



## Arsenic Exposure and Toxicology: A Historical Perspective

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The metalloid arsenic is a natural environmental contaminant to which humans are routinely exposed in food, water, air, and soil. Arsenic has a long history of use as a homicidal agent, but in the past 100 years arsenic, has been used as a pesticide, a chemotherapeutic agent and a constituent of consumer products. In some areas of the world, high levels of arsenic are naturally present in drinking water and are a toxicological concern. There are several structural forms and oxidation states of arsenic because it forms alloys with metals and covalent bonds with hydrogen, oxygen, carbon, and other elements. Environmentally relevant forms of arsenic are inorganic and organic existing in the trivalent or pentavalent state. Metabolism of arsenic, catalyzed by arsenic (+3 oxidation state) methyltransferase, is a sequential process of reduction from pentavalency to trivalency followed by oxidative methylation back to pentavalency. Trivalent arsenic is generally more toxicologically potent than pentavalent arsenic. Acute effects of arsenic range from gastrointestinal distress to death. Depending on the dose, chronic arsenic exposure may affect several major organ systems. A major concern of ingested arsenic is cancer, primarily of skin, bladder, and lung. The mode of action of arsenic for its disease endpoints is currently under study. Two key areas are the interaction of trivalent arsenicals with sulfur in proteins and the ability of arsenic to generate oxidative stress. With advances in technology and the recent development of animal models for arsenic carcinogenicity, understanding of the toxicology of arsenic will continue to improve.

**Key Words:** arsenic; cancer; exposure.

The word “arsenic” elicits a fearful response in most people. This is because arsenic has a long history of being a poison, both intentional and unintentional, to humans. However, most laymen do not know or understand that we are constantly exposed to arsenic because it is naturally present in the environment, is used in commercial products, and has medical applications. Although most typical environmental exposures to arsenic do not pose a health risk, several areas of the world contain arsenic from natural or anthropogenic sources at levels that create a toxicological concern. Many of these areas have been identified, and efforts are being made to either remediate these areas or limit access to them.

Arsenic is the number one substance in the most recent (ATSDR, 2007a) Comprehensive, Environmental, Response, Compensation and Liability Act (CERCLA) Priority List of Hazardous Substances published by the Agency for Toxic Substances and Disease Registry (ATSDR). This list is comprised of substances found at hazardous waste sites on the National Priorities List. The substances are ranked on frequency or occurrence, toxicity, and potential for human exposure.

An understanding of the chemistry of arsenic is needed to appreciate the toxicology of this metalloid, which shares properties of metals and nonmetals. (A metal has luster, conducts heat and electricity, and is malleable and ductile. Elemental arsenic tends to be nonductile.) In the environment, arsenic is found in inorganic and organic forms and in different valence or oxidation states. The valence states of arsenic of environmental interest are the trivalent (III) and pentavalent (V) states. Elemental arsenic has a valence state of (0). Arsine and arsenides have a valence of (–III). In this review, we will be focused on the arsenicals in the trivalent and pentavalent states that are found in the environment and to which humans are exposed. A list of relevant environmental arsenicals is shown in Table 1. The structure of some of these arsenicals is shown in Figure 1.

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**TABLE 1**  
**Arsenic Compounds of Environmental and Human Relevance**

Trivalent oxidation state	Pentavalent oxidation state
Arsenite	Arsenate
Arsenic trioxide	Arsenic pentoxide
Monomethylarsonous acid	Monomethylarsonic acid
Dimethylarsinous acid	Dimethylarsinic acid
	Trimethylarsine oxide
	Arsanilic acid
	Arsenobetaine

The most toxicologically potent arsenic compounds are in the trivalent oxidation state. This has to do with their reactivity with sulfur containing compounds and generation of reactive oxygen species (ROS). However, humans are exposed to both trivalent and pentavalent arsenicals. In this review, we will discuss in a historical context the exposure of these compounds, how we have learned that the metabolism of arsenic is a critical determinant of its toxic effects, and potential modes of action (MOA), animal carcinogenicity, and the epidemiology of this metalloid. Table 2 highlights some of the historical aspects of arsenic over the past 250 years.

#### Arsenic as an Intentional Homicidal and Suicidal Poison

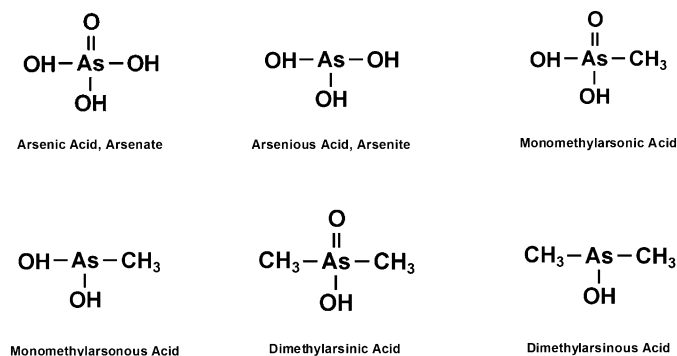
Arsenic is a naturally occurring element that an individual typically encounters every day in food, water, soil, and air. While understanding how environmental exposures may affect human health, especially at low levels, is currently an active area of research, humans have known on some level about the toxicity of arsenic for centuries.

In the Middle Ages, arsenic gained notoriety as an effective homicidal and suicidal agent, both because of the frequency of its use and because of its involvement in many high-profile murders. In fact, arsenic is often referred to as the “king of poisons” and the “poison of kings” because of its potency and the discreetness, by which it could be administered, particularly

with the intent of removing members of the ruling class during the Middle Ages and Renaissance (Vahidnia *et al.*, 2007). For example, it is well documented that arsenic was among the poisons used by the Medici and Borgia families to eradicate rivals (Cullen, 2008). Arsenic continued to enjoy its reputation as a high-profile poison and was implicated in several other prominent murder cases, most famously in the death of Napoleon Bonaparte in 1851, which some conspiracy theorists claim was a political assassination (Cullen, 2008).

Up until the mid-1850s, arsenic remained a popular poison for several reasons. Arsenic was readily available and because it is odorless and tasteless, it was undetectable in food or beverages (Bartrip, 1992). The most visible symptoms of acute arsenic poisoning—nausea, vomiting, diarrhea, and abdominal pain—could be easily confused with other common diseases at the time (e.g., cholera and pneumonia) (ATSDR, 2007b). Also, importantly, for a long time, there was no reliable analytical method for detecting, much less measuring, arsenic in tissue or other media, although early tests for arsenic were introduced in the mid-1700s. Interestingly, in the first trial ever recorded to present forensic evidence, a woman was sentenced to death because a white powder recovered by a servant was “proven” to be arsenic, based on appearance, texture, behavior in water, and garlic-like odor when burned (Caudill, 2009; Cullen, 2008). The detection of arsenic took a leap forward in 1832 when James Marsh decided to investigate analytical methods to provide juries with more reliable evidence of “visible arsenic” (Cullen, 2008). His test method was first used in the trial of Marie LaFarge in France in 1840, in which Mme. LaFarge was charged with poisoning her husband with arsenic-laden cakes (Cullen, 2008). Generally, the tests involved mixing the sample of interest with zinc and acid and heating the vessel with a flame, which would cause a silvery substance to accumulate on the glass vessel; this was considered diagnostic for arsenic in amounts as low as 0.02 mg (Marsh, 1837; Newton, 2007). Although this method would be considered primitive by today’s standards, the Marsh test represented a turning point in arsenic analytics and the beginning of the end of undetected arsenic poisonings.

Although stories of murder by arsenic appeal to the morbid interests of the public, these murders provided important insights that advanced the knowledge of arsenic toxicology. For example, information on the acute effects of arsenic and the target organs involved was obtained by studying poisonings. Importantly, these cases also precipitated the development of analytical methods for different media, including biological samples, which eventually led to an increased understanding of metabolism of arsenic. Due to improved understanding of arsenic measurement, one cannot readily “get away with murder” by using arsenic anymore. Nonetheless, incidents do still occur. As recently as 2003, arsenic poisoning made headlines when arsenic was detected in coffee served at a church meeting in Maine (Maine Rural Health, 2008; Zernike, 2003).



**FIG. 1.** Chemical structure of some relevant arsenic compounds.

**TABLE 2**  
**Timeline of Some Historic Events in the Toxicology of Arsenic**

1786	Fowler's solution (1% potassium arsenite)
1836	Marsh Test for detection of arsenic developed
1842	Dimethylarsinic acid detected in the environment
1867	Paris green (copper acetoarsenite) used as insecticide in United States
1887	Hutchison proposes arsenic is a human skin carcinogen
1910	Salvarsan used as a chemotherapeutic agent
1940s	British antilewisite is developed
1942	U.S. arsenic drinking water interim standard set at 50 µg/l
1968	Tseng <i>et al.</i> publish findings on prevalence of skin cancer in an arsenic-exposed Taiwanese population
1975	EPA adopts drinking water interim standard at 50 µg/l
1993	WHO recommends drinking water standard of 10 µg/l
1995	Dimethylarsinic acid a tumor promoter in four rat organs
1998	Dimethylarsinic acid a complete carcinogen in rat urinary bladder
2000	FDA approves arsenic trioxide for leukemia chemotherapy
2001	EPA lowers U.S. arsenic drinking water standard to 10 µg/l (ruling delayed to 2002)
2001	Inorganic arsenic a complete carcinogen in adult mice after transplacental exposure
2002	Arsenic (+3 oxidation state) methyltransferase isolated in rat liver cytosol
2010	Inorganic arsenic a complete carcinogen in adult mice after whole life exposure

### Medicinal Uses of Arsenic

Despite its toxicity—or perhaps because of it—arsenic has been used beneficially to treat certain ailments. Documented cases of arsenic as a therapeutic agent date back to before 2000 BCE (Antman, 2001; Hyson, 2007). The Father of Medicine, Hippocrates, is thought to have used an arsenic paste to treat ulcers and abscesses (Riethmiller, 2005; Waxman and Anderson, 2001). Other pioneering physicians (e.g., Aristotle and Paracelsus) are also reported to have used arsenic medicinally (Cullen, 2008; Jolliffe, 1993).

Although arsenic has been used throughout history, more detailed documentation of its use began in the late 18th century. Fowler's solution, which was discovered in 1786, is a 1% solution of potassium arsenite that was used in the treatment of various diseases, including malaria, syphilis, asthma, chorea, eczema, and psoriasis (Rohe, 1896; Scheindlin, 2005). In 1910, Paul Ehrlich introduced a new arsenic-based drug called Salvarsan, which became known as the “magic bullet” for

treating syphilis and was used until the use of penicillin became more prevalent in the 1940s (Riethmiller, 2005; Yarnell, 2005).

Arsenic also has a rich history as a cancer chemotherapeutic. As reported by Antman (2001), pharmacology texts from the 1880s described the use of arsenical pastes for the treatment of skin and breast cancer. In 1878, it was found that Fowler's solution could be effective in lowering the white blood cell count in leukemia patients (Antman, 2001, 210-9361). Although the use of Fowler's solution eventually declined over time due to its overt toxicity, a more detailed understanding of arsenic mechanism of action has allowed arsenic trioxide to emerge as an effective chemotherapeutic drug for treating acute promyelocytic leukemia (Rust and Soignet, 2001; Zhang *et al.*, 2001). With the success of this drug, the treatment of other cancers with arsenic trioxide is also being investigated (Murgo, 2001; Sekeres, 2007).

### Arsenic as a Pigment and Use in Other Products

Arsenic's use as a pigment (e.g., Paris Green or copper acetoarsenite) in the 1800s was suspected as a major source of unintentional arsenic poisonings. Although the arsenic-based pigment was used in many consumer products (e.g., toys, candles, and fabric), its use in wallpaper was particularly linked to widespread sickness and death during this period (Scheindlin, 2005; Wood, 1885). Concerns associated with the use of wallpaper containing arsenic-based pigment were reported as early as 1839, and the theory was eventually proposed that illnesses from wallpaper were related to the biotransformation of the arsenic compounds by mold to a toxic arsenic gas (Gosio gas) (Cullen and Bentley, 2005). This theory gained momentum, and in 1893, Bartolomeo Gosio, an Italian physician, demonstrated that arsenic could be volatilized from arsenic-containing compounds, including Paris Green (Buck and Stedman, 1904; Cullen and Bentley, 2005). Although it became widely accepted at the time that arsenic gas from the wallpaper was responsible for the deaths and illnesses, this notion has been challenged recently by scientists who believe that there were insufficient quantities of the gas generated (now known to be trimethylarsine) to cause the reported effects; and possibly the mold, itself, was the responsible agent (Cullen and Bentley, 2005). Regardless of the toxicity of the wallpaper, the work conducted by Gosio and later by Frederick Challenger (in the late 1930s), laid the groundwork for today's understanding of arsenic metabolism, namely that the metabolism of arsenic involves sequential reduction and oxidative methylation steps (Cullen and Bentley, 2005).

Although arsenic use has been phased out of pigment products, it is still used in the production of glass and semiconductors (ATSDR, 2007b).

