on Toxicology.

Mary E. Davis is a professor in the Department of Physiology and Pharmacology of the West Virginia University Health Sciences Center. Her research interests are in the toxicology of environmental and occupational pollutants, including water-disinfection byproducts, halogenated solvents, and arsenic. She is particularly interested in mechanisms of toxicity in the liver, kidneys, and vascular system. Dr. Davis was treasurer of the Society of Toxicology and is a former president of the society's Allegheny–Erie Regional Chapter. She received her PhD in pharmacology from Michigan State University. She was a member of the National Research Council Committee on Assessing Human Health Risks of Trichloroethylene and Committee on Tetrachloroethylene and currently serves on the Committee on Toxicology.

Yvonne P. Dragan is associate director of US safety assessment and head of molecular and investigative toxicology at AstraZeneca Pharmaceuticals. Before joining AstraZeneca, she worked at the National Center for Toxicological Research of the Food and Drug Administration, where she was program director of the Hepatic Toxicology Center and director of the Division of Systems Toxicology. Dr. Dragan also held positions at Ohio State University and the University of Wisconsin–Madison, where her research focused on chemical carcinogenesis. She has been active in the Society of Toxicology, having served as an elected member of the Executive Council and as president of the Carcinogenesis Specialty Section and currently serving as a member of the elected council of the Carcinogenesis Specialty Section. She received her PhD in pharmacology and toxicology from the Medical College of Virginia.

Rebecca C. Fry is an assistant professor in the Department of Environmental Sciences and Engineering of the University of North Carolina School of Global Public Health. She uses environmental toxicogenomics, toxicoepigenomics, and systems-biology approaches to understand the mechanisms of arsenic-induced carcinogenesis. A broad goal is to identify the genes and their encoded proteins that protect humans against or sensitize them to arsenic-induced disease. State-of-the-art technologies are used, including next-generation sequencing to understand genomewide consequences of arsenic exposure. Her laboratory studies arsenic-exposed populations and varied disease outcomes to identify signaling pathways that are differentially modulated in response to exposure. A major goal of the research is to identify mechanisms of prevention of arsenic-induced disease. Dr. Fry received her MS and PhD in biology from Tulane University.

Chris Gennings is professor of biostatistics at the Virginia Commonwealth University and director of the research incubator for the Center for Clinical and Translational Research. Her research interests include nonlinear regression modeling, categorical data analysis, analysis of complex mixtures, and statistical issues in mixture toxicology. She has a research project on empirical approaches for evaluating sufficiently similar complex mixtures and is the director of a training grant focused on the integration of mixture toxicology, toxicogenomics, and statistics. Dr. Gennings received her PhD in biostatistics from the Virginia Commonwealth University. She was a member of the National Research Council Committee on the Health Risks of Phthalates.

Gary L. Ginsberg is a senior toxicologist in the Connecticut Department of Public Health Division of Environmental and Occupational Health Assessment. He is involved in the use of toxicology and risk-assessment principles to evaluate human exposure to chemicals in air, water, soil, food, and the workplace. He has published in toxicology, carcinogenesis, physiologically based pharmacokinetic modeling, interindividual variability, and children's risk assessment. He also holds an adjunct faculty position at the Yale School of Medicine and is an assistant clinical professor at the University of Connecticut School of Medicine. Dr. Ginsberg received his PhD in toxicology from the University of Connecticut. He was a member of the National Research Council Committee on Improving Risk Analysis Approaches Used by the Environmental Protection Agency.

Margaret R. Karagas is professor and section head of biostatistics and epidemiology in the Department of Community and Family Medicine of the Geisel School of Medicine at Dartmouth. She also holds appointments as codirector of epidemiology and chemoprevention at the Norris Cotton Cancer Center and as director of the research design, epidemiology, biostatistics, and ethics component of the Dartmouth SYNERGY. She is director of the formative Children's Environmental Health and Disease Prevention Research Center at Dartmouth. She conducts epidemiologic studies of human malignancies, in particular melanoma and nonmelanoma skin cancer, bladder cancer, and large bowel neoplasms. Recent work has focused on multiple routes of exposure to arsenic and risks of cancer, maternal and infant infection, and other birth and childhood outcomes. Collaborative efforts entail the design and application of novel biomarkers. Dr. Karagas received her PhD from the University of Washington. She was a member of the European Food Safety Authority Working Group on Arsenic and of the National Research Council Committee on the Analysis of Cancer Risks in Populations Near Nuclear Facilities.

