ENVIRONMENTAL CARCINOGENESIS

# Arsenic in Drinking Water and Bladder Cancer

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## INTRODUCTION

Inorganic arsenic is a metallic element found throughout the earth's crust. Human exposure to high levels of this element is primarily through inhalation of contaminated dusts in occupational settings or ingestion of drinking water contaminated with arsenic from naturally occurring sources. Despite limited findings in animal testing, there is extensive human epidemiologic evidence that inhaled arsenic causes lung cancer and ingested arsenic causes skin cancer. Based on this evidence, the International Agency for Research on Cancer has classified arsenic as an established human carcinogen (1).

In addition to skin cancer, mounting evidence shows that ingested arsenic is also carcinogenic to various internal organs. Results from highly exposed populations in Taiwan and other countries have shown that ingested arsenic may cause cancer of the bladder, lung, and kidney, which are cancers that have a greater impact on mortality than skin cancer (2–8). Moreover, these results provide evidence that significant cancer risks may be associated with arsenic exposures at or below the current U.S. drinking water standard of 50 µg/l (9,10). Because millions of people throughout the world are currently drinking water contaminated with arsenic at or above this level, ingested arsenic may be causing extensive preventable cancer mortality.

Research that links drinking water arsenic to internal cancers has not been without controversy (11), and the use of this research to estimate cancer risks at low doses and to establish a potentially costly new arsenic drinking water standard has been hotly debated. After a brief overview of the general pharmacokinetics of arsenic, this article reviews the major epidemiologic evidence linking ingested arsenic to bladder cancer and discusses the controversy surrounding this research. Our present focus is on bladder cancer because evidence indicates that it has higher mortality ratios associated with ingested arsenic than other target organs (2,6). We then review the current controversy regarding the estimated cancer risks from ar-

senic exposures at or near the current U.S. drinking water standard and the potentially large economic and health implications of lowering this standard. We close this review with a brief discussion about the use of genetic biomarkers in determining the mechanism of arsenic-induced carcinogenesis and in low-dose risk assessment.

## GENERAL TOXICOLOGY OF ARSENIC

Arsenic exists in several different chemical forms in the environment, and the particular chemical form of arsenic to which one is exposed determines the associated toxicity. For example, arsenobetaine, an organic form of arsenic commonly found in shellfish and other seafood, is easily excreted by humans and is considered essentially nontoxic. Inorganic arsenic, on other hand, either in the trivalent or pentavalent state is associated with acute and chronic toxic effects.

Exposure to inorganic arsenic occurs most commonly from one of two sources: inhalation from industrial sources or ingestion of contaminated drinking water. Occupational exposures have historically been seen in workers exposed to arsenical pesticides or smelter workers exposed to arsenic fumes produced as a byproduct of the smelting of copper or lead ores. Exposures to high levels of ingested arsenic are primarily due to drinking water contamination resulting from the leaching of naturally occurring arsenic from soils and rock into nearby groundwater.

Several areas throughout the world have been found to contain high levels of groundwater arsenic, including parts of Taiwan, Chile, Japan, China, Argentina, Mexico, India, Finland, Hungary, and Bangladesh. Several groundwater supplies in the United States also have high levels of arsenic, including areas in California, Nevada, Alaska, and Utah. In fact, it has been estimated that over 300,000 people in the United States have been drinking water contaminated with arsenic above the current U.S. drinking water standard (12).

Once ingested, inorganic arsenic is rapidly absorbed and distributed to various organs. Because this distribution is rapid, serum arsenic is a poor indicator of exposure and urinary arsenic levels are more accurate for documenting arsenic intake. Once arsenic is absorbed, it is metabolized in a two-step methylation process, first to monomethylarsonic acid (MMA) and then to dimethylarsinic acid (DMA). These methylated metabolites are considerably less acutely toxic than inorganic arsenic. Unfortunately, this detoxification mechanism is typically incomplete and approximately 5–25% of inorganic arsenic is excreted unmethylated. It is this unmethylated form, which passes through the urinary tract and is briefly stored in the bladder, that is thought to be responsible for the increased rates of bladder cancer seen in exposed populations.