### Arsenic as a Pesticide

Although it was recognized that the arsenic used in pigments could be toxic to humans, Paris Green was used as an

insecticide from 1867 to 1900; it was effective in controlling Colorado potato beetles and mosquitoes (Cullen, 2008; Peryea, 1998). In the late 1800s, another arsenic-based pesticide, lead arsenate, became extensively used; it was an effective pesticide but was less toxic to plants than Paris Green (Peryea, 1998). Lead arsenate was widely used as a pesticide for apple and cherry orchards through the early 1900s. By 1960, most uses of lead arsenate were phased out after it was recognized that its use was associated with health effects in orchard workers and an increasing concern that arsenic residues on fruits were a public health concern (Frisbie, 1936; Nelson *et al.*, 1973). The official United States ban in lead arsenate use did not take place until 1988 (Peryea, 1998).

Up to the early 1900s, concerns about arsenic toxicity had focused on the acute effects of arsenic; the use of arsenic in insecticides furthered the understanding of how low levels of exposure over longer periods might affect public health. Studies in orchard workers were some of the first to propose a link between arsenic exposure and cancer (lung cancer in this case) (Mabuchi *et al.*, 1980; Nelson *et al.*, 1973; Tollestrup *et al.*, 1995). Also, because arsenic was released to the environment in concentrated amounts, the use of arsenic-based pesticides helped develop knowledge of arsenic's fate and transport (Peryea, 1998).

The use of lead arsenate pesticides has been effectively eliminated for over 50 years. However, because of the pesticide's environmental persistence, it is estimated that millions of acres of land are still contaminated with lead arsenate residues. This presents a potentially significant public health concern in some areas of the United States (e.g., New Jersey, Washington, and Wisconsin), where large areas of land used historically as orchards have been converted into residential developments (Hood, 2006).

Some modern uses of arsenic-based pesticides still exist. Chromated copper arsenate (CCA) has been registered for use in the United States since the 1940s as a wood preservative, protecting wood from insects and microbial agents. In 2003, CCA manufacturers instituted a voluntary recall of residential uses of CCA-treated wood. CCA is still approved for use in nonresidential applications, such as in marine facilities (pilings and structures), utility poles, and sand highway structures (U.S. EPA, 2008; WPSC, 2008).

The use of organic arsenical pesticides began in the 1950s and has continued into the present day. Overall, organic arsenicals in the pentavalent oxidation state are much less toxic than inorganic arsenicals because, unlike inorganic arsenic, these ingested organic arsenicals are not readily taken up into cells and undergo limited metabolism (Cohen *et al.*, 2006). Key pesticides based on organic arsenicals include monosodium methanearsonate (MSMAs<sup>V</sup>) and dimethylarsinic acid (DMAs<sup>V</sup>), also known as cacodylic acid. The use of these compounds as pesticides has given scientists the opportunity to comparatively study organic and inorganic arsenic toxicity and carcinogenicity, including investigations on metabolites

of organic and inorganic arsenic compounds (e.g., the trivalent methylated arsenic compounds). In particular, the study of cacodylic acid has helped elucidate a potential mode of carcinogenic action for arsenic. In light of this, the U.S. Environmental Protection Agency (EPA) (2006) chose not to evaluate DMAs<sup>V</sup> as carcinogenic to humans in the reregistration decision for this pesticide (U.S. EPA, 2006). Similarly, in 2007, a U.S. EPA Science Advisory Board (SAB) determined that DMAs<sup>V</sup> had the potential to cause cancer in humans only when doses are high enough to cause bladder cell cytotoxicity (U.S. EPA, 2007). Some uses of these compounds as herbicides were discontinued in 2009 (U.S. EPA, 2009). At that time, EPA reregistered MSMAs<sup>V</sup> for use on cotton but decided to stop the use of it on golf courses, sod farms, and right-of-ways in 2013. However, EPA agreed to reevaluate new information on the carcinogenicity of inorganic arsenic, the environmental degradation product of the organic arsenical products in 2012. EPA will consider the results of the reevaluation in make decisions regarding new applications for registration of MSMAs<sup>V</sup>.

### Copper Smelting

Studies of orchard workers have provided a basis for understanding some of the long-term effects of occupational exposure to arsenic. However, occupational exposure studies in the copper smelting industry are much more extensive and have established definitive links between arsenic, a by-product of copper smelting, and lung cancer via inhalation (e.g., Enterline *et al.*, 1995; Jarup *et al.*, 1989; Pinto *et al.*, 1977; Welch *et al.*, 1982). Dermal and neurological effects were also increased in some of these studies (Dunlap, 1921; Feldman *et al.*, 1979; Lagerkvist *et al.*, 1986). Although exposures to arsenic changed over time (i.e., as time went on, occupational controls became more stringent and workers were exposed to reduced arsenic concentrations), the arsenic exposures measured from these studies ranged from about 0.05 to 0.3 mg/m<sup>3</sup> and are significantly higher than airborne environmental exposures to arsenic (which range from 0 to 0.000003 mg/m<sup>3</sup>) (ATSDR, 2007b; European Commission, 2000). The pathway of exposure for these workers was mainly *via* inhalation of arsenic dusts but could also be from arsenic trioxide vapors (Yager *et al.*, 1997).

Communities near smelters have also been studied (Marsh *et al.*, 1997; Tollestrup *et al.*, 2003). Exposure in these studies was often not well characterized but might have been *via* inhalation or from the ingestion of arsenic-contaminated soil, depending on the activity of the smelter. Overall, no clear association has been established between environmental exposure to arsenic and adverse health effects in communities residing near smelters (as reviewed in European Commission, 2000).

## Arsenic in the Environment

Historical uses of arsenic in products, pharmaceuticals, and industry have led to important insights on arsenic's toxicity. In the modern era, most interest in arsenic toxicology comes from naturally occurring background exposures in food, water, and soil. Understanding the environmental levels that can cause a public health concern is a key area of research.

### *Arsenic in Drinking Water*

Arsenic found in water is almost entirely in the inorganic form and can be stable as both arsenite and arsenate, trivalent and pentavalent inorganic arsenicals, respectively (Saxe *et al.*, 2006). The U.S. Geological Survey estimates that the median groundwater concentration is 1  $\mu\text{g/l}$  or less, although some groundwater aquifers, particularly in the western United States, can contain much higher levels (Focazio *et al.*, 1999). For example, median levels in Nevada were about 8  $\mu\text{g/l}$  (Focazio *et al.*, 1999) but levels of naturally occurring arsenic as high as 1000  $\mu\text{g/l}$  have been measured in the United States in drinking water (Lewis *et al.*, 1999; Steinmaus *et al.*, 2003).

Several regions outside the United States also have high levels of arsenic in groundwater, averaging several hundred  $\mu\text{g/l}$ , resulting in significant human exposures *via* drinking water (for review, see Nordstrom, 2002). An extensive body of research studying the health effects associated with arsenic in drinking water both within and outside the United States has been published. It is through these studies that ingestion of arsenic has been definitively linked to increased incidence of cancer in lung, bladder, skin, kidney, liver, and potentially prostate. A number of noncancer effects also are linked to exposure in drinking water, including skin lesions, cardiovascular disease, neurological effects, and diabetes (ATSDR, 2007b; NRC, 1999, 2001). A historical perspective on the important contributions that drinking water studies have made to our understanding of oral chronic toxicity from arsenic is described in more detail later in this paper.

### *Arsenic in the Diet*

Although inorganic arsenic was added to food as a preservative in the late 1800s and early 1900s, today, inorganic arsenic is not intentionally added to food (Buck and Stedman, 1904; Taylor, 1873). Nonetheless, because arsenic is ubiquitous in the environment, diet is the largest source of both inorganic and organic arsenic for typical individuals. Estimates of dietary inorganic arsenic intakes vary. In the United States, Schoof *et al.* (1999) estimated an average adult intake of 3.2  $\mu\text{g/day}$ , with a range of 1–20  $\mu\text{g/day}$ . Estimates for children were similar (Yost *et al.*, 2004). Recently, the European Food Safety Authority (EFSA) estimated a higher intake level, although estimates depended on simplifying assumptions regarding the ratio of inorganic arsenic to total arsenic in food. The analysis estimated a typical intake of 0.13–0.56  $\mu\text{g/kg/day}$

for average consumers (9.1–39.2  $\mu\text{g/day}$  for a 70 kg adult) (EFSA, 2009).

The possibility that arsenic is an essential nutrient has received some research attention, especially in the 1970s and 1980s, although some interest extends into the present day (Uthus, 1992, 2003). In 1988, the U.S. EPA convened a scientific panel to specifically evaluate the potential essentiality of arsenic (U.S. EPA, 1988). Based on an extensive review of the literature, this panel concluded, "information from experimental studies with rats, chicks, minipigs, and goats demonstrates the plausibility that arsenic, at least in inorganic form, is an essential nutrient." Since 1988, very little new research on the essentiality of arsenic has been conducted. In 1999, a National Research Council report stated that essentiality of arsenic in humans had not been tested to date and there was no known biochemical process for which arsenic was essential (NRC, 1999). Later, an EPA arsenic science advisory panel cited similar arsenic essentiality evidence as the 1988 panel and determined that arsenic essentiality is in "need of further research" (U.S. EPA, 2007).

Food also contains many organic arsenic compounds, which are generally considered to have low toxicity, although toxicity does vary among the individual compounds. Developing analytical methods to identify these compounds has been important for distinguishing these compounds from the more toxic inorganic forms. The key organic arsenic compounds that can be routinely found in food (depending on food type) include monomethylarsonic acid (MMAs<sup>V</sup>), DMAs<sup>V</sup>, arsenobetaine, arsenocholine, arsenosugars, and arsenolipids. DMAs<sup>V</sup> or MMAs<sup>V</sup> can be found in various types of fin fish, crabs, and mollusks, but often at very low levels (Borak and Hosgood, 2007). Arsenobetaine is the major form of arsenic in marine animals, and, by all accounts, it is considered a compound that is nontoxic under conditions of human consumption (ATSDR, 2007b; EFSA, 2009). Although arsenobetaine is little studied, available information indicates it is not mutagenic, immunotoxic, or embryotoxic (Borak and Hosgood, 2007). Arsenocholine, which is mainly found in shrimp, is chemically similar to arsenobetaine, and is considered to be "essentially nontoxic" (ATSDR, 2007b).

Arsenosugars and arsenolipids have recently been identified. Exposure to these compounds and toxicological implications are currently being studied. Arsenosugars are detected mainly in seaweed but are also found to a lesser extent in marine mollusks (EFSA, 2009). Concerns about the potential toxicity of arsenosugars have been raised because there is evidence that arsenosugars are metabolized to DMAs<sup>V</sup> (Andrewes *et al.*, 2004). Studies addressing arsenosugar toxicity, however, have largely been limited to *in vitro* studies, which show that arsenosugars are significantly less toxic than both inorganic arsenic and trivalent methylated arsenic metabolites (Kaise *et al.*, 1996). Arsenolipids, which are a component of fish oil, have only been recently characterized; their toxicity has not been studied (Schmeisser *et al.*, 2006).

### Arsenic in Soil

The natural content of arsenic in soils globally ranges from 0.01 to over 600 mg/kg, with an average of about 2–20 mg/kg (Yan-Chu, 1994). In the United States, a nationwide survey conducted in areas that were judged not to have anthropogenic sources of arsenic reported that natural background concentrations in soil ranged from less than 1 to 97 mg/kg (Shacklette and Boerngen, 1984). Arsenic in soil is almost entirely in the inorganic form, except in areas with intentional organic arsenic application, where higher levels of organic compounds can be found (Saxe *et al.*, 2006). In soils, pentavalent arsenic predominates due to oxidation of trivalent arsenicals (Gong *et al.*, 2001).

Exposure to arsenic in soil can occur through multiple pathways. Incidental ingestion is typically the most significant exposure pathway for soil. Compared with the intake of naturally occurring arsenic from water and the diet, soil arsenic constitutes only a small fraction of intake (Petito Boyce *et al.*, 2008); this is a reflection of the relatively small amounts of inorganic arsenic in soil that is typically ingested on a daily basis as well as the reduced bioavailability of arsenic in soil compared with water. Overall, a large number of studies have shown that the relative oral bioavailability of arsenic in soils to be less than 50% (Roberts *et al.*, 2002).

Other potential exposure pathways for soil arsenic include dermal absorption and inhalation of wind-blown soil particles (i.e., fugitive dust). However, arsenic is not readily absorbed through the skin from soil (U.S. EPA, 2001), and, as discussed below, the amount of arsenic measured in ambient air is low.

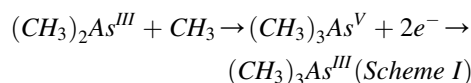
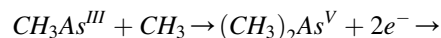
### Arsenic in Air

Compared with arsenic exposure from food and water, exposure to arsenic in air, which is almost entirely as inorganic arsenic, is generally very low. The European Commission (2000) reports that levels of arsenic in air range 0–1 ng/m<sup>3</sup> in remote areas, 0.2–1.5 ng/m<sup>3</sup> in rural areas, 0.5–3 ng/m<sup>3</sup> in urban areas, and up to about 50 ng/m<sup>3</sup> in the vicinity of industrial sites. Based on these data, the European Commission (2000) estimated that in relation to food, cigarette smoking, water, and soil, air contributes less than 1% of total arsenic exposure, even when assuming an arsenic air exposure that is significantly above typical background (i.e., 20 ng/m<sup>3</sup>).

