

Arsenic: Health Effects, Mechanisms of Actions, and Research Issues

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A meeting on the health effects of arsenic (As), its modes of action, and areas in need of future research was held in Hunt Valley, Maryland, on 22–24 September 1997. Exposure to As in drinking water has been associated with the development of skin and internal cancers and non-carcinogenic effects such as diabetes, peripheral neuropathy, and cardiovascular diseases. There is little data on specific mechanism(s) of action for As, but a great deal of information on possible modes of action. Although arsenite [As(III)] can inhibit more than 200 enzymes, events underlying the induction of the noncarcinogenic effects of As are not understood. With respect to carcinogenicity, As can affect DNA repair, methylation of DNA, and increase radical formation and activation of the protooncogene *c-myc*, but none of these potential pathways have widespread acceptance as the principal etiologic event. In addition, there are no accepted models for the study of As-induced carcinogenesis. At the final meeting session we considered research needs. Among the most important areas cited were *a*) As metabolism and its interaction with cellular constituents; *b*) possible bioaccumulation of As; *c*) interactions with other metals; *d*) effects of As on genetic material; *e*) development of animal models and cell systems to study effects of As; and *f*) a better characterization of human exposures as related to health risks. Some of the barriers to the advancement of As research included an apparent lack of interest in the United States on As research; lack of relevant animal models; difficulty with adoption of uniform methodologies; lack of accepted biomarkers; and the need for a central storage repository for stored specimens. **Key words:** S-adenosylmethionine, arsenic, arsenate, arsenite, dimethylarsinic acid, heat shock proteins, heme oxygenase, monomethylarsonic acid. *Environ Health Perspect* 107:593–597 (1999). [Online 14 June 1999]

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Arsenic (As) is a naturally occurring element, is present in food, soil, air, and water, and all human populations are exposed to it in one form or another. The major sources of exposure for the majority of the U.S. population are from food and water. Food contains both organic and inorganic As, whereas drinking water contains primarily inorganic forms of As. For most people exposure from air and soil is minimal, but in certain instances, soil may be an important route for exposure in children. In addition, there are industrial exposures for workers. For example, semiconductor workers are exposed to gallium arsenide and some farmers handle arsenical herbicides. A variety of adverse health effects, e.g., skin and internal cancers, cardiovascular, and neurological effects, have been attributed to As exposure, primarily from drinking water. Of the various arsenical compounds, current evidence indicates that based on acute lethality data, the inorganic arsenicals are more acutely toxic than most organic forms. There are suggestions that the chronic toxicity of many organic forms of As, especially those found in fish and shellfish, is also less than the inorganic As; the available information, however, is from indirect sources. For example, an organic arsenical from fish,

arsenobetaine, is absorbed and excreted as the parent chemical and appears to exert little acute toxicity. However, no chronic bioassays in animals or surveys in humans have been conducted. Conversely, the inorganic compounds are acutely toxic and long-term exposure in humans has been associated with various cancers. There is no generally accepted animal model for As-induced cancer.

On 22–24 September 1997, the scientific meeting “Arsenic: Health Effects, Mechanisms of Action and Research Issues” was held at Marriott’s Hunt Valley Inn in Hunt Valley, Maryland. The meeting was cosponsored by the National Cancer Institute, the National Institute of Environmental Health Sciences, and the U.S. Environmental Protection Agency (EPA). Impetus for this meeting was provided by the upcoming 1 January 2000 EPA As rule promulgation and the recent worldwide reports of adverse effects resulting from exposure to As in drinking water. The purposes of this meeting were to gather scientists conducting research on As or in related areas, listen to their research presentations, discuss problems, and recommend research areas. The meeting was divided into six lecture–discussion sessions: Chemistry,

Toxicology and Exposure, Metabolism, Epidemiology, Biodynamics, Molecular Mechanisms of Carcinogenesis, and Dose–Response Considerations. A seventh session (Summary Discussion) was convened to recommend research areas, discuss barriers to research advancement, and define critical resource needs.