## CONTROVERSY REGARDING THE TAIWAN STUDIES

The most extensive studies to date on the effects of ingested arsenic and bladder cancer have been conducted in populations from the southwest coast of Taiwan. In the 1920s, residents of this area began using water from deep artesian wells to avoid the high salinity of shallower wells. Unfortunately, the artesian wells contained high levels of arsenic and the use of these wells eventually led to endemic rates of blackfoot disease (BFD), a unique peripheral vascular disease caused by arsenic (13). Residents of this area were also found to have high rates of the pigmented and hyperkeratotic skin lesions characteristic of arsenicosis, and studies as early as the 1960s found high rates of skin cancer among people of the BFD endemic area (13,14). Eventually, high rates of other cancers were found. In fact, at the highest level of exposure (around 800 µg/l), relative risk estimates for bladder cancer mortality of 28.7 for men and 65.4 for women were reported (Table 1) (6,10).

Despite the large magnitude of these associations, the presence of clear dose-response trends (15), and the consistency of the findings among several investigations (7,16–18), the data from Taiwan have been extensively questioned for the following reasons. First, it was suggested that arsenic may not be the sole cause of these cancers. Instead, it was postulated that certain fluorescent humic acidlike substances were responsible for at least some portion of these effects because these substances were also found in high levels in the artesian wells and they could cause a vascular disease similar to BFD (19,20). A second criticism of the Taiwanese findings was that they were not supported by toxicologic tests on animals; despite repeated tests in multiple species at very high doses, animal testing has generally failed to detect carcinogenic effects of inorganic arsenic on the bladder (21). Additionally, the Taiwan findings were criticized for being primarily based on investigations using ecologic study designs in which exposures are based on large group averages rather than on direct individual exposure data.

*Table 1*

*Age Standardized Mortality Rates (per 100,000) and Relative Risk Estimates for Bladder Cancer in a Taiwanese Population Exposed to Arsenic in Drinking Water*

| Exposure group | Mortality rates | | Relative risk estimates | |
|---|---|---|---|---|
| | Male | Female | Male | Female |
| | 3.1 | 1.4 | 1.0 | 1.0 |
| | 15.7 | 16.7 | 5.1 | 11.9 |
| | 37.8 | 35.1 | 12.2 | 25.1 |
| | 89.1 | 91.5 | 28.7 | 65.4 |

[a] Median arsenic well water concentration ($\mu$g/l).
From refs. 6 and 10.

The results of several recent investigations have addressed most of these criticisms and have provided substantial supportive evidence that ingested arsenic does indeed cause bladder cancer (Table 2). Of these studies, the largest are two ecologic mortality studies from South America. In the first one, mortality rates for bladder cancer were found to be twice the national average in two counties of Cordoba, a province in Argentina where a portion of the population was exposed to contaminated well water at average arsenic levels of 178 $\mu$g/l (4). In the second study, bladder cancer mortality was six to eight times higher than national rates in a population of approximately 400,000 in Northern Chile where most people had been exposed to naturally contaminated river water with average arsenic levels around 600 $\mu$g/l (2).

In addition to these large ecologic studies, two cohort investigations have identified associations between ingested arsenic and bladder cancer, although the numbers of cases in both of these studies was small. In Nami-kicho, Nakajo-machi, Japan, an area contaminated by waste water released from a small arsenic trisulfide factory, a cohort study of 113 exposed residents identified three urinary cancer deaths where only 0.1 was expected (3). And in a study of patients treated with Fowler's solution, an arsenical medication used to treat a variety of skin conditions, bladder cancer mortality rates were three times higher than national averages (8).