## Arsenic Metabolism

Study of the metabolism of inorganic arsenic in mammals is rooted in 19th century studies of arsenic metabolism in microorganisms (Cullen, 2008). As discussed above, identification of Gosio gas as trimethylarsine, a volatile product emitted by microorganisms exposed to inorganic arsenic, presaged work on microbial conversion of inorganic arsenic into methylated metabolites. Based on these studies, Challenger

(1947, 1951) provided a chemically plausible scheme (I) for the methylation of inorganic arsenic.



(As<sup>V</sup>, arsenate; As<sup>III</sup>, arsenite; CH<sub>3</sub>As<sup>V</sup>, monomethylarsonic acid; CH<sub>3</sub>As<sup>III</sup>, monomethylarsonous acid; (CH<sub>3</sub>)<sub>2</sub>As<sup>V</sup>, dimethylarsinic acid; (CH<sub>3</sub>)<sub>2</sub>As<sup>III</sup>, dimethylarsinous acid; (CH<sub>3</sub>)<sub>3</sub>As<sup>V</sup>, trimethylarsine oxide; (CH<sub>3</sub>)<sub>3</sub>As<sup>III</sup>, trimethylarsine).

For Challenger's scheme of arsenic biotransformation (i.e., alternate steps involving reduction of arsenic from pentavalency to trivalency and subsequent oxidative methylation of trivalent arsenicals) to move from a plausible theory to a well-accepted biological principle, a variety of chemical and biochemical approaches were explored. Over the past half century, research has revealed a remarkable unity in patterns of arsenic metabolism in organisms as diverse as simple prokaryotes and members of the deuterostomal superphylum, including humans. Elucidating the pathway for arsenic metabolism has provided new insights into production of metabolites of inorganic arsenic. Advancements in our understanding suggest that these metabolites may mediate some of the toxic and carcinogenic effects associated with exposure to inorganic arsenic. From these efforts has emerged a fuller picture of the complex relations between the metabolism and toxicity of arsenic and the role that interindividual variation in capacity to metabolize arsenic may play as a determinant of risk. Continuing exploration of the metabolic pathway will likely provide more insights into the linkage of metabolism and toxicity. Here, we review our evolving understanding of arsenic metabolism over the past 5 decades and describe some of the challenges for future research.

The possibility that inorganic arsenic undergoes chemical conversion in higher organisms was noted at least 60 years ago in a study that found "discrepancies in analytical data" in mice and rats treated with arsenic-containing water and sludge samples (Clements *et al.*, 1951). Because analysis of arsenic in tissues and excreta by then-standard colorimetric assays did not account for all the administered arsenic, the authors conjectured that some of the missing mass of arsenic might be present in tissues and excreta in previously unknown and undetected forms. Improvement in analytical techniques over the next 2 decades made possible detection of methylated arsenicals in environmental samples and human urine. Braman and Foreback (1973) used hydride generation–cryotrapping–column chromatography–atomic absorption spectrometry to demonstrate that natural waters and human urine contained both inorganic and organic (methylated) arsenicals. In this study of

four individuals, inorganic arsenic accounted for about 25%, methyl arsenic accounted for about 8%, and dimethyl arsenic for about 66% of the total arsenic in urine. Remarkably, despite the many refinements and improvements in analytical techniques for arsenic speciation over intervening years, these estimates of fractional distribution of arsenic metabolites in urine are consistent with currently reported values. Using a single volunteer, Crecelius (1977) explored the metabolism of arsenic after ingestion of wine or water that contained inorganic arsenic. After ingestion of wine or water, the concentration of inorganic arsenic in urine peaked within 24 h; peak concentrations of methyl and dimethyl arsenic in urine occurred at later time points.

The temporal pattern for the appearance of inorganic and methylated arsenicals in urine reported by Crecelius was broadly compatible with the pattern predicted by the Challenger scheme, suggesting that internal dosimetry of inorganic arsenic and its metabolites would follow a similar pattern. These findings were the impetus for a large body of work on the distribution, retention, and clearance of arsenicals in humans and other species. These dosimetric data have supported development of several pharmacokinetic models for humans and other species (El-Masri and Kenyon, 2008; Evans *et al.*, 2008; Gentry *et al.*, 2004; Mann *et al.*, 1996a, 1996b; Yu, 1999). Such models are useful for estimating tissue exposures and can be used in risk assessment (Liao *et al.*, 2008).

Three decades ago, the biochemical reactions underlying arsenic methylation were unknown. Braman and Foreback (1973) suggested that methylation of arsenic was due to "methylcobalamin-methionene (*sic*) reactions in the body." This hypothesis reflected then-current ideas about methylation of metals and metalloids as exemplified by studies of microbial methylation of inorganic mercury (Landner, 1972). Wood (1974) formally stated this chemical scheme for arsenic methylation in a review of toxic element methylation processes.

Early work on formation of methylated arsenicals in mammals used tissue homogenates and subcellular fractions to explore the requirements for *in vitro* methylation. Buchet and Lauwerys (1985) showed addition of S-adenosylmethionine (AdoMet) and glutathione (GSH) to reaction mixtures containing rat liver cytosol supported conversion of inorganic arsenic to methylated products. Focus on AdoMet as a methyl group donor for arsenic methylation was consistent with the then-contemporary finding that enzymatically catalyzed methylation of selenium used AdoMet for methyl group donation (Mozier *et al.*, 1988). Thus, by the late 1980's, it was possible to define the operational characteristics of the enzyme that catalyzed the conversion of inorganic arsenic to methylated metabolites. It was likely to be a cytosolic AdoMet-dependent methyltransferase that required the presence of a reductant. Although it was unclear whether the reductant protected the enzyme against oxidation or functioned as a cofactor in enzyme catalysis, an absolute requirement for a reductant imposed a number of limitations on any strategy used to isolate and characterize the protein.

Purification of a mammalian arsenic methyltransferase was the object of intensive research in the 1990s. In a series of studies summarized by Aposhian (1997), a ~60 kDa protein purified from rabbit liver cytosol catalyzed AdoMet-dependent methylation of arsenite and monomethylarsonous acid (MMAs<sup>III</sup>). The presence of a monothiol (e.g., cysteine, GSH) or a dithiol (e.g., dithiothreitol) was required for these activities (Zakharyan *et al.*, 1999). In related work, Lin *et al.*, (2002) purified a ~43 kDa protein from mouse liver cytosol that catalyzed AdoMet-dependent methylation of arsenite and MMAs<sup>III</sup>. A partial amino acid sequence of this protein showed that it was the product of the *cyt19* gene previously annotated as a methyltransferase of unknown function. Subsequently, this mouse gene was annotated as arsenic (+3 oxidation state) methyltransferase (*As3mt*) and cloned (Walton *et al.*, 2003). Mouse *As3mt* proved to be a prototype for arsenic methyltransferases in many genomes. Genes encoding proteins closely related to mouse *As3mt* occur in genomes of organisms ranging from sea squirts to humans (Thomas *et al.*, 2007). These proteins share conserved sequences commonly found in nonnucleic acid methyltransferases and cysteinyl residues required for catalytic function as an arsenic methyltransferase (Fomenko *et al.*, 2007). Given differences in molecular masses of proteins isolated from rabbit liver and mouse liver, they are unlikely to be identical. In the absence of full sequencing of the rabbit liver protein, it has proven difficult to pursue the characteristics of this putative arsenic methyltransferase. Indeed, subsequent research has focused on the role of *As3mt* and its protein products in the metabolism of arsenicals.

Although the presence of GSH in buffers preserved enzymatic activity during purification, dithiol-containing reductants (thioredoxin, Tx; glutaredoxin, Gx; reduced lipoic acid, LA) were found to support high rates of catalysis *in vitro* by recombinant rat *As3mt* (Waters *et al.*, 2004b). Although GSH modulated production of mono-, di-, and trimethylated arsenicals from inorganic arsenic, it could be replaced by Tx, Gx, or LA, indicating that its presence was not required for catalysis (Waters *et al.*, 2004a). These findings suggested that redox cycling of physiological dithiol-containing reductants was required for catalytic activity of *As3mt*. Because all intermediates predicted by the Challenger scheme, including methylated arsenicals containing trivalent or pentavalent arsenic, can be found in reaction mixtures with recombinant rat *As3mt*, a Tx/Tx reductase/NADPH cycling system, and AdoMet, *As3mt* may catalyze both oxidative methylation of trivalent arsenic-containing substrates and reduction of pentavalent arsenic in methylated products. The fusing of two intimately related catalytic functions in one enzyme would assure that metabolic transformation proceeded without accumulation of reactive and toxic intermediates. Notably, there is evidence for separate pathways for reduction of pentavalent arsenic to trivalent arsenic in mammalian cells. For example, GSH transferase omega (GSTO) catalyzes *in vitro* reduction of monomethylarsonic acid (MMAs<sup>V</sup>) to MMAs<sup>III</sup>

(Zakharyan *et al.*, 2001). Whether GSTO contributes significantly to arsenic reduction in intact organisms is unclear; studies in GSTO knockout mice failed to detect differences in patterns of arsenic methylation and excretion (Chowdhury *et al.*, 2006). Phosphorylative-arsenolytic enzymes such as purine nucleoside phosphorylase (PNP) can function as catalysts in reaction schemes in which an activated arsenate ester is reduced by thiols to arsenite (Gregus *et al.*, 2009; Nemeti *et al.*, 2010). Existence of several mechanisms for reduction of pentavalent arsenic could be an example of functional redundancy in which independent processes mediate a single important biochemical reaction (Wang and Zhang, 2009). Understanding interrelations between catalysis of methylation and of reduction in the cell will be critical to more fully comprehend the molecular basis for arsenic metabolism.

Studies in which altered *As3mt* expression leads to changes in capacity for arsenic methylation confirm its critical role. Heterologous expression of rat *As3mt* in human uroepithelial cells that do not methylate arsenic confers capacity to methylate arsenic (Drobna *et al.*, 2005). Silencing of *AS3MT* expression in human hepatoma cells by RNA interference diminishes, but does not eliminate, capacity to methylate arsenic (Drobna *et al.*, 2006). The fate of arsenic differs radically in *As3mt* knockout mice and wild-type mice. Following single or repeated oral doses of arsenate, tissues from *As3mt* knockout mice attain higher concentrations of inorganic arsenic than do those from wild-type mice and the rate of whole body clearance of arsenic is much slower in knockout than in wild-type mice (Drobna *et al.*, 2009; Hughes *et al.*, 2010). A lower rate of whole body clearance of inorganic arsenic and its methylated metabolites in *As3mt* knockout mice is consistent with earlier findings that methylated and dimethylated arsenic clear faster than inorganic arsenic (Buchet *et al.*, 1981; Marafante *et al.*, 1987). These phenotypic changes in *As3mt* knockout mice are also associated with increased susceptibility to damage to the uroepithelial cells following exposure to inorganic arsenic (Yokohira *et al.*, 2010).

Capacity to produce methylated metabolites of inorganic arsenic can depend on environmental or genetic factors. For example, environmental factors such as selenium nutrition or protein and lipotrope deficiency affect patterns of urinary metabolites in mice treated with inorganic arsenic (Kenyon *et al.*, 1997; Vahter and Marafante, 1987). Similarly, there is evidence that genotypic variation in the *AS3MT* gene in humans alters the capacity to methylate inorganic arsenic. Changes in the capacity to methylate inorganic arsenic can be referred to as the arsenic methylation phenotype. Thus, it is possible to look for connections between *AS3MT* genotype and arsenic methylation phenotype.

A linkage between *AS3MT* genotype and arsenic methylation phenotype was first noted in a study of arsenite metabolism by cultured primary human hepatocytes (Drobna *et al.*, 2004). Sequencing of cDNA from one of eight hepatocyte donors detected a single missense allelic mutation in exon 9 that would

change amino acid 287 in wild-type *AS3MT* from methionine to threonine (M287T), indicating that this donor was a heterozygote expressing both wild-type *AS3MT* and M287T *AS3MT*. This genotypic difference was associated with an altered arsenic methylation phenotype. Cells from the affected donor showed a higher capacity for production of monomethylarsenic at medium concentrations of arsenite compared with cells from donors homozygous for wild-type *AS3MT* (which showed reduced mono- and dimethylarsenic production). In populations worldwide, the nonsynonymous single nucleotide polymorphism (SNP) for the M287T mutation occurs at an allele frequency of 0.01 to 0.1 (Fujihara *et al.*, 2007; Wood *et al.*, 2006). Hence, under Hardy-Weinberg conditions, the frequency of *AS3MT* heterozygotes (wild-type/M287T) would range from 0.02 to 0.18 and the frequency of M287T *AS3MT* homozygotes from 0.0001 to 0.01. COS-1 cells expressing M287T *AS3MT* had 350% higher levels of arsenic methylation activity and 190% higher levels of immunoreactive *AS3MT* than did cells expressing wild-type *AS3MT* (Wood *et al.*, 2006). This finding suggested that regulation of *AS3MT* expression or turnover of *AS3MT* was affected by the M287T mutation.