James S. MacDonald is founder and president of Chrysalis Pharma Consulting, a firm focused on bringing new molecular entities from the lead optimization stage to proof-of-concept in patients. Over a period of 31 years before founding the company, he held several leadership positions at Merck and Schering-Plough, retiring as executive vice president of preclinical development. In the latter role, he oversaw the company's Department of Drug Safety and Department of Drug Metabolism/Pharmacokinetics and led efforts to move new molecular entities from discovery into clinical trials. Dr. MacDonald is an adjunct professor of pharmacology and toxicology at the Indiana University School of Medicine. His research interests lie in assessing strategies for identifying potential human cancer hazards and in using mode-of-action data in assessing human relevance. Dr. MacDonald received his PhD in toxicology from the University of Cincinnati and is a diplomate of the American Board of Toxicology.

Ana Navas-Acien is associate professor in the Department of Environmental Health Sciences of the Johns Hopkins Bloomberg School of Public Health. She is a physician–epidemiologist with a specialty in preventive medicine and public health. Her research interests are in cardiovascular effects and diabetes related to chronic exposure to arsenic in drinking water and food. She is currently the principal investigator in a large prospective cohort study of arsenic exposure and metabolism in American Indian communities. Other research interests include the cardiometabolic and renal effects of cadmium and lead and characterization of secondhand-smoke exposure in indoor public places. Dr. Navas-Acien received her MD from the University of Granada School of Medicine in Spain, her MPH from the National School of

Health in Madrid, and her PhD in epidemiology from the Johns Hopkins University Bloomberg School of Public Health. She was a member of the National Research Council Committee on Science for EPA's Future and serves on the Committee on Emerging Science for Environmental Health Decisions.

Marie E. Vahter is professor at the Institute of Environmental Medicine of the Karolinska Institutet in Sweden and head of the institute's Unit of Metals and Health. Her research interests are in the human health effects and associated mechanisms of arsenic, cadmium, and lead and in factors that influence susceptibility to these metals, such as metabolism, genetic predisposition, and nutrition. Her recent work focuses on early-life metal exposure. She has also been involved in health risk assessments for a variety of metals throughout her career. Dr. Vahter received her PhD in toxicology from the Karolinska Institute. She was a member of the two National Research Council Committees on Arsenic in Drinking Water.

Robert O. Wright is professor of pediatrics and preventive medicine and director of the Division of Environmental Health at Mount Sinai School of Medicine. His research interests are in effect modifiers of metal toxicity, including gene–environment interactions in neurodevelopment and fetal growth. The role of epigenetic biomarkers in reproductive health is a particular interest. Before joining Mount Sinai, Dr. Wright was associate professor of pediatrics at Harvard Medical School, associate professor of environmental health at the Harvard School of Public Health, and an attending physician at Children's Hospital in Boston. Dr. Wright received his MD from the University of Michigan and his MPH in epidemiology and biostatistics from the Harvard School of Public Health.

Appendix B

Workshop Agenda

Inorganic Arsenic: Scientific Considerations for Hazard Identification and Dose–Response Analysis

April 4, 2013
National Research Council, Lecture Room
2101 Constitution Avenue, NW, Washington, DC

7:30 am	REGISTRATION
7:45 am	**Welcome and Introduction** *Joseph Graziano, Committee Chair*
8:00	**Update on EPA's Inorganic Arsenic Activities** *Kenneth Olden and Vincent Cogliano, U.S. Environmental Protection Agency*
Strengths and Weaknesses of Recent Epidemiologic Studies of Inorganic Arsenic	
8:15 am	Cancer Evidence and Dose-Response Relationships *Kenneth Cantor, National Cancer Institute*
8:45 am	Noncancer Evidence and Dose–Response Relationships *Craig Steinmaus, California Environmental Protection Agency, University of California at Berkeley*
Integration of Metabolism and Mode of Action Information in Hazard Identification and Dose–Response Analyses	
9:15 am	Metabolism, Its Consequences, and Implications for Low Dose Assessments *David Thomas, U.S. Environmental Protection Agency*
9:40 am	Mode of Action and Mechanism Identification and Implications for Low Dose Assessments *Samuel Cohen, University of Nebraska Medical Center*
10:05 am	Interplay Between One-carbon Metabolism, Arsenic Metabolism and Epigenetics *Mary Gamble, Columbia University*
10:30 am	BREAK
10:45 am	Mode of Action for Lung and Cardiovascular Effects *R. Clark Lantz, University of Arizona*
11:10 am	Impact of In Utero and Whole Life Exposure and Implications for Dose Response *Michael Waalkes, National Institute for Environmental Health Sciences*
11:35 am	**Panel Discussion:** Moderators: *Aaron Barchowsky and Rebecca Fry* 1. Are there data that support low dose mechanisms and modes of action? 2. Is there a continuum of common thread in cancer and noncancer mechanisms and modes of action?