The results of these four studies resolved several of the controversies surrounding the causal association between arsenic and bladder cancer. First, none of these studies involved areas or conditions where the presence of fluorescent humic substances has been documented. Second, both the Japanese study and Fowler's solution study used retrospective cohort designs where exposures were based on individual rather than grouped data. In addition, at least two studies in which individual exposure data were collected have been performed on residents of the BFD endemic regions of Taiwan (7,15). These studies have

*Table 2*

*Results of Selected Epidemiologic Studies of Ingested Arsenic and Bladder Cancer in Areas Outside of Taiwan*

| Reference | Study design | Study area | No. of cases | Arsenic source | Standardized mortality ratio | 95% confidence interval |
|---|---|---|---|---|---|---|
| Cuzick et al. (8) | | | 5 | Fowler's solution | 3.1 | 1.0–7.3 |
| Tsuda et al. (3)[a] | | | 3 | Well water | 31.2 | 8.6–91.7 |
| Hopenhayn-Rich et al. (4) | | | 158 | Well water | 2.1 (men) | 1.8–2.5 |
| | | | | | 1.8 (women) | 1.2–2.6 |
| Smith et al. (2) | Ecologic | Chile | 157 | River water | 6.0 (men) | 4.8–7.4 |
| | | | | | 8.2 (women) | 6.3–10.5 |

[a] Cases include all urinary tract tumors.

confirmed the associations between ingested arsenic and bladder cancer that were identified by the earlier ecologic analyses. Thus, the possibility of substantial bias due to grouped exposure classification can be excluded.

The other criticism of the Taiwan data was that their results were generally not supported by laboratory animal research. Although it is true that most animal testing has not shown arsenic to be a potent carcinogen, this does not mean that arsenic is not a human carcinogen. Rather, failure to find positive results in most animal testing more likely reflects the variability of arsenic metabolism among different species. In rats for example, arsenic is sequestered in the red blood cells (21), a process that may protect this species from arsenic-induced cancers. Moreover, arsenic is not the only chemical with limited carcinogenic potential in animal testing known to cause bladder cancer in humans. Benzidine, for example, a well-documented and highly potent bladder carcinogen in humans, does not induce bladder cancer in rats or mice (21).

In summary, the human epidemiologic evidence linking ingested arsenic to bladder cancer is extensive, and the controversy surrounding this association has, for the most part, been resolved. What remains controversial, however, is the use of this research in estimating the dose-response relationship of arsenic at low doses and the use of these estimates to establish a new U.S. drinking water standard. These controversies have worldwide implications because many countries base their drinking water standards on U.S. regulations.

## ESTABLISHING A NEW DRINKING WATER STANDARD

The drinking water standard for arsenic of 50 μg/l was first established in 1942 and has remained unchanged (22). At the time the standard was set, little was known about the carcinogenic effects of ingested arsenic. However, as information became available on these effects, amendments to the Safe Drinking Water Act were passed that required that the U.S. Environmental Protection Agency (EPA) revise this standard. Unfortunately, this revision has not been a smooth process, primarily due to the lack of data on the risks associated with low exposures and the high costs that it is claimed would be incurred if the standard is substantially lowered.

Based on several national surveys, the EPA estimated that approximately 350,000 people in this country might drink water containing more than 50 μg/l of arsenic and about 2.5 million people drink water containing more than 25 μg/l (12). As part of its standard setting process,

*Table 3*

Estimated Lifetime Risks of Dying from Cancer due to Exposure to Different Environmental Carcinogens in the United States

| Carcinogen | Risk |
|---|---|
| Environmental tobacco smoke (passive smoking) | |
|   Low exposure (not married to a smoker) | 4/1000 |
|   High exposure (married to a smoker) | 10/1000 |
| Radon in homes | |
|   Average exposure | 3/1000 |
|   High exposure (1–3% of homes) | 20/1000 |
| Arsenic in drinking water | |
|   2.5 μg/l (U.S. estimated average) | 1/1000 |
|   50 μg/l (U.S. water standard) | 21/1000 |

From ref. 10.

the EPA must provide a quantitative estimate of the risk associated with these exposures. As with most environmental carcinogens, the exact risks of ingesting arsenic at low to moderate levels is unknown because most data available on arsenic and cancer have come from populations involving much higher exposures. Although several studies have attempted to assess the cancer risks in populations exposed to low to moderate levels of arsenic, these have usually involved small sample sizes or other study design factors that have limited their ability to identify true effects (23–25) . Therefore, as it has done with many other environmental contaminates, the EPA has extrapolated results from high-dose studies to estimate risks at lower exposures. In the case of ingested arsenic, the EPA used the original Taiwanese study on skin cancer (14), the only data available at the time for quantitative risk assessment, to estimate a lifetime risk of skin cancer of 2.5/1000 at the current drinking water standard (26). It should be noted that this estimate is only for skin cancer. Subsequent risk analyses by other authors using Taiwanese data on internal cancers have produced estimates of lifetime cancer risks on the order of 1/100 for arsenic exposures at 50 μg/l, risks that may be higher than those of environmental tobacco smoke or household radon (Table 3) (9,10).