Population-based studies have shown that M287T genotype and genotype differences at other exonic and intronic sites affect arsenic methylation phenotype. Compared with the wild-type genotype, M287T genotype is associated with a significant increase in the odds ratio for percentage of urinary arsenic present as % monomethylarsenic (%MMAs) in men working in a copper smelter (Hernandez *et al.*, 2007, 2008). A similar increase in %MMAs was associated with the M287T genotype in men, but not women, who were exposed to inorganic arsenic from drinking water and food (Lindberg *et al.*, 2007). An altered sequence in exon 1 encoding part of the 5' untranslated region of variable number tandem repeats is associated with a significant increase in the odds ratio for percentage of urinary arsenic present as monomethylarsenic in men working in a copper smelter (Hernandez *et al.*, 2007). In contrast to the effects of exonic changes, several sequence changes in introns 6, 7, and 9 of *AS3MT* result in decreased %MMAs and increased % dimethylarsenic in urine compared with those found in individuals with wild-type *AS3MT* (Agusa *et al.*, 2009; Chung *et al.*, 2009; Schläwicke Engström *et al.*, 2007).

The concept of genotype-phenotype correlations can be extended to determine if changes in *AS3MT* genotype can be linked to evidence of altered susceptibility to adverse health effects associated with chronic exposure to inorganic arsenic. In a Mexican population that chronically uses drinking water contaminated with inorganic arsenic, M287T *AS3MT* genotype was associated with significantly increased %MMAs (Valenzuela *et al.*, 2009). A marginal difference ( $p < 0.055$ ) was found in frequencies of the M287T genotype and occurrence of pre-malignant skin lesions, suggesting that a genotype change associated with a change in the arsenic methylation profile could be linked to increased susceptibility to a type of skin lesion



commonly associated with chronic exposure to arsenic. Another study in this Mexican population found that the percentage of DNA damage in peripheral blood leukocytes (PBLs) of children (measured by the comet assay) and the level of arsenic exposure was significantly influenced ( $p < 0.034$ ) by M287T genotype (Sampayo-Reyes *et al.*, 2010). A follow-up study in a Taiwanese population that examined the relation between urinary profiles of arsenicals and cancer risk over a 15 year period during which exposure to inorganic arsenic decreased found associations between earlier cancer incidence and higher baseline urinary %MMAs or smaller temporal change in urinary %MMAs (Chung *et al.*, 2009). This finding suggests that persistently high levels of monomethylarsenic in urine related to the M287T genotype are associated with increased cancer risk. Taken together, these studies show that the M287T genotype that affects the arsenic methylation phenotype can also be associated with increased risk of either noncancer or cancer health effects. Additional studies will show whether other *AS3MT* polymorphisms produce these or other phenotypic effects.

The effects of polymorphisms in *AS3MT* should be evaluated in the context of polymorphisms in other genes that may affect the arsenic methylation phenotype or influence susceptibility to the adverse health effects associated with chronic exposure to inorganic arsenic. Polymorphisms in two genes encoding enzymes used in AdoMet production, methylenetetrahydrofolate reductase, and cystathione  $\beta$  synthase, affect the profile of arsenicals found in urine (Porter *et al.*, 2010; Steinmaus *et al.*, 2007). An exon 3 polymorphism in PNP, a putative arsenate reductase, increases the risk of skin cancer in individuals exposed to inorganic arsenic in drinking water (De Chaudhuri *et al.*, 2008). Associations have been identified between polymorphisms in several genes encoding members of the GSH transferase (GST) family and urinary metabolite profiles or risk for arsenic-induced cancers (Lin *et al.*, 2007; McCarty *et al.*, 2007a, 2007b; Paiva *et al.*, 2010; Wang *et al.*, 2009). Although the single-gene strategy for identifying modifiers is effective, a more heuristic approach to understanding the role of genes in control of arsenic metabolism and toxicity has looked for haplotypes associated with altered metabolism or response. For example, three SNPs in *AS3MT* are in linkage disequilibrium (LD) and associated with an altered arsenic methylation phenotype (Meza *et al.*, 2005). A LD cluster on human chromosome 10 near the *AS3MT* locus indicates that several genes may be acting to modify the arsenic methylation phenotype or to alter susceptibility (Gomez-Rubio *et al.*, 2010). Further studies of gene associations and of the role of members of the LD cluster will be needed to identify the modifiers.

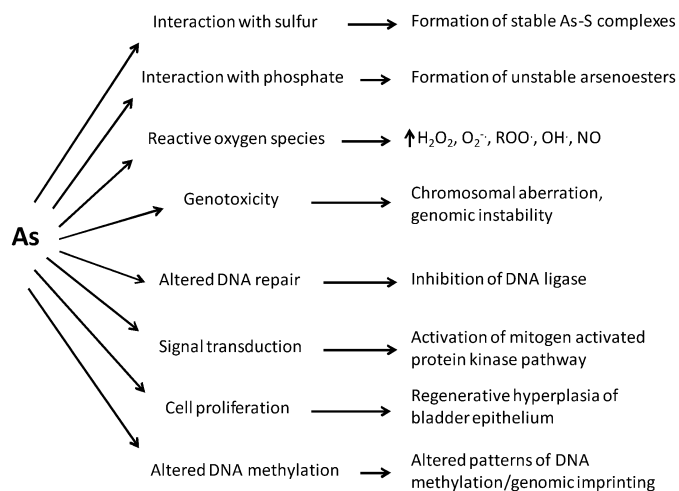
Although this review has focused on metabolism of arsenicals mediated by the host organism, particularly the central role of *AS3MT* as a determinant of metabolic capacity and susceptibility, the organisms that compose the microbiome of the gastrointestinal tract also have the capacity to convert inorganic arsenic to methylated species. The microbiome of the human gastrointestinal tract consists of nearly 10-times as

many cells as does the body of the host and contains about 3-times as many genes as does the human genome (Zhu *et al.*, 2010). Given the size and genetic diversity of organisms of the gut microbiome, it is probably not surprising that a complex interaction has evolved between these cells and cells that constitute the host component of the gastrointestinal system (Spor *et al.*, 2011). Evidence for a role of the microbiota of the gastrointestinal tract in the metabolism of arsenicals comes primarily from *in vitro* studies in which the microbiota of the mouse cecum is incubated under strictly anaerobic conditions with arsenicals. Studies with arsenate and DMAs<sup>V</sup> found that these arsenicals were rapidly converted to methylated metabolites (Kubachka *et al.*, 2009; Pinyayev *et al.*, 2011). In addition to oxyarsenical metabolites, the products of these reactions included thioarsenicals in which O is replaced with S. The prevalence of thioarsenicals among the products of metabolism of anaerobic microorganisms presumably reflects low O tension and the abundance of H<sub>2</sub>S in cultures. In terms of risk, the significance of metabolism of arsenic by the microbiota of the gastrointestinal tract (preabsorptive metabolism) depends on whether the bioavailabilities of the metabolites are materially different from those of the parent compounds. Additional studies are needed to resolve this issue.

Over the past half century, the study of arsenic metabolism has progressed from descriptive studies of kinetic behavior of inorganic arsenic and its metabolites to an effort to understand the molecular basis of metabolism and the interactions among genes that affect the capacity for metabolism and modify the biological response to this arsenic. It has recently been suggested that arsenic is a toxicant that could profitably be studied by newly available techniques in genomics, metabolomics, and proteomics (Vlaanderen *et al.*, 2010). It will be interesting to see what new insights these technologies will bring to our understanding of arsenic metabolism and toxicity.

### Arsenic Mode of Action

The precise MOA for the many disease endpoints following acute and chronic arsenic exposure are not known, although research in this area has been ongoing for many years. A clear understanding of the MOA for arsenic, indeed for any toxic chemical, will facilitate selection of the appropriate human risk assessment model (e.g., linear vs. nonlinear). The most appropriate dose-response model for low environmental exposures to arsenic is the subject of debate. It is likely that several MOAs account for the adverse effects of arsenic; indeed, these MOAs may be interdependent. Although inorganic arsenic's MOA for all disease endpoints is not fully elucidated, it is apparent that there are clear differences in the toxicities of arsenicals, largely due to valence state. Trivalent arsenicals (e.g., arsenite, monomethylarsonous acid, dimethylarsinous acid) are more potent toxicants than pentavalent arsenicals (e.g., arsenate, monomethylarsonic acid, dimethylarsinic acid). Several review articles have been published



**FIG. 2.** Proposed MOA for arsenic and examples of biochemical effects that result from this action.

recently on the MOA for arsenic (Druwe and Vaillancourt, 2010; Kitchin and Conolly, 2010; Kitchin and Wallace, 2008; Kumagi and Sumi, 2007; Platanius, 2009; Rossman, 2003; Schoen *et al.*, 2004; Schumacher-Wolz *et al.*, 2009; Tapio and Grosche, 2006). Several proposed MOA for arsenic and examples of the biochemical effects that occur are shown in Figure 2.

### Interactions with Sulfur

The chemistry of arsenic is an important aspect of its MOA. Observations in the late 19th century noted that arsenic exists in the body in pentavalent and trivalent oxidation states and that it interacts with sulfur (Parascandola, 1977). One of the first proposed MOAs for arsenic, suggested by Binz and Schulz in 1879 (Parascandola, 1977), was the interference of cellular oxidation from the cycling of oxygen during the interconversion of arsenate and arsenite. This would suggest that both arsenicals are equally potent, but as it became apparent, arsenite is more potent than arsenate, so this proposal was soon disfavored.

Early mechanistic studies centered on an aromatic arsenic compound used to treat syphilis and trypanosomiasis. This compound, Salvarsan, was synthesized by Paul Ehrlich's laboratory in the early 1900s and was the first man-made antibiotic. (See Riethmiller, 2005 for a review of the discovery of Salvarsan and Lloyd *et al.*, 2005 for determination of its structure.) Ehrlich proposed that the chemotherapeutic (toxic) effect of Salvarsan involved its binding to a chemoreceptor in the microorganism and that this receptor might contain a hydroxyl or sulfhydryl group (Parascandola, 1977). Carl Voegtlin and colleagues conducted a series of studies investigating the MOA of Salvarsan and similarly structured arsenicals (reviewed in Voegtlin (1925)). They determined that the most potent arsenicals were in the trivalent arsenious oxide form (R-As=O, 3-amino-4-hydroxyphenyl arsenious oxide, "arsenoxide") and that Salvarsan must be converted to this in

the body to be therapeutically effective. Voegtlin *et al.* (1923), knowing that arsenic reacts chemically with sulfur, investigated the effect of a recently found protein-like substance with a sulfhydryl group, "glutathione," on the trypanocidal effect of arsenoxide (note at this time, the glutathione used was a dipeptide containing glutamic acid and cysteine). *In vitro* and *in vivo* studies showed that glutathione and other sulfhydryl compounds such as cysteine and thioglycollate antagonized the trypanocidal effect of arsenoxide. Chemicals without a sulfhydryl group, such as glucose, were ineffective. It was later determined that trivalent arsenic, not pentavalent arsenic, binds to "fixed" sulfhydryl groups of tissue proteins (Peters, 1949; Rosenthal, 1932; Voegtlin *et al.*, 1923). Voegtlin *et al.* (1923) hypothesized that the MOA for Salvarsan involved a chemical binding between arsenoxide and the sulfhydryl group of glutathione or other cellular sulfhydryl compounds.

A general hypothesis in the 1920s and 1930s was that toxic chemicals selectively inhibited enzymes, which would lead to a pathologic effect. Rudolph Peters and colleagues (Peters *et al.*, 1945), at the outset of World War II, were tasked to find an antidote for the arsenical warfare agent,  $\beta$ -chlorovinyl-dichloroarsine, also known as lewisite. Peters *et al.* (1945) had hypothesized that the pyruvate oxidase system was sensitive to arsenicals and that sulfhydryl groups within this system were attacked. Previously, Cohen *et al.* (1931) had shown that aromatic arsenicals bound to sulfhydryl groups were dissociable, which suggested the removal of arsenic from enzymes was achievable, thus decreasing its toxic effect. It was thought that excess monothiols could reverse the effect of arsenic on pyruvate oxidation. However, monothiols such as glutathione were ineffective antagonists. Stocken and Thompson (1946) incubated kerateine, a form of keratin with the disulfide linkages reduced (forming free sulfhydryl groups) with lewisite and arsenite. It was determined that the arsenic in lewisite was bound to two thiol groups, forming a stable 5-membered ring, whereas arsenite was bound to only one sulfhydryl group. From this work, a dithiol antidote to lewisite, 2,3-dimercapropanol (British anti-lewisite, BAL) was developed. Later it was determined that in the pyruvate oxidase system (later termed the pyruvate dehydrogenase complex [PDH]), that lipoic acid, which contains vicinal dithiols, was the sensitive moiety to which arsenicals would bind. Although arsenite can inhibit PDH by binding to vicinal sulfhydryl groups within this enzyme, as does phenylarsine oxide, studies by Samikkannu *et al.* (2003) suggest that arsenite inhibits the enzyme by generating ROS that inactivate the protein. This occurs at concentrations of arsenite much lower than concentrations required for inhibition by direct binding to the sulfhydryl group.