Chemistry, Toxicology, and Exposure

The integral relationships between arsenical exposure, *in vivo* methylation of As, and mechanisms of inorganic/methylated arsenical toxicity are central to providing appropriate risk assessment calculations for human populations exposed to this element. Exposure of human populations to elevated concentrations of inorganic As in drinking water from wells in Taiwan, Inner Mongolia, and China has resulted in usual dermatological manifestations that included raindrop pigmentation, hyperkeratoses, and skin cancer (1,2). In addition, subsequent analyses indicated the presence of the characteristic As-induced uroporphyrinuria, coproporphyrinuria, and a tubular proteinuria. The probable mechanisms of *in vivo* As methylation involve a 2-electron reduction of arsenate [As(V)] to arsenite [As(III)] and a methyl transfer reaction involving S-adenosylmethionine (SAM) to generate the methylarsonic acid (MMA) and dimethylarsinic acid (DMA) metabolites (3,4). The inhibitory action of various arsenicals and arsenothiols on glutathione reductase were discussed in relation to the prooxidant effects of arsenicals. The variety of inhibitory effects of arsenicals on

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nicotine adenine dinucleotide-linked substrates—e.g., support of mitochondrial respiration, decrease in adenosine triphosphate (ATP) synthesis, and increased phosphorylation of other intracellular biochemical species—were discussed in relation to the generation of reactive oxygen species (ROS), oxidative stress, and altered cellular gene regulation (5). In particular, the roles of oxidative insult in inducing the stress protein response, including members of the heat shock protein 70 family and the 32-kD stress protein (heme oxygenase), which may be involved in reducing the potential of intracellular heme and porphyrins to participate in the formation of ROS. Other stress proteins such as those in the 27, 60, and 90 kD families are also induced by As, indicating a broad proteotoxic effect following cellular exposure to this element (6,7). The relationship(s) between arsenical-induced cell injury/cell death and the carcinogenic response require further study.

Metabolism

Reports were presented on the diversity of the methods that mammals use to detoxify inorganic As. Although many organisms can methylate inorganic As, some mammals, including the marmoset monkey, chimpanzee, guinea pig, and many South American monkeys lack or are deficient in liver arsenite methyltransferases (3). At present, this is the best experimental evidence for polymorphism of these methyltransferase genes. A major question raised by these studies is whether methylation is the primary detoxification pathway of inorganic As(III). Diversity was also shown in how different tissues vary in arsenite methyltransferase activity. For the mouse, the greatest activity is in mouse testis, which is greater than kidney > lung > liver (3,8). These data show that the liver is not the only site of inorganic As(III) methylation, as has been claimed in the past. When the chelating agent DIMAVAL (2,3-dimercapto-1-propanesulfonic acid) was given to 24 subjects in Chile, there was a marked change in MMA and DMA excretion in the urine. The MMA excretion before DIMAVAL was 14%; after oral administration, MMA increased to 42% of total As excretion (9).

Variations in As metabolism in humans were also discussed because all groups of humans studied methylate inorganic As (10). Future research on factors affecting As methylation is needed. However, the patterns of As metabolites in urine have substantial interindividual variation. For example, a small number of women in the Argentina Andes excrete little MMA in their urine (11). Factors that appear to influence the methylation of inorganic As are the species of As that the individuals are exposed to, dose levels, routes of exposure, and type of diet. Arsenic metabolism in bacteria

was discussed and some bacteria were resistant to As. For example, the resistance of *Escherichia coli* to inorganic As was due to the products of five genes. The genes and their products are ArsR-metal binding protein with a domain for As(III) and Sb(III); ArsD-metal binding protein with three pairs of vicinal cysteine residues; ArsA, a catalytic unit of arsenite-translocating ATPase; ArsB membrane sector of ArsA gene product and ArsC, an arsenate reductase—the product is arsenite, which is the substrate of the pump. Such studies need to be extended to mammalian systems.