These risk estimates suggest that a substantial lowering of the current arsenic standard is needed to avoid undue cancer risks. In fact, the World Health Organization (WHO) has already lowered their recommended standard for drinking water arsenic to 10 μg/l (27). Unfortunately, the issuance of a new standard in the United States is being delayed, primarily due to criticisms aimed at the EPA risk assessment process. Specifically, some

authors have suggested that certain potential inaccuracies in this process may have led to an overestimation of the risks of low arsenic exposure. Thus, doubts have surfaced about the need for a strict arsenic standard, and questions have been raised about whether the costs incurred from a stringent drinking water standard might be better spent on other public health issues (28). Although several aspects of the EPA risk assessment process have been questioned, two areas seem to have received the most criticism: The EPA's assumption that cancer risks are linearly associated with arsenic exposure at low doses and the relevance of cancer risks in the Taiwanese to other populations, such as the United States. In the following section, we review the validity of these criticisms and discuss the importance of establishing a new standard despite lingering doubts regarding these issues.

## DOSE-RESPONSE RELATIONSHIP

Perhaps the most criticized aspect of the EPA risk assessment process is the method the EPA used to extrapolate the risks from the high-dose Taiwanese data to lower exposures. That is, it has been argued that the dose-response relationship between ingested arsenic and cancer may not be linear as the EPA has assumed but rather a threshold or sublinear response may exist (19,29,30). If this is the case, the EPA risk assessment would overestimate risks. Currently, the evidence that a threshold or significant sublinear dose-response mechanism exists for ingested arsenic and cancer is limited.

The idea that a threshold exists for the carcinogenic effects of arsenic is most commonly based on the methylation process and the relatively lower toxicities of MMA and DMA compared with inorganic arsenic. Supporters of the threshold hypothesis postulated that for inorganic arsenic to exert a carcinogenic effect, it would have to exceed the level of exposure below which most of the absorbed inorganic arsenic is methylated and thus detoxified. To examine this hypothesis, a comprehensive analysis of all published reports on arsenic methylation compared the results of numerous studies from different populations under a wide variety of exposure conditions (31). On average, the results indicated that regardless of the internal or absorbed dose, the average proportions of inorganic arsenic, MMA, and DMA (around 20%, 15%, and 65%, respectively) remained quite constant across different exposure levels. Even at very low doses, a portion of ingested arsenic remains unmethylated. Subsequent studies on arsenic methylation in exposed and un-

exposed populations in Chile, Finland, Nevada, and Taiwan have confirmed these findings and have provided substantial evidence that a threshold for arsenic methylation does not exist (31–34).

Although the original methylation threshold hypothesis has been refuted, several other issues have been raised that may affect the dose-response relationship between ingested arsenic and cancer. For example, some investigators believe that at high exposures the conversion of MMA to DMA may be inhibited or saturated and that MMA or a reactive intermediate (MMA +3) may be considerably more toxic than DMA (35). If this is true, highly exposed populations, such as those involved in the Taiwanese studies, would not be able to detoxify arsenic as effectively as people with lower exposures, and linear extrapolation from highly exposed populations would overestimate risks. Although some studies have shown that the conversion of MMA to DMA may be inhibited at high exposures (36,37), the association between elevated proportions of MMA and cancer have not been firmly established. Thus, based on our current knowledge, a sublinear dose-response relationship that would significantly affect the EPA risk estimate cannot be substantiated.