### Interactions with Phosphate

Arsenate was observed to affect *in vitro* phosphate turnover in the early 1930s (Harden, 1932). Over the years, additional

*in vitro* studies have shown that arsenate has inherent toxicological properties because of its interaction with phosphate. However, it is not known if there are *in vivo* effects that are a result of this interaction between arsenate and phosphate.

Arsenic and phosphorus are in Group 15 of the periodic table (nitrogen or nitrogen group) and have similar physicochemical properties. Arsenic acid ( $\text{H}_3\text{AsO}_4$ ) and phosphoric acid ( $\text{H}_3\text{PO}_4$ ), the fully protonated forms of arsenate and phosphate, respectively, have comparable structure and similar acid dissociation constants. Because of their similar properties, arsenate can substitute for phosphate in several biochemical reactions (Dixon, 1997). Like phosphate, arsenate forms ester linkages with its hydroxyl groups. However, the As–O bond is about 10% longer than the P–O bond (Dixon, 1997), rendering it less stable; the arsenate ester bond can easily dissociate. Arsenate uncouples formation of adenosine-5'-triphosphate (ATP) *in vitro* by a mechanism termed "arsenolysis." Doudoroff *et al.* (1947) were the first to use this term when they observed that glucose-1-phosphate was hydrolyzed *in vitro* with addition of arsenate and sucrose phosphorylase. Arsenolysis can occur during glycolysis and oxidative phosphorylation in the presence of arsenate (Crane and Lipmann, 1953; Gresser, 1981). In the glycolytic pathway, arsenate can form the intermediate anhydride, 3-phosphoglyceroyl arsenate. In oxidative phosphorylation, arsenate can couple with adenosine-5'-diphosphate. Both reactions form unstable arsenate anhydrides, which hydrolyze easily. The overall result is that formation of ATP is diminished. At the cellular level, arsenate depletes ATP in rabbit (Delnomdedieu *et al.*, 1994) and human (Winski and Carter, 1998) erythrocytes. The human erythrocytes died a few hours after arsenic exposure, presumably because of the loss of both ATP and plasma membrane integrity. Arsenite is ineffective in depleting ATP in human erythrocytes.

#### Reactive Oxygen Species

The formation of reactive oxygen and nitrogen species by arsenic is one of the most studied MOAs for arsenic toxicity today (Hughes and Kitchin, 2006; Kitchin and Ahmad, 2003; Kitchin and Conolly, 2010; Lantz and Hays, 2006; Shi *et al.*, 2004). This has come about from studies in the 1980s that reported on the induction of "stress" proteins *in vitro* by arsenite (Johnston *et al.*, 1980; Keyse and Tyrrell, 1989) and DNA strand breaks in mammals by DMAs<sup>V</sup> (Yamanaka *et al.*, 1989). ROS formed by arsenic are involved in several of the proposed MOAs including genotoxicity, signal transduction, cell proliferation, and inhibition of DNA repair.

Reactive species are formed *in vitro* and *in vivo* in the presence of arsenic and include superoxide anion, hydroxyl radical, hydrogen peroxide, reactive nitrogen species, and arsenic-centered and arsenic peroxy radicals (Kitchin 2001; Shi *et al.*, 2004). Production of these species have been detected by measuring oxidative DNA damage (8-hydroxy-

2'-deoxyguanosine), lipid peroxidation and stress response genes, the loss of antioxidant defenses (e.g., glutathione), and induction of heat shock proteins or the use of electron spin resonance and fluorescence spectroscopy (Del Razo *et al.*, 2001; Liu *et al.*, 2001; Nesnow *et al.*, 2002; Shi *et al.*, 2004; Yamanaka *et al.*, 1990). Several studies have shown that the addition of antioxidants and radical scavengers decrease arsenic-induced ROS formation and related toxicity (Shi *et al.*, 2004). However, this antagonism is not always observed *in vivo* (Wei *et al.*, 2005). The mechanism of ROS formation by arsenic is not clear. It may occur during oxidation of arsenite to arsenate (Del Razo *et al.*, 2001), by formation of arsine during arsenic metabolism (Yamanaka and Okada, 1994), stimulation of NADH or NADPH oxidase (Lynn *et al.*, 2000; Smith *et al.*, 2001; Straub *et al.*, 2008), ferritin iron release by arsenic with active oxygen generated by the Fenton reaction (Ahmad *et al.*, 2000), inhibition of redox enzymes such as glutathione reductase and thioredoxin reductase (Lin *et al.*, 2001; Styblo *et al.*, 1997), or may be secondary to arsenic-induced cytotoxicity (Wei *et al.*, 2005).

#### Genotoxicity

Reports on the genotoxic effects of arsenic were first published in the mid-1970s. Nishioka (1975) screened the mutagenic activities of metal compounds using an assay (rec-assay) that detected inhibition of growth in wild-type ( $\text{Rec}^+$ ) and recombination-deficient ( $\text{Rec}^-$ ) strains of *Bacillus subtilis*. Arsenite, and less so arsenate, inhibited growth in  $\text{Rec}^-$  relative to  $\text{Rec}^+$  cells, which suggested that arsenic induced DNA damage. However, in this same study, arsenite did not induce tryptophan reversions in *Escherichia coli* strains. Most studies published since the 1970s indicate that arsenic does not directly interact with DNA to cause point mutagens in bacterial or mammalian reversion assays (Basu *et al.*, 2001). However, arsenic is comutagenic. Arsenite enhances the mutagenic effect of ultraviolet (UV) radiation in bacterial (Rossman, 1981) and mammalian cells (Li and Rossman, 1991) and that of direct-acting mutagens such as methyl methanesulfonate (Lee *et al.*, 1986) and N-methyl-N-nitrosourea (Li and Rossman, 1989a) in mammalian cells. Although not directly mutagenic, arsenic is genotoxic, inducing effects including deletion mutations, oxidative DNA damage, DNA strand breaks, sister chromatid exchanges, chromosomal aberrations, aneuploidy, and micronuclei (Basu *et al.*, 2001; Hei *et al.*, 1998; Rossman, 2003). Other effects of arsenic related to genotoxicity include gene amplification, transforming activity, and genomic instability (Rossman, 2003). These genotoxic effects of arsenic are observed *in vitro* in mammalian cells and *in vivo* in laboratory animals and humans (Basu *et al.*, 2001; Rossman, 2003). For example, Beckman *et al.* (1977) observed chromosomal aberrations in lymphocytes in workers exposed to arsenic. Trivalent arsenicals, both inorganic and organic, are more potent genotoxins than the pentavalent arsenicals (Kligerman *et al.*, 2003; Mass *et al.*, 2001). The mechanism of genotoxic

action of arsenic may result from generation of ROS, inhibition of DNA repair, and altered DNA methylation that may lead to genomic instability (Rossman, 2003).

#### Altered DNA Repair

Arsenic inhibits DNA repair in bacterial and mammalian cells. This inhibitory effect may account for the cogenotoxic effect of arsenic with N-methyl-N-nitrosourea and UV radiation (Rossman, 2003). One of the first studies examining the effect of arsenic on DNA repair used strains of *E. coli* with different DNA repair phenotypes (Rossman *et al.*, 1977). Cells were exposed to UV radiation and then plated with or without arsenite. Cells competent for postreplicative DNA repair in the presence of arsenite were the most sensitive to the lethal effects of UV radiation. In nuclear extracts from Chinese hamster V79 cells, arsenite inhibited participation of DNA ligase II in N-methyl-N-nitrosourea induced DNA repair (Li and Rossman, 1989a). The inhibition, which required a high concentration of arsenite (50% decrease at 10mM) may have been due to binding of arsenite directly to ligase. However, later studies using human cell nuclear extracts suggested inhibition of DNA repair was an indirect effect of arsenic that occurred at a much lower concentration (50% decrease at 10 $\mu$ M), due to induction of ROS or altered cell signaling that changed gene expression (Hu *et al.*, 1998).

Enzymes involved in nucleotide excision repair (NER) and base excision repair (BER) are also affected by arsenic (Hartwig *et al.*, 2003; Rossman, 2003; Schoen *et al.*, 2004; Sykora and Snow, 2008). In a small group of individuals exposed to arsenic in drinking water, toenail arsenic levels (a biomarker of arsenic exposure) were inversely correlated with the expression of three NER genes (Andrew *et al.*, 2003). Trivalent arsenicals inhibit BER and NER activity by interacting with zinc finger motifs of proteins in these two DNA repair systems (Ding *et al.*, 2009; Piatek *et al.*, 2008). The catalytic function of zinc finger containing proteins depends on binding of zinc to cysteinyl residues. Arsenic may disrupt protein function by displacing zinc from its binding site or by inactivating the sulfhydryl groups of cysteine by oxidation.

In a recent review, Gentry *et al.* (2010) highlighted the role of inhibition of DNA repair by arsenic as a mode of action for its carcinogenic effect. They analyzed data on *in vitro* cellular and *in vivo* gene expression changes following exposure to inorganic arsenic. The analysis of the data suggests the key events in carcinogenicity of arsenic include inhibition of DNA repair under conditions of oxidative stress, inflammation, and proliferative signaling. This may lead to a condition in which mitosis proceeds without maintaining the integrity of the cellular DNA.

#### Signal Transduction

Signal transduction pathways transmit extracellular signals, via an intracellular series of signaling molecules (e.g., protein

kinases), into alterations in gene expression. Cellular processes such as proliferation, differentiation, and apoptosis are directed and managed by these pathways or cascades. Arsenic can alter signal transduction, which leads to activation or inhibition of transcription factors, regulatory proteins which bind to DNA and regulate gene transcription (Bode and Dong, 2002; Druwe and Vaillancourt, 2010; Huang *et al.*, 2004; Kumagi and Sumi, 2007; Leonard *et al.*, 2004; Plataniias, 2009). In the 1990s, some of the first studies examining the effects of arsenic on signal transduction were published. Rouse *et al.* (1994) observed that p38, a protein in the mitogen-activated protein kinase (MAPK) cascade, was activated by arsenite *in vitro*. The other arms of the MAPK pathway, extracellular-regulated protein kinases (ERKs), and the *c-Jun* N-terminal kinases (JNKs) are also activated by arsenic (Bode and Dong, 2002; Yang and Frenkel, 2002). Liu *et al.* (1996) reported that arsenite *in vitro* strongly activated JNK and p38 but to a lesser extent ERK. The activation process appeared to involve generation of oxidative stress, as the free radical scavenger N-acetylcysteine inhibited activation of the kinases (Liu *et al.*, 1996).

Signal transduction via the MAPK pathway activates the transcription factor activator protein-1 (AP-1) (Bode and Dong, 2002; Kumagi and Sumi, 2007). Arsenite activates JNK and p38 in HeLa cells, which in turn stimulates AP-1 transcriptional activity, leading to increased expression of the proto-oncogenes *c-jun* and *c-fos* (Cavigelli *et al.*, 1996). In mice exposed to arsenite in drinking water, activation of the MAPK pathway was correlated with hyperproliferation of bladder epithelium (Luster and Simeonova, 2004). This appeared to result from increased activation of AP-1 followed by expression of AP-1-related genes that have a role in cell proliferation. The pathway for this activation appears to involve c-Src and epidermal growth factor receptor (EGFR) signaling cascades (Simeonova *et al.*, 2002). c-Src is activated in primary porcine aortic endothelial cells at low arsenite concentrations (<5 $\mu$ M), which are also sufficient to induce cell proliferation and increase H<sub>2</sub>O<sub>2</sub> and superoxide accumulation, H<sub>2</sub>O<sub>2</sub>-dependent tyrosine phosphorylation and NF- $\kappa$ B-dependent transcription (Barchowsky *et al.*, 1999). MAP kinases, ERK, and p38 are not activated at these levels in the endothelial cells but at higher concentrations that can result in cell death. More recently, Andrew *et al.* (2009) reported that arsenic at levels relevant to human exposure activates the EGFR pathway signaling in the lung. In human bronchial epithelial cells, it appears that arsenic increases levels of EGFR ligand, heparin binding-EGF, and activates EGFR phosphorylation (downstream of EGFR effects included increased pERK and the cell cycle promoter cyclin D1 levels). In human lung tumor biopsies, levels of phosphorylated EGFR were higher in specimens from subjects with elevated toenail arsenic levels compared with those with lower exposures.