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3. Are there gender and species differences in metabolism or modes of action that impact dose response and disease susceptibility?
4. Do the kinetics and dynamics of inorganic arsenic and its metabolites in different organs promote organ-specific disease?

12:30 pm BREAK

Probabilistic Dose–Response and Harmonization Approaches for Cancer and Noncancer Effects

1:30 pm
- Lessons Learned from Lead and Particulate Matter
  Joel Schwartz, Harvard School of Public Health

2:00 pm
- Extrapolation of Mode of Action Data to Dose–Response Modeling of Human Health End Points
  Harvey Clewell, The Hamner Institutes of Health Sciences

2:30 pm **Panel Discussion:**
Moderators: *Gary Ginsberg and Robert Wright*
Discussant: *Daniel Axelrad, U.S. Environmental Protection Agency*

1. Do the available gene expression data define a coherent mechanism for cancer and noncancer effects?
2. Do these data adequately describe the array of effects at low dose?
3. How might the mode of action based dose response be affected by population variability?
4. How would one construct a probabilistic assessment of noncancer dose response from which the probability of an adverse effect can be estimated at any dose?
5. What are the implications of mode of action based and probabilistic-based assessments for risk–benefit analysis?

3:00 pm BREAK

Risk Assessment Approaches and Application of IRIS Values

3:15 pm
- Systematic Review and Evidence Integration for Literature-Based Environmental Health Assessments
  Andrew Rooney, U.S. National Toxicology Program

3:45 pm
- Perspectives of Risk Assessors and Users of IRIS Values
  Question: Given your experience in risk assessment activities, what aspects of the Toxicological Review of Inorganic Arsenic would be critical for maximizing the utility and credibility of the review for the risk assessments that you undertake?
  Barbara Beck, Gradient Corporation
  Michael Hansen, Consumers Union
  Kate Sande, Minnesota Department of Health
  Joyce Tsuji, Exponent, Inc.

4:10 pm **Panel Discussion:**
Moderators: *Sandra Baird and Hugh Barton*

Question 1: *Joyce Tsuji and Michael Hansen*
1a. "Science and Decisions" (NRC 2009) recommended that EPA adopt a unified dose–response assessment framework for cancer and noncancer end points. It has been suggested that an arsenic IRIS assessment might provide:

a. Risk estimates for noncancer end points (rather than or in addition to a concentration assumed to be health protective, such as an RfD)
b. Nonlinear cancer assessment
c. Multiple risk estimates for a single toxicity end point (e.g., alternative mode of action hypotheses, estimates from different studies, multiple dose–response models fitted to the same data)
d. Risk estimates for many toxicity end points. What recommendations do you have for EPA on use of these approaches?

1b. If the toxicological review of inorganic arsenic contains risk estimates derived from the dose-response approaches described in Question 1a, how would that impact the practice of risk assessment in the activities you are involved in?

Question 2: *Barbara Beck and Kate Sande*
2a. EPA has been asked by stakeholders to explicitly include consideration of populations that may have increased susceptibility to the adverse effects from arsenic (e.g., life stages, genetics, pre-existing disease, and environmental stressors such as co-exposures and nutritional deficiencies). What type of quantitative estimates of susceptibility would be useful in your risk assessment activities? If quantitative estimates cannot be derived, how do you recommend EPA provide information on susceptibility so that it can be used to inform your risk assessment activities?
2b. What types of documentation (and level of detail) are necessary to assist you and other users of an IRIS assessment if one of these approaches (which are not currently standard methods) were used?

5:00 pm **Additional Questions for Workshop Speakers and Panelists from Committee**

5:20 pm OPEN MICROPHONE
Each speaker has a maximum time limit of 5 minutes. Accompanying written materials are encouraged.

6:00 pm ADJOURN