Because methylation is important in As metabolism, methylation reactions, which involve interactions, inhibitors, and selenium-As interactions, were also discussed. This is of particular importance because of the antagonism between As and selenium in many biologic systems. In addition, many investigators do not realize the various pathways available for mammals to methylate a substrate and the complex interactions and relationships of these pathways.

In South America, variations of human responses to As exposure have been reported (11). This area of variation in response to As is rapidly expanding and may need newer approaches and different methods of analysis. One potential tool involves using pharmacokinetic (PK) models based on ingestion of As(III), As(V), MMA, DMA, or any mixture of these four compounds in the human (12). Such models describe tissue burdens of As species as a function of time and dose. The PK model is being used as the framework to test a receptor-mediated mechanism of action for inorganic As. In addition, the potential importance of polymorphism in As toxicology was examined. Basic biochemical and molecular biology studies on the mechanisms of As metabolism are scarce.

Epidemiology

Two studies of As exposure and cancer in Chile were presented. The first, an ecological cohort study, used community estimates of As in water and age-specific ingestion rates (13). This study found an association between As in drinking water at 50 µg/L and skin cancer and four internal cancers. The second study, a case-control study, found an association between bladder cancer and As exposure, as reflected in statistically significant changes in odds ratios with an estimated lifetime cumulative exposure to As (13).

Studies involving cancer and noncancer end points after As exposure in Taiwan in the Blackfoot disease endemic areas were described (14). The study types included ecological correlation, case-control, and cohort studies. Key findings included an association between As and multiple health effects—in particular, diabetes and hypertension—as well

as several cancers. Skin lesions were the most sensitive sign of As exposure. Several factors, such as inadequate nutrition and genetics, appeared to enhance susceptibility to As-induced disease (15,16).

Analyses of differences in skin cancer cell types as a function of a racial group and As exposure were presented. These analyses showed that among Euro-Americans, basal cell carcinoma was the most frequent type of both As-induced skin cancer and skin cancers from other causes. Among Asians, Bowen disease was the most frequent type of As-induced skin cancer, but not in skin cancers from other causes. These studies suggest an interaction between skin melanin content, ultraviolet (UV) light, and As-induced skin cancer.

In India, individuals exposed to elevated inorganic As levels in drinking water showed a range of health effects including peripheral vascular disease, noncirrhotic portal fibrosis, lung symptoms, and polyneuropathy. Arsenic in Taiwan, skin pigmentation changes and hyperkeratoses were the most sensitive indicators of inorganic As exposure (17).

A recent study in Thailand found that skin manifestations, including skin cancer, were associated with elevated levels of inorganic As in drinking water; females were more affected than males. Tests of mental function also indicated an association between As and several indicators of function, such as intelligence quotient tests. Several factors, such as employment status and type, appeared to affect susceptibility to inorganic As toxicity (18).

A case-control study of skin cancer, specifically basal cell carcinomas, in Hungary found an increased risk of a basal cell carcinoma associated with elevated levels of atmospheric As from coal combustion. This was particularly apparent in cases diagnosed before 1982 as compared to cases diagnosed after 1986. It is important to note that air concentrations of As began to decline in 1971. In addition, the same investigators reported that basal cell carcinomas were associated with elevated occupational exposure to airborne As.

In the United States, preliminary results based on community estimates of exposure from a Utah cohort mortality study were presented (19). These results indicated an association between inorganic As exposure and certain cancers as well as noncancer effects, including hypertension and diabetes. A study design for an analysis of As metabolism in U.S. populations was presented. Finally, efforts to identify U.S. communities with elevated levels of As in drinking water were described (19).

Arsenic Biodynamics

This session addressed the potential beneficial effects of As. Whether it is nutritionally essential for human health is being debated.