The transcription factors nuclear factor- $\kappa$ B (NF- $\kappa$ B) and nuclear factor erythroid-2-related factor 2 (Nrf2) are also affected by arsenic (Bode and Dong, 2002; Kumagi and Sumi, 2007). Inactive NF- $\kappa$ B is bound in the cytosol to the protein

inhibitory  $\kappa\beta$  ( $I\kappa\beta$ ). Upon a stimulus,  $I\kappa\beta$  is phosphorylated by  $I\kappa\beta$  kinase and NF- $\kappa\beta$  is released. NF- $\kappa\beta$  is translocated to the nucleus, binds to its promoter region, and expression of genes for cytokines and growth factors is stimulated. Arsenite appears to suppress tumor necrosis factor- $\alpha$  induced NF- $\kappa\beta$  activation by modifying a reactive thiol in  $I\kappa\beta$  kinase (Roussel and Barchowsky, 2000; Schumilla *et al.*, 1998). However, arsenite has also been reported to activate NF- $\kappa\beta$  via generation of ROS (Felix *et al.*, 2005; Hu *et al.*, 2002; Wijeweera *et al.*, 2001). Barchowsky *et al.* (1996) were the first to demonstrate in cultured porcine aortic endothelial cells that noncytotoxic concentrations of arsenite ( $\leq 5\mu\text{M}$ ) stimulated generation of ROS and the activation of NF- $\kappa\text{B}$ . For example,  $5\mu\text{M}$  arsenite activates NF- $\kappa\text{B}$  in endothelial cells (Barchowsky *et al.*, 1996), whereas  $500\mu\text{M}$  arsenite in human bronchial epithelial or embryonic kidney cells inhibits NF- $\kappa\text{B}$  activation by directly blocking  $I\kappa\text{B}$  kinase and subsequent phosphorylation and degradation of the inhibitor  $I\kappa\text{B}\alpha$  (Roussel and Barchowsky, 2000). These differing effects of arsenic on NF- $\kappa\text{B}$  activation appear to be cell-specific and dose-dependent. Nrf2, by regulating gene expression, controls the cellular antioxidant response to exogenous insult. Activation of Nrf2 *in vitro* by *tert*-butylhydroquinone (tBHQ) and sulforaphane (SF) can protect a cell from trivalent arsenic-induced toxicity (Wang *et al.*, 2007). Nrf2 null mice are more susceptible to arsenite-induced liver and bladder toxicity than Nrf2 homozygous mice (Jiang *et al.*, 2009). Wang *et al.* (2008) reported that arsenite and MMAs<sup>III</sup> activate Nrf2 by mechanism distinct from that of tBHQ or SF.

Arsenic is well known for its carcinogenic properties, but interestingly, this metalloid is also used to treat a specific form of cancer. Arsenic trioxide (which solubilizes to arsenite in water) is a treatment for cancer patients with all trans-retinoic acid-resistant acute promyelocytic leukemia (Bode and Dong, 2002; Platanias, 2009). Arsenic trioxide generates ROS, which activates JNK and upregulates pro-apoptotic proteins and downregulates anti-apoptotic proteins. Thus, the leukemic cells undergo arsenic-induced apoptosis and the patients enter a state of remission to the cancer. However, the cancer treatment with arsenic needs to be carefully monitored because of its acute toxicological effects.

### Cellular Proliferation

A hallmark of arsenic toxicity in humans is hyperkeratosis. Cells may proliferate from mitogenic stimuli or compensatory regeneration due to cell toxicity and death (Cohen and Ellwein, 1990). Germolec and colleagues in the 1990s observed that inorganic arsenic stimulates overexpression of growth factors that could potentially mediate skin neoplasia. Primary human keratinocytes incubated with arsenite overexpress growth factors including granulocyte macrophage-colony stimulating factor (GM-CSF) and transforming growth factor- $\alpha$  (TGF- $\alpha$ ) and the proinflammatory cytokine tumor necrosis factor- $\alpha$  (Germolec *et al.*, 1996, 1997). These cells also proliferate in

the presence of arsenite. The skin of Tg.AC mice (transgenic mouse, see below for details) displays hyperkeratosis following a 10-week drinking water exposure to arsenite (200 ppm). The expression of mRNAs for GM-CSF and TGF- $\alpha$  in skin of these arsenite-treated mice was significantly elevated (Germolec *et al.*, 1998). More recently, Waalkes *et al.* (2008) have shown a role for Rac1 in epidermal hyperproliferation and skin carcinogenesis in arsenic-exposed animals. Rac1 is a signaling G protein that regulates the cell cycle, maintains epidermal stem cells, and other cellular processes. *Rac1* gene transcripts and its protein are overexpressed in skin and tumors of Tg.AC mice treated with arsenite during gestation (days 8–18) followed by topical application of TPA through adulthood. The results suggest that the cancer response is associated with distorted signaling between skin tumor stem cells and altering population dynamics. Rac1 also appears to have a role in arsenite-induced NADPH oxidase generation of oxidants in porcine aortic endothelial cells (Smith *et al.*, 2001) and arsenite-stimulated mouse hepatic sinusoidal endothelial cell differentiation and dysfunction (Straub *et al.*, 2008).

The rat bladder urothelium shows increased cytotoxicity and cell proliferation following exposure to DMAs<sup>V</sup> in drinking water (Wanibuchi *et al.*, 1996) or the diet (Arnold *et al.*, 1999). Both studies showed increased bromodeoxyuridine labeling index, which is indicative of cell proliferation. The cytotoxicity and hyperplasia of the urothelium were reversible after removal of dietary DMAs<sup>V</sup>. Morphological examination of the rat urothelium following treatment of rats with dietary DMAs<sup>V</sup> (100 ppm) for 1–3 days showed focal cellular necrosis and after 7 days widespread necrosis (Cohen *et al.*, 2001). Increased cell proliferation was observed in the urothelium after 7 days of exposure to DMAs<sup>V</sup>. Coadministering 2,3-dimercapto-1-propanesulfonic acid, a chelator of trivalent arsenic, in the diet with DMAs<sup>V</sup> to rats inhibited the necrosis and regenerative proliferation of the urothelium (Cohen *et al.*, 2002). This and other evidence suggested to Cohen and colleagues that DMAs<sup>III</sup> might be the toxic species. *In vitro* studies with rat urothelial cells suggest that the cytotoxicity of trivalent arsenicals may be a result of, at least in part, oxidative damage (Wei *et al.*, 2005). Simeonova *et al.* (2000) observed hyperplasia of the bladder urothelium in female mice following exposure to 100 ppm sodium arsenite in drinking water for 4 weeks. After 16 weeks of exposure to arsenite, the hyperplasia was accompanied by an increase in DNA binding of the activating protein (AP)-1 transcription factor and inorganic arsenic in bladder tissue. *In vitro* studies with UROTSA cells, an immortalized human bladder epithelial cell line, showed increased proliferation and AP-1 DNA binding following a 72 h exposure to arsenite (2 $\mu\text{M}$ ). A follow-up study by Simeonova *et al.* (2002) showed that arsenite induced a c-Src-dependent activation of the EGFR and mitogen-activated protein kinase pathway in UROTSA cells. *In vivo* exposure to arsenite (50 ppm, 8 weeks) to mice resulted in EGFR and extracellular signal-regulated kinase (ERK) activation with the involvement

of c-Src interacting with EGFR. More recent work in *As3mt* knockout mice (Yokohira *et al.*, 2010) and rat (Suzuki *et al.*, 2010) also suggest that inorganic arsenic administered in the diet may be associated with a similar MOA (cytotoxicity and cell proliferation) for urothelial cytotoxicity.

#### Altered DNA Methylation

Epigenetic mechanisms such as altered DNA methylation have a role in arsenic toxicity and carcinogenicity. Gene transcription is regulated by DNA methylation. In the late 1990s, two separate laboratories reported that arsenic alters DNA methylation, with both hypo- and hypermethylation of DNA observed. Zhao *et al.* (1997) incubated a rat liver epithelial cell line (TRL 1215) with arsenite up to 18 weeks and reported global DNA hypomethylation, aberrant gene expression, and malignant transformation of the cells. Mass and Wang (1997) observed hypermethylation of a portion of the p53 promoter region of human lung adenocarcinoma A549 cells treated with arsenite. Arsenate was also effective but required higher doses; DMAs<sup>V</sup> was ineffective in A549 cells. The tumor suppressor protein p53 has a role in cell cycle regulation. Inhibition of its expression by hypermethylation of its promoter region could potentially lead to the development of cancer. A follow-up study showed that hypomethylation and hypermethylation of genomic DNA was associated with arsenite exposure *in vitro* in human cells using a method sensitive to DNA methylation alterations (Zhong and Mass, 2001). This suggests that the absolute level of genomic DNA methylation is less important than methylation within a specific DNA sequence. Benbrahim-Tallaa *et al.* (2005) observed genomic DNA hypomethylation in a human prostate epithelial cell line following a long-term exposure to arsenite that resulted in malignant transformation of the cells.

In some individuals chronically exposed to arsenic in drinking water, the p53 promoter region of DNA from whole blood shows a dose-dependent hypermethylation relative to control subjects (Chanda *et al.*, 2006). However, some of the arsenic-exposed individuals showed hypomethylation of this promoter region. In this same study, the tumor suppressor gene p16 was also hypermethylated in individuals exposed to high levels of arsenic.

The mechanism of the effect of arsenic on DNA methylation is not clear. Hypomethylation may be due to nutritional factors or inhibition of DNA methyltransferase. Also, S-adenosylmethionine, which is the methyl donor for the methylation of both DNA and arsenic in its metabolism, may potentially be shunted to the latter with increased exposure to arsenic.

Pilsner *et al.* (2007, 2009, 2011) reported in several studies of Bangladeshi adults exposed to arsenic in drinking water on its effect on genomic methylation of PBL DNA. They observed that genomic PBL DNA methylation was positively associated with exposure to arsenic in a dose-dependent manner (Pilsner *et al.*, 2007). Plasma folate modified this effect, needing to be  $\geq 9$  nmol/l for the hypermethylation to occur. Folate is an

important cofactor in the transfer of methyl groups from SAM to DNA, arsenic, and other substrates. In a group of arsenic-exposed individuals that developed skin lesions, it was found that folate deficiency, hyperhomocysteinemia, low urinary creatinine, and hypomethylation of PBL DNA were risk factors for the arsenic-induced skin lesions (Pilsner *et al.*, 2009). Pilsner and colleagues suggested that the hypermethylation of PBL DNA that is associated with increased arsenic exposure might be an adaptive response because the hypomethylation of PBL DNA is associated with the risk for development of skin lesions. This same group reported that plasma selenium is inversely associated with genomic PBL DNA methylation. In addition, selenium may reduce the body of arsenic. Overall, the effect of arsenic on genomic DNA methylation is still not clear and requires further investigation.

#### Carcinogenicity of Arsenic in Animal Models

The use of animals for the study of cancer causing agents has been a scientific foundation for many years. This is so that chemicals with unknown carcinogenicity in humans can be tested or that a potential mechanism can be studied for chemicals known to be carcinogenic. Leitch and Kennaway (1922) conducted some of the first animal carcinogenicity studies with arsenic. They fed bread containing potassium arsenite (Fowler's solution) to mice and rats 3 days per week. Most of the animals died from the treatment, and no tumors were detected. In a second experiment, the arsenical solution was directly applied to mouse skin three times per week. About two-thirds of the initial 100 animals died from the treatment, but a wart on one animal developed at the application site after 85 days of treatment. This wart eventually developed into a metastasizing squamous cell epithelioma. A follow-up study under similar conditions produced negative results (Leitch, 1923). These results by Leitch and Kennaway, of no carcinogenic effect with arsenic in one experiment, followed by a limited positive result, are similar to what has been published up to the 1990s. Many studies of experimental arsenic carcinogenesis using oral, dermal, or parenteral administration followed over the years. These studies included mice and rats (Baroni *et al.*, 1963; Byron *et al.*, 1967; Heuper and Payne, 1962; Kanisawa and Schroeder, 1969; Neubauer, 1947; Schroeder *et al.*, 1968), dogs (Byron *et al.*, 1967), rabbits and chickens (Neubauer, 1947), and monkeys (Thorgeirsson *et al.*, 1994). Some studies involved classical initiation/promotion experiments with arsenic used as either the initiator (with the promoter croton oil) or promoter (with the initiator dimethylbenz(a)anthracene) (Boutwell, 1963). The results from these studies were largely negative or inconclusive. These carcinogenicity results as well as other findings up to this time led some to question the carcinogenic potency of arsenic in humans (Frost, 1967).

In the late 1970s and early 1980s, the potential for development of lung tumors in rats (Ishinishi *et al.*, 1977)

and Syrian Golden hamsters (Ishinishi *et al.*, 1983; Pershagen *et al.*, 1984; Pershagen and Bjorklund, 1985; Yamamoto *et al.*, 1987) administered arsenic intratracheally was examined. Epidemiological studies had shown that workers in the nonferrous metal smelting and arsenical pesticide manufacturing industries were at risk of developing lung cancer (Lee and Franmeni, 1969; Ott *et al.*, 1974). The experimental procedure was to instill arsenic (arsenic trioxide, arsenic trisulfide, or calcium arsenate) suspended in saline into the trachea of the animals. Animals received one dose per week for 15 weeks (total dose, 3.75 or 5.25 mg As) and then were monitored through their entire life span. Results for arsenic trioxide were problematic because up to 50% of the treated animals died. There was less mortality (10–14%) in animals treated with the other arsenicals. The results for arsenic trisulfide were inconclusive. Calcium arsenate and arsenic trioxide were weakly tumorigenic. The International Agency for Research on Cancer (IARC, 2004) considers that these studies provided limited evidence for carcinogenicity of inorganic arsenicals.