In addition to criticisms aimed at the EPA dose-response model, questions have been raised about the comparability of the Taiwanese study population to citizens of the United States and other countries. Although several issues have been raised, such as differences in water consumption patterns and the presence of certain concomitant exposures, most criticisms have been aimed at the dietary patterns of the Taiwanese study populations. Specifically, some authors have hypothesized that the low-protein Taiwanese diet may not have provided adequate methyl sources to detoxify arsenic (19). If this is true, the Taiwanese would be more susceptible to the carcinogenic effects of arsenic, and any risk assessment based on this population would overestimate risks in relatively well-fed groups. In support of this hypothesis, it has been found that animals fed low-protein diets are not able to methylate arsenic as well as those on normal diets (38). To what degree this effect occurs in humans, however, is unknown. Elevated cancer risks have been found in arsenic-exposed populations such as in Cordoba, Argentina, a major beef-producing area, where protein deficiencies are unlikely (4). In addition, Mushak and Crocetti (39) have estimated that less than 1% of the daily intake of dietary methyl donors is required to completely methylate the amount of arsenic ingested by the Taiwanese. Finally, questions have been raised as to whether

the Taiwanese diet is truly low in protein. A subsequent reanalysis of the original Taiwanese dietary data (40) found that although the Taiwanese diet is indeed lower in protein and methyl sources than U.S. averages, these levels are still above recommended daily values (41). Thus, dietary deficiencies may not have altered the susceptibility of the Taiwanese, and the effect of protein intake on arsenic methylation needs to be more thoroughly evaluated before the relevance of the Taiwanese data is discounted on this basis.

In summary, several aspects of the EPA risk assessment process have been questioned, and the use of this information to enact a new drinking water standard in the United States has been criticized. Unfortunately, despite the limited evidence and controversial nature of these criticisms, they have caused a delay in the establishment of a new arsenic standard. Currently, several studies in the United States are underway to investigate the risks of ingested arsenic at doses under 100 µg/l. For example, we are currently performing a case-control study in two areas in the western United States in which large numbers of people have consistently been exposed to drinking water arsenic in the range of 50–100 µg/l. In this study, detailed information on residential history and drinking water sources and information on various potential confounding and susceptibility factors will be collected. Unfortunately, a study such as this takes years to complete. Meanwhile, thousands of people in the United States and elsewhere continue to be exposed to levels of arsenic that may be associated with substantial cancer risks. The WHO has already recognized the potentially large public health impacts of low to moderate doses of ingested arsenic and has recommended a drinking water standard of 10 µg/l. Considering the high cancer risks that are predicted, it would seem prudent for the United States to follow suit and quickly establish a similar standard.

## GENETIC BIOMARKERS AND ARSENIC TOXICOLOGY

Much of the uncertainty in the standard setting process is related to the lack of well-designed epidemiologic studies in populations who drink water with arsenic concentrations in the 20- to 150-µg/l range. In addition, there is a lack of knowledge on the mechanisms by which arsenic exerts its effects and the issue of whether certain individuals or groups may be more susceptible to ingested arsenic than others. Genetic biomarker studies have not

only been useful in establishing the link between ingested arsenic and genetic damage, but they are currently being used to provide information into the mechanistic and susceptibility issues of arsenic carcinogenesis as well.

Several studies have used one particular genetic biomarker, the micronucleus (MN) assay, to establish the association between drinking water arsenic and genetic damage in the bladder. This assay measures the frequency with which chromosomes and chromosomal fragments are lost from the nucleus during cell division. Studies done on arsenic-exposed and unexposed populations in Nevada, Chile, and Mexico have all shown higher prevalence of MN cells in the urine of exposed subjects compared with unexposed subjects (34,42,43). In one study, an increase in MN cells was seen at urinary arsenic levels of 54 µg/l, a level similar to that attained from drinking water containing 50 µg/l. Thus, this study provides evidence that genetic damage occurs at exposures near the current U.S. drinking water standard. Interestingly, this study also found an exposure-dependent increase in MN cells up to urinary arsenic levels of 729 µg/l but not above 729 µg/l (42). The authors of this study postulated that the lack of MN cells at the highest exposure levels may have been due to the cytotoxic or cytostatic effects of arsenic at very high doses.