Arsenic is a growth factor for animal nutrition, particularly chickens, although data demonstrating potential beneficial effects for humans are lacking (20). The definition of nutritionally essential trace elements (ETEs) has evolved over the past 50 years and may be expected to expand as the result of future research. The criteria for essentiality for human health are that withdrawal or absence of the ETE from the diet produces either functional or structural abnormalities and that the abnormalities are related to or a consequence of specific biochemical changes that can be reversed by the presence of the ETE. In this regard, As essentiality has not been convincingly demonstrated. However, the influence of As on human health may not be easily definable. Recent studies show that anomalies or imbalances in trace metals can influence human health and well-being without necessarily producing overt effects. In addition, it is now recognized that clinical expression of a latent deficiency or excess is often contingent on variables such as changes in general malnutrition or challenges such as stress, infection, or injury. Some signs of As deprivation in animals include perinatal mortality, enlarged spleens, osmotic fragility of erythrocytes, and rough hair coats. In addition, a low intake of inorganic As reportedly reduces SAM levels, alters production of polyamines, and affects methylation pathways. It was hypothesized that inorganic As played a role in methionine metabolism. Arsenic reportedly protects against selenium toxicity; one study suggested a mechanism that involved enhanced biliary excretion of selenium (21,22).

Molecular Mechanisms of Carcinogenesis

Arsenic does not directly react with DNA or cause gene mutations, except to a small extent at high doses. However, it does cause gene amplification and chromosomal damage at lower doses and can enhance mutagenesis by other agents, apparently by inhibiting DNA repair. Because no DNA repair enzyme is sensitive to inhibition by arsenite, it is likely that arsenite can interfere with the control of DNA repair rather than with the repair enzymes (5). Arsenite can also cause aneuploidy. Unlike spindle poisons, arsenite does not inhibit spindle fiber formation; instead, it deranges the spindle apparatus, possibly by accelerating microtubule polymerization. In work on a new cell transformation system using rat liver epithelial cell line TRL 1215, chronic exposure to arsenite-induced malignant transformation was associated with global DNA hypomethylation, decreased DNA methyltransferase activity, and activation (overexpression) of the protooncogene *c-myc*. These results suggest that arsenite may act as a

carcinogen by causing DNA hypomethylation leading to aberrant gene expression (23). Opposite results on DNA methylation by arsenite were seen on the *p53* promoter in human lung A549 carcinoma cells. Chronic exposure to arsenite caused a progressive increase in CpG methylation within the *p53* promoter, which would be expected to block transcription of the *p53* gene (24). The *p53* gene is an important tumor-suppressor gene whose protein product plays an important role in cell cycle control, apoptosis, and control of DNA repair. Altered expression of *p53* and changes in cell cycle distribution were reported in three cell lines 24 hr after treatment with arsenite. Cells transfected with a mutant *p53* gene showed increased arsenite sensitivity. Administration of the inorganic As metabolite, dimethylarsinic acid (DMA), in large doses caused DNA damage to the mouse lung. In cultured human alveolar L-132 cells, DMA also caused induction of DNA strand breaks, DNA-protein cross-links and alkali-labile sites. It also acted as a tumor promoter in mouse lung initiated with 4-nitroquinoline 1-oxide. The mechanism of action is thought to be via a dimethyl arsine radical formed in cells from DMA (25).

Dose-Response Concentrations

Several hypotheses (e.g., gene amplification, alteration of methylation patterns, and radical formation) concerning the potential mechanisms by which inorganic As could induce cancer were presented. None of these possible pathways have received widespread acceptance, probably for two reasons. First, mechanistic studies of inorganic As have been the subject of fairly recent investigations, and second, there are no universally accepted animal models for the study of As-induced carcinogenesis. Although not unanimous, there was a lot of support, based on known genetic effects of As, for a nonlinear dose-response curve for the carcinogenic effects of As (26). Comments on the shape of the dose response elicited comments on how to model such a response. No conclusions were reached on this aspect.