In the 1990s, carcinogenicity studies in animals shifted from inorganic arsenic to its main mammalian metabolite, dimethylarsinic acid (DMAs<sup>V</sup>). DMAs<sup>V</sup> is a clastogen and induces chromosomal aberrations *in vitro* (Dong and Lou, 1993; Endo *et al.*, 1992). Yamamoto *et al.* (1995) assessed DMAs<sup>V</sup> in a multiorgan carcinogenesis bioassay. Rats were pretreated with five known carcinogens and then administered DMAs<sup>V</sup> in drinking water (50–400 ppm) for 24 weeks. DMAs<sup>V</sup> promoted tumors in urinary bladder, kidney, liver, and thyroid gland. Urinary bladder showed the strongest response. This result was important because urinary bladder is one of the target organs for arsenic in humans (NRC 1999, 2001). Studies continued investigating the tumor promoting effects of DMAs<sup>V</sup> in rats and mice in urinary bladder and lung, respectively (Yamanaka *et al.*, 1996; Wanibuchi *et al.*, 1996). DMAs<sup>V</sup> in drinking water (rats, 2–100 ppm; mice, 200–400 ppm) promoted the tumorigenic effects of two initiators (N-butyl-N-(4-hydroxybutyl) nitrosamine and 4-nitroquinolone 1-oxide). DMAs<sup>V</sup> alone in drinking water (400 ppm, 50 weeks) promoted lung tumor development in A/J mice, which spontaneously develop lung tumors (Hayashi *et al.*, 1998). Dose-dependent cellular proliferation of urinary bladder epithelium was observed in rats administered DMAs<sup>V</sup> in drinking water (10–100 ppm) up to 23 weeks (Wanibuchi *et al.*, 1996).

Studies then followed that assessed the complete carcinogenicity of DMAs<sup>V</sup>. Wei *et al.* (1999, 2002) administered DMAs<sup>V</sup> in drinking water (12.5–200 ppm) to male F344 rats for 2 years. They observed dose-related development of bladder tumors. Tumors were not observed in other organs or in the control or 12.5 ppm DMAs<sup>V</sup> groups. Arnold *et al.* (2006) administered DMAs<sup>V</sup> in the feed of B6C3F1 mice (8–500 ppm) and F344 rats (2–100 ppm) of both sexes for 2 years. Although no treatment-related tumors were observed in mice, rats developed urinary bladder tumors. Female rats were more sensitive than male rats to the effects of DMAs<sup>V</sup> in the diet.

A 2-year dietary exposure of monomethylarsonic acid, a metabolic precursor of DMAs<sup>V</sup>, to F344 rats (50–1300 ppm) and B6C3F1 mice (10–400 ppm) yielded no tumors (Arnold *et al.*, 2003). Cohen *et al.* (2006) proposed that the carcinogenic effect of DMAs<sup>V</sup> is a result of metabolism of DMAs<sup>V</sup> to dimethylarsinous acid (DMAs<sup>III</sup>), which reacts with critical proteins in the urothelium or induces oxidative stress in the cells, resulting in cytotoxicity. This initiates cellular proliferation in the urothelium and eventual tumor development. A criticism of the DMAs<sup>V</sup> promoting and carcinogenicity studies are the high doses used and that there are species differences in arsenic pharmacokinetics. Rat hemoglobin uniquely binds DMAs<sup>III</sup> (Lu *et al.*, 2007), which extends the pharmacokinetic half-life of this arsenical relative to other mammals (Vahter *et al.*, 1984). It is not known if the retention of arsenic by the rat affects development of urinary bladder tumors following exposure to DMAs<sup>V</sup>. Although these findings have been taken as evidence that DMAs<sup>V</sup> operates as a nonlinear carcinogen (Cohen *et al.*, 2006), it has been noted that humans are unlikely to experience doses of DMAs<sup>V</sup> in the diet sufficient to induce cytotoxicity and subsequent carcinogenicity (U.S. EPA, 2006). As mentioned above, there is newer research investigating the role of inorganic arsenic-induced cytotoxicity and regenerative hyperplasia as an MOA for arsenic carcinogenesis. Suzuki *et al.* (2010) measured dose-dependent changes in the bladder epithelium of female F344 rats exposed to 0, 1, 10, 25, and 50 ppm sodium arsenite in feed (no effects were observed at 1 ppm or below). In this study, both trivalent methylated and other thiol-containing arsenic metabolites were hypothesized to account for the observed bladder cell cytotoxicity and regenerative hyperplasia. More recent studies find that even in mice that do not methylate arsenic (*As3mt* knockout mice), bladder cell cytotoxicity can be measured after exposure to inorganic arsenic. These studies indicate that inorganic arsenic itself, in the absence of metabolism to methylated trivalent species, is capable of causing cytotoxic damage and regenerative hyperplasia (Yokohira *et al.*, 2010). However, there is a higher body burden of arsenic in the bladder of *As3mt* knockout mice than wild-type mice at steady state during repeated treatment with arsenate (Hughes *et al.*, 2010). Inorganic arsenic was the predominant arsenic species in this tissue of *As3mt* knockout mice. In wild-type mice, the bladder contained a mixture of inorganic arsenic and methylated arsenicals.

The skin is a target organ in humans exposed to inorganic arsenic. Dermal effects of arsenic in humans include altered pigmentation, hyperkeratosis, and cancer. The transgenic mouse model, Tg.AC, contains the *v-Ha ras* oncogene. This strain has the characteristics of genetically initiated skin with a predisposition to papillomas. In an experiment conducted in the late 1990s, such transgenic mice were exposed to arsenite (200 ppm) in drinking water for 4 weeks. Then the mice were treated topically (two times per week for 2 weeks) with the promoter 12-*O*-tetradecanoyl phorbol-13-acetate (TPA). The

number of papillomas increased in the arsenite plus TPA-treated Tg.AC mice (Germolec *et al.*, 1997, 1998). Papillomas were not observed in Tg.AC mice treated with arsenite alone, control mice, or wild-type mice treated with arsenite and TPA. Results from this study and others led Germolec *et al.* (1997) to suggest that arsenic is a tumor enhancer.

*In vitro* studies have shown that arsenic is comutagenic with several chemicals and UV radiation (Li and Rossman, 1989a, 1989b, 1991). Rossman and colleagues examined the cocarcinogenic effect of arsenite in drinking water (1.25–10 ppm, 26 weeks) and exposure to solar UV radiation (1–1.7 KJ/m<sup>2</sup>, three times/week) in hairless Skh1 mice (Burns *et al.*, 2004; Rossman *et al.*, 2001). No tumors were observed in untreated mice or those exposed to arsenite alone. However, skin tumors including invasive squamous cell carcinomas developed in the arsenite plus UV radiation exposed group. Studies with a mouse keratinocyte cell line suggested that arsenite decreases the rate of DNA repair of some photoproducts and inhibits apoptosis induced by UV radiation (Wu *et al.*, 2005). Skin carcinogenesis by UV radiation may be enhanced by arsenite decreasing DNA repair and inhibiting apoptosis of damaged cells.

The developing fetus can be highly sensitive to the adverse effects of chemicals. Metals such as nickel and lead are transplacental carcinogens in rodents (Diwan *et al.*, 1992; Waalkes *et al.*, 1995). The fetus can be exposed to arsenic because it crosses the placenta (Lindgren *et al.*, 1984). With this information, Waalkes *et al.* (2003) conducted a gestational exposure study of arsenite in mice. Dams were exposed to arsenite at two dose levels (42.5 and 85 ppm) in drinking water from gestation day 8 to 18. The treatment did not affect the dams. Male and female offspring were observed through adulthood. There was an increase in hepatocellular carcinoma (dose-related) and liver tumor multiplicity in arsenic-exposed male offspring. In female offspring, there were dose-related increases in adrenal tumor incidence and multiplicity and incidence of ovarian tumors and lung carcinomas. The incidence of proliferative lesions in the uterus and oviduct were also increased by arsenite. This study was repeated (Waalkes *et al.*, 2004) and included a group of offspring treated on the skin with TPA. In male offspring, similar results were observed with the 2003 study, and TPA treatment had no effect on the liver and adrenal tumors. TPA also had no effect on the hyperplasia of uterus and oviduct caused by arsenic. TPA promoted liver tumors in females and lung tumors in male and female mice. No skin tumors were detected. Gestational exposure of arsenic was also conducted with Tg.AC transgenic mouse (Waalkes *et al.*, 2008). Following *in utero* exposure to arsenite in drinking water (42.5 and 85 ppm), offspring were treated with TPA through adulthood. Arsenic-alone treated animals were not affected. Papillomas and squamous cell carcinomas were observed in TPA-alone treated mice (no arsenite exposure). In arsenic and TPA-treated mice, there was a 30-fold increase in squamous cell carcinoma multiplicity. These carcinomas were also more invasive. Distorted

skin tumor stem cell signaling and stem cell population dynamics were detected. Waalkes *et al.* (2008) suggested that arsenic targets stem cells as the fetal basis of skin cancer in adulthood.

The standard cancer bioassay is to treat animals with the chemical after they are weaned. However, humans are exposed to many chemicals, throughout their lifetime. Several recent epidemiology studies have reported that exposure to inorganic arsenic at an early age results in increased susceptibility to cancer in adulthood (Smith *et al.*, 2006; Yuan *et al.*, 2010). From this evidence and the results of their transplacental arsenic model, Waalkes and colleagues (Tokar *et al.*, 2011a) conducted a lifetime arsenite drinking water (6–24 ppm) exposure study in mice. The exposure began before breeding (to the parents), during pregnancy and lactation and after weaning through adulthood. In offspring of both sexes, dose-related lung adenocarcinomas, hepatocellular carcinomas and adrenal tumors developed. Gall bladder tumors were detected in males and uterine tumors in females. In the whole life study, target sites were similar to the *in utero* alone exposures but occurred at lower doses. The tumors in the lifetime study were generally more aggressive and frequent. Also, an overabundance of cancer stem cells was found in the liver and lung tumors. This suggests that stem cells may be a target for arsenic. Based on the experimental results, it is also likely that interference with the normal functioning of the estrogen receptor by arsenic may be involved in carcinogenesis.

Overall, animal models, especially recently developed models, have advanced our understanding of arsenic carcinogenicity. A representative set of the oral arsenic carcinogenicity studies in animals is summarized in Table 3. As with many chemicals, differences in pharmacokinetics and pharmacodynamics among different animal species and the doses used in animal bioassays necessitates an examination of the human relevance of findings from animal studies.

### Epidemiologic Studies of Arsenic Exposure from Drinking Water

Elevated arsenic concentrations in water have been indicated as a public health hazard in many countries of the world where individuals rely on groundwater for drinking purposes (Smith *et al.*, 2000). Since 1968, a series of epidemiologic reports from a small area in southwestern Taiwan have offered data on harmful effects of high levels (>300 µg/l) of arsenic in drinking water. Blackfoot disease, a unique peripheral disease in lower extremities that could result in spontaneous gangrene in the toes and feet, has been known in the area since the 1920s (Ch'i and Blackwell, 1968). In a landmark study, Tseng *et al.* (1968) documented in a cross-sectional study that high level of arsenic exposures at 300 µg/l and 600 µg/l were related to a high prevalence of skin cancer. Using the median well arsenic concentration within a village among 42 villages and ICD-8 codes on death certificates, Chen *et al.* (1988) subsequently



TABLE 3  
Representative Oral Carcinogenicity Studies of Arsenic in Animals

Study	Species	Sex	Arsenical species	Dose	Exposure period	Outcome	Notes
Kanisawa and Schroeder (1969)	Rat	Female and Male	Arsenite	5 ppm, drinking water	Lifetime, started at weaning	No treatment-related effect	
Yamamoto <i>et al.</i> (1995)	Rat	Male	DMAs <sup>V</sup>	50–100 ppm, drinking water	6–30 weeks of age	Promoter of urinary bladder, kidney, liver, and thyroid gland tumors	Animals pretreated with five carcinogens
Wei <i>et al.</i> (1999)	Rat	Male	DMAs <sup>V</sup>	12.5–200 ppm, drinking water	10–104 weeks of age	Urinary bladder carcinogen	
Rossmann <i>et al.</i> (2001)	Mouse	Female	Arsenite	10 ppm, drinking water	3–29 weeks of age	2.4-fold increase in skin tumor yield in UV + arsenite-treated compared with UV alone; no tumors in arsenite alone	Mice irradiated with UV light, 3×/week
Waalkes <i>et al.</i> (2003)	Mouse	Female and male	Arsenite	42.5 and 85 ppm, drinking water	Gestation day 8–18	Female—ovarian and lung tumors Male—liver and adrenal tumors	Transplacental exposure
Arnold <i>et al.</i> (2006)	Rat	Female and male	DMAs <sup>V</sup>	Rat: 2–100 ppm, diet	104 weeks	Rat—urinary bladder carcinogen in both sexes	Female rats more sensitive than male rats
	Mouse			Mouse: 8–500 ppm, diet		Mouse—no treatment-related tumors	
Tokar <i>et al.</i> (2011a)	Mouse	Female and male	Arsenite	6–24 ppm, drinking water	Two weeks prior to breeding through adulthood	Female—lung, liver, adrenal, uterine, ovarian tumors Male—lung, liver, gall bladder, and adrenal tumors	“Whole-life” exposure

revealed that there was a dose-response relationship of arsenic concentration and mortality due to internal cancers, including cancer of the lung, liver, bladder, and kidney, as well as all vascular diseases, peripheral vascular diseases, and cardiovascular diseases (Tseng *et al.*, 1996). In areas of Argentina and Chile where drinking water was also naturally contaminated, studies have confirmed the associations between high levels of arsenic concentration with the risk of mortality from cancers of the bladder, lung, and kidney (Bates *et al.*, 2004; Ferreccio *et al.*, 2000; Hopenhayn-Rich *et al.*, 1996, 1998; Marshall *et al.*, 2007; Smith *et al.*, 1998). Similar to the study conducted by Tseng *et al.*, arsenic exposure was measured at the group level with an ecologic or partial ecologic study design in these studies. However, it is unlikely that biases or confounding could account for the observed associations, as the associations were strong and consistent across different populations, and the distribution of the exposure was not related to individuals' lifestyles. In particular, the data from Taiwan have been used by the U.S. EPA for risk assessment; based in part on these data, in 2001, the U.S. EPA adopted a new standard for arsenic in drinking water at 10 µg/l, replacing the old standard of 50 µg/l (U.S. EPA, 2001).