To further investigate the relationship between arsenic ingestion and MN cells, an intervention study was performed in which the prevalence of these cells in a group of highly exposed Chilean men were measured before and after these men were supplied with water low in arsenic (44). After 8 weeks of drinking low-arsenic water, the prevalence of MN cells fell from 2.63 to 1.79 per 1000 cells, adding further evidence that ingested arsenic causes genetic injury to the bladder. Despite these findings, it should be noted that the relevance of the MN cell biomarker to cancer, as with many genetic biomarkers, remains to be elucidated.

Other genetic biomarkers are currently being used to develop insight into the actual mechanisms by which arsenic exerts its effects and some of the factors that may determine individual susceptibility to arsenic. For example, we are currently conducting a bladder cancer case-control study in Argentina in which we are collecting oral epithelial cells from cases and controls as a source of DNA for genotype analysis. Two metabolic enzymes, glutathione S-transferase µ (GSTM1) and glutathione S-transferase θ (GSTT1) are important in cancer susceptibility because they may regulate an individual's ability to methylate arsenic (45–47). Carriers of homozygous deletions in these genes (null genotypes) have an absence

of this enzyme activity and may be more susceptible to potential carcinogens. Hence, the results of genetic susceptibility analysis can be used to determine if GST genotype influences methylation capabilities and susceptibility to the genotoxic effects of arsenic.

In addition to the GST genotype analysis of oral epithelial cells, we are also collecting biopsies from bladder tumors and will compare the tumors from patients with a history of arsenic exposure to the tumors from patients who do not have a history of arsenic exposure. Tumors from exposed and unexposed cases will be compared biologically, histologically, and genetically to gain insight into the causal pathway of arsenic-induced carcinogenesis. For example, comparative genomic hybridization (CGH) will be used to screen for genetic aberrations throughout the genome of these tumors. This method is based on a competitive hybridization of differentially labeled tumor and normal DNA to normal metaphase chromosomes and reveals deletions and gains of DNA sequences in the tumor genome. CGH has previously been used to survey tumor DNA for gains and losses of chromosomes associated with specific types of cancer but has never been used to examine the same types of tumors of different etiologies. In this study, by comparing the DNA of arsenic-exposed and unexposed tumors, gross changes related to arsenic exposure may be found. For example, chromosomal areas that show losses could be areas where tumor suppressor genes involved in arsenic-induced carcinogenesis may lie. Similarly, oncogenes that are amplified by arsenic exposure may be located within areas showing gains. Demonstrating a difference in the distribution of genetic events between arsenic-exposed and unexposed tumors would illustrate that these two groups of bladder tumors arise from distinct etiologies.

The MN assay and CGH are just a few of the genetic biomarkers that are being used in the study of arsenic toxicology. Use of the MN assay has provided evidence that genetic damage occurs in the bladder at arsenic exposures near the current U.S. standard. Incorporating these and other genetic biomarkers into current and future research should provide insight into the mechanism by which arsenic causes cancer and the factors that determine individual susceptibility.

## CONCLUSION

Millions of people throughout the world are drinking water containing inorganic arsenic. Although initially controversial, the association between high exposures to ingested arsenic and bladder cancer is now well established. Unfortunately, the dose-response relationship, especially at low to moderate doses such as those found in the United States, remains unclear. Attempts to define these risks and establish new drinking water regulations have been and remain controversial, primarily due to questions regarding the risk assessment process used to establish these standards. Epidemiologic studies involving low to moderate dose exposures should help to more precisely define these risks and aid in the establishment of appropriate drinking water regulations. In addition, genetic biomarker studies may provide information on the mechanistic and susceptibility issues of arsenic-induced carcinogenesis and thus may also help elucidate dose-response relationships at low doses. However, until a new arsenic drinking water standard is implemented, most evidence suggests that populations who are currently exposed to arsenic in their drinking water will continue to have substantially elevated cancer risks. Waiting for more precise data before a new standard is applied will only prolong these risks. Therefore, until further research can be completed, an interim drinking water arsenic standard similar to the WHO recommendation of 10 µg/l may be appropriate.

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