Summary Discussion and Recommendations

At the end of the workshop, questions and recommendations were developed by the attendees and the session chairs. Broadly speaking, these included both basic and human research initiatives that were felt to be of importance in the goal of more fully assessing the risk presented by As in the environment. Several important barriers to the advancement of research in As carcinogenicity and toxicology were perceived, involving both funding and technical elements. These barriers were discussed in the hopes of providing some direction toward resolution of these issues.

Research needs assessment. Some of the most important basic research needs that were discussed included several aspects of As metabolism and its interactions with living cells. Inorganic As can often be methylated by cells, which appears to reduce its acute toxic effects in many cases. Although it is methylated in many tissues, this enzyme activity in most tissues has not been well defined and such tissue specificity in methylation may have important implications in toxicity and carcinogenesis. The metabolism of inorganic As in various cell lines, including human cell lines, would be an important aspect of these studies. Methylation of As appears to reduce its acute toxic effects in many cases. With respect to its chronic effects, there are some data that the methylated forms (MMA and DMA) can also be toxic: this deserves further research. Some species show a minimal capacity for methylation of As and it is an open question how these nonmethylator animals survive in the face of As exposure.

The question of As bioaccumulation was considered an important area for additional effort and the means by which the various As species enter and leave cells were considered an important element of this effort. These studies should focus on mammalian cells and include human cells. Arsenobetaine and arsenocholine accumulate in various seafoods, although knowledge of their effects in mammals is scant. If As does in fact bioaccumulate, what is the storage form? The interaction of the inorganic As with cellular proteins may be the key. Many times cells can show native or acquired resistance to metals and this resistance, at times, involves protein binding. The existence and mechanisms of resistance to As need more in-depth exploration. Comparative studies of nonhuman primate species that vary in methylation capacity would be an important step in answering some of these questions. Abnormal methylation patterns may also occur and may be based in genetic variation or may be due to disease states. Additionally, how intestinal microflora may vary in its ability to process inorganic As in human and animals may be important in defining the actual form and extent of exposure to As.

Several issues were mentioned in the Methods Development session. The development of methods to determine As bioavailability was considered important in defining risk from environmental As. Separation of some of the various forms of As is currently considered problematic; method development would be important. Development of biomarkers of As exposure and toxicity is also a clear need.

Gender and age differences in susceptibility to As are poorly defined and such differences could explain the basis of sensitivity in certain subpopulations. Immunotoxicity,

including immunosuppression, is often one of the most subtle effects of a toxic agent but has not been well defined for As.

Metal-metal interactions are important in the toxicity of many metals. The role of essential metals such as chromium, cobalt, zinc, and selenium in As toxicity has not been adequately explored. Nutrition studies should define the interactions of As with essential metals and proteins. Similarly, the effect of other toxic inorganics, such as cadmium, lead, and mercury on the toxic potential of As has not been defined. In an environment where multiple exposures are the rule rather than the exception, such interactions may be critical in defining toxic potential. Any synergistic and additive toxic effects of arsenicals in cellular systems should be defined. In addition, some metals are redox active and will generate ROS. There is some evidence that methylated As compounds may cause peroxidative damage to cellular components at high doses, although this aspect of As toxicity needs to be more fully explored. For instance, the chemistry of As radicals is poorly understood. Questions remain concerning the formation of ROS by As at low doses, a factor of critical importance in defining mechanistic significance. The development of noninvasive methods to assay oxidative damage would greatly facilitate such studies.

On the molecular level there is clear evidence that As can induce aberrant gene expressions. The effects of As on signal transduction may be critical to the modification of gene expression. Whether specific gene expressions induced by As are transitory or permanent could have important impact for toxic mechanisms and should be explored.