In the two most recent decades, epidemiologic studies of arsenic exposure from drinking water have evolved using more modern epidemiology study designs and methodologies. Several case-control and cross-sectional studies have included measures of arsenic exposure at the level of the individual, including studies in the United States in states such as New Hampshire (Karagas *et al.*, 2001, 2004) and Nevada (Steinmaus *et al.*, 2003), as well as other countries such as West Bengal of India (Haque *et al.*, 2003), Bangladesh (Ahsan *et al.*, 2006; McCarty *et al.*, 2006; Rahman *et al.*, 1999; Sohel *et al.*, 2009), Inner Mongolia of China (Mumford *et al.*, 2007), and northeastern Taiwan (Chiou *et al.*, 2001). These studies shed light on the chronic effects of arsenic, including total and cancer mortality (Sohel *et al.*, 2009), bladder and skin cancer (Chen *et al.*, 2010; Karagas *et al.*, 2001, 2004; Steinmaus *et al.*, 2003), skin lesions (Ahsan *et al.*, 2006; Haque *et al.*, 2003; McCarty *et al.*, 2006), respiratory diseases (Mazumder *et al.*, 2000; Parvez *et al.*, 2010; von Ehrenstein *et al.*, 2005), and cardiovascular effects (Mumford *et al.*, 2007; Rahman *et al.*, 1999), at levels of inorganic arsenic exposure <500 µg/l. The ascertainment of exposure at the individual level reduces exposure misclassification and enhances internal validity of the

study; these are crucial considerations for studies in areas with varying levels of arsenic exposure, especially levels of arsenic concentrations  $<500 \mu\text{g/l}$ . Specifically, in some studies, the use of arsenic concentrations measured in urine (Ahsan *et al.*, 2006; Navas-Acien *et al.*, 2008), blood (Hall *et al.*, 2006), and toenail samples (Karagas *et al.*, 2001, 2004) as biomarkers for the internal dose of arsenic exposure have opened new research opportunities in molecular epidemiology as these studies often involved collection of biospecimens and detailed information on lifestyle factors. For instance, the case-control studies in New Hampshire gave suggestions of increased risk of bladder cancer and skin cancer associated with toenail arsenic, especially among smokers (Karagas *et al.*, 2001, 2004). Another case-control study in this part of the United States, with information on specific cancer histologic types found that elevated toenail arsenic concentrations ( $\geq 0.114 \mu\text{g/g}$ ) were associated with the risk of small-cell carcinomas of the lung (Heck *et al.*, 2009). These studies, with collection of biospecimens, have also provided platforms for subsequent studies investigating the effects of arsenic on signal transduction, gene expression, and DNA repair functions using human samples (Andrew *et al.*, 2006, 2008, 2009). The Health Effects of Arsenic Longitudinal Study (HEALS), a prospective cohort involving more than 20,000 participants in a rural area in Bangladesh, has recently reported that arsenic exposure, measured in both water (0.01–864  $\mu\text{g As/l}$ ) and urine (7–5000  $\mu\text{g As/g}$ ) samples, is related to all-cause, chronic-disease (Argos *et al.*, 2010), and cardiovascular disease especially heart disease mortality in the population (Chen *et al.*, 2011). The HEALS also creates an infrastructure for comprehensive evaluations of other health effects of arsenic exposure using an array of study designs (Chen *et al.*, 2009), including case-controls, case-cohort, and intervention studies to evaluate genetic (Ahsan *et al.*, 2007) and nutritional susceptibility (Chen *et al.*, 2007; Gamble *et al.*, 2006). These studies have provided valuable information on potential exposure-disease mechanisms using human samples.

Recent epidemiologic studies have extended the scope of research to outcomes beyond incidence or mortality of chronic diseases in adulthood (Smith and Steinmaus, 2009). In several comparisons between the arsenic-exposed and unexposed populations, studies in Chile indicated that individuals born in a high arsenic-exposure period with probable exposure *in utero* and early childhood experienced elevated risk of deaths due to acute myocardial infarction (Yuan *et al.*, 2007), lung cancer, and bronchiectasis (Smith *et al.*, 2006), suggesting the importance of early childhood or *in utero* exposure in disease causation. Several studies conducted in Bangladesh and West Bengal found that elevated arsenic concentration in groundwater was associated with increased risks of fetal loss and infant death (Rahman *et al.*, 2007), spontaneous abortion and stillbirth (von Ehrenstein *et al.*, 2006), decreased birth weight (Rahman *et al.*, 2009), and decrements in intellectual testing in school-aged children (von Ehrenstein *et al.*, 2007;

Wasserman *et al.*, 2004, 2007), although results were inconsistent and require further study. More recently, Yorifuji *et al.* (2010) reported elevated deaths and mortality ratios from several cancer types (pancreatic, leukemia) in an adult Japanese population following neonatal exposure to arsenic-contaminated milk powder. These studies give new insight on the potential early-life effects of arsenic exposure.

Taken together, over the past 50 years, epidemiologic studies have provided abundant data on health effects of arsenic exposure from drinking water. A representative set of these studies is summarized in Table 4. Over time, accuracy and reliability of measurements for the exposure in studies have been improved, with possibilities of using a life course approach to investigate the effects of *in utero* and prenatal exposure. Although early studies were mostly retrospective or cross-sectional in design, prospective studies with biorepository resources have been conducted to confirm previous associations and extended the assessment to the molecular level. Besides cancer, evidence has emerged for the adverse effects of arsenic exposure on cardiovascular disease, respiratory disease, neurological functions, reproductive outcomes, and children's intelligence. In addition, studies began to identify mechanisms using biomarkers and preclinical abnormalities as endpoints and to assess susceptibility due to genetic, nutritional, and lifestyle factors. Although challenges of studying effects of lower level exposure relative to arsenicosis-endemic areas (e.g., Bangladesh) remain, we expect that epidemiologic studies in years to come will continue elucidating the etiology of arsenic-related health effects as well as providing information for prevention, risk assessment, and intervention.

### Summary and Looking Forward

The knowledge base of the exposure and toxicological effects of arsenic has expanded greatly, particularly in the past 10–20 years. We know that exposure to arsenic for most people is an everyday occurrence because it is a natural component of the environment. The exposure pathways of arsenic to most people are dietary and drinking water and these exposures occur at relatively low levels. However, there are areas of the world, such as India, Bangladesh, and others, where the levels of arsenic in drinking water are naturally excessive, which has led to toxic manifestations in these populations. The effects of arsenic in drinking water on the U.S. population are less clear, which may be due to a lower arsenic exposure than in other areas of the world such as Bangladesh. Data from Karagas *et al.* (2001, 2004) has suggested, especially among smokers, an increased risk of bladder and skin cancer is associated with toenail arsenic.

Other types of exposure can come from soil contaminated with arsenic, from its occupational use as a pesticide or a by-product of metal ore smelting, from its use as a chemotherapeutic agent,

TABLE 4  
Representative Human Epidemiology Studies in Arsenic Drinking Water-Exposed Populations

Study	Population	Dose	Findings
Tseng <i>et al.</i> (1968)	Taiwan, >40,000 in survey	0.01–1.82 ppm	Skin cancer (10.6/1000), <sup>a</sup> hyperpigmentation (183.5/1000), keratoses (71/1000), Blackfoot disease (8.9/1000); prevalence rate of effects increased with age; Blackfoot disease associated with hyperpigmentation, keratosis, and skin cancer
Chen <i>et al.</i> (1988)	Taiwan, ~900,000 person-years	0-30 ppm, 30-59 ppm, ≥ 60 ppm	Significant dose-response relationship between As and age-adjusted mortality for bladder, kidney, skin, prostate, lung and liver cancer
Smith <i>et al.</i> (1998)	Chile, >400,000 person-years	<100 to 570 µg/l	SMR for bladder cancer: men, 6.0; women, 8.2; lung cancer: men 3.8; women 3.1; increased mortality for bladder, lung, kidney, and skin cancer
Karagas <i>et al.</i> (2001)	United States, ~800 skin cancer cases + controls	Based on toenail As concentration (overall range, 0.01–2.57 µg/g)	Risk of skin cancer did not appear elevated at toenail As concentrations detected for most study subjects; odds ratios with toenail As concentration above 97th percentile were 2.07 for SCC and 1.44 for BCC.
Smith <i>et al.</i> (2006)	Antofagasta, Chile	~1 ppm for 12 years	<i>In utero</i> and early childhood exposure occurred between 1959 and 1971; lung cancer SMR: early childhood, 7.0; <i>in utero</i> + early childhood, 6.1; Bronchiectasis SMR: early childhood, 12.4, <i>in utero</i> + early childhood, 46.2
Mumford <i>et al.</i> (2007)	Inner Mongolia, China; 313 subjects	≤21–690 µg/l	Dose-dependent relationship between prevalence rates of QT prolongation and water As concentrations; measuring QT interval may detect early cardiac As cardiac toxicity
Argos <i>et al.</i> (2010)	Bangladesh, >10,000 in survey	0.01–864 ppb	Chronic As exposure via drinking water associated with increased mortality rate. Mortality increased with As concentration in drinking water, As dose, and total As in urine

Note. BCC, basal cell carcinoma; SCC, squamous cell carcinoma; SMR, standardized mortality rate.

<sup>a</sup>Note. Prevalence rate.

and what interests many people, but occurs rarely, as a homicidal agent. With increases in analytical technology, what most likely will occur is the discovery of presently unknown forms of arsenic (e.g., arsenolipids) that we are exposed to, particularly in our diet.

The metabolic pathway of arsenic is now more clearly but not exactly defined. The discovery of arsenic (+3 oxidation state) methyltransferase has been a major breakthrough, particularly with the findings that there are polymorphisms in this enzyme. Several of these polymorphisms are associated with the toxic effects that develop from exposure to arsenic. Experimental use of the *As3mt* knockout mouse in the investigation of the metabolism and toxicity of arsenic may provide new knowledge. Finally, elucidation of the pathway of formation of the thiolated arsenic metabolites (e.g., dimethyl-dithioarsinic acid), some of which are toxicologically potent *in vitro*, will aid in the understanding of the toxicology of arsenic.

We know that arsenic causes acute and chronic dose-dependent effects, in many organ systems. A major unknown is the mode of action for the toxic effects of arsenic. Certainly, metabolism of arsenic has a role in this effect. However, if one or several metabolites are the putative toxic species is not known. Many MOA have been studied including oxidative stress, genotoxicity, altered DNA methylation, and others. Several of these MOA may be interrelated. With the advent of the “omics” age, toxic pathways of arsenic may soon be elucidated.

Other recent advances are the development of an animal (mouse) model for arsenic carcinogenicity following transplacental and whole-life exposure to arsenic and findings in mice that arsenic may impact stem cell population dynamics, which ultimately lead to transformed cells (Tokar *et al.*, 2011b). Also, there is important work in animal models that support a role for cytotoxicity and regenerative hyperplasia in the carcinogenic MOA for DMAs<sup>V</sup>.

More research is still needed to understand arsenic exposure, metabolism, effects, and MOA for cancer. Nevertheless, with recent findings and advances in technology, many of the unanswered questions regarding the toxicology of arsenic may soon be answered. This knowledge will lead to better protection of populations at risk from arsenic-related illnesses.

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