Much of the discussion on research needs focused on the carcinogenic potential of As. The absence of clear animal models for this important human carcinogen was considered a major issue. Establishing animal models should be encouraged because so many of the mechanistic studies that need to be conducted cannot be done in humans. The development of whole-animal and cell culture systems for defining the carcinogenic process for As was considered critical to advancing our knowledge in this area. Further mechanistic studies defining the genotoxic effects (such as aneuploidy, comutagenesis, and chromosome aberrations) at the molecular level were proposed. It is possible that different mechanisms of actions for As may apply at different stages of carcinogenesis or in different target sites. The genetic disruptions induced by As during transformation need further elucidation. Defining the genetic changes in As-induced tumors and in As-transformed cells would assist in defining the molecular events in As carcinogenesis. Rodent model systems need to be relevant to human exposures to the extent

possible and consideration of diet and exposure to other potential carcinogens (i.e., UV, other metals) need to be assessed. The model systems of cell transformation also need to be relevant with regard to humans in both the use of reasonable dosages and cell types that would be representative of target sites. Defining the basis for the apparent differences in susceptibility between human and rodents for cancer would be a great step forward. Model systems for various stages of carcinogenesis may be important in this regard and the existence of multiple mechanisms of carcinogenesis should not be excluded for As. The investigation of how preneoplastic lesions that develop in As exposures are associated with tumors may also allow the use of these as biomarkers.

Several research needs that involved human study were discussed. Overall, a better characterization of human As exposure is required to more precisely define actual health risks. This should include defining actual doses and durations of exposure. Host modifiers of the response to As, including diet and genetic polymorphisms that may be of etiological significance in development of disease, should also be studied. There is evidence that susceptibility to As toxicity is higher in people who are nutritionally deficient, although the specific nutritional deficiencies need to be defined. The exploration and validation of mechanistically based biomarkers are a key element in defining adverse effects of As exposure. An important question about the reversibility of the effects of As in humans remains. Locations that have substantially reduced As levels in their drinking water should be evaluated to identify any correlated reduction in adverse health effects. Determination of the lowest concentrations at which dermal signs of As toxicity are observed in international studies would help establish the lower dose limit in the United States. All of these factors point to the need to identify mechanistic components of some dose-response models to provide valid risk assessment. Arsenic always, ethical issues for human studies need to be carefully considered.

Specific epidemiology studies were recommended that considered the following elements: characterization of dose-response relationships; characterization of the duration-response relationships; linkage to mechanistic studies; and evaluation of population variability, especially as related to mechanisms. The use of multicenter comparative epidemiology studies could facilitate these studies and these should have the following attributes: *a*) consistent quality control measures; *b*) standardized questionnaires (e.g., describing host factors, etc.); *c*) different populations with overlapping exposures; *d*) a well-characterized exposure, including As

species, bioavailability, etc., in all exposure media (food, air, water); *e*) characterization of individual exposure; *f*) use of standard reference materials; and *g*) consider the use of a centralized analytical laboratory. To enhance the comparability and validity of such studies, the effects of As must be well defined using consistent criteria. This would include the standardization of types of skin lesions and a focus on known target sites (lung, skin, and bladder for cancer). Investigation of non-cancer effects should also be considered, including such effects as hypertension, cardiovascular disease, diabetes, and possibly intelligence quotient effects from exposure during development. Maternal to fetal transport and the potential actions of As during development, especially of the nervous system, were also considered priority areas for research. Mechanistic studies involving biologically plausible exposures should be performed in conjunction with epidemiology studies.

Factors influencing inorganic As metabolism in humans are largely undefined. We should begin looking for factors, in addition to exposure, that may correlate with adverse effects in humans which are now attributed primarily to As. There are several potentially important modifying factors in As toxicity that should be assessed. Nutritional factors such as dietary selenium or methionine and general nutritional status should be analyzed for an effect on the toxic potential of As in humans. The presence of other disease can often modify toxic potential; for example, liver disease may modify the toxic effects of As. The presence of other carcinogenic compounds that may act as cocarcinogens with As is a possibility and should be studied. Genetic polymorphisms in As toxicity and carcinogenesis may exist but have received relatively little attention.

The conditions of exposure to As in humans need to be more fully characterized, including defining the contribution of As-containing herbicides as well as other arsenicals. The definition of how hair and nail As levels may serve to define levels of As exposure, in comparison to urine levels, could provide additional data to define dose-effect relationships. Exposures associated with As due to cooking should be explored, including what species of As are produced by the specific processes and whether As could be concentrated in foodstuffs in such cases. In addition, can therapeutic intervention, such as with the chelators, reduce the adverse effects of environmental As exposure or in fact provide some form of preventive treatment?

Barriers to research advancement. There are several important barriers to the advancement of As research. Difficulty in obtaining funding for As research was considered a major issue. One contributing factor seems

to be a general lack of interest in As, e.g., it does not seem to be relevant. For example, in a review of a grant application, one scientist commented on the exotic enzymes involved in the metabolism of As. A lack of funding for programs that incorporate multidisciplinary research efforts was also considered to be an important barrier. Funding for innovative pilot projects concerning mechanisms in As research could prove invaluable in defining the mechanism(s) of this important environmental carcinogen.

Technical issues that inhibit progress include the poor availability of radioactive forms of As that are critical to many metabolism studies. In addition, the lack of a reliable animal model for inorganic As-induced carcinogenesis has not been addressed. Support for the international logistics involved in the communication of research methodology results and for the exchange of samples are needed. The means for communication among researchers and between researchers and regulatory personnel, e.g., the EPA, should be developed.

Important future resources. Important current and future resources that may assist in defining the hazards posed by As exposure were also discussed. Enhancement of communications was an important issue and it became clear during the meeting that it was useful to invite participants who are, or have been, traditionally outside the field of As research. The enhanced communication from the introduction of fresh perspectives was evident. Other means to increase fruitful communication could include the development of e-mail lists of As researchers. A repository of data appearing in foreign-language journals available in translated form would greatly assist in international communications. Additionally, a meeting to establish conformity to As epidemiology studies and to assist in the incorporation of mechanistic and molecular aspects into epidemiology studies is a critical need.

Resources that could be developed to assist research efforts would include several unified protocols and methods to allow confidence when comparing data from different sources. Such standard protocols would include urine analysis of As, including sample preparation; analytical methods for assessment of As in food, water, and air; standardized classification system for As-induced skin lesions; and standardized questionnaires for epidemiologic studies.

Proposed unified research facilities that would be valuable in the advancement of As research included a facility for growing human cell lines. Enhanced availability of radioisotopic As compounds and other materials is important. The development of knockout mice unable to methylate inorganic

As would help in many mechanistic and metabolism studies.

Biomarkers of exposure and effect should be carefully evaluated. This should include urinary metabolites and forms of As in biologic fluids. Measurement of genetic effects, such as clastogenic effects in lymphocytes or other cells, should also be determined. In this regard, studies to facilitate biomarker development and the storage of tissue specimens from populations with significant As exposure should be carried out in preparation for future studies. Although what may be the best end point(s) for assessment is unclear, certain target tissues and easily accessible tissues like skin samples and lymphocytes would be valuable. Additionally, DNA and tumor specimens should prove invaluable in defining aberrant gene expression induced by As and may help in defining carcinogenic mechanisms. The use of a central repository concept would be appropriate for such stored specimens.

Several meetings to discuss specific aspects of As toxicology were proposed. This included a meeting to develop a standardized As-induced skin lesion classification. A meeting to determine the most appropriate biomarkers of exposure and effects for As would also be valuable, as would one to discuss the development of animal model(s) for As-induced carcinogenesis. Additional information on the effects of As in the human health and environmental areas has been published (27–30